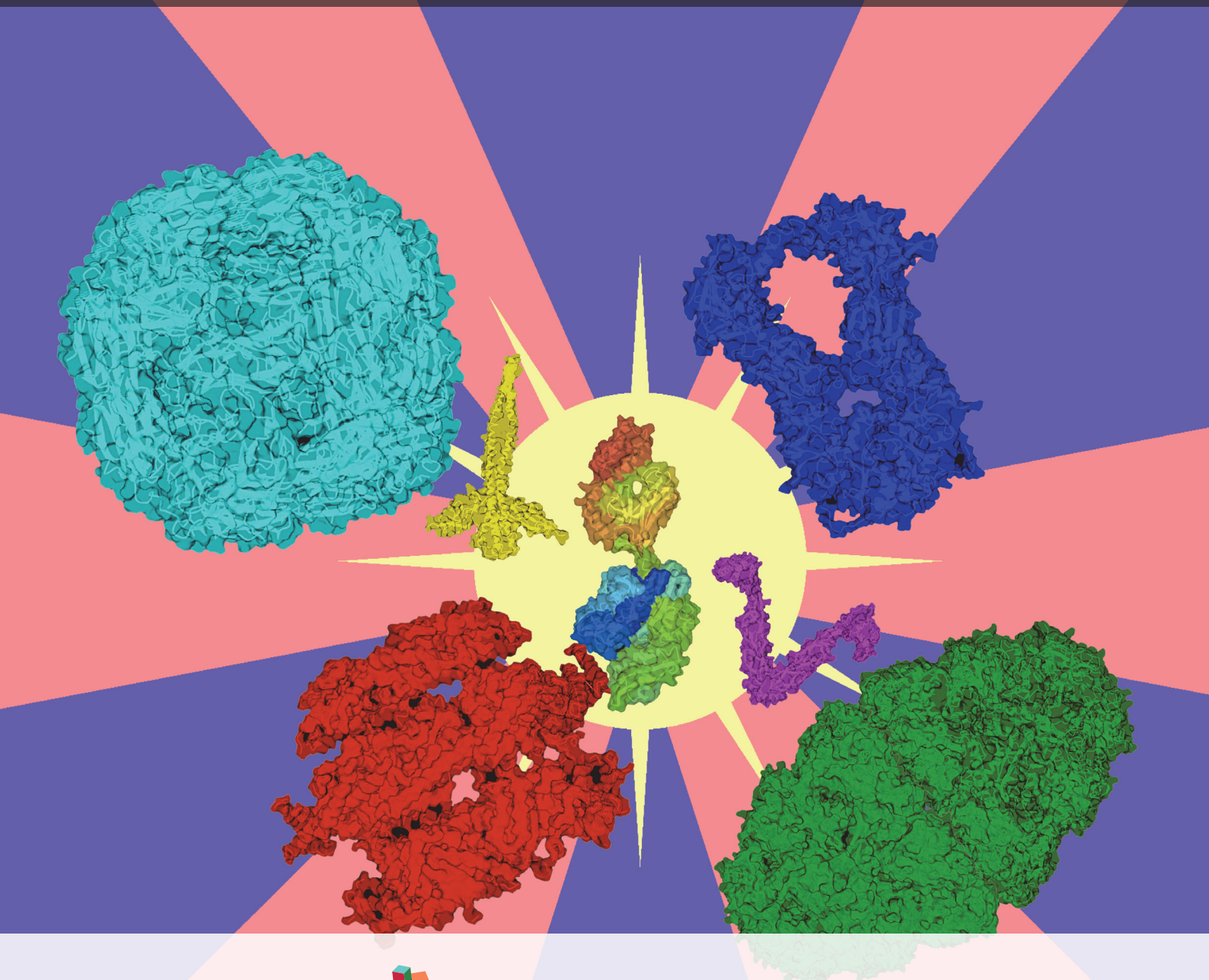
THE HSP70 MOLECULAR CHAPERONE MACHINES

EDITED BY: Matthias P. Mayer and Pierre Goloubinoff
PUBLISHED IN: Frontiers in Molecular Biosciences



frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88945-125-8
DOI 10.3389/978-2-88945-125-8

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

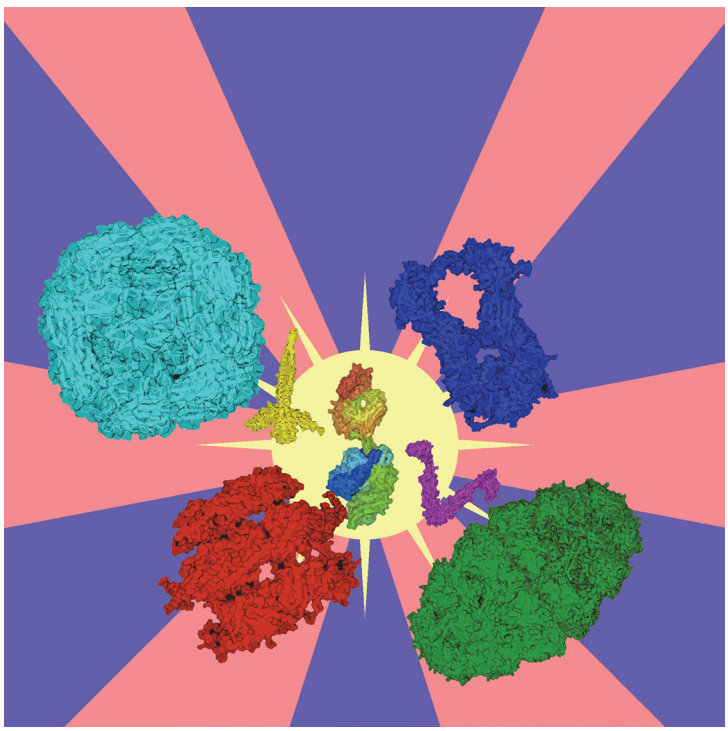
Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view.
By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: **researchtopics@frontiersin.org**

THE HSP70 MOLECULAR CHAPERONE MACHINES

Topic Editors:
Matthias P. Mayer, Ruprecht-Karls-Universität Heidelberg, Germany
Pierre Goloubinoff, University of Lausanne, Switzerland



HSP70 is a central hub of the chaperone network (center). It uses the energy of ATP-hydrolysis to modify the conformation and the function of various polypeptides. It is targeted onto its misfolded or alternatively folded substrates by J-domain co-chaperones (HSP40s, magenta). It is dissociated from its modified polypeptide substrates by ADP to ATP exchange, which is accelerated by ADP-release factors (GrpE, yellow, in prokaryotes and mitochondria and plastides of eukaryotic cells; HspBP1-family proteins, Bag-domain proteins, and Hsp110/Hsp170 proteins in cytosol and endoplasmic reticulum of eukaryotic cells). Small HSPs (cyan) and HSP90s (blue) can maintain various misfolded polypeptides in a nonaggregated conformation poised to be further processed by HSP70. Upon activation by HSP70, the AAA+ disaggregases (HSP104, ClpB, red) can use ATP to forcefully solubilize stable protein aggregates and, together with HSP70, revert them into the native functional state. Some proteins are handed over to GroEL/GroES (green) or Hsp90 (blue) for further maturation or to be kept inactive until activation by cellular signals.
Cover figure by Pierre Goloubinoff

Members of the HSP70 family form a central hub of the molecular chaperone network, controlling protein homeostasis in prokaryotes and in the ATP-containing compartments of the eukaryotic cells. The heat-inducible form HSPA1A (HSP70), its constitutive cytosolic cognate HSPA8 (Hsc70), its endoplasmic reticulum form HSPA5 (BiP), and its mitochondrial form HSPA9 (Mortalin), as well as the more distantly related HSPHs (HSP110s), make up 1-2 % of the total mass of proteins in human cells. They use the energy of ATP-hydrolysis to prevent and forcefully revert the process of protein misfolding and aggregation during and following various stresses, presumably by working as unfoldases to lift aberrant conformers out of kinetic traps. As such, HSP70s, in cooperation with their J-domain co-chaperones and nucleotide exchange factors (NEFs) and co-disaggregases, form an efficient network of cellular defenses against the accumulation of cytotoxic misfolded protein conformers, which may cause degenerative diseases such as Parkinson's and Alzheimer's disease, diabetes, and aging in general. In addition to their function in repair of stress-induced damage, HSP70s fulfill many housekeeping functions, including assisting the de novo folding and maturation of proteins, driving the translocation of protein precursors across narrow membrane pores into organelles, and by controlling the oligomeric state of key regulator protein complexes involved in signal transduction and vesicular trafficking. For reasons not well understood, HSP70s are also found on the surface of some animal cells, in particular cancer cells where they may serve as specific targets for cancer immunotherapy. Here, we gathered seven mini reviews, each presenting a complementary aspect of HSP70's structure and function in bacteria and eukaryotes, under physiological and stressful conditions. These articles highlight how, the various members of this conserved family of molecular chaperones, assisted by their various J-domain and NEF cochaperones and co-disaggregases, harness ATP hydrolysis to perform a great diversity of life-sustaining cellular functions using a similar molecular mechanism.

Citation: Mayer, M. P., Goloubinoff, P., eds. (2017). The HSP70 Molecular Chaperone Machines. Lausanne: Frontiers Media. doi: 10.3389/978-2-88945-125-8

Table of Contents

frontiers in Molecular Biosciences

EDITORIAL
published: 24 January 2017
doi: 10.3389/fmolb.2017.00001

Editorial: The HSP70 Molecular Chaperone Machines

Pierre Goloubinoff *

Department of Plant Molecular Biology, Faculty of Biology and Medicine, University of Lausanne, Lausanne, Switzerland

Keywords: unfoldase, disaggregase, HSC70, J- domain cochaperones, nucleotide exchanges factors, Hsp110s, protein misfolding, protein aggregation

Editorial on the Research Topic

The HSP70 Molecular Chaperone Machines

The HSP70s belong to a small family of highly conserved ~70 kDa enzymes that can use the energy of ATP-hydrolysis to modify the structure, and consequently the function, of specific native proteins, and to unfold, solubilize, and thereby reduce the cellular concentration of harmful misfolded proteins (Finka et al., 2016). Particular HSP70s are expressed constitutively in the cytosol of bacteria and in all the ATP-containing compartments of eukaryotic cells. In unstressed bacteria, plant, and animal cells, HSP70s are 0.5–2% of the total protein mass (Finka and Goloubinoff, 2013). They form the central hub of the chaperone network that controls all aspects of cellular protein homeostasis: protein *de novo* synthesis, protein translocation across membranes, native folding, and oligomer assembly. HSP70s also participate in the active removal of toxic protein aggregates by actively converting them into harmless degraded peptides, or into natively refolded functional proteins (Calamini and Morimoto, 2012; Finka et al., 2016).

In immortalized human cancer cells and in naïve rat livers cells for example, HSPA8 is the major HSP70 species that is constitutively expressed in the cytosol, accounting for about half of the total mass of the HSP70s (Finka and Goloubinoff, 2013). In heat-shocked cells, particular HSP70s accumulate. Hence, following a 4 h mild fever-like heat-shock at 41°C, the total mass of the HSP70s in Jurkat cells increases 1.6-folds, from ~0.7% (at 37°C) to ~1.1% (Finka et al., 2015). Thus, although sharing 90% sequence identity with HSPA8, the cytosolic HSPA1A, which generally remains undetected in unstressed tissues, strongly accumulates during various abiotic stresses (Finka et al., 2015). Noticeably, non-heat-shocked cancer cells generally express constitutively abnormally elevated levels of HSPA1A that may even exceed the naturally high amounts of HSPA8. High expression levels of HSPA1A in otherwise unstressed tissues is thus a hallmark of malignancy and of poor survival outcome (Feder et al., 1992; Finka and Goloubinoff, 2013; Yang et al., 2015).

The *endoplasmic reticulum* HSP70, HSPA5 (called BIP), and the mitochondrial HSP70, HSPA9 (called mortalin) are the next most abundant HSP70 species. As in the case of cytosolic HSPA8, their concentration may also increase in response to heat-shock (Finka et al., 2015). In addition to their function in protein quality control, they act as pulling motors that import cytosolic polypeptides into their respective organelles.

Assisted by over 30 different J-domain cochaperones in eukaryotes (Dekker et al.) and various nucleotide exchange factors (NEFs) (Bracher and Verghese), the HSP70s can control a great number of housekeeping cellular processes. They can use the energy of ATP-hydrolysis to convert active (alter)native protein complexes into differently active native polypeptides. Thus,

Abbreviations: SBD, Substrate binding domain; NBD, Nucleotide binding domain; NEF, Nucleotide Exchange Factor; Natively folded proteins, proteins folded in a functional native conformation; (Alter)natively folded proteins, proteins folded in a different, albeit still native conformation.

OPEN ACCESS

Edited by:
Anat Ben-Zvi,
Ben-Gurion University of the Negev, Israel

Reviewed by:
Martin Lothar Duennwald,
University of Western Ontario, Canada

***Correspondence:**
Pierre Goloubinoff
pierre.goloubinoff@unil.ch

Specialty section:
This article was submitted to Protein Folding, Misfolding and Degradation, a section of the journal Frontiers in Molecular Biosciences

Received: *14 December 2016*
Accepted: *05 January 2017*
Published: *24 January 2017*

Citation:
Goloubinoff P (2017) Editorial: The HSP70 Molecular Chaperone Machines. Front. Mol. Biosci. 4:1. doi: 10.3389/fmolb.2017.00001

at the exit of ribosomes, specific HSP70s can pull and regulate the *de-novo* folding of nascent polypeptides. Others can pull and unfold cytosolic polypeptides necessary for their translocation across membranes into organelles. Yet other HSPAs can regulate the post-translocation folding and assembly of (alter)natively folded protein complexes and coordinate their timely disassembly into differently active sub-complexes, as in the case of clathrin cages and triskalions (Sousa and Lafer; De Los Rios and Goloubinoff, 2016). HSP70s can also determine the lifespan of native cellular proteins by timely targeting them to chaperone-gated proteases, as in the case of the bacterial transcription factor σ32, which is targeted by DnaK to the FtsH protease (Rodriguez et al., 2008; VanPelt and Page, 2017). HSP70s also target misfolded proteins to the lysosome in a process called chaperone-mediated-autophagy (Cuervo and Wong, 2014).

Some HSP70s can act as ATP-fuelled molecular motors that, together with specific cochaperones, such as their eukaryotic HSP110 cognates (Mattoo et al., 2013; Nillegoda and Bukau) or the ClpB/HSP104s, which are non-cognate AAA+ ATPases (Mogk et al., 1999), can solubilize stable aggregates and convert them into harmless natively refolded or degraded polypeptides. Thus, together, the HSP70s form a powerful cellular defense network against harmful misfolded proteins. HSP70s failure to be expressed, or to function in aging metazoan cells, directly correlates with the late age onset of severe human degenerative diseases, such as Parkinson, Alzheimer, and type 2 diabetes that are associated to the accumulation of misfolded proteins in the damaged tissues (Hartl, 2016).

In this research topic, several reviews tackled from different complementing angles the central question: how may the members of a small class of relatively simple two-domain 70 kDa molecules use ATP to drive dramatic conformational changes, in so diverse aggregated, misfolded, and alternatively folded polypeptides, and thus control such a great number of different housekeeping and stress related biological functions in the cell?

In their review "Insight into the molecular mechanism of allostery in HSP70s," Mayer and Kityk described the intricate molecular paths of various allosteric signals during the HSP70 ATPase cycle, leading to dramatic movements of the two subdomains that bind the polypeptide substrates. They focused on specific residues that are involved in various key allosteric signals in the chaperone molecule, such as the signal that is issued by the substrate upon binding to the base of the substrate binding domain (SBD) to induce ATP hydrolysis in the nucleotide binding domain (NBD). Another signal is issued by the NBD to induce the closure of the SBD's lid onto its base, and yet another signal that is issued by the NEF-triggered NBD to induce the sequential release of the ADP from the NBD and of the structurally modified polypeptide from the SBD.

In their review "Multi-layered molecular mechanisms of polypeptide holding, unfolding and disaggregation by HSP70/HSP110chaperones" Finka et al. addressed the apparent consequences of the coordinated subdomain movements in the HSP70 molecule during the ATPase cycle, on the bound misfolded or (alter)natively folded polypeptide substrates, leading to their conversion into native or degraded polypeptides. Two complementing mechanisms are being discussed: (1) unfolding by direct clamping, in which the closure of the SBD lid onto a bulky misfolded protein segment and its consequent squeezing against the SBD base would result in the local unfolding of the bound misfolded segment. (2) Unfolding by entropic pulling, in which the distal binding of several HSP70 molecules onto the same misfolded polypeptide, and their ensuing independent dangling motions would generate a force capable of unfolding by entropic pulling the misfolded segments that are in between two HSP70-bound sites (Goloubinoff and De Los Rios, 2007).

The various allosteric paths leading to sub-domain movements and their possible effects on a bound misfolded substrate can be summarized as follow: In an ATP-bound HSP70, there is a tight crosstalk between a J-domain from a DNAJ co-chaperone anchored in the NBD, and a hydrophobic misfolded polypeptide substrate newly bound to the wide-open SBD. Both then concomitantly trigger ATP hydrolysis in the NBD and the closing of the SBD's lid that applies a local pressure onto a bulky misfolded segment in the bound substrate leading, upon tight engulfment by the SBD, to the extensive unfolding of the substrate (Sharma et al., 2010; Mashaghi et al., 2016). The subsequent transient binding of a NEF to the distal lobes of the NBD then triggers ADP release from the NBD and the opening of the protein-binding lid, allowing the newly unfolded polypeptide to dissociate and spontaneously refold to the native state.

However effective an unfolding nanomachine may the HSP70 molecule be, it remains quiet ineffective at choosing its substrates among the many different polypeptides in need to be structurally modified. Substrate discrimination by the HSP70 system is predominantly achieved by the J-domain co-chaperones that initially interact with the different substrates and at the same time bind the HSP70s through their J-domain.

In their review "DNAJs: more than substrate delivery to HSPA," Dekker et al. highlighted the crucial role of J-domain cochaperones to the protein quality control of metazoan cells. DNAJs can target HSP70s onto misfolded, aggregated, or specific alternatively folded protein substrates, to be unfolded, refolded, or ultimately degraded by chaperone-gated proteases. In addition, specific DNAJs are involved in the unidirectional translocation of cytosolic pre-proteins across membranes to the ER, the mitochondria, the lysosome, and in plants also the chloroplast and the peroxisome. Other DNAJs carry unique anti-aggregation functions of their own, as in the case of mammalian DNAJB6, which is a very potent anti-aggregation chaperone that prevents the formation of toxic polyglutamine species that cause severe degenerative diseases, such as Huntington's chorea.

In their review, "Metazoan HSP70-based protein disaggregases: emergence and mechanisms," Nillegoda and Bukau further dwelt on the key role of J-domain cochaperones of HSP70, in particular the human DNAJA2 and DNAJB1, which seem to specifically recruit HSC70 and its sequence-related HSP110 co-chaperone, to form powerful disaggregating hetero-oligomers capable of solubilizing denatured reporter proteins, among them, stable alfa-synuclein fibrils (Gao et al., 2015; Finka et al., 2016). Two putative "clamp and walk" and

"metazoan nucleation" models are discussed for the HSP70- and HSP110-mediated disaggregation mechanism.

In contrast to misfolded protein aggregates serving as substrates to the HSC70-HSP110 disaggregation machinery, in their review "The role of molecular chaperones in clathrin mediated vesicular trafficking," Sousa and Lafer discuss the role of a specific J-domain cochaperone, auxilin, in the recruitment of HSC70 and HSP110 to a specific position onto a natively assembled protein complex: the clathrin cage. There, HSC70 uses ATP to pull and disassemble clathrin coats by a "steric wedge" mechanism, which is very similar to entropic pulling. Recently, the same authors brought evidence that loose HSC70 multimers might form and thus join their "steric wedge" entropic pulling forces to disassemble clathrin cages more efficiency. HSC70 also sequesters the depolymerized clathrin, implying that triskelion release must be regulated by HSP110 (Sousa et al., 2016). Interestingly, in neurons, HSP110 activity as a specific NEF for HSC70 is regulated upon phosphorylation by a Calcium-regulated kinase (Ishihara et al., 2003). Thus, following the vesicle fusion with the plasma membrane and the discharge of their neuro-transmitters content in the synapse, the transient entry of Ca^{2+} ions in the cytosol would be able to precisely signal the onset of clathrin cage dismantling for triskelion recycling.

In their review "The nucleotide exchange factors of HSP70 molecular chaperones" (Bracher and Verghese), Bracher and Verghese discussed the large diversity of the eukaryotic NEFs. In addition to the HSP110s, which are sequence wise-, structure wise-, and function wise-related to the HSP70s (Mattoo et al., 2013), there are unrelated HSPBP1/Sil1, BAG domain proteins and the M-domain of the HSP104 disaggregase cochaperone (Carroni et al., 2014; Doyle et al., 2015), and the GrpE type of NEFs to be found in prokaryotes and eukaryotic organelles of eubacteria origin. Despite their great disparity of structure, the various NEFs, interact with the same surfaces on the HSP70s: the tips of the two NBD subdomains embracing the ADP-binding pocket.

It should be noted that BAG domain proteins and GrpE do not have nucleotide binding sites. Moreover, both GrpE and HSP110 were shown to induce polypeptide release, respectively, from DnaK and HSP70, in the total absence of ATP (Sharma et al., 2010; Mattoo et al., 2013). Thus, these versatile regulatory co-chaperones should be called ADP- and substrate-release factors rather than, nucleotide exchange factors.

Within cells, HSP70s can use ATP to drive structural changes in other cellular proteins. Yet, extracellular HSP70 is also to be found at the surface of cells facing the ATP-less outward milieu. In mammals extracellular HSP70 can stimulate innate immune responses and facilitate the cross-presentation of immunogenic peptides, in association with major histocompatibility complex (MHC) antigens (Shevtsov and Multhoff, 2016).

Providing biological context to this research topic, Przyborski et al. (Bracher and Verghese) discussed in their review "Plasmodial HSP70s are functionally adapted to the malaria parasite life cycle" the role HSP70 in a severe human disease: malaria. The parasite *Plasmodium falciparum* encodes for half a dozen different HSP70s, which are key to the survival and the pathology of the parasite in its various stages of its development, in particular for its resistance to high temperatures episodes during human fever, as well as of low ambient temperature when dwelling in the salivary glands of the mosquitos.

In conclusion, whereas the lack of an appropriate level of HSP70 expression in metazoan neural and muscle tissues, without stress and following biotic and abiotic stresses, has been associated to the onset of excessive damaging apoptosis, leading to degenerative tissue loss and accelerated aging (Kimura et al., 2007; Ben-Zvi et al., 2009; Morimoto and Cuervo, 2014), excessive accumulation of HSP70s has been linked to therapeutic resistance of carcinomas, increased metastasis, and poor survival outcomes to anti-cancer therapies (Sherman and Gabai, 2015; Figure 1). Yet, compared to terminally differentiated cells, rapidly growing embryonic cells that are not cancerous, also express high HSP70 and HSP90 levels (Jensen et al., 2013). Thus, the effectiveness of the various HSP70s at maintaining cellular protein homeostasis and controlling life span likely depends on yet to be characterized, fine-tuned qualitative differences in the expression and activation levels of the various HSP70s and their many J-domain, NEF, and disaggregase cochaperones.

AUTHOR CONTRIBUTIONS

The manuscript was written solely by PG.

FUNDING

Research is supported by Grant 31003A_156948 from the Swiss National Fund.

REFERENCES

Ben-Zvi, A., Miller, E. A., and Morimoto, R. I. (2009). Collapse of proteostasis represents an early molecular event in *Caenorhabditis elegans* aging. *Proc. Natl. Acad. Sci. U.S.A.* 106, 14914–14919. doi: 10.1073/pnas.0902882106

Calamini, B., and Morimoto, R. I. (2012). Protein homeostasis as a therapeutic target for diseases of protein conformation. *Curr. Top. Med. Chem.* 12, 2623–2640. doi: 10.2174/1568026611212220014

Carroni, M., Kummer, E., Oguchi, Y., Wendler, P., Clare, D. K., Sinning, I., et al. (2014). Head-to-tail interactions of the coiled-coil domains regulate ClpB activity and cooperation with Hsp70 in protein disaggregation. *Elife* 3:e02481. doi: 10.7554/eLife.02481

Cuervo, A. M., and Wong, E. (2014). Chaperone-mediated autophagy: roles in disease and aging. *Cell Res.* 24, 92–104. doi: 10.1038/cr.2013.153

De Los Rios, P., and Goloubinoff, P. (2016). Hsp70 chaperones use ATP to remodel native protein oligomers and stable aggregates by entropic pulling. *Nat. Struct. Mol. Biol.* 23, 766–769. doi: 10.1038/nsmb.3283

Doyle, S. M., Shastry, S., Kravats, A. N., Shih, Y. H., Miot, M., Hoskins, J. R., et al. (2015). Interplay between *E. coli* DnaK, ClpB and GrpE during protein disaggregation. *J. Mol. Biol.* 427, 312–327. doi: 10.1016/j.jmb.2014.10.013

Feder, J. H., Rossi, J. M., Solomon, J., Solomon, N., and Lindquist, S. (1992). The consequences of expressing hsp70 in Drosophila cells at normal temperatures. *Genes Dev.* 6, 1402–1413. doi: 10.1101/gad.6.8.1402

Finka, A., and Goloubinoff, P. (2013). Proteomic data from human cell cultures refine mechanisms of chaperone-mediated protein homeostasis. *Cell Stress Chaperones* 18, 591–605. doi: 10.1007/s12192-013-0413-3

Finka, A., Mattoo, R. U., and Goloubinoff, P. (2016). Experimental milestones in the discovery of molecular chaperones as polypeptide unfolding enzymes.

Annu. Rev. Biochem. 85, 715–742. doi: 10.1146/annurev-biochem-060815-014124

Finka, A., Sood, V., Quadroni, M., Rios Pde, L., and Goloubinoff, P. (2015). Quantitative proteomics of heat-treated human cells show an across-the-board mild depletion of housekeeping proteins to massively accumulate few HSPs. *Cell Stress Chaperones* 20, 605–620. doi: 10.1007/s12192-015-0583-2

Gao, X., Carroni, M., Nussbaum-Krammer, C., Mogk, A., Nillegoda, N. B., Szlachcic, A., et al. (2015). Human Hsp70 disaggregase reverses Parkinson's-linked alpha-synuclein amyloid fibrils. *Mol. Cell* 59, 781–793. doi: 10.1016/j.molcel.2015.07.012

Goloubinoff, P., and De Los Rios, P. (2007). The mechanism of Hsp70 chaperones: (entropic) pulling the models together. *Trends Biochem. Sci.* 32, 372–380. doi: 10.1016/j.tibs.2007.06.008

Hartl, F. U. (2016). Cellular homeostasis and aging. *Annu. Rev. Biochem.* 85, 1–4. doi: 10.1146/annurev-biochem-011116-110806

Ishihara, K., Yamagishi, N., and Hatayama, T. (2003). Protein kinase CK2 phosphorylates Hsp105 alpha at Ser509 and modulates its function. *Biochem. J.* 371(Pt 3), 917–925. doi: 10.1042/bj20021331

Jensen, P. L., Beck, H. C., Petersen, J., Hreinsson, J., Wånggren, K., Laursen, S. B., et al. (2013). Proteomic analysis of human blastocoel fluid and blastocyst cells. *Stem Cells Dev.* 22, 1126–1135. doi: 10.1089/scd.2012.0239

Kimura, K., Tanaka, N., Nakamura, N., Takano, S., and Ohkuma, S. (2007). Knockdown of mitochondrial heat shock protein 70 promotes progeria-like phenotypes in caenorhabditis elegans. *J. Biol. Chem.* 282, 5910–5918. doi: 10.1074/jbc.M609025200

Mashaghi, A., Bezrukavnikov, S., Minde, D. P., Wentink, A. S., Kityk, R., Zachmann-Brand, B., et al. (2016). Alternative modes of client binding enable functional plasticity of Hsp70. *Nature* 539, 448–451. doi: 10.1038/nature20137

Mattoo, R. U., Sharma, S. K., Priya, S., Finka, A., and Goloubinoff, P. (2013). Hsp110 is a bona fide chaperone using ATP to unfold stable misfolded polypeptides and reciprocally collaborate with Hsp70 to solubilize protein aggregates. *J. Biol. Chem.* 288, 21399–21411. doi: 10.1074/jbc.M113.479253

Mogk, A., Tomoyasu, T., Goloubinoff, P., Rüdiger, S., Röder, D., Langen, H., et al. (1999). Identification of thermolabile *Escherichia coli* proteins: prevention and reversion of aggregation by DnaK and ClpB. *EMBO J.* 18, 6934–6949. doi: 10.1093/emboj/18.24.6934

Morimoto, R. I., and Cuervo, A. M. (2014). Proteostasis and the aging proteome in health and disease. *J. Gerontol. A Biol. Sci. Med. Sci.* 69(Suppl. 1), S33–S38. doi: 10.1093/gerona/glu049

Rodriguez, F., Arsène-Ploetze, F., Rist, W., Rudiger, S., Schneider-Mergener, J., Mayer, M. P., et al. (2008). Molecular basis for regulation of the heat shock transcription factor σ32 by the DnaK and DnaJ chaperones. *Mol. Cell* 32, 347–358. doi: 10.1016/j.molcel.2008.09.016

Sharma, S. K., De los Rios, P., Christen, P., Lustig, A., and Goloubinoff, P. (2010). The kinetic parameters and energy cost of the Hsp70 chaperone as a polypeptide unfoldase. *Nat. Chem. Biol.* 6, 914–920. doi: 10.1038/nchembio.455

Sherman, M. Y., and Gabai, V. L. (2015). Hsp70 in cancer: back to the future. *Oncogene* 34, 4153–4161. doi: 10.1038/onc.2014.349

Shevtsov, M., and Multhoff, G. (2016). Heat shock protein-peptide and Hsp-based immunotherapies for the treatment of cancer. *Front. Immunol.* 7:171. doi: 10.3389/fimmu.2016.00171

Sousa, R., Liao, H. S., Cuéllar, J., Jin, S., Valpuesta, J. M., Jin, A. J., et al. (2016). Clathrin-coat disassembly illuminates the mechanisms of Hsp70 force generation. *Nat. Struct. Mol. Biol.* 23, 821–829. doi: 10.1038/nsmb.3272

VanPelt, J., and Page, R. C. (2017). Unraveling the CHIP:Hsp70 complex as an information processor for protein quality control. *Biochim. Biophys. Acta* 1865, 133–141. doi: 10.1016/j.bbapap.2016.11.005

Yang, Z., Zhuang, L., Szatmary, P., Wen, L., Sun, H., Lu, Y., et al. (2015). Upregulation of heat shock proteins (HSPA12A, HSP90B1, HSPA4, HSPA5 and HSPA6) in tumour tissues is associated with poor outcomes from HBV-related early-stage hepatocellular carcinoma. *Int. J. Med. Sci.* 12, 256–263. doi: 10.7150/ijms.10735

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Molecular Biosciences

MINI REVIEW
published: 20 October 2015
doi: 10.3389/fmolb.2015.00058

Insights into the molecular mechanism of allostery in Hsp70s

Matthias P. Mayer and Roman Kityk*

Zentrum für Molekulare Biologie der Universität Heidelberg (ZMBH), DKFZ/ZMBH Alliance, Ruprecht-Karls-Universität Heidelberg, Heidelberg, Germany

Hsp70s chaperone an amazing number and variety of cellular protein folding processes. Key to their versatility is the recognition of a short degenerate sequence motif, present in practically all polypeptides, and a bidirectional allosteric intramolecular regulation mechanism linking their N-terminal nucleotide binding domain (NBD) and their C-terminal polypeptide substrate binding domain (SBD). Through this interdomain communication ATP binding to the NBD and ATP hydrolysis control the affinity of the SBD for polypeptide substrates and substrate binding to the SBD triggers ATP hydrolysis. Genetic screens for defective variants of Hsp70s and systematic analysis of available structures of the isolated domains revealed some residues involved in allosteric control. Recent elucidation of the crystal structure of the Hsp70 homolog DnaK in the ATP bound open conformation as well as numerous NMR and mutagenesis studies bring us closer to an understanding of the communication between NBD and SBD. In this review we will discuss our current view of the allosteric control mechanism of Hsp70 chaperones.

Keywords: Hsp70 heat-shock proteins, allostery, interdomain communication, conformational dynamics, structure-function relationships

OPEN ACCESS

Edited by:
Paolo De Los Rios, Ecole Polytechnique Fédérale de Lausanne, Switzerland

Reviewed by:
Dejana Mokranjac, University of Munich, Germany
Eileen M. Lafer, University of Texas Health Science Center at San Antonio, USA

****Correspondence:***
Matthias P. Mayer
m.mayer@zmbh.uni-heidelberg.de

Specialty section:
This article was submitted to Protein Folding, Misfolding and Degradation, a section of the journal Frontiers in Molecular Biosciences

Received: *20 August 2015*
Accepted: *05 October 2015*
Published: *20 October 2015*

Citation:
Mayer MP and Kityk R (2015) Insights into the molecular mechanism of allostery in Hsp70s. Front. Mol. Biosci. 2:58. doi: 10.3389/fmolb.2015.00058

INTRODUCTION

Hsp70s are involved in a large variety of cellular processes. Thereby they interact with substrate proteins that are in many different conformations: with completely extended polypeptides such as nascent chains at the ribosome (Deuerling and Bukau, 2004; Hartl et al., 2011) or during translocation into organelles (Neupert and Herrmann, 2007; Chacinska et al., 2009); with partially folded and misfolded conformations in late folding intermediates, or upon disaggregation and refolding of stress denatured proteins (Tyedmers et al., 2010); and with native regulatory proteins to control their activity and stability (e.g., heat shock transcription factor σ^{32} in *E. coli* or transcription factors, receptors, and kinases in eukaryotes) (Wegele et al., 2004), and while assisting oligomerization or disassembly of oligomeric structures (e.g., clathrin, Sousa and Lafer, 2015). Hsp70s are ATP dependent chaperones that consist of an N-terminal 45 kDa nucleotide binding domain (NBD) and a 25 kDa substrate polypeptide binding domain (SBD). They do not work alone but interact with cochaperones of the J-domain protein (DnaJ, Hsp40) family, which target Hsp70s to substrate proteins, and several families of nucleotide exchange factors. Hsp70s also cooperate with chaperones of other families like small HSPs and Hsp100s for protein disaggregation, with Hsp90 for regulation of native proteins, with ribosome bound chaperones like trigger factor in prokaryotes and specialized Hsp70s (RAC) in eukaryotes and with Hsp60s for *de novo* folding of proteins. Thus, Hsp70 is probably the most versatile of all chaperones, constituting a central hub of the cellular protein folding network.

One reason for this versatility is most likely the degenerate recognition motif of Hsp70s, which consists of a core of five preferentially hydrophobic amino acids flanked by regions in which positively charged residues are favorable for binding (Rüdiger et al., 1997). Such motifs occur frequently in most proteins. In the folded state these motifs are generally found in the hydrophobic core of the proteins and are exposed only during synthesis when emerging from the ribosomal exit tunnel, during translocation through membranes or during stress denaturation. This explains why Hsp70s interact with most proteins when they are in the denatured but not in the native state. Substrate proteins which interact with Hsp70 in their native conformation apparently expose such sequence motifs even in the completely folded state. Recognition of a short degenerative motif in substrate proteins eliminates any size limitations and Hsp70 can interact with very large proteins and protein complexes, like clathrin cages, or protein aggregates. Another reason for the versatility of the Hsp70 system is certainly the number of J-domain proteins which has increased in the course of evolution from six in *E. coli* and 22 in *S. cerevisiae* to 47 in humans (Kampinga and Craig, 2010). J-domain proteins either bind prospective protein substrates themselves or bind to structures like the ribosomal exit tunnel (e.g., zuotin) or translocation pores (e.g., Sec63, Pam18) where substrates for Hsp70 emerge, and recruit Hsp70 for specific protein folding tasks. Similarly, the different nucleotide exchange factors of three distinct families in the eukaryotic cytosol—the modular multidomain Bag family, the HspBP1 family and the Hsp110 family, which are Hsp70 homologs themselves (Bracher and Verghese, 2015)—may harness Hsp70s for diverse functions.

Finally and most importantly, the intricate mechanism of the Hsp70 machine itself makes it such a versatile tool. In contrast to ATP-independent chaperones, the affinity of Hsp70 for substrates is regulated by nucleotide, the substrate itself, the J-domain cochaperones and nucleotide exchange factors (**Figure 1**). In a

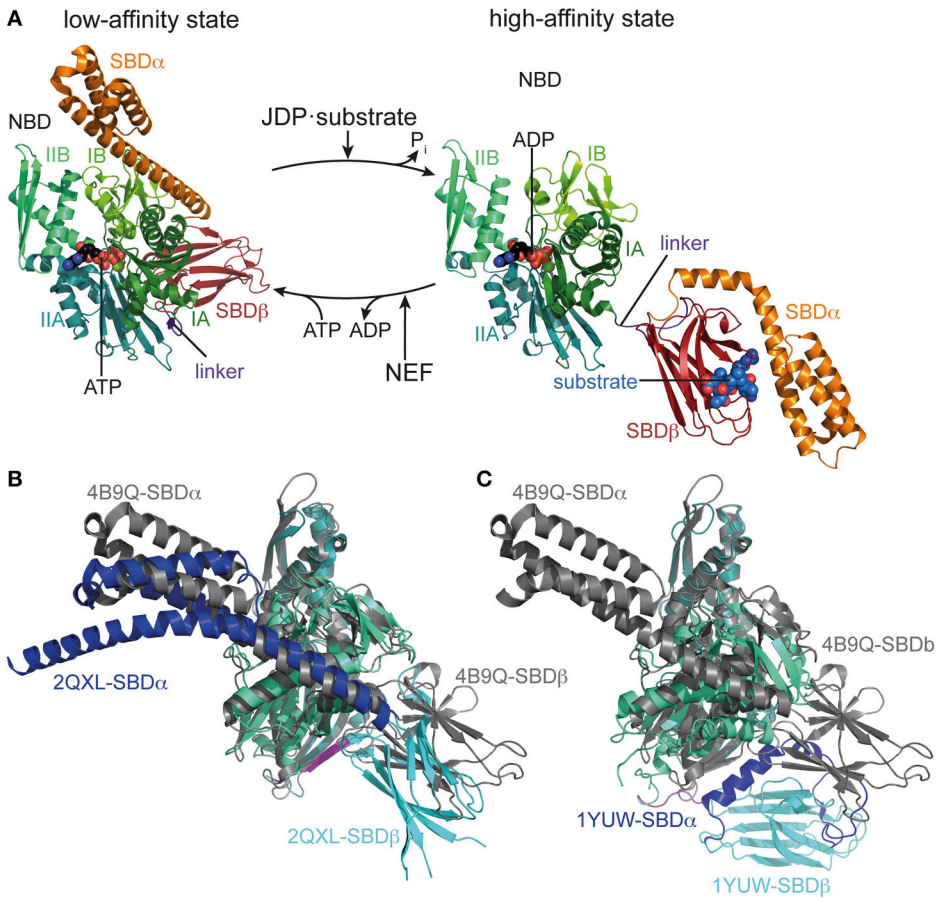


FIGURE 1 | Conformational cycle of Hsp70s. (A) Structural changes associated with the ATPase cycle of *E. coli* DnaK. Left, crystal structure of DnaK in the ATP bound open conformation (low-affinity state, PDB ID 4B9Q, Kityk et al., 2012) in cartoon representation with NBD subdomains IA, IB, IIA, IIB in different shades of green, SBDβ in dark red and SBDα in orange. Right, solution structure of DnaK in the ADP bound/nucleotide-free state as derived from residual dipolar coupling NMR experiments and crystal structures of the isolated domains (high-affinity state, PDB IDs 2KHO, Bertelsen et al., 2009) colored as in the ATP bound state and NBDs in identical orientation. **(B)** Overlay of the crystal structures of DnaK·ATP (PDB ID 4B9Q; gray) and Sse1 (PDB ID 2QXL, Liu and Hendrickson, 2007); NBD, deep teal; SBDβ, cyan; SBDα, blue. **(C)** Overlay of the crystal structures of DnaK·ATP (PDB ID 4B9Q; gray) and bovine Hsc70(1-554) (PDB ID 1YUW, Jiang et al., 2005) NBD, deep teal; SBDβ, cyan; SBDα, blue.

nutshell, in the ATP bound state Hsp70 has a low affinity for substrates but high substrate association and dissociation rates. Upon ATP hydrolysis, substrate association and dissociation rates decrease some 100 and 1000-fold, respectively, leading to an increase in affinity of 10 to 50-fold (Schmid et al., 1994; Mayer et al., 2000). However, ATP hydrolysis rates are very low but stimulated synergistically by the substrate itself and the J-domain cochaperone (Karzai and McMacken, 1996; Barouch et al., 1997; Misselwitz et al., 1998; Laufen et al., 1999; Silberg et al., 2004). Thus, Hsp70 acts like a mouse-trap where the substrate itself triggers its capture. The synergism of substrate with J-domain proteins in triggering ATP hydrolysis allows J-domain proteins to target Hsp70 to the proper substrate. At physiological ATP concentrations, nucleotide exchange is rate-limiting for substrate release and thus allows nucleotide exchange factors to regulate the residence time of substrates bound to Hsp70. This mechanism of association of substrates with Hsp70·ATP at high rates and subsequent ATP hydrolysis and transition to the high affinity state creates a non-equilibrium situation resulting in ultra-high affinity that so far has not been found in any other chaperone (De Los Rios and Barducci, 2014). In the following we will discuss the current knowledge of the structural basis for this allosteric mechanism.

STRUCTURAL BASIS FOR ALLOSTERY IN Hsp70s

A thorough understanding of the structural basis for allostery in Hsp70s was hampered for many years by the lack of a structure of the full-length protein. Structures of isolated domains (Flaherty et al., 1990; Zhu et al., 1996) were available for many years but information on the assembly of NBD and SBD in ADP or ATP states is rather recent (Jiang et al., 2005; Chang et al., 2008; Bertelsen et al., 2009; Kityk et al., 2012; Qi et al., 2013). The NBD shares structural homology with actin and sugar kinases (Flaherty et al., 1991), and can be divided into two lobes (I and II) with two subdomains each (IA, IB, IIA, IIB), which form a deep cleft at the bottom of which nucleotides bind, contacting all four subdomains (Flaherty et al., 1990, **Figure 1**). The SBD is composed of a two-layered β-sandwich subdomain (SBDβ), which contains the substrate binding channel with a central pocket capable of binding a single hydrophobic residue of the substrate; an α-helical subdomain (SBDα), consisting of five helices; and a C-terminal intrinsically disordered segment of about 30 residues of unclear function, which seems to be involved in chaperone activity and, in the eukaryotic cytosol, contains the EEVD motif at the very C-terminus, serving as docking site for the cochaperones Hop/Sti1 and Chip (Zhu et al., 1996; Demand et al., 1998; Ballinger et al., 1999; Scheufler et al., 2000; Zhang et al., 2005; Smock et al., 2011). In the isolated SBD, helices A and B of the SBDα are tightly packed onto the SBDβ, enclosing the substrate binding channel like a lid. This also seems to be the most prevalent conformation of the full-length protein in the nucleotide-free and ADP bound states (Jiang et al., 2005; Chang et al., 2008; Bertelsen et al., 2009; Marcinowski et al., 2011; Schlecht et al., 2011). The crystal structure of *Geobacillus kaustophilus* DnaK and NMR studies on *E. coli* DnaK suggest that NBD and SBD are rather separated, independently tumbling units in the nucleotide-free and ADP states only connected by the flexible linker (Swain et al., 2007; Chang et al., 2008; Bertelsen et al., 2009; Zhuravleva et al., 2012). In contrast, the crystal structure of nucleotide-free bovine Hsc70 shows NBD and SBD in a docked conformation (Jiang et al., 2005).

Recently, the crystal structure of DnaK in the ATP-bound open conformation was solved, which significantly broadened our knowledge about allostery in Hsp70s (Kityk et al., 2012; Qi et al., 2013). Comparison of the DnaK·ATP structure with the solution structure of DnaK·ADP indicates that ATP binding leads to dramatic structural rearrangements in the protein (**Figure 1A**). DnaK·ATP has a more compact structure; SBDα and SBDβ are completely detached from each other and docked onto two sides of the NBD; and the interdomain linker is buried in the lower crevice of the NBD. This structure is similar to the structure of the Hsp110 Sse1, which serves as nucleotide exchange factor for Hsp70s (Dragovic et al., 2006; Raviol et al., 2006; Liu and Hendrickson, 2007; Polier et al., 2008; Schuermann et al., 2008) but has clear differences in the structure and orientation of the SBDβ and SBDα (**Figure 1B**). Differences are more striking when compared to the structure of a two-domain construct of bovine Hsc70, which was crystallized in the nucleotide-free state (Jiang et al., 2005) (**Figure 1C**). An overlay of the NBD of DnaK·ATP with all previously solved crystal structures of isolated NBDs in complex with different nucleotides and the solution structure of the full-length protein in the ADP state (e.g., Flaherty et al., 1990; Wilbanks et al., 1994; O'Brien et al., 1996; Jiang et al., 2005; Bertelsen et al., 2009) reveals that ATP binding leads to the rotation of the NBD lobes toward each other (**Figure 2A** and **Supplemental Movie 1**). This leads to a widening of the lower crevice of the NBD, enabling the linker to insert between subdomains IA and IIA. The surface rearrangements of the NBD allow SBDα and SBDβ docking on the NBD. A number of residues (e.g., Arg151, Arg167, Asp326, Asp393, Lys414, Asp481; all numbers refers to residues in *E. coli DnaK*), which are part of an extensive H-bond network at the NBD-SBDβ interface, were found in genetic and biochemical studies to be important for allosteric signal transmission between the two domains (Montgomery et al., 1999; Vogel et al., 2006a,b; Smock et al., 2010; Kityk et al., 2015) (**Figure 2B**). Thus, interface stabilization by an H-bond network plays a pivotal role in interdomain communication in Hsp70s. In particular, Asp481, which contacts the NBD subdomain IA, and K414, which contacts NBD subdomain IIA, act like a clamp, fixing the NBD in the ATP bound state and strongly reducing basal ATPase activity in the absence of a trigger provided by substrate binding and interaction with a J-domain protein (Kityk et al., 2015).

ATP-induced docking of the SBD to the NBD leads to the stabilization of the open conformation of the SBD. In SBDα, detached from SBDβ, helices A and B form a continuous helix. The substrate binding cleft in SBDβ is wider as compared to the structure of the isolated DnaK-SBD with bound peptide substrate (PDB code 1DKX; **Figures 2C,D**), which is consistent with low affinity for polypeptide substrates and high substrate

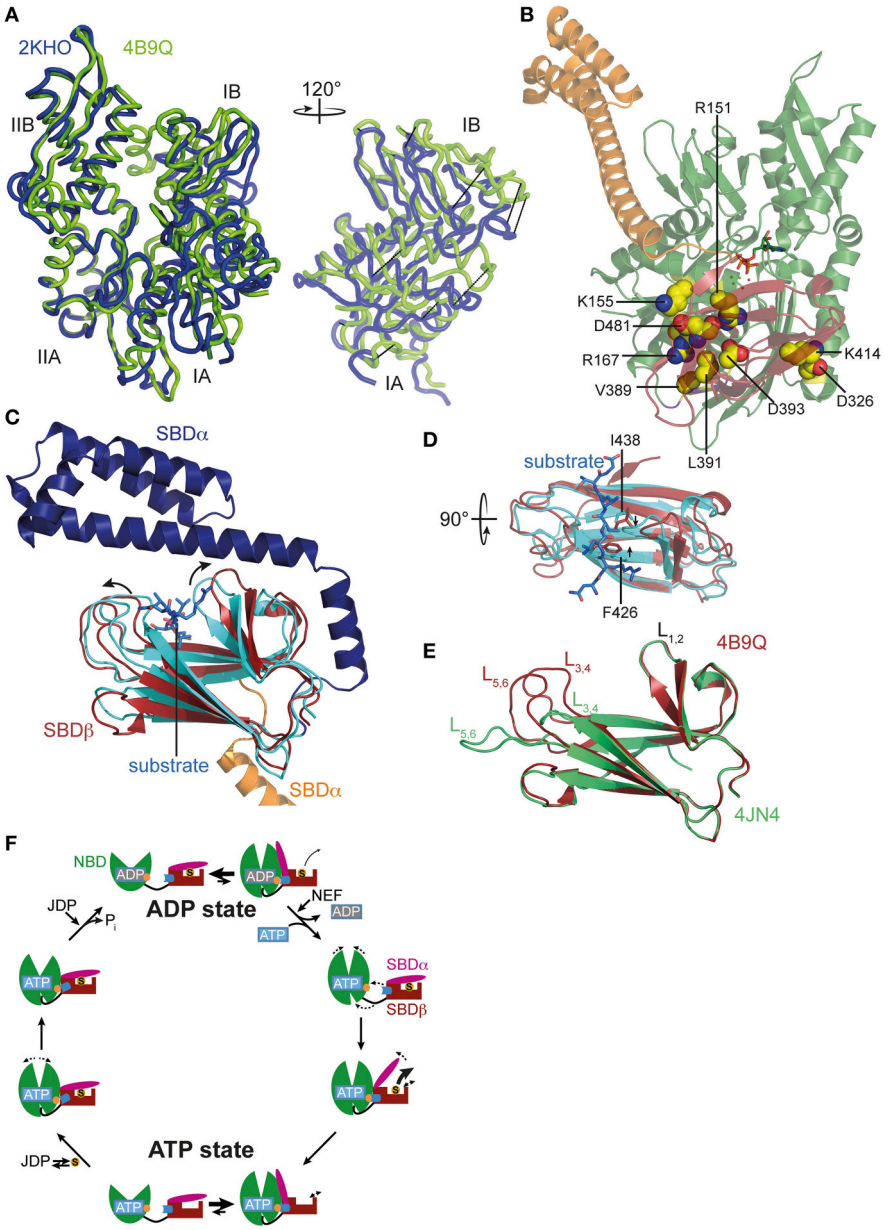


FIGURE 2 | ATP-induced changes in NBD and SBD and allosteric cycle of Hsp70s. (A) Overlay of the NBD of *E. coli* DnaK in the ATP bound open conformation (PDB ID 4B9Q, Kityk et al., 2012; green) and of DnaK in the nucleotide-free/ADP bound state (PDB ID 2KHO, Bertelsen et al., 2009; blue) in tube representation. Left, standard view; right, only subdomains IA and IB rotated by 120° as indicated. **(B)** Structure of DnaK in the ATP bound open conformation with residues known to be involved in interdomain communication and found in the NBD-SBD interface in space-filling representation with carbon atoms in yellow, oxygen atoms in red and nitrogen atoms in blue. **(C)** Overlay of the SBD of DnaK in the ATP bound open conformation; SBDβ, dark red; SBDα, orange and cut for space reasons and the structure of the isolated SBD in complex with a substrate peptide (PDB ID 1DKX, Zhu et al., 1996); SBDβ, cyan; SBDα, dark blue; peptide in light blue and stick representation. Arrows indicate ATP-induced changes visible in this orientation. **(D)** Overlay of SBDβ as in **(A)**, but rotated by 90° as indicated. Arrows indicate ATP-induced narrowing of the central substrate binding pocket. **(E)** Overlay of the SBDβ of the two available structures of DnaK in the ATP bound open conformation (PDB IDs 4B9Q, Kityk et al., 2012; dark red; 4JN4, Qi et al., 2013; green). Indicated are the substrate enclosing loops $L_{1,2}$, $L_{3,4}$, and $L_{5,6}$. **(F)** In the ADP state Hsp70s are in equilibrium between the closed conformation with NBD (green) and SBD (dark red) only connected via the conserved interdomain linker (black) and substrate (S) tightly enclosed in the substrate binding pocket and a very transient open conformation with NBD and SBDβ (dark red) docked. Since the open conformation is very transient, substrates only dissociate from this state at low rates. Nucleotide exchange factors (NEFs) catalyze ADP dissociation. Subsequent ATP binding to Hsp70 induces rotation of the NBD lobes toward each other, opening of the lower cleft of the NBD, insertion of the conserved interdomain linker, and docking of SBDβ to the NBD, resulting in opening of the α-helical lid (SBDα, magenta) and release of the substrate with high rates. In the ATP state Hsp70s are also in equilibrium between the open and very transient closed conformation. The outer loops of the SBDβ are highly dynamic. Substrates associate with J-domain proteins (JDP) and bind with high rates to the open conformation of Hsp70. Substrate binding induces closing of the SBDα and dissociation of the SBDβ from the NBD, which allows rotation of the NBD lobes to a position optimal for ATP hydrolysis. Substrates stimulate ATP hydrolysis through a distinct pathway (blue) involving a trigger on the NBD (orange). How JDPs act in synergism with substrates is currently not known. Dashed arrows indicate domain movement/dynamics.

association and dissociation rates in the ATP-state (Schmid et al., 1994; Mayer et al., 2000). The substrate binding cleft is also more open than the NMR structure of the substrate-free isolated SBDβ (PDB code 1DG4) (Pellecchia et al., 2000) This observation suggests that the conformational changes in SBDβ of the DnaK·ATP structure are induced by SBDβ-NBD interactions, and not by the absence of substrate or detachment of SBDα, as was demonstrated recently (Zhuravleva and Gierasch, 2015). Notably, the outer loops of the SBDβ are the only parts which differ significantly between the two crystal structures of DnaK·ATP (PDB codes 4B9Q and 4JN4; **Figure 2E**) (Kityk et al., 2012; Qi et al., 2013). While in one structure the outer loops $L_{3,4}$ and $L_{5,6}$ protrude upward from the β-sandwich forming a cradle for the substrate (4B9Q), in the other structure they extend the β-strands horizontally (4JN4) (**Figure 2E**). Although the outer loops seem to be very flexible in the first DnaK·ATP structure as well, as indicated by the high b-factor, it is not clear whether the SBDβ opens to the same extent seen in the second structure. The construct used in the second structure had the outer loop $L_{3,4}$ replaced by a short MGG-motif, and, in the crystal $L_{5,6}$ makes extensive contacts with other molecules that stabilize the extended conformation.

Comparison of the SBDβ of the DnaK·ATP structure with the structure of the isolated SBD with bound substrate (PDB code 1DKX) also suggests a mechanism for how substrates stimulate the ATPase activity. Substrate binding is accompanied by pronounced conformational rearrangements in the SBDβ: (I) the substrate enclosing loop $L_{1,2}$ moves toward the substrate and away from the NBD, (II) binding of the central hydrophobic residue requires an expansion of the substrate binding pocket as compared to the ATP-bound state, leading to an overall reorganization of the SBDβ. These changes are transmitted toward the interface through a defined pathway involving residues Val440, Leu484, and Asp148, presumably leading to disruption of some interdomain contacts and release of the linker from the lower crevice of the NBD (Kityk et al., 2015). As a consequence, the NBD subdomains are able to rotate to a degree where the catalytic residues in the ATPase active center reach the optimal position for γ-phosphate cleavage. Such a model is consistent with NMR measurements on a full-length DnaK construct with bound ATP and substrate peptide, which suggest that substrate binding causes dissociation of the SBDβ from the NBD (Zhuravleva et al., 2012).

Although all of these details on the mechanics of allosteric regulation have been elucidated mainly in *E. coli* DnaK, they are believed to be valid for all Hsp70s due to the high evolutionary conservation of this protein family, albeit, variations of this theme certainly exist, in particular in respect to kinetics of conformational changes.

INTERPLAY BETWEEN CONFORMATIONAL DYNAMICS AND ALLOSTERY IN Hsp70s

Many different studies have demonstrated that Hsp70s are highly dynamic and undergo transitions between open and closed conformations independent of the nucleotide status (Mapa et al., 2010; Marcinowski et al., 2011; Schlecht et al., 2011; Kityk et al., 2012). Thus, each nucleotide state consists of an ensemble of different conformations as originally proposed (Mayer et al., 2000) and nucleotides modulate the frequency of structural transitions and affect the equilibrium between different conformers of Hsp70s. A recent NMR and molecular dynamics study revealed that the SBDβ is much more dynamic in the ATP state than in the ADP state, and that the SBDβ-NBD contacts influence the dynamics of the substrate binding pocket (Zhuravleva and Gierasch, 2015). Based on their data, the authors propose that the substrate binding loops and SBDβ-NBD interface are dynamically coupled and that this coupling is part of the allosteric mechanism.

The conformational equilibrium is also influenced by substrates. Binding of a substrate to Hsp70·ATP seems to induce the closure of the α-helical lid before ATP hydrolysis occurs. Consistent with this notion is the observation that substrate binding reduces the ATP-induced blueshift of Trp102 fluorescence in DnaK, indicating the undocking of the α-helical lid from the NBD even in the absence of ATP hydrolysis (Slepenkov and Witt, 1998; Vogel et al., 2006a). On the other hand, bound substrates were demonstrated to slow ATP-induced docking of the α-helical lid onto the NBD (Kityk et al., 2012). **Figure 2F** summarizes the current view of allostery and the conformational cycle of Hsp70s.

PERSPECTIVES

Recent years have brought about significant progress in understanding the underlying mechanisms of interdomain communication in Hsp70s. Despite these advances many questions are still not solved. For example, due to the relative scarcity of structural information, the details of the Hsp70-Hsp40 interaction remain elusive. Hence it is not clear how Hsp40s alone, or together with the substrate, influence allosteric signal transmission between the domains. In eukaryotes Hsp70 additionally interacts with other co-chaperones like HOP and CHIP, linking the Hsp70 machinery to the Hsp90 system and the proteasome degradation pathway, respectively. It is not clear whether they alter, either alone or in cooperation with other cochaperones like nucleotide exchange factors, the interdomain communication in Hsp70s to facilitate transfer of the substrate to Hsp90 or to stabilize the Hsp70-substrate complex for timely release at the proteasome. Lastly, it is not known whether interdomain communication is subject to modulation by the post-translational modifications of Hsp70s that occur in eukaryotes (Muller et al., 2012; Truman et al., 2012; Morgner et al., 2015). Interest in the molecular mechanisms of interdomain communication in the Hsp70s is also driven by the prospect of medical applications. Taking into account the important role of Hsp70s in different pathophysiological processes, including cancer, neurodegenerative diseases and autoimmunity, one of the key research areas is development of Hsp70 modulators. The nucleotide binding pocket of Hsp70 was classified as poor inhibitor binding site due to the mostly electrostatic and polar interactions with nucleotide (Halgren, 2009). The polypeptide substrate binding site may be unsui for inhibitors and activators of Hsp70 function and only inhibitors

with limited potency have been found so far (Otvos et al., 2000; Otaka et al., 2007; Yamamoto et al., 2010; Knappe et al., 2011). Therefore, allosteric control of the ATPase cycle appears as an attractive target and the first allosteric modulators have already been found (Wisén and Gestwicki, 2008; Kang et al., 2014; Taldone et al., 2014; Hassan et al., 2015).

ACKNOWLEDGMENTS

The work of the authors was funded by the Deutsche Forschungsgemeinschaft (MA1278/4-1, SFB638 TP13).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fmolb.2015.00058

Supplemental Movie 1 | Lobe rotation in the NBD of Hsp70 upon transition between the ATP and ADP bound state. The structure of the NBD of *E. coli* DnaK in the ATP bound conformation (PDB ID 4B9Q) was morphed into the structure of the ADP bound conformation (PDB ID 2KHO) using the Yale Morph Server (Krebs and Gerstein, 2000; Flores et al., 2006). Subdomains of the NBD in different shades of green (IA, dark green; IB chartreuse; IIA, dark teal; IIB, lime); NBD-SBD-linker in purple.

REFERENCES

Ballinger, C. A., Connell, P., Wu, Y., Hu, Z., Thompson, L. J., Yin, L. Y., et al. (1999). Identification of CHIP, a novel tetratricopeptide repeat-containing protein that interacts with heat shock proteins and negatively regulates chaperone functions. *Mol. Cell. Biol.* 19, 4535–4545.

Barouch, W., Prasad, K., Greene, L., and Eisenberg, E. (1997). Auxilin-induced interaction of the molecular chaperone Hsc70 with clathrin baskets. *Biochemistry* 36, 4303–4308. doi: 10.1021/bi962727z

Bertelsen, E. B., Chang, L., Gestwicki, J. E., and Zuiderweg, E. R. P. (2009). Solution conformation of wild-type *E. coli* Hsp70 (DnaK) chaperone complexed with ADP and substrate. *Proc. Nat. Acad. Sci. U.S.A.* 106, 8471–8476. doi: 10.1073/pnas.0903503106

Bracher, A., and Verghese, J. (2015). The nucleotide exchange factors of Hsp70 molecular chaperones. *Front. Mol. Biosci.* 2:10. doi: 10.3389/fmolb.2015.00010

Chacinska, A., Koehler, C. M., Milenkovic, D., Lithgow, T., and Pfanner, N. (2009). Importing mitochondrial proteins: machineries and mechanisms. *Cell* 138, 628–644. doi: 10.1016/j.cell.2009.08.005

Chang, Y.-W., Sun, Y.-J., Wang, C., and Hsiao, C.-D. (2008). Crystal structures of the 70-kDa heat shock proteins in domain disjoining conformation. *J. Biol. Chem.* 283, 15502–15511. doi: 10.1074/jbc.M708992200

De Los Rios, P., and Barducci, A. (2014). Hsp70 chaperones are non-equilibrium machines that achieve ultra-affinity by energy consumption. *Elife* 3:e02218. doi: 10.7554/elife.02218

Demand, J., Lüders, J., and Höhfeld, J. (1998). The carboxy-terminal domain of Hsc70 provides binding sites for a distinct set of chaperone cofactors. *Mol. Cell. Biol.* 18, 2023–2028.

Deuerling, E., and Bukau, B. (2004). Chaperone-assisted folding of newly synthesized proteins in the cytosol. *Crit. Rev. Biochem. Mol. Biol.* 39, 261–277. doi: 10.1080/10409230490892496

Dragovic, Z., Broadley, S. A., Shomura, Y., Bracher, A., and Hartl, F. U. (2006). Molecular chaperones of the Hsp110 family act as nucleotide exchange factors of Hsp70s. *EMBO J.* 25, 2519–2528. doi: 10.1038/sj.emboj.7601138

Flaherty, K. M., DeLuca-Flaherty, C., and McKay, D. B. (1990). Three-dimensional structure of the ATPase fragment of a 70K heat-shock cognate protein. *Nature* 346, 623–628. doi: 10.1038/346623a0

Flaherty, K. M., McKay, D. B., Kabsch, W., and Holmes, K. C. (1991). Similarity of the three-dimensional structures of actin and the ATPase fragment of a 70-kDa heat shock cognate protein. *Proc. Natl. Acad. Sci. U.S.A.* 88, 5041–5045. doi: 10.1073/pnas.88.11.5041

Flores, S., Echols, N., Milburn, D., Hespenheide, B., Keating, K., Lu, J., et al. (2006). The database of macromolecular motions: new features added at the decade mark. *Nucleic Acids Res.* 34, D296–D301. doi: 10.1093/nar/gkj046

Halgren, T. A. (2009). Identifying and characterizing binding sites and assessing druggability. *J. Chem. Inf. Model.* 49, 377–389. doi: 10.1021/ci800324m

Hartl, F. U., Bracher, A., and Hayer-Hartl, M. (2011). Molecular chaperones in protein folding and proteostasis. *Nature* 475, 324–332. doi: 10.1038/nature10317

Hassan, A. Q., Kirby, C. A., Zhou, W., Schuhmann, T., Kityk, R., Kipp, D. R., et al. (2015). The novolactone natural product disrupts the allosteric regulation of Hsp70. *Chem. Biol.* 22, 87–97. doi: 10.1016/j.chembiol.2014.11.007

Jiang, J., Prasad, K., Lafer, E. M., and Sousa, R. (2005). Structural basis of interdomain communication in the Hsc70 chaperone. *Mol. Cell* 20, 513–524. doi: 10.1016/j.molcel.2005.09.028

Kampinga, H. H., and Craig, E. A. (2010). The HSP70 chaperone machinery: J proteins as drivers of functional specificity. *Nat. Rev. Mol. Cell Biol.* 11, 579–592. doi: 10.1038/nrm2941

Kang, Y., Taldone, T., Patel, H. J., Patel, P. D., Rodina, A., Gozman, A., et al. (2014). Heat shock protein 70 inhibitors. 1. 2,5′-thiodipyrimidine and 5-(phenylthio)pyrimidine acrylamides as irreversible binders to an allosteric site on heat shock protein 70. *J. Med. Chem.* 57, 1188–1207. doi: 10.1021/jm401551n

Karzai, A. W., and McMacken, R. (1996). A bipartite signaling mechanism involved in DnaJ-mediated activation of the *Escherichia coli* DnaK protein. *J. Biol. Chem.* 271, 11236–11246. doi: 10.1074/jbc.271.19.11236

Kityk, R., Kopp, J., Sinning, I., and Mayer, M. P. (2012). Structure and dynamics of the ATP-bound open conformation of Hsp70 chaperones. *Mol. Cell* 48, 863–874. doi: 10.1016/j.molcel.2012.09.023

Kityk, R., Vogel, M., Schlecht, R., Bukau, B., and Mayer, M. P. (2015). Pathways of allosteric regulation in Hsp70 chaperones. *Nat. Commun.* 6, 8308. doi: 10.1038/ncomms9308

Knappe, D., Zahn, M., Sauer, U., Schiffer, G., Sträter, N., and Hoffmann, R. (2011). Rational design of oncocin derivatives with superior protease stabilities and antibacterial activities based on the high-resolution structure of the oncocin-DnaK complex. *Chembiochem* 12, 874–876. doi: 10.1002/cbic.201000792

Krebs, W. G., and Gerstein, M. (2000). The morph server: a standardized system for analyzing and visualizing macromolecular motions in a database framework. *Nucleic Acids Res.* 28, 1665–1675. doi: 10.1093/nar/28.8.1665

Laufen, T., Mayer, M. P., Beisel, C., Klostermeier, D., Mogk, A., Reinstein, J., et al. (1999). Mechanism of regulation of hsp70 chaperones by DnaJ cochaperones. *Proc. Natl. Acad. Sci. U.S.A.* 96, 5452–5457. doi: 10.1073/pnas.96.10.5452

Liu, Q., and Hendrickson, W. A. (2007). Insights into Hsp70 chaperone activity from a crystal structure of the yeast Hsp110 Sse1. *Cell* 131, 106–120. doi: 10.1016/j.cell.2007.08.039

Mapa, K., Sikor, M., Kudryavtsev, V., Waegemann, K., Kalinin, S., Seidel, C. A. M., et al. (2010). The conformational dynamics of the mitochondrial Hsp70 chaperone. *Mol. Cell* 38, 89–100. doi: 10.1016/j.molcel.2010.03.010

Marcinowski, M., Höller, M., Feige, M. J., Baerend, D., Lamb, D. C., and Buchner, J. (2011). Substrate discrimination of the chaperone BiP by autonomous and cochaperone-regulated conformational transitions. *Nat. Struct. Mol. Biol.* 18, 150–158. doi: 10.1038/nsmb.1970

Mayer, M. P., Schröder, H., Rüdiger, S., Paal, K., Laufen, T., and Bukau, B. (2000). Multistep mechanism of substrate binding determines chaperone activity of Hsp70. *Nat. Struct. Biol.* 7, 586–593. doi: 10.1038/76819

Misselwitz, B., Staeck, O., and Rapoport, T. A. (1998). J proteins catalytically activate Hsp70 molecules to trap a wide range of peptide sequences. *Mol. Cell* 2, 593–603. doi: 10.1016/S1097-2765(00)80158-6

Montgomery, D. L., Morimoto, R. I., and Gierasch, L. M. (1999). Mutations in the substrate binding domain of the *Escherichia coli* 70 kDa molecular chaperone, DnaK, which alter substrate affinity or interdomain coupling. *J. Mol. Biol.* 286, 915–932. doi: 10.1006/jmbi.1998.2514

Morgner, N., Schmidt, C., Beilsten-Edmands, V., Ebong, I.-O., Patel, N. A., Clerico, E. M., et al. (2015). Hsp70 forms antiparallel dimers stabilized by post-translational modifications to position clients for transfer to Hsp90. *Cell Rep.* 11, 759–769. doi: 10.1016/j.celrep.2015.03.063

Muller, P., Ruckova, E., Halada, P., Coates, P. J., Hrstka, R., Lane, D. P., et al. (2012). C-terminal phosphorylation of Hsp70 and Hsp90 regulates alternate binding to co-chaperones CHIP and HOP to determine cellular protein folding/degradation balances. *Oncogene* 32, 3101–3110. doi: 10.1038/onc.2012.314

Neupert, W., and Herrmann, J. M. (2007). Translocation of proteins into mitochondria. *Annu. Rev. Biochem.* 76, 723–749. doi: 10.1146/annurev.biochem.76.052705.163409

O'Brien, M. C., Flaherty, K. M., and McKay, D. B. (1996). Lysine 71 of the chaperone protein Hsc70 Is essential for ATP hydrolysis. *J. Biol. Chem.* 271, 15874–15878. doi: 10.1074/jbc.271.27.15874

Otaka, M., Yamamoto, S., Ogasawara, K., Takaoka, Y., Noguchi, S., Miyazaki, T., et al. (2007). The induction mechanism of the molecular chaperone HSP70 in the gastric mucosa by Geranylgeranylacetone (HSP-inducer). *Biochem. Biophys. Res. Commun.* 353, 399–404. doi: 10.1016/j.bbrc.2006.12.031

Otvos, L., O, I., Rogers, M. E., Consolvo, P. J., Condie, B. A., Lovas, S., et al. (2000). Interaction between heat shock proteins and antimicrobial peptides. *Biochemistry* 39, 14150–14159. doi: 10.1021/bi0012843

Pellecchia, M., Montgomery, D. L., Stevens, S. Y., Vander Kooi, C. W., Feng, H. P., Gierasch, L. M., et al. (2000). Structural insights into substrate binding by the molecular chaperone DnaK. *Nat. Struct. Biol.* 7, 298–303. doi: 10.1038/74062

Polier, S., Dragovic, Z., Hartl, F. U., and Bracher, A. (2008). Structural basis for the cooperation of Hsp70 and Hsp110 chaperones in protein folding. *Cell* 133, 1068–1079. doi: 10.1016/j.cell.2008.05.022

Qi, R., Sarbeng, E. B., Liu, Q., Le, K. Q., Xu, X., Xu, H., et al. (2013). Allosteric opening of the polypeptide-binding site when an Hsp70 binds ATP. *Nat. Struct. Mol. Biol.* 20, 900–907. doi: 10.1038/nsmb.2583

Raviol, H., Sadlish, H., Rodriguez, F., Mayer, M. P., and Bukau, B. (2006). Chaperone network in the yeast cytosol: Hsp110 is revealed as an Hsp70 nucleotide exchange factor. *EMBO J.* 25, 2510–2518. doi: 10.1038/sj.emboj.7601139

Rüdiger, S., Germeroth, L., Schneider-Mergener, J., and Bukau, B. (1997). Substrate specificity of the DnaK chaperone determined by screening cellulose-bound peptide libraries. *EMBO J.* 16, 1501–1507. doi: 10.1093/emboj/16.7.1501

Scheufler, C., Brinker, A., Bourenkov, G., Pegoraro, S., Moroder, L., Bartunik, H., et al. (2000). Structure of TPR domain-peptide complexes: critical elements in the assembly of the Hsp70-Hsp90 multichaperone machine. *Cell* 101, 199–210. doi: 10.1016/S0092-8674(00)80830-2

Schlecht, R., Erbse, A. H., Bukau, B., and Mayer, M. P. (2011). Mechanics of Hsp70 chaperones enables differential interaction with client proteins. *Nat. Struct. Mol. Biol.* 18, 345–351. doi: 10.1038/nsmb.2006

Schmid, D., Baici, A., Gehring, H., and Christen, P. (1994). Kinetics of molecular chaperone action. *Science* 263, 971–973. doi: 10.1126/science.8310296

Schuermann, J. P., Jiang, J., Cuellar, J., Llorca, O., Wang, L., Gimenez, L. E., et al. (2008). Structure of the Hsp110:Hsc70 nucleotide exchange machine. *Mol. Cell* 31, 232–243. doi: 10.1016/j.molcel.2008.05.006

Silberg, J. J., Tapley, T. L., Hoff, K. G., and Vickery, L. E. (2004). Regulation of the HscA ATPase reaction cycle by the co-chaperone HscB and the iron-sulfur cluster assembly protein IscU. *J. Biol. Chem.* 279, 53924–53931. doi: 10.1074/jbc.M410117200

Slepenkov, S. V., and Witt, S. N. (1998). Peptide-induced conformational changes in the molecular chaperone DnaK. *Biochemistry* 37, 16749–16756. doi: 10.1021/bi981738k

Smock, R. G., Blackburn, M. E., and Gierasch, L. M. (2011). Conserved, disordered C terminus of DnaK enhances cellular survival upon stress and DnaK *in vitro* chaperone activity. *J. Biol. Chem.* 286, 31821–31829. doi: 10.1074/jbc.M111.265835

Smock, R. G., Rivoire, O., Russ, W. P., Swain, J. F., Leibler, S., Ranganathan, R., et al. (2010). An interdomain sector mediating allostery in Hsp70 molecular chaperones. *Mol. Syst. Biol.* 6, 414. doi: 10.1038/msb.2010.65

Sousa, R., and Lafer, E. M. (2015). The role of molecular chaperones in clathrin mediated vesicular trafficking. *Front. Mol. Biosci.* 2:26. doi: 10.3389/fmolb.2015.00026

Swain, J. F., Dinler, G., Sivendran, R., Montgomery, D. L., Stotz, M., and Gierasch, L. M. (2007). Hsp70 chaperone ligands control domain association via an allosteric mechanism mediated by the interdomain linker. *Mol. Cell* 26, 27–39. doi: 10.1016/j.molcel.2007.02.020

Taldone, T., Kang, Y., Patel, H. J., Patel, M. R., Patel, P. D., Rodina, A., et al. (2014). Heat shock protein 70 inhibitors. 2. 2,5'-thiodipyrimidines, 5-(phenylthio)pyrimidines, 2-(pyridin-3-ylthio)pyrimidines, and 3-(phenylthio)pyridines as reversible binders to an allosteric site on heat shock protein 70. *J. Med. Chem.* 57, 1208–1224. doi: 10.1021/jm401552y

Truman, A. W., Kristjansdottir, K., Wolfgeher, D., Hasin, N., Polier, S., Zhang, H., et al. (2012). CDK-dependent Hsp70 phosphorylation controls G1 cyclin abundance and cell-cycle progression. *Cell* 151, 1308–1318. doi: 10.1016/j.cell.2012.10.051

Tyedmers, J., Mogk, A., and Bukau, B. (2010). Cellular strategies for controlling protein aggregation. *Nat. Rev. Mol. Cell Biol.* 11, 777–788. doi: 10.1038/nrm2993

Vogel, M., Bukau, B., and Mayer, M. P. (2006a). Allosteric regulation of Hsp70 chaperones by a proline switch. *Mol. Cell* 21, 359–367. doi: 10.1016/j.molcel.2005.12.017

Vogel, M., Mayer, M. P., and Bukau, B. (2006b). Allosteric regulation of Hsp70 chaperones involves a conserved interdomain linker. *J. Biol. Chem.* 281, 38705–38711. doi: 10.1074/jbc.M609020200

Wegele, H., Müller, L., and Buchner, J. (2004). Hsp70 and Hsp90–a relay team for protein folding. *Rev. Physiol. Biochem. Pharmacol.* 151, 1–44. doi: 10.1007/s10254-003-0021-1

Wilbanks, S. M., DeLuca-Flaherty, C., and McKay, D. B. (1994). Structural basis of the 70-kilodalton heat shock cognate protein ATP hydrolytic activity. I. kinetic analyses of active site mutants. *J. Biol. Chem.* 269, 12893–12898.

Wisén, S., and Gestwicki, J. E. (2008). Identification of small molecules that modify the protein folding activity of heat shock protein 70. *Anal. Biochem.* 374, 371–377. doi: 10.1016/j.ab.2007.12.009

Yamamoto, S., Nakano, S., Owari, K., Fuziwara, K., Ogawa, N., Otaka, M., et al. (2010). Gentamicin inhibits HSP70-assisted protein folding by interfering with substrate recognition. *FEBS Lett.* 584, 645–651. doi: 10.1016/j.febslet.2009.12.021

Zhang, M., Windheim, M., Roe, S. M., Peggie, M., Cohen, P., Prodromou, C., et al. (2005). Chaperoned ubiquitylation–crystal structures of the CHIP U box E3 ubiquitin ligase and a CHIP-Ubc13-Uev1a complex. *Mol. Cell* 20, 525–538. doi: 10.1016/j.molcel.2005.09.023

Zhu, X., Zhao, X., Burkholder, W. F., Gragerov, A., Ogata, C. M., Gottesman, M. E., et al. (1996). Structural analysis of substrate binding by the molecular chaperone DnaK. *Science* 272, 1606–1614. doi: 10.1126/science.272.5268.1606

Zhuravleva, A., Clerico, E. M., and Gierasch, L. M. (2012). An interdomain energetic tug-of-war creates the allosterically active state in Hsp70 molecular chaperones. *Cell* 151, 1296–1307. doi: 10.1016/j.cell.2012.11.002

Zhuravleva, A., and Gierasch, L. M. (2015). Substrate-binding domain conformational dynamics mediate Hsp70 allostery. *Proc. Nat. Acad. Sci.* 112, E2865–E2873. doi: 10.1073/pnas.1506692112

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Molecular Biosciences

REVIEW
published: 05 June 2015
doi: 10.3389/fmolb.2015.00029

Multi-layered molecular mechanisms of polypeptide holding, unfolding and disaggregation by HSP70/HSP110 chaperones

Andrija Finka[1,2], *Sandeep K. Sharma*[3] *and Pierre Goloubinoff*[1]*

[1] *Department of Plant Molecular Biology, Faculty of Biology and Medicine, University of Lausanne, Lausanne, Switzerland,* [2] *Laboratoire de Biophysique Statistique, School of Basic Sciences, École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland,* [3] *Department of Chemistry, Umeå University, Umeå, Sweden*

OPEN ACCESS

Edited by:
James Shorter,
University of Pennsylvania, USA

Reviewed by:
Kevin Anthony Morano,
The University of Texas Health Science Center at Houston, USA
Martin Lothar Duennwald,
University of Western Ontario, Canada

***Correspondence:**
Pierre Goloubinoff,
Department of Plant Molecular Biology, Faculty of Biology and Medicine, University of Lausanne, Biophore Building, Lausanne CH-1015, Switzerland
pierre.goloubinoff@unil.ch

Specialty section:
This article was submitted to Protein Folding, Misfolding and Degradation, a section of the journal Frontiers in Molecular Biosciences

Received: *13 April 2015*
Accepted: *19 May 2015*
Published: *05 June 2015*

Citation:
Finka A, Sharma SK and Goloubinoff P (2015) Multi-layered molecular mechanisms of polypeptide holding, unfolding and disaggregation by HSP70/HSP110 chaperones. Front. Mol. Biosci. 2:29. doi: 10.3389/fmolb.2015.00029

Members of the HSP70/HSP110 family (HSP70s) form a central hub of the chaperone network controlling all aspects of proteostasis in bacteria and the ATP-containing compartments of eukaryotic cells. The heat-inducible form HSP70 (HSPA1A) and its major cognates, cytosolic HSC70 (HSPA8), *endoplasmic reticulum* BIP (HSPA5), mitochondrial mHSP70 (HSPA9) and related HSP110s (HSPHs), contribute about 3% of the total protein mass of human cells. The HSP70s carry out a plethora of housekeeping cellular functions, such as assisting proper *de novo* folding, assembly and disassembly of protein complexes, pulling polypeptides out of the ribosome and across membrane pores, activating and inactivating signaling proteins and controlling their degradation. The HSP70s can induce structural changes in alternatively folded protein conformers, such as clathrin cages, hormone receptors and transcription factors, thereby regulating vesicular trafficking, hormone signaling and cell differentiation in development and cancer. To carry so diverse cellular housekeeping and stress-related functions, the HSP70s act as ATP-fuelled unfolding nanomachines capable of switching polypeptides between different folded states. During stress, the HSP70s can bind (hold) and prevent the aggregation of misfolding proteins and thereafter act alone or in collaboration with other unfolding chaperones to solubilize protein aggregates. Here, we discuss the common ATP-dependent mechanisms of holding, unfolding-by-clamping and unfolding-by-entropic pulling, by which the HSP70s can apparently convert various alternatively folded and misfolded polypeptides into differently active conformers. Understanding how HSP70s can prevent the formation of cytotoxic protein aggregates, pull, unfold, and solubilize them into harmless species is central to the design of therapies against protein conformational diseases.

Keywords: entropic pulling, HSP40 heat-shock proteins, chaperone proteins, misfolded proteins, conformational diseases, DnaK, DNAJ homologs

HSP70/HSP110 Evolution and Cellular Functions

Together with the HSP90s, members of the conserved HSP70/HSP110 family (HSP70s) are prevailing ATP-hydrolyzing chaperones that control all aspects of cellular proteostasis. The HSP70s and co-chaperones may constitute up to 3% of the total protein mass of unstressed human cells (Finka and Goloubinoff, 2013; Finka et al., 2015). In the test tube, proper mixtures of purified HSP70s and co-chaperones can prevent the aggregation of artificially unfolded proteins. This is, however, a rather ineffective process in the absence of ATP, which necessitates a large molar excess of chaperones over their protein substrates (Sharma et al., 2011). In stressed cells, owing to the presence of ATP and J-domain co-chaperones, the HSP70s are much more effective "holding" chaperones. Under regular physiological conditions, they may further use ATP-hydrolysis to warrant the proper *de novo* folding of nascent polypeptides, drive the post-translational translocation of cytosolic polypeptides across membranes and promote their assembly into active oligomers, or drive their disassembly into reversibly inactive conformers (Schuermann et al., 2008; Mattoo et al., 2013). Importantly, HSP70s can forcefully disaggregate stable stress- or mutation-induced misfolded proteins, which may be toxic to animal cells and cause the apoptosis of old neurons in particular, leading to neural tissue degeneration (Bellotti and Chiti, 2008). Moreover, the HSP70s can mediate the refolding of solubilized misfolded polypeptides into harmless native proteins, or control their degradation by chaperone-gated proteases (Mattoo and Goloubinoff, 2014; Cho et al., 2015).

With the exception of exotic thermophilic and hyperthermophilic *archaea*, genes encoding for HSP70s (DnaK in bacteria) are present in all living organisms (Macario and De Macario, 1999). In over 1200 sequenced bacterial genomes, only two members of the order *Aquificales* lack HSP70 genes (Warnecke, 2012). Likewise, all eukaryotic genomes encode for at least half a dozen HSP70 genes and three or four related HSP110 (HSPHs) genes, predicted to be expressed in all the ATP-containing compartments of the cell: cytosol, nucleus, lumen of the *endoplasmic reticulum* (ER), mitochondrial matrix and in plants, the chloroplast stroma and the glyoxisome (Schlicher and Soll, 1996; Wimmer et al., 1997). Moreover, HSP70s can be secreted and exposed on the extra-cellular surface, were they carry a yet unclear ATP-independent immunogenic function, particularly important for cancer detection and therapy (Tytell, 2005).

A bioinformatic search in the human genome can identify 13 HSP70 and 4 HSP110 genes, which are also actively transcribed. In addition, there are 50 J-domain co-chaperones, known to target the various HSP70s onto their substrates, and at least six nucleotide exchange factors (NEFs) called BAG and GrpE (Kampinga et al., 2009; Finka et al., 2011; Mayer, 2013; Clerico et al., 2015). Some genes are poorly expressed and may remain below detection thresholds of current mass spectrometry methods, or they may be conditionally expressed only in particular tissues, or under various stresses (Finka et al., 2011) such as heat shock (Finka et al., 2015). In Hela cells, high throughput mass spectrometry proteomic using stable isotope labeling of amino acids in cell cultures (SILAC) identified and precisely quantified ten different HSP70/HSP110 proteins, eighteen J-domain cochaperones and five NEFs (Geiger et al., 2012; Finka and Goloubinoff, 2013) (**Figure 1A**) that summed into 3.2% of the total protein mass. In comparison, a medium resolution mass spectrometry proteomic analysis of human Jurkat cells, identified and quantified ten different HSP70/HSP110 proteins, fifteen J-domain cochaperones and five NEFs (Finka et al., 2015) that summed into 2.7% of the total protein mass.

When exposed to various abiotic or chemical stresses, such as treatments with heat, the HSP90 inhibitor geldanamycin, or various non-steroidal anti-inflammatory drugs, the mRNA and protein levels of cytosolic HSP70 (mainly HSPA1A, HSPA8, and HSPA4) may increase many folds, whereas levels of ER HSP70 (also named HSPA5, BIP) remain unchanged (Saidi et al., 2007; Finka et al., 2011, 2015). Reciprocally, under a ER stress, such as treatments with deoxyglucose or tunicamycin causing a typical Unfolded Protein Response (Ryno et al., 2013), the mRNA levels predominantly of ER BIP may increase many folds, whereas the mRNA levels of cytosolic HSP70 and HSC70 remains unchanged (Finka et al., 2011). The quantitative observation that without stress, HSC70 and BIP are very abundant chaperones, implies that they both carry central house-keeping physiological functions (Finka et al., 2015), beyond their well-characterized defensive role during and following stress (Ben-Zvi et al., 2004; Shorter, 2011; Rampelt et al., 2012; Mattoo et al., 2013).

HSP70s Can Assist Translation by Pulling Polypeptides out of Ribosomes

At the exit of eukaryotic ribosomes, the L31 protein can bind a special J-domain cochaperone called zuotin (DNAJC2), which in turn can anchor a special HSP70 (SSZ1) and cytosolic HSC70. The presence of several consecutive bulky hydrophobic residues in the sequence of some nascent polypeptides might slow down their translocation through the narrow channel of the ribosome. Remarkably, ATP-hydrolysis by SSZ1 and HSC70 and their consequent clamping onto the emerging polypeptides, alongside their dissociation from their zuotin anchor, can exert a pulling force to relieve the translocation stalling (Otto et al., 2005; Fiaux et al., 2010; Koplin et al., 2010; Shalgi et al., 2013, 2014; Zhang et al., 2014).

ER HSP70s Can Pull Polypeptides into the ER Lumen

Not all ER proteins are synthetized and co-translocated on the SRP-bound ribosomes. Some pre-polypeptides are first synthetized on cytosolic ribosomes. For thermodynamic reasons they may have to reach a near-native fold before being presented to the ER surface to be further translocated across narrow import channels into the ER lumen. On the ER lumen side, BIP (HSPA5) in the ATP-bound state can spontaneously associate to a specific anchoring J-domain cochaperone (SEC63) and thus become poised in the opened state to bind incoming polypeptides from the cytosol. Upon ATP hydrolysis, luminal BIP may "lock" on a threaded polypeptide emerging from the pore and, owing to the

A

Gene symbols	Mass proportion (%)		Copy number per μm³		Subcellular localization
	HeLa	Jurkat	HeLa	Jurkat	
HSPA1A/B; HSP70	0.5697	0.0015	2814	29	Cytosol and nucleus
HSPA5; GRP78	0.5562	0.28	2661	548	Endoplasmic reticulum
HSPA8; HSC70	0.9364	1.6111	4579	12101	Cytosol and nucleus
HSPA9, Mortalin	0.4304	0.2192	2023	4304	Mitochondria
HSPA13	0.0015	0.0003	10	3	Endoplasmic reticulum
HSPA14	0.0065	0.0055	41	53	Cytosol and nucleus
HSPH1; HSP105	0.1092	0.0017	391	3	Cytosol and nucleus
HSPH2; HSPA4;APG2	0.1677	0.2398	618	360	Cytosol and nucleus
HSPH3; HSPA4L	0.0268	0.0477	99	1005	Cytosol and nucleus
HYOU1; GRP170	0.0619	0.0816	193	91	Endoplasmic reticulum
DNAJA1	0.0457	0.0455	353	540	Cytosol and nucleus
DNAJA2	0.0225	0.0071	171	83	Cytosol and nucleus
DNAJA3	0.0253	0.0063	167	64	Mitochondria
DNAJB1	0.0244	0.0122	223	171	Cytosol and nucleus
DNAJB4	0.008	n.d.	73	n.d.	Cytosol and nucleus
DNAJB6	0.0017	0.0012	17	23	Cytosol and nucleus
DNAJB11	0.0198	0.0123	170	162	Endoplasmic reticulum
DNAJB12	0.0037	0.0006	31	8	Cytosol and nucleus
DNAJB14	0.0001	0.0044	1	71	Cytosol and nucleus
DNAJC2; ZRF	0.0074	n.d.	36	n.d.	Cytosol and nucleus
DNAJC10	0.0019	0.0005	7	3	Endoplasmic reticulum
DNAJC11	0.0044	0.0048	24	41	Cytosol and nucleus
DNAJC13	0.0061	0.0036	8	8	Cytosol and nucleus
DNAJC15	0.0007	0	16	0	Cytosol and nucleus
DNAJC17	0.0011	0.0008	11	13	Cytosol and nucleus
DNAJC19; TIMM14	0.001	0.0009	28	5	Mitochondria
DNAJC21; DNAJA5	0.0025	n.d.	13	n.d.	Cytosol and nucleus
DNAJC23; SEC63	0.0227	0.0026	89	120	Endoplasmic reticulum
DNAJC26; GAK	0.002	0.0039	5	34	Cytosol and nucleus
BAG1	0.004	n.d.	33	n.d.	Cytosol and nucleus
BAG2	0.019	0.026	275	582	Cytosol and nucleus
BAG3	0.018	0.0002	103	2	Cytosol and nucleus
BAG4	0.0001	n.d.	1	n.d.	Cytosol and nucleus
BAG5	0.001	0.0018	8	17	Cytosol and nucleus
BAG6;BAT3	n.d.	0.0364	n.d.	66	Cytosol and nucleus
GRPEL1	0.009	0.023	132	505	Mitochondria
Total	3.1631	2.7482			

B

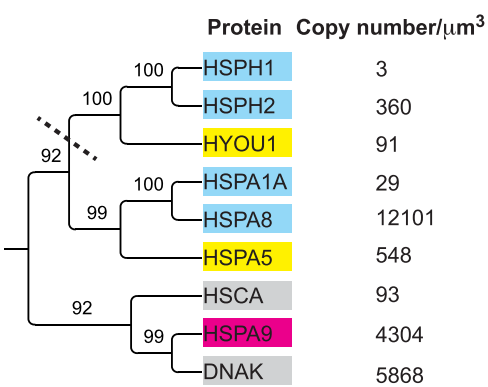


FIGURE 1 | Amounts, stoichiometries and phylogenetic relationships among HSP70s and their main cochaperones in human cells. (A) List of the significantly detected HSP70 and HSP110 cognate proteins (light green), J-domain cochaperones (orange) and NEFs (blue) in Hela and Jurkat cells and their presumed sub-cellular compartments. **(B)** Phylogenetic tree (neighbor joining method) from the protein sequences of the most abundant HSP70 and HSP110 proteins in Jurkat cells, using *E. coli* DnaK and HSCA as out-groups. Presumed subcellular localization: CYT; cytoplasmic (cyan), ER; Endoplasmic reticulum (yellow). MIT; mitochondria (magenta). The copy number per micron cube are from human (Jurkat) cells (Finka and Goloubinoff, 2013; Finka et al., 2015) and from *E. coli* (gray) (Arike et al., 2012).

concomitant dissociation of ADP-bound BIP from its J-anchor SEC63, the chaperone can exert an entropic pulling force on the locked-upon incoming polypeptide. Pulling of the translocating polypeptide from inside the lumen may compel bulky structured domains that are still on the cytosolic side to collide with the pore entry, leading to their unfolding and unidirectional translocation into the lumen. There, the protein can reach the native state, assisted by soluble DNAJB11 and the ATP-fuelled unfoldases BIP (HSPA5) and HYOU (Matlack et al., 1997; Goloubinoff and De Los Rios, 2007; Griesemer et al., 2014; Melnyk et al., 2015).

HSP70s can Pull Polypeptides into the Mitochondria

Similarly, in the mitochondrial matrix, a special HSP70 (mortalin, HSPA9) can associate in the ATP-bound state to two anchoring J-domain cochaperones named PAM16/18, and also to a special anchor named TIM44 (Slutsky-Leiderman et al., 2007) and thus await for incoming polypeptides from the cytosol. Given that a steep proton gradient needs to be maintained across the inner mitochondrial membrane, import pores must be as narrow as possible. When a typical hydrophobic polypeptide segment emerges, HSPA9 is triggered to clam upon it, while hydrolyzing ATP and concomitantly dissociating from its membrane anchors. This exerts an entropic pulling force that can unfold the polypeptide on the cytosolic side and unidirectionally translocate it to into the mitochondrial stroma (Goloubinoff and De Los Rios, 2007).

HSP70s can Control Protein Degradation

HSP70s also control protein degradation by chaperoning various native polypeptides toward specific proteases. Thus, without heat stress, bacterial DnaK, together with DnaJ can specifically bind the native σ32 transcription factor and present it to the protease FtsH for rapid degradation (Arsene et al., 2000). In eukaryotes, HSP70s can direct ubiquitin E3 ligases onto a polypeptide target to be subsequently degraded by the proteasome (Demand et al., 2001; Cyr et al., 2002; Kettern et al., 2010). In addition to chaperone-assisted proteasomal degradation, HSP70s are involved in selective chaperone-assisted autophagy and chaperone-mediated autophagy (Kettern et al., 2010).

The HSP70s Substrates

HSP70s can preferentially bind and process misfolded or alternatively-folded polypeptides, as opposed to (natively-)unfolded and natively-folded polypeptides for which they may have a lower affinity (Priya et al., 2013). By alternatively folded proteins, we intend to emphasize that such substrates can be natively folded and active proteins that also expose specific hydrophobic surfaces with a high affinity for J-domain co-chaperones and HSP70s. Under different biological circumstances, the alternatively folded proteins may acquire differently active, native states without exposed hydrophobic surfaces, thereby having low affinity for the chaperones. Examples of alternatively folded active proteins that can bind chaperones: clathrin cages (Schuermann et al., 2008; Mattoo et al., 2013), IκB oligomers (Weiss et al., 2007), nuclear heat-shock factor-1 trimers (Voellmy and Boellmann, 2007b), nuclear steroid hormone receptors (Echeverria and Picard, 2010), bacterial transcription factor σ32 (Rodriguez et al., 2008); their corresponding, differently active native conformers being, respectively, the clathrin triskelions, IkB monomers, inactive cytosolic heat-shock factor-1, cytosolic steroid hormone receptor and unfolded bacterial transcription factor σ32.

Given the general unfolding activity of HSP70s, it is unclear whether the so called intrinsically unfolded proteins are HSP70s substrates. The intrinsically unfolded α-synuclein protein does not bind DnaK or DnaJ, although it optimally exposes an ideal putative DnaK binding site (Rudiger et al., 1997). In contrast, once α-synuclein is stably aggregated into small oligomers, it strongly and specifically binds DnaJ, which in turn recruits DnaK on the stable misfolded α-synuclein oligomers. Upon ATP-hydrolysis and locking, the oligomers become partially unfolded and disaggregated (Hinault et al., 2010). DnaJ thus guides DnaK specifically onto the misfolded substrate, but not onto the unfolded polypeptide, suggesting that in general, the unfolded conformers might behave as the low-affinity products of the chaperone unfolding reactions.

HSP70s Can Control Biological Switches

HSP70s are key regulators of protein complexes involved in vesicular trafficking. Thus, HSC70 and HSP110, both assisted by the particular J-domain cochaperone auxilin, mediate the ATP-dependent dismantling of clathrin cages for recycling (Augustine et al., 2006; Morgan et al., 2013). Together with the HSP90s, the HSP70s can also control the activation of the steroid hormone receptors (Dittmar et al., 1998) and their own expression under heat stress, by regulating the activity, the oligomeric and phosphorylation state of heat-shock transcription factor-1 (Voellmy and Boellmann, 2007a). It has been reported that the pathological form of the cystic fibrosis transmembrane conductance regulator (CFTR) is stalled with a HSC70/HSP90 complex during protein maturation, suggesting that the HSP70/HSP90 chaperone complexes act transiently more on the early folding steps of the mutant CFTR than in the case of wild type CFTR (Coppinger et al., 2012). HSP70 over-expression in TNF-treated mammalian cells may cause the deoligomerization and thus the inactivation of large active IκB complexes, thereby inhibiting apoptosis (Aschkenasy et al., 2011). In parallel, HSP70s, especially HSPA1A can also inhibit caspases (Sabirzhanov et al., 2012) and are thus attractive drug targets for pro-inflammatory diseases suffering from unchecked apoptosis, such as Parkinson and Alzheimer's diseases. Therapies based on the over-expression of HSP70s, HSPA1A and HSP110 (HSPH1, Apg-1) in particular (Mattoo et al., 2013), might arrest pro-inflammatory degenerative diseases caused by toxic protein aggregates (Hinault et al., 2006). Yet, HSP70s are also pro-oncogenic factors against, which specific inhibitors need to be developed for cancer therapies (Schilling et al., 2015).

HSP70s appear to use a similar mechanism to process both randomly structured aggregates and alternatively folded oligomers: A J-domain cochaperone is first observed to target a HSP70 molecule onto the substrate. This may then trigger ATP-hydrolysis by the HSP70 and its "locking" onto the target polypeptide, thereby causing the local unfolding of the clamped-upon secondary structures and leading to the disaggregation and/or deoligomerization of the substrate. Upon NEF-mediated

release, the unfolded substrate may then refold into a differently active polypeptide or be further degraded by an ATP-consuming protease (Goloubinoff and De Los Rios, 2007).

The large diversity of the above-described cellular functions, raises the question of how the HSP70s, which are simple ~640 residue molecules sharing a simple common mechanism, may carry out so diverse cellular tasks? HSP70s are relatively simple two-domain individual 70 kDa polypeptides, which in order to act as effective polypeptide unfolding nanomachines, do not need to assemble into large cage-like oligomers, as in the case of the GroEL/CCT chaperonins, or ring-shaped hexamers in the case of ClpB/HSP104 (Priya et al., 2013; Mattoo and Goloubinoff, 2014). Here, we discuss various non-exclusive molecular mechanisms by which the HSP70s may carry so many diverse housekeeping and stress-damage control tasks in the cells.

HSP70s Nomenclature and Phylogeny

As in the case of gene families that have been studied for four decades, the nomenclature of the HSP70s is very complex and confusing with historical layers of terms. For example, the most abundant HSP70 is called DnaK in bacteria, SSA in yeast, HSC70 in humans, whereas they should be called HSPA8 according to the last agreed-upon nomenclature (Kampinga et al., 2009). Similarly, the main ER HSP70, which was traditionally called BIP or Grp78, recently became HSPA5. ER HSP110, which was traditionally called Grp170, is now called HYOU1, although sequence-wise it clearly belongs to the same clade as the cytoplasmic HSPH1, which was traditionally called HSP105 or Apg1 (**Figure 1A**).

A phylogenetic tree generated from the amino acid sequences of the most abundant HSP70s in Hela cells, HSPA8 (cytosolic HSC70), HSPA1A (cytosolic HSP70), HSPA9 (mitochondrial mortalin) HSPA5 (ER BIP), HSPH1, HSPH2 (cytosolic HSP110s) and HYOU (ER HSP110), shows two large subclasses: The DnaK-like HSP70s, that are both present in bacteria and eukaryotes and the HSP110s (Mattoo et al., 2013), which are only in the cytosol and the ER lumen of eukaryotes (**Figure 1B**).

HSP70s Structures and Functions

HSP70 and HSP110 are composed of a highly conserved two-lobbed 40-kDa nucleotide-binding domain (NBD) (**Figure 2A**, light beige) and of a more variable 30–50 kDa protein-binding domain (PBD) (**Figure 2A**, orange). The highest degree of evolutionary conservation is in the 40 kDa N-terminal NBD, which functions as ATP-fuelled molecular motor regulating dramatic structural changes in the 30 kDa C-terminal PDB (Mayer, 2013). The PDB resembles a crocodile jaw composed of a beta-strand subdomain, acting as a rigid base and a flexible α-helical subdomain acting as flexible lid, across which, bulky misfolded polypeptide substrates can bind and thus become "prays" to be chewed upon. In the apo- and ATP-bound states, the lid is widely open, with its back rear sticking to the NBD, allowing bulky misfolded, or structured alternatively folded polypeptides (such as the transcription factor σ32) with exposed hydrophobic surfaces, to freely bind the hydrophobic surfaces in the wide-open chaperone jaws (Schlecht et al., 2011; Clerico et al., 2015).

The Multilayered Molecular Mechanisms of the HSP70s

Mechanism of Passive Prevention of Aggregation

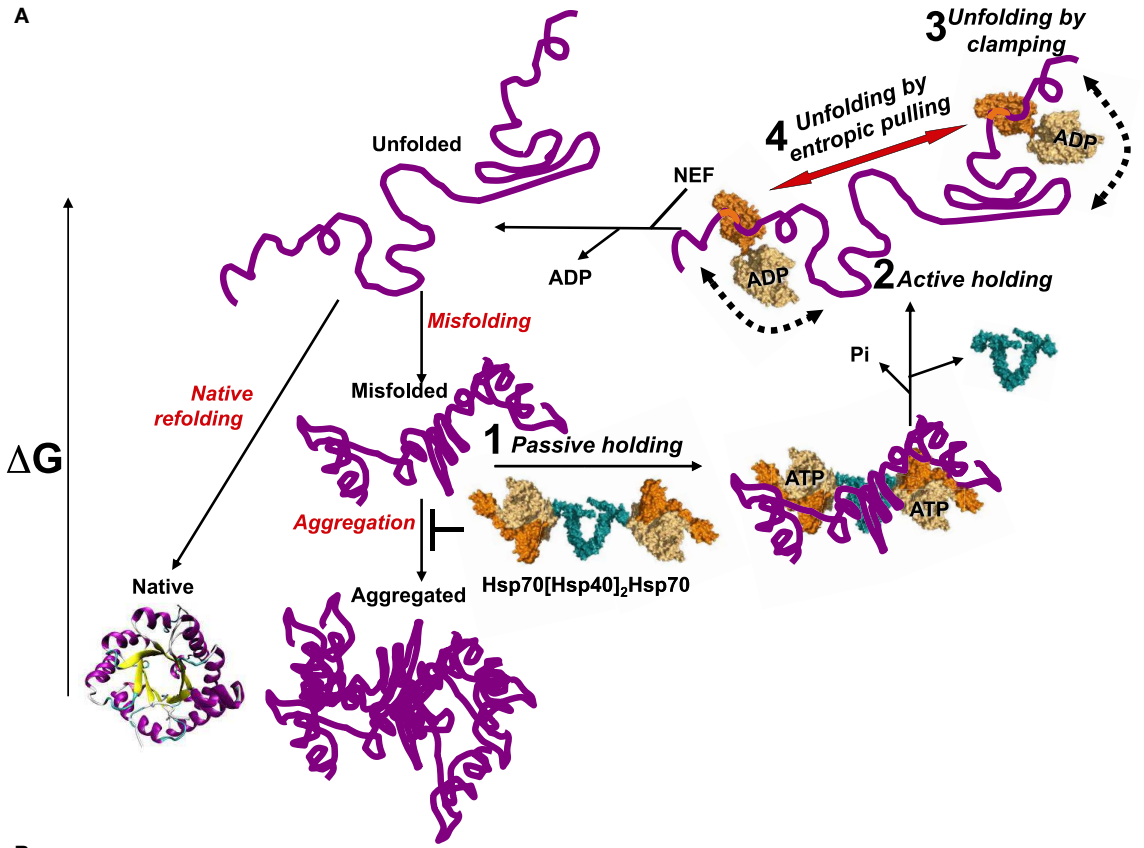
Once a protein is artificially denatured in the presence of a molar excess of HSP70 (and HSP40) but without ATP, it can weakly bind the chaperones and thus become partially prevented from forming large aggregates that scatter light. Hence, many *in vitro* chaperone assays measure the decrease in the net light scattering signal of an aggregating polypeptide in the presence of increasing concentrations of chaperones (Haslbeck and Buchner, 2015). But light scattering intensities are not quantitative and moreover don't reflect the ability of HSP70 chaperone molecules to use the energy of ATP-hydrolysis to change the conformational fate and thus the biological activity of hundreds different polypeptides in the cell.

Mechanisms of Active Unfolding by Clamping

Upon ATP hydrolysis, the rear of the lid detaches from the NBD and undergoes a closing movement toward the base in a wide rotation motion around the hinge (**Figure 2A**, step 3). There is experimental evidence that this mere clamping motion may already apply a destabilizing pressure on the misfolded structures of a bound substrate (Sharma et al., 2010). This motion may be futile, either when it is blocked by an over-resistant bulky polypeptide (Schlecht et al., 2011), or when the substrate has escaped the jaws before complete closure has occurred (Sousa, 2012). Importantly, however, this motion may be productive when it reaches complete closure, or "clamping," allowing the entrapment of a small, fully extended segment of the substrate, typically composed of five-six non-bulky hydrophobic residues preferably flanked by positive charges (Rudiger et al., 2000). Indicative of the complete unfolding of the clamped-upon segment, the ATP-dependent closure of DnaK's PDB has been shown to cause the peptide bonds of the substrate to undergo cis-trans isomerization of their secondary amides, reminiscent of peptidyl prolyl cis/trans isomerase activity (Schiene-Fischer et al., 2002). In the clamped state, the substrate is tightly bound to the ADP-containing HSP70 with an affinity estimated to be about 1000 fold higher than in the ATP bound or apo states, which both display wide-opened PBDs (Goloubinoff and De Los Rios, 2007).

Mechanisms of Active Holding

When clamping occurs under heat-shock conditions, substrate dissociation from HSP70s may come to a halt. This is the classic "holdase" activity of the chaperone (Slepenkov and Witt, 2002; Haslbeck and Buchner, 2015). The holding mode of HSP70s may be mediated by the NEFs, in particular bacterial GrpE, which can undergo a reversible thermal denaturation and thus reversibly loose its physiological ability to accelerate ADP- and substrate release from the HSP70s (Diamant and Goloubinoff, 1998; Grimshaw et al., 2001). This "holding" activity of the HSP70s under an ongoing heat-shock makes biological sense, as the untimely release of stress-labile polypeptides would obligatorily lead to their misfolding and aggregation, ultimately into species becoming increasingly resistant to the HSP70s unfolding activity (Sharma et al., 2011) (**Figure 2A**, step 2). The



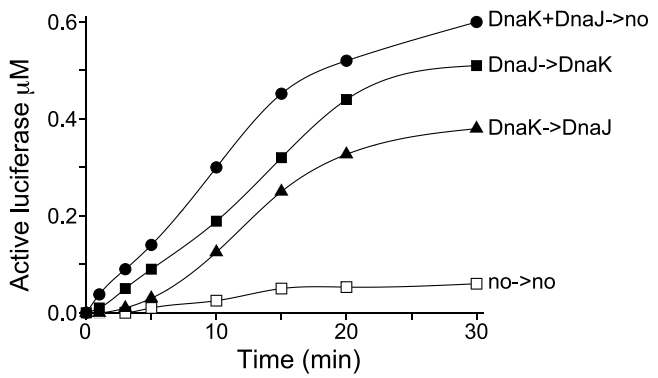


FIGURE 2 | The various modes of HSP70s action on their polypeptide substrates. (A) A nascent or a stress-unfolded polypeptide (upper left) with high free energy may readily fold to the native state (lower left) or misfold (middle center) and form stable aggregates with a low free energy. HSP70s, possibly pre-associated to HSP40 dimers, can spontaneously bind the misfolding species and thus passively prevent their aggregation, albeit rather inefficiently (step 1). ATP hydrolysis by HSP70 may actively lock the chaperone on the polypeptides and thus effectively prevent their aggregation (step 2). Further, ATP-hydrolysis can cause a forceful clamping motion of the lid toward the base of the PBD and apply an unfolding force that can cause local unfolding of misfolded segments in the substrate (step 3). The concomitant clamping of two distal HSP70s on the same misfolded polypeptide may further cause unfolding by entropic pulling of the intervening misfolded segments between two bound chaperones (step 4). Catalyzed by NEFs, ADP release from HSP70 unclamps the lid from its base. This in turn releases the locally unfolded polypeptide in solution (upper leftward arrow) where is may refold spontaneously to the native state with the lowest free energy. **(B)** Effect of order of addition of DnaK or DnaJ on luciferase refolding. DnaKJ->no (circles): DnaK (0.8 μM) and DnaJ (0.4 μM) were first preincubated for 5 min' with ATP at 22°C and at $T = 0$', FT-Luciferase (1 μM) and GrpE (0.7 μM) were added. DnaK-> DnaJ (triangles): DnaK was first preincubated 5 min' with FT-Luciferase and ATP, and at $T = 0$', DnaJ and GrpE were added. DnaJ-> DnaK (plain squares): DnaJ was first preincubated 5 min' with FT-Luciferase and ATP and, at $T = 0$', DnaK and GrpE were added. no->no (empty squares): spontaneous Luciferase refolding without chaperones.

differences between HSP70-mediated passive and active holding activities were shown in online light scattering measurements during the gradual denaturation and aggregation of a heat-labile luciferase in the presence of prokaryotic DnaK + DnaJ (Sharma et al., 2011), or human HSP70 + HSP40 and HSP110 + HSP40 (Mattoo et al., 2013), in the absence or presence of ATP. Although both chaperone and co-chaperone have specific hydrophobic surfaces that can bind misfolding polypeptides, without ATP, they were poorly effective at preventing the aggregation of the thermolabile luciferase, or in other words, much more of them was needed to prevent luciferase aggregation. In contrast, with ATP, both displayed a strong synergic holding activity at low concentrations, implying that when the conditions are unfavorable, DnaK, Hsp70 or HSP110 can use the energy of ATP hydrolysis to tightly clamp upon the heat-labile polypeptides to actively prevent aggregation and halt their release as long as heat-shock persists. Exactly the same ATP-dependent "holding" behavior was observed with HSP70 (HSPA1A) and HSP40 (Mattoo et al., 2013). The need under a prolonged heat-stress for very large amounts of non-catalytic holding HSP70s to prevent the aggregation of as many polypeptide substrates, could explain why the HSP70s are among the most abundant proteins in human cells (Finka and Goloubinoff, 2013). ATP-fuelled HSP70 clamping may not only cause the local unfolding of the clamped-upon segment but also maintain unfolded also some distal segments that are protruding away from the chaperone jaws.

Remarkably, at physiological temperatures, the clamping by a single DnaK molecule embracing at most 8–10 residues, at any of the dozen or so putative DnaK binding sites (Rudiger et al., 1997) along the 572 residue-long luciferase polypeptide, sufficed to maintain the latter nearly completely unfolded. This was revealed by partial protease treatments: The clamped-upon luciferase species were significantly more sensitive to a limited amount of trypsin, compared to the much more compact resistant misfolded substrates and natively folded products of the chaperone reaction (Sharma et al., 2010). The particular ability of the firefly luciferase to be maintained nearly completely unfolded by a single HSP70 molecule might be attributed to the fact that it folds mostly as a large single domain. In a multi-domain polypeptide, except for the clamped-upon domain that would be maintained unfolded by the chaperone, the other independent domains could be either misfolding or natively folded (Fitter, 2009). Thus, at variance with monomeric misfolded luciferase, aggregation-prone misfolded multi-domain polypeptides, are expected to necessitate the cooperative action of several HSP70 bound at different hydrophobic clusters along the same polypeptide, in order for it to become sufficiently unfolded, to reach after release the native state (Ben-Zvi and Goloubinoff, 2002).

Mechanism of Active Catalytic Unfolding/Refolding

In contrast to heat shock conditions, where the chaperone can be acting as a holding protein platform to prevent aggregation, upon returning to physiological temperatures and GrpE renaturation, the chaperone can switch back into a more effective catalytic mode of action: It can turn into an unfolding enzyme capable to iteratively bind, unfold and release polypeptides, until many substrates become converted into many native products.

Strong evidence that mere ATP-fuelled clamping by a single DnaK molecule may suffice to cause extensive unfolding, leading thereafter to native refolding of the 63 kDa luciferase, was presented by Sharma et al. (2010). Even in the presence of an unprecedented 15 fold molar excess of misfolded substrates over the chaperone, DnaK could effectively regenerate several native luciferase molecules in several consecutive cycles of DnaJ-mediated binding, ATP-fuelled unfolding and GrpE-mediated release (Sharma et al., 2010). At such stoichiometric ratios, chances of concomitant binding of two DnaK molecules to the same polypeptide are excessively low, excluding an obligate cooperative action between them. Given that each stable misfolded luciferase polypeptide that was successfully converted into a native luciferase monomer must have been produced by the action of a single DnaK molecule, ATP-fuelled unfolding by clamping was likely the central mechanism at work in this particular case.

This reaction did set a low consumption record of merely five ATP hydrolyzed per refolded luciferase protein. This strategy, whereby an inactive misfolded polypeptide could be "rehabilitated" by an ATP-consuming unfoldase into a functional one, was estimated to be about a thousand times more economic than the alternative, consisting of degrading it first by an ATP-consuming unfolding protease and then synthetizing a new polypeptide at the cost of three ATPs per peptide bond.

This notwithstanding, the hydrolysis of five ATPs implied that the chaperone may have undergone five productive clamping/unfolding events, but upon HSP70 dissociation, four luciferases spontaneously misfolded again and only one succeeded to reach the native state (Sharma et al., 2010). Yet, there was an alternative scenario, in which the chaperone could have undergone five ATP-hydrolysis events accompanied by five attempted clamping events, but only one would have succeed to reach the native state, whereas the other four would have caused only partial and ineffective unfolding (Sharma et al., 2010). This second scenario was confirmed by an order of addition experiment in which the misfolded luciferase monomers were first interacted with an excess of DnaK, DnaJ and alpha-labeled radioactive ATP for 2 min, to allow initial binding and clamping. Then, apyrase was added to readily convert all the ATP into AMP. Following the separation of the proteins from the nucleotides by a quick spin column chromatography, an inactive chaperone-luciferase complex was isolated that was found to contain 0.6 μM of apyrase-resistant ADP. Remarkably, subsequent addition of GrpE to this inactive complex, readily yield up to 590 nM of native luciferase, although this happened in the total absence of ATP. This experiment produced two important clues about HSP70s mechanism. (1) Whereas the unfolding of a stable misfolded substrate by HSP70s necessitates ATP-hydrolysis, the refolding of the chaperone-released unfolded products does not necessitate ATP and is thus spontaneous. This confirmed Anfinsen's seminal observations (Anfinsen, 1973) that unfolded polypeptides should, in principle, be able the reach their native state unassisted by other proteins. (2) The excellent match between the amounts of entrapped ADP in the isolated inactive

chaperone-luciferase complexes, and the subsequently refolded luciferase, implies that upon release, the native refolding was nearly 95% efficient, excluding scenario one, in which only 20% of the released unfolded species would have reached the native state while the other would have misfolded again. Rather, this experiment indicated that four out of five clamping events by the chaperone ended up being unproductive. The possibility that upon ATP-hydrolysis, the ADP-bound HSP70 might undergo only a partial but incomplete clamping and unfolding motion was shown by Mayer and collaborators, who used fluorescence spectroscopy to demonstrate that following ATP hydrolysis, some ADP-bound DnaK molecules need not obligatorily end up being with tightly closed SBDs that clamp around fully extended polypeptides (Schlecht et al., 2011).

Relevant to this chaperone mechanism, quantitative proteomics of human Jurkat cells (**Figure 1A**) showed copy numbers of 18500:1350:1180, representing an overall stoichiometry of about 16:1:1 for HSP70s, J-domain cochaperones and NEFs (Bags and GrpE), respectively. Assuming an equal distribution of Hsp70s through the cytosol of Jurkat cells, this suggests that neither J-domain nor NEF cochaperones can be persistent structural constituents of the core unfoldase machineries. Rather, they may both need to act as substoichiometric catalysts (Pierpaoli et al., 1998; Laufen et al., 1999; Hinault et al., 2010), which in iterative cycles of binding and dissociation, can accelerate respectively, the ATP-fuelled unfolding of the misfolded substrates by the chaperone and the release of the unfolded products from the chaperone (Sharma et al., 2010).

Mechanism of Active Unfolding by Entropic Pulling

In contrast to chaperone-amenable substrates, such as monomeric misfolded luciferase, which could be productively unfolded by the solitary clamping action of individual DnaK molecules, larger soluble aggregates of the same misfolded luciferase (Mattoo et al., 2013), or of heat-denatured G6PDH (Diamant et al., 2000; Ben-Zvi et al., 2004), or of freeze-thawed denatured rhodanese monomers (Natalello et al., 2013), stringently required the presence of a large molar excess of chaperone (DnaK), in order to become productively unfolded and renatured. This suggested that the productive unfolding of severely damaged misfolded polypeptides may require the concomitant binding of several DnaKs at different hydrophobic binding sites along same misfolded polypeptide (Rudiger et al., 1997, 2000). Together with the observed unfolding by pulling effect of mitochondrial HSP70, which is distally applied on translocating polypeptides from within the mitochondria, a common mechanism of unfolding by entropic pulling was proposed that described the forces applied on a misfolded substrate by two or more clamped ADP-DnaK molecules on a misfolded segment situated in between two chaperone-bound segments in an aggregate. Due to the excluded volumes of the two clamped chaperones and their bumping into each other and into the aggregate during a very long time in molecular terms (0.1-1 minute), they were found to apply unfolding-pulling forces, which can pull away and unfold vicinal misfolded segments (De Los Rios et al., 2006; Goloubinoff and De Los Rios, 2007). Thus, in the case of more damaged protein aggregates, the active local clamping of the HSP70s may not suffice to productively unfold a multidomain polypeptide and an additional cooperative mechanism of unfolding by entropic pulling may be necessary (**Figure 2A**, step 4).

One major limitation of unfolding by entropic pulling is that in order to be effective, more than one HSP70 molecule has to be concomitantly bound to the same misfolded polypeptide. Although as many as 12,000 HSC70 molecules may be present in a micron cube of human cytoplasm (**Figure 1**) (Finka et al., 2015), some of the remaining 1,200,000 polypeptides may misfold. Once these would exceed 6000, none of them would be reverted to the native state by the 12,000 HSC70s, even at the futile cost of several times 12,000 hydrolyzed ATP.

One possible way for HSP70s to overcome dilution by their own substrate would be to assume that they don't necessarily have to be equally distributed in the cytosol. Moreover they may have a natural tendency to form, at rest, low affinity oligomers (Motohashi et al., 1994). When challenged by misfolded polypeptides, these would readily dissociate and, because of their slow diffusion rate, readily bind nearby each other, increasing the chances of productive multiple binding on the same misfolded polypeptide, despite the unfavorable stoichiometry.

A second way for HSP70s to overcome this limitation would be to function with dimeric J-cochaperone. Indeed all DNAJA/B and HSP40 are dimers associated by a small dimerization domain through their C-terminal ends (Mattoo et al., 2014). Thus, a dimeric DnaJ may optimally present its two N-terminal J-domains for the binding of the NBDs of two ATP-bound HSP70s, while still keeping the two far apart enough so that, after binding to the substrate, they might optimally exclude each other and apply a cooperative unfolding force by entropic pulling. Given that cells have a large excess of ATP over ADP and that the cytosolic HSP70s are about 10 times more abundant than their J-domain cochaperones, it is tempting to speculate that without a stress, most DNAJA/B dimers in the cell will be associated to two ATP-bound HSP70s. Once challenged by a stress-induced misfolded polypeptide, these loose hetero-tetramers could dissociate and the two HSP70s would be thus able to lock onto nearby regions on the same misfolded polypeptide substrate. Simple order of addition kinetics of DnaK-DnaJ-GrpE-mediated refolding of inactive FT-luciferase, illustrates this. When DnaK and DnaJ was first pre-incubated (for 5 min) with ATP and at $T = 0$', supplemented by FTluc and GrpE (**Figure 2B**, DnaKJ->no), luciferase refolding readily started without a delay and at a rapid rate of 140 nM per 5 min. When DnaJ alone was first preincubated with TFluc and ATP at $T = 0$', DnaK and GrpE were added (**Figure 2B**, DnaJ->DnaK), refolding was slightly delayed and the refolding rate was slightly lower (90 nM in the first 5 min). In contrast, when DnaK alone was first preincubated with ATP and FTluc, and at $T = 0$' incubated with DnaJ and GrpE (**Figure 2B**, DnaK->DnaJ), luciferase refolding was strongly delayed and reached less than a net 15 nM active Luciferase in the first 5 min of the reaction. These results indicate that, as initially shown by Bukau and colleagues (Gamer et al., 1992), the DnaJ dimer may indeed be the first to bind the

substrate and thereafter effectively recruit two DnaKs onto the substrate. Noticeably, when DnaK was compelled to pre-interact first with the substrate, the strong delay in the refolding rate suggests that some time was needed for the wrongly associated DnaKs to first dissociate from the substrate and for DnaJ to properly bind and only then, optimally recruit DnaK on the substrate. Moreover, the fastest rate that we observed with preincubated DnaK + DnaJ + ATP prior they were presented to the substrate and GrpE, suggest that in the presence of ATP, DnaK and DnaJ may form a loose hetero-oligomer, likely a $DnaJ_2DnaK_2$, optimally poised to bind cooperatively to the same misfolded substrate and engage in the HSP70-unfoldase cycle (**Figure 2A**, step 1).

A third way to overcome the limitation for unfolding by entropic pulling caused upon dilution of the HSP70s by their own substrate, would be the spontaneous formation of loose hsp70dimers. Thus, HSP110:HSP70 heterodimers associated by their N-terminal domains can apparently form and act reciprocally as NEFs on each other. Somehow, together they can efficiently disaggregate large stable aggregates (Schuermann et al., 2008; Mattoo et al., 2013). Similarly, bacterial DnaKs might also form loose homodimers, which would favor their ability to act upon resistant substrates by entropic pulling (Sarbeng et al., 2015).

Finally, one elegant way in which evolution may have chosen to overcome HSP70 limitation by substrate dilution, was recruitment of powerful disaggregating nanomachines, such as ClpB in *E. coli* or HSP104 in yeast. These are AAA+ type hexameric cylinders capable of applying ATP-fuelled power-stokes on inserted misfolded polypeptide loops from the aggregates in the hexamer cavity and unfold them by stretching strokes (Goloubinoff et al., 1999; Winkler et al., 2012). Once activated by HSP70, ClpB or HSP104 can convert large protein aggregates into lower oligomeric species amenable to HSP70-mediated unfolding by clamping.

The multilayer mechanisms of HSP70 as holding and unfolding chaperones can be summarized in four steps (**Figure 2A**). Without ATP, the wide opened protein-binding domain may weakly bind to hydrophobic patches on the surface of misfolding polypeptides, thereby passively and rather inefficiently preventing aggregation (**Figure 2A**, step 1). But passive binding is probably irrelevant to the physiological situation in cells. ATP-hydrolysis, by driving the closure of the PBD in the substrate and causing local unfolding, can dramatically increase the affinity of the misfolded polypeptides for the chaperone, thereby actively and efficiently prevent aggregation (**Figure 2A**, step 2). Owing to the transient thermal inactivation of the NEF (GrpE), dissociation during heat-shock is prevented. Upon returning to the physiological conditions, GrpE may become functional again and trigger the release of the unfolded substrate, leading to efficient spontaneous refolding. Catalytic cycles of misfolded substrate binding, ATP-fuelled unfolding by clamping, release and spontaneous refolding may thus convert non-aggregated misfolded species (**Figure 2A**, step 3), such as FT-luciferase into native proteins (Sharma et al., 2010). In case the misfolded species are tightly associated into large insoluble aggregates, the binding and local unfolding by clamping of a single Hsp70 molecule may not suffice to unfold distal misfolded segments in the same polypeptide. The binding and locking of additional HSP70 molecules elsewhere on the same polypeptide would add another unfolding effect by way of the Brownian movements of the locked chaperones and their volume exclusion from each other, applying unfolding forces on the misfolded segments located in between the HSP70-bound sites (**Figure 2A**, step 4).

This additional mode of ATP-dependent unfolding by distal entropic pulling of the HSP70, can predominate in the case of polypeptide precursors that are partially folded in the cytosol and need to be imported across the narrow pores of the mitochondria (Elsner et al., 2009), the ER (Matlack et al., 1997) and chloroplasts (Shi and Theg, 2011). Here, the organellar ATP-bound HSP70s are uniquely targeted to the import pores by specific pore-associated J-domain cochaperones to clamp upon the entering polypeptides segments, which are unstructured. In these cases, the unfolding action of the organellar HSP70s is not local but distal, by pulling on the bulky structures that are still on the cytoplasmic side of the precursor protein and are forced to collide onto the narrow pore entry and thereby to unfold prior translocation. Moreover, ATP-dependent unfolding by entropic pulling is likely to be the mechanism by which HSC70 and HSP110, targeted by their specific J-domain cochaperone auxilin, actively deoligomerize clathrin cages into triskelions (Zhuo et al., 2010).

Conclusion

HSP70s are a central hub of the chaperone network carrying very diverse physiological functions in proteostasis, such as polypeptide folding/unfolding, protein assembly/disassembly, protein activation/inactivation, polypeptide translocation, and degradation. Moreover, HSP70s act as polypeptide-unfolding nanomachines that serve as defenses against stress- and mutation-induced formation and accumulation of cytotoxic misfolded protein conformers. Assisted by J-domain cochaperones, the HSP70s can specifically detect among a large excess of low-affinity native proteins a minority of high-affinity misfolding intermediates on their way to form toxic aggregates. HSP70s may further collaborate with each other (DnaK-DnaK, HSP70-HSP110) and with AAA+ unfoldases (HSP100/ClpB), at disentangling already formed aggregates and convert them into natively refoldable, or protease-degradable polypeptides. The pharmacological upregulation, or adenoviral-mediated over-expression of specific HSP70s, HSP110s and their specific J-domain and NEF cochaperones, are attractive avenues for therapies against degenerative protein conformational diseases and aging (Ebrahimi-Fakhari et al., 2013). Moreover, the development of specific inhibitors of HSP70s and J-domain co-chaperones, is a promising approach to develop therapies against aggressive cancers (Patury et al., 2009; Chang et al., 2011).

Acknowledgments

Research was financed by Swiss National Science Foundation Grants 125502/1 and 140512/1.

References

Anfinsen, C. B. (1973). Principles that govern folding of protein chains. *Science* 181, 223–230. doi: 10.1126/science.181.4096.223

Arike, L., Valgepea, K., Peil, L., Nahku, R., Adamberg, K., and Vilu, R. (2012). Comparison and applications of label-free absolute proteome quantification methods on *Escherichia coli*. *J. Proteomics* 75, 5437–5448. doi: 10.1016/j.jprot.2012.06.020

Arsene, F., Tomoyasu, T., and Bukau, B. (2000). The heat shock response of *Escherichia coli*. *Int. J. Food Microbiol.* 55, 3–9. doi: 10.1016/S0168-1605(00)00206-3

Aschkenasy, G., Bromberg, Z., Raj, N., Deutschman, C. S., and Weiss, Y. G. (2011). Enhanced Hsp70 expression protects against acute lung injury by modulating apoptotic pathways. *PLoS ONE* 6:e26956. doi: 10.1371/journal.pone.0026956

Augustine, G. J., Morgan, J. R., Villalba-Galea, C. A., Jin, S., Prasad, K., and Lafer, E. M. (2006). Clathrin and synaptic vesicle endocytosis: studies at the squid giant synapse. *Biochem. Soc. Trans.* 34, 68–72. doi: 10.1042/BST0340068

Bellotti, V., and Chiti, F. (2008). Amyloidogenesis in its biological environment: challenging a fundamental issue in protein misfolding diseases. *Curr. Opin. Struct. Biol.* 18, 771–779. doi: 10.1016/j.sbi.2008.10.001

Ben-Zvi, A., De Los Rios, P., Dietler, G., and Goloubinoff, P. (2004). Active solubilization and refolding of stable protein aggregates by cooperative unfolding action of individual Hsp70 chaperones. *J. Biol. Chem,* 279, 37298–37303. doi: 10.1074/jbc.M405627200

Ben-Zvi, A. P., and Goloubinoff, P. (2002). Proteinaceous infectious behavior in non-pathogenic proteins is controlled by molecular chaperones. *J. Biol. Chem.* 277, 49422–49427. doi: 10.1074/jbc.M209163200

Chang, L., Miyata, Y., Ung, P. M., Bertelsen, E. B., Mcquade, T. J., Carlson, H. A., et al. (2011). Chemical screens against a reconstituted multiprotein complex: myricetin blocks DnaJ regulation of DnaK through an allosteric mechanism. *Chem. Biol,* 18, 210–221. doi: 10.1016/j.chembiol.2010.12.010

Cho, Y., Zhang, X., Pobre, K. F., Liu, Y., Powers, D. L., Kelly, J. W., et al. (2015). Individual and collective contributions of chaperoning and degradation to protein homeostasis in *E. coli*. *Cell Rep.* 11, 321–333. doi: 10.1016/j.celrep.2015.03.018

Clerico, E. M., Tilitsky, J. M., Meng, W., and Gierasch, L. M. (2015). How Hsp70 molecular machines interact with their substrates to mediate diverse physiological functions. *J. Mol. Biol.* 427, 1575–1588. doi: 10.1016/j.jmb.2015.02.004

Coppinger, J. A., Hutt, D. M., Razvi, A., Koulov, A. V., Pankow, S., Yates, J. R. III, et al. (2012). A chaperone trap contributes to the onset of cystic fibrosis. *PLoS ONE* 7:e37682. doi: 10.1371/journal.pone.0037682

Cyr, D. M., Hohfeld, J., and Patterson, C. (2002). Protein quality control: U-box-containing E3 ubiquitin ligases join the fold. *Trends Biochem. Sci.* 27, 368–375. doi: 10.1016/S0968-0004(02)02125-4

De Los Rios, P., Ben-Zvi, A., Slutsky, O., Azem, A., and Goloubinoff, P. (2006). Hsp70 chaperones accelerate protein translocation and the unfolding of stable protein aggregates by entropic pulling. *Proc. Natl. Acad. Sci. U.S.A.* 103, 6166–6171. doi: 10.1073/pnas.0510496103

Demand, J., Alberti, S., Patterson, C., and Hohfeld, J. (2001). Cooperation of a ubiquitin domain protein and an E3 ubiquitin ligase during chaperone/proteasome coupling. *Curr. Biol.* 11, 1569–1577. doi: 10.1016/S0960-9822(01)00487-0

Diamant, S., Ben-Zvi, A. P., Bukau, B., and Goloubinoff, P. (2000). Size-dependent disaggregation of stable protein aggregates by the DnaK chaperone machinery. *J. Biol. Chem.* 275, 21107–21113. doi: 10.1074/jbc.M001293200

Diamant, S., and Goloubinoff, P. (1998). Temperature-controlled activity of DnaK-DnaJ-GrpE chaperones: protein-folding arrest and recovery during and after heat shock depends on the substrate protein and the GrpE concentration. *Biochemistry* 37, 9688–9694. doi: 10.1021/bi980338u

Dittmar, K. D., Banach, M., Galigniana, M. D., and Pratt, W. B. (1998). The role of DnaJ-like proteins in glucocorticoid receptor center dot hsp90 heterocomplex assembly by the reconstituted hsp90 center dot p60 center dot hsp70 foldosome complex. *J. Biol. Chem.* 273, 7358–7366. doi: 10.1074/jbc.273.13.7358

Ebrahimi-Fakhari, D., Saidi, L. J., and Wahlster, L. (2013). Molecular chaperones and protein folding as therapeutic targets in Parkinson's disease and other synucleinopathies. *Acta Neuropathol. Commun.* 1, 79. doi: 10.1186/2051-5960-1-79

Echeverria, P. C., and Picard, D. (2010). Molecular chaperones, essential partners of steroid hormone receptors for activity and mobility. *Biochim. Biophys. Acta* 1803, 641–649. doi: 10.1016/j.bbamcr.2009.11.012

Elsner, S., Simian, D., Iosefson, O., Marom, M., and Azem, A. (2009). The mitochondrial protein translocation motor: structural conservation between the human and yeast Tim14/Pam18-Tim16/Pam16 co-chaperones. *Int. J. Mol. Sci.* 10, 2041–2053. doi: 10.3390/ijms10052041

Fiaux, J., Horst, J., Scior, A., Preissler, S., Koplin, A., Bukau, B., et al. (2010). Structural analysis of the ribosome-associated complex (RAC) reveals an unusual Hsp70/Hsp40 interaction. *J. Biol. Chem.* 285, 3227–3234. doi: 10.1074/jbc.M109.075804

Finka, A., and Goloubinoff, P. (2013). Proteomic data from human cell cultures refine mechanisms of chaperone-mediated protein homeostasis. *Cell Stress Chaperones* 18, 591–605. doi: 10.1007/s12192-013-0413-3

Finka, A., Mattoo, R. U., and Goloubinoff, P. (2011). Meta-analysis of heat- and chemically upregulated chaperone genes in plant and human cells. *Cell Stress Chaperones* 16, 15–31. doi: 10.1007/s12192-010-0216-8

Finka, A., Sood, V., Quadroni, M., Rios, P., and Goloubinoff. P. (2015). Quantitative proteomics of heat-treated human cells show an across-the-board mild depletion of housekeeping proteins to massively accumulate few HSPs. *Cell Stress Chaperones*. doi: 10.1007/s12192-015-0583-2. [Epub ahead of print].

Fitter, J. (2009). The perspectives of studying multi-domain protein folding. *Cell. Mol. Life Sci.* 66, 1672–1681. doi: 10.1007/s00018-009-8771-9

Gamer, J., Bujard, H., and Bukau, B. (1992). Physical interaction between heat shock proteins DnaK, DnaJ, and GrpE and the bacterial heat shock transcription factor sigma 32. *Cell* 69, 833–842. doi: 10.1016/0092-8674(92)90294-M

Geiger, T., Wehner, A., Schaab, C., Cox, J., and Mann, M. (2012). Comparative proteomic analysis of eleven common cell lines reveals ubiquitous but varying expression of most proteins. *Mol. Cell. Proteomics* 11, M111.014050. doi: 10.1074/mcp.M111.014050

Goloubinoff, P., and De Los Rios, P. (2007). The mechanism of Hsp70 chaperones: (entropic) pulling the models together. *Trends Biochem. Sci.* 32, 372–380. doi: 10.1016/j.tibs.2007.06.008

Goloubinoff, P., Mogk, A., Ben Zvi, A. P., Tomoyasu, T., and Bukau, B. (1999). Sequential mechanism of solubilization and refolding of stable protein aggregates by a bichaperone network. *Proc. Natl. Acad. Sci. U.S.A.* 96, 13732–13737. doi: 10.1073/pnas.96.24.13732

Griesemer, M., Young, C., Robinson, A. S., and Petzold, L. (2014). BiP clustering facilitates protein folding in the endoplasmic reticulum. *PLoS Comput. Biol.* 10:e1003675. doi: 10.1371/journal.pcbi.1003675

Grimshaw, J. P., Jelesarov, I., Schonfeld, H. J., and Christen, P. (2001). Reversible thermal transition in GrpE, the nucleotide exchange factor of the DnaK heat-shock system. *J. Biol. Chem.* 276, 6098–6104. doi: 10.1074/jbc.M009290200

Haslbeck, M., and Buchner, J. (2015). Assays to characterize molecular chaperone function *in vitro*. *Methods Mol. Biol.* 1292, 39–51. doi: 10.1007/978-1-4939-2522-3_3

Hinault, M. P., Ben-Zvi, A., and Goloubinoff, P. (2006). Chaperones and proteases - Cellular fold-controlling factors of proteins in Neurodegenerative diseases and aging. *J. Mol. Neurosci.* 30, 249–265. doi: 10.1385/JMN:30:3:249

Hinault, M. P., Cuendet, A. F., Mattoo, R. U., Mensi, M., Dietler, G., Lashuel, H. A., et al. (2010). Stable alpha-synuclein oligomers strongly inhibit chaperone activity of the Hsp70 system by weak interactions with J-domain co-chaperones. *J. Biol. Chem.* 285, 38173–38182. doi: 10.1074/jbc.M110.127753

Kampinga, H. H., Hageman, J., Vos, M. J., Kubota, H., Tanguay, R. M., Bruford, E. A., et al. (2009). Guidelines for the nomenclature of the human heat shock proteins. *Cell Stress Chaperones* 14, 105–111. doi: 10.1007/s12192-008-0068-7

Kettern, N., Dreiseidler, M., Tawo, R., and Hohfeld, J. (2010). Chaperone-assisted degradation: multiple paths to destruction. *Biol. Chem.* 391, 481–489. doi: 10.1515/bc.2010.058

Koplin, A., Preissler, S., Ilina, Y., Koch, M., Scior, A., Erhardt, M., et al. (2010). A dual function for chaperones SSB-RAC and the NAC nascent polypeptide-associated complex on ribosomes. *J. Cell Biol.* 189, 57–68. doi: 10.1083/jcb.200910074

Laufen, T., Mayer, M. P., Beisel, C., Klostermeier, D., Mogk, A., Reinstein, J., et al. (1999). Mechanism of regulation of Hsp70 chaperones by DnaJ cochaperones. *Proc. Natl. Acad. Sci. U.S.A.* 96, 5452–5457. doi: 10.1073/pnas.96.10.5452

Macario, A. J. L., and De Macario, E. C. (1999). The archaeal molecular chaperone machine: peculiarities and paradoxes. *Genetics* 152, 1277–1283.

Matlack, K. E. S., Plath, K., Misselwitz, B., and Rapoport, T. A. (1997). Protein transport by purified yeast sec complex and Kar2p without membranes. *Science* 277, 938–941. doi: 10.1126/science.277.5328.938

Mattoo, R. H., and Goloubinoff, P. (2014). Molecular chaperones are nanomachines that catalytically unfold misfolded and alternatively folded proteins. *Cell. Mol. Life Sci.* 71, 3311–3325. doi: 10.1007/s00018-014-1627-y

Mattoo, R. U., Sharma, S. K., Priya, S., Finka, A., and Goloubinoff, P. (2013). Hsp110 is a bona fide chaperone using ATP to unfold stable misfolded polypeptides and reciprocally collaborate with Hsp70 to solubilize protein aggregates. *J. Biol. Chem.* 288, 21399–21411. doi: 10.1074/jbc.M113.479253

Mattoo, R. U. H., Farina Henriquez Cuendet, A., Sujatha, S., Finka, A., Priya, S., Sharma, S. K., et al. (2014). Synergism between a foldase and an unfoldase: reciprocal dependence between the thioredoxin-like activity of DnaJ and the polypeptide-unfolding activity of DnaK. *Front. Mol. Biosci.* 1:7. doi: 10.3389/fmolb.2014.00007

Mayer, M. P. (2013). Hsp70 chaperone dynamics and molecular mechanism. *Trends Biochem. Sci.* 38, 507–514. doi: 10.1016/j.tibs.2013.08.001

Melnyk, A., Rieger, H., and Zimmermann, R. (2015). Co-chaperones of the mammalian endoplasmic reticulum. *Subcell. Biochem.* 78, 179–200. doi: 10.1007/978-3-319-11731-7_9

Morgan, J. R., Jiang, J. W., Oliphint, P. A., Jin, S. P., Gimenez, L. E., Busch, D. J., et al. (2013). A Role for an Hsp70 nucleotide exchange factor in the regulation of synaptic vesicle endocytosis. *J. Neurosci.* 33, 8009–8021. doi: 10.1523/JNEUROSCI.4505-12.2013

Motohashi, K., Taguchi, H., Ishii, N., and Yoshida, M. (1994). Isolation of the stable hexameric DnaK.DnaJ complex from Thermus thermophilus. *J. Biol. Chem.* 269, 27074–27079.

Natalello, A., Mattoo, R. U. H., Priya, S., Sharma, S. K., Goloubinoff, P., and Doglia, S. M. (2013). Biophysical characterization of two different stable misfolded monomeric polypeptides that are chaperone-amenable substrates. *J. Mol. Biol.* 425, 1158–1171. doi: 10.1016/j.jmb.2012.12.025

Otto, H., Conz, C., Maier, P., Wolfle, T., Suzuki, C. K., Jeno, P., et al. (2005). The chaperones MPP11 and Hsp70L1 form the mammalian ribosome-associated complex. *Proc. Nat. Acad. Sci. U.S.A.* 102, 10064–10069. doi: 10.1073/pnas.0504400102

Patury, S., Miyata, Y., and Gestwicki, J. E. (2009). Pharmacological targeting of the Hsp70 chaperone. *Curr. Top. Med. Chem.* 9, 1337–1351. doi: 10.2174/156802609789895674

Pierpaoli, E. V., Sandmeier, E., Schonfeld, H. J., and Christen, P. (1998). Control of the DnaK chaperone cycle by substoichiometric concentrations of the co-chaperones DnaJ and GrpE. *J. Biol. Chem.* 273, 6643–6649. doi: 10.1074/jbc.273.12.6643

Priya, S., Sharma, S. K., and Goloubinoff, P. (2013). Molecular chaperones as enzymes that catalytically unfold misfolded polypeptides. *FEBS Lett.* 587, 1981–1987. doi: 10.1016/j.febslet.2013.05.014

Rampelt, H., Kirstein-Miles, J., Nillegoda, N. B., Chi, K., Scholz, S. R., Morimoto, R. I., et al. (2012). Metazoan Hsp70 machines use Hsp110 to power protein disaggregation. *Embo J.* 31, 4221–4235. doi: 10.1038/emboj.2012.264

Rodriguez, F., Arsene-Ploetze, F., Rist, W., Rudiger, S., Schneider-Mergener, J., Mayer, M. P., et al. (2008). Molecular basis for regulation of the heat shock transcription factor sigma32 by the DnaK and DnaJ chaperones. *Mol. Cell* 32, 347–358. doi: 10.1016/j.molcel.2008.09.016

Rudiger, S., Germeroth, L., Schneider-Mergener, J., and Bukau, B. (1997). Substrate specificity of the DnaK chaperone determined by screening cellulose-bound peptide libraries. *EMBO J.* 16, 1501–1507. doi: 10.1093/emboj/16.7.1501

Rudiger, S., Mayer, M. P., Schneider-Mergener, J., and Bukau, B. (2000). Modulation of substrate specificity of the DnaK chaperone by alteration of a hydrophobic arch. *J. Mol. Biol.* 304, 245–251. doi: 10.1006/jmbi.2000.4193

Ryno, L. M., Wiseman, R. L., and Kelly, J. W. (2013). Targeting unfolded protein response signaling pathways to ameliorate protein misfolding diseases. *Curr. Opin. Chem. Biol.* 17, 346–352. doi: 10.1016/j.cbpa.2013.04.009

Sabirzhanov, B., Stoica, B. A., Hanscom, M., Piao, C. S., and Faden, A. I. (2012). Over-expression of HSP70 attenuates caspase-dependent and caspase-independent pathways and inhibits neuronal apoptosis. *J. Neurochem.* 123, 542–554. doi: 10.1111/j.1471-4159.2012.07927.x

Saidi, Y., Domini, M., Choy, F., Zryd, J. P., Schwitzguebel, J. P., and Goloubinoff, P. (2007). Activation of the heat shock response in plants by chlorophenols: transgenic Physcomitrella patens as a sensitive biosensor for organic pollutants. *Plant Cell Environ.* 30, 753–763. doi: 10.1111/j.1365-3040.2007.01664.x

Sarbeng, E. B., Liu, Q., Tian, X., Yang, J., Li, H., Wong, J. L., et al. (2015). A functional DnaK dimer is essential for the efficient interaction with Hsp40 heat shock protein. *J. Biol. Chem.* 290, 8849–8862. doi: 10.1074/jbc.M114.596288

Schiene-Fischer, C., Habazettl, J., Schmid, F. X., and Fischer, G. (2002). The hsp70 chaperone DnaK is a secondary amide peptide bond cis-trans isomerase. *Nat. Struct. Biol.* 9, 419–424. doi: 10.1038/nsb804

Schilling, D., Kuhnel, A., Konrad, S., Tetzlaff, F., Bayer, C., Yaglom, J., et al. (2015). Sensitizing tumor cells to radiation by targeting the heat shock response. *Cancer Lett.* 360, 294–301. doi: 10.1016/j.canlet.2015.02.033

Schlecht, R., Erbse, A. H., Bukau, B., and Mayer, M. P. (2011). Mechanics of Hsp70 chaperones enables differential interaction with client proteins. *Nat. Struct. Mol. Biol.* 18, 345–U135. doi: 10.1038/nsmb.2006

Schlicher, T., and Soll, J. (1996). Molecular chaperones are present in the thylakoid lumen of pea chloroplasts. *Febs Lett.* 379, 302–304. doi: 10.1016/0014-5793(95)01534-5

Schuermann, J. P., Jiang, J. W., Cuellar, J., Llorca, O., Wang, L. P., Gimenez, L. E., et al. (2008). Structure of the Hsp110: Hsc70 nucleotide exchange machine. *Mol. Cell* 31, 232–243. doi: 10.1016/j.molcel.2008.05.006

Shalgi, R., Hurt, J. A., Krykbaeva, I., Taipale, M., Lindquist, S., and Burge, C. B. (2013). Widespread regulation of translation by elongation pausing in heat shock. *Mol. Cell* 49, 439–452. doi: 10.1016/j.molcel.2012.11.028

Shalgi, R., Hurt, J. A., Lindquist, S., and Burge, C. B. (2014). Widespread inhibition of posttranscriptional splicing shapes the cellular transcriptome following heat shock. *Cell Rep.* 7, 1362–1370. doi: 10.1016/j.celrep.2014.04.044

Sharma, S. K., De Los Rios, P., Christen, P., Lustig, A., and Goloubinoff, P. (2010). The kinetic parameters and energy cost of the Hsp70 chaperone as a polypeptide unfoldase. *Nat. Chem. Biol.* 6, 914–920. doi: 10.1038/nchembio.455

Sharma, S. K., De Los Rios, P., and Goloubinoff, P. (2011). Probing the different chaperone activities of the bacterial HSP70-HSP40 system using a thermolabile luciferase substrate. *Proteins* 79, 1991–1998. doi: 10.1002/prot.23024

Shi, L. X., and Theg, S. M. (2011). The motors of protein import into chloroplasts. *Plant Signal. Behav.* 6, 1397–1401. doi: 10.4161/psb.6.9.16916

Shorter, J. (2011). The mammalian disaggregase machinery: Hsp110 synergizes with Hsp70 and Hsp40 to catalyze protein disaggregation and reactivation in a cell-free system. *PLoS ONE* 6:e26319. doi: 10.1371/journal.pone.0026319

Slepenkov, S. V., and Witt, S. N. (2002). The unfolding story of the *Escherichia coli* Hsp70 DnaK: is DnaK a holdase or an unfoldase? *Mol. Microbiol.* 45, 1197–1206. doi: 10.1046/j.1365-2958.2002.03093.x

Slutsky-Leiderman, O., Marom, M., Iosefson, O., Levy, R., Maoz, S., and Azem, A. (2007). The interplay between components of the mitochondrial protein translocation motor studied using purified components. *J. Biol. Chem.* 282, 33935–33942. doi: 10.1074/jbc.M704435200

Sousa, R. (2012). A dancer caught midstep: the structure of ATP-bound Hsp70. *Mol. Cell* 48, 821–823. doi: 10.1016/j.molcel.2012.12.008

Tytell, M. (2005). Release of heat shock proteins (Hsps) and the effects of extracellular Hsps on neural cells and tissues. *Int. J. Hyperthermia* 21, 445–455. doi: 10.1080/02656730500041921

Voellmy, R., and Boellmann, F. (2007a). "Chaperone regulation of the heat shock protein response," in *Molecular Aspects of the Stress Response: Chaperones, Membranes and Networks,* eds P. Csermely and L. Vígh, (New York, NY: Springer), 89–99.

Voellmy, R., and Boellmann, F. (2007b). Chaperone regulation of the heat shock protein response. *Adv. Exp. Med. Biol.* 594, 89–99. doi: 10.1007/978-0-387-39975-1_9

Warnecke, T. (2012). Loss of the DnaK-DnaJ-GrpE chaperone system among the Aquificales. *Mol. Biol. Evol.* 29, 3485–3495. doi: 10.1093/molbev/mss152

Weiss, Y. G., Bromberg, Z., Raj, N., Raphael, J., Goloubinoff, P., Ben-Neriah, Y., et al. (2007). Enhanced heat shock protein 70 expression alters proteasomal degradation of IkappaB kinase in experimental acute respiratory distress syndrome. *Crit. Care Med.* 35, 2128–2138. doi: 10.1097/01.CCM.0000278915.78030.74

Wimmer, B., Lottspeich, F., Vanderklei, I., Veenhuis, M., and Gietl, C. (1997). The glyoxysomal and plastid molecular chaperones (70-kDa heat shock protein) of watermelon cotyledons are encoded by a single gene. *Proc. Natl. Acad. Sci. U.S.A.* 94, 13624–13629. doi: 10.1073/pnas.94.25.13624

Winkler, J., Tyedmers, J., Bukau, B., and Mogk, A. (2012). Chaperone networks in protein disaggregation and prion propagation. *J. Struct. Biol.* 179, 152–160. doi: 10.1016/j.jsb.2012.05.002

Zhang, Y., Ma, C., Yuan, Y., Zhu, J., Li, N., Chen, C., et al. (2014). Structural basis for interaction of a cotranslational chaperone with the eukaryotic ribosome. *Nat. Struct. Mol. Biol.* 21, 1042–1046. doi: 10.1038/nsmb.2908

Zhuo, Y., Ilangovan, U., Schirf, V., Demeler, B., Sousa, R., Hinck, A. P., et al. (2010). Dynamic interactions between clathrin and locally structured elements in a disordered protein mediate clathrin lattice assembly. *J. Mol. Biol.* 404, 274–290. doi: 10.1016/j.jmb.2010.09.044

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Molecular Biosciences

MINI REVIEW
published: 30 June 2015
doi: 10.3389/fmolb.2015.00035

DNAJs: more than substrate delivery to HSPA

*Suzanne L. Dekker, Harm H. Kampinga and Steven Bergink**

Department of Cell Biology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands

Proteins are essential components of cellular life, as building blocks, but also to guide and execute all cellular processes. Proteins require a three-dimensional folding, which is constantly being challenged by their environment. Challenges including elevated temperatures or redox changes can alter this fold and result in misfolding of proteins or even aggregation. Cells are equipped with several pathways that can deal with protein stress. Together, these pathways are referred to as the protein quality control network. The network comprises degradation and (re)folding pathways that are intertwined due to the sharing of components and by the overlap in affinity for substrates. Here, we will give examples of this sharing and intertwinement of protein degradation and protein folding and discuss how the fate of a substrate is determined. We will focus on the ubiquitylation of substrates and the role of Hsp70 co-chaperones of the DNAJ class in this process.

Keywords: DNAJ, ubiquitin E3 ligases, HSP70 heat-shock proteins, degradation, protein folding

OPEN ACCESS

Edited by:
Pierre Goloubinoff, University of Lausanne, Switzerland

Reviewed by:
Nico P. Dantuma, Karolinska Institutet, Sweden
Krzysztof Liberek, University of Gdansk, Poland
Pierre Genevaux, Centre National de la Recherche Scientifique, France

****Correspondence:***
Steven Bergink, Department of Cell Biology, FB30, University Medical Center Groningen, University of Groningen, Antonius Deusinglaan 1, Groningen 9700AD, Netherlands
s.bergink@umcg.nl

Specialty section:
This article was submitted to Protein Folding, Misfolding and Degradation, a section of the journal Frontiers in Molecular Biosciences

Received: *30 April 2015*
Accepted: *12 June 2015*
Published: *30 June 2015*

Citation:
Dekker SL, Kampinga HH and Bergink S (2015) DNAJs: more than substrate delivery to HSPA. Front. Mol. Biosci. 2:35. doi: 10.3389/fmolb.2015.00035

Protein Stress

The capacity of the protein quality control network is limited and collapse occurs when the system is overwhelmed. This can occur after sudden massive stress including temperature shifts, nutrient deprivation or pathogenic infection (Lee, 1992; Scheuner et al., 2001; Kaufman et al., 2002; Schelhaas et al., 2007; Morimoto, 2008). These types of stresses are usually sensed and the organism responds with the activation of stress pathways, such as the heat shock response after temperature elevation (Morimoto, 2008).

Also "normal" conditions in the cell, for example the increased synthesis of proteins during cell cycle progression (Morimoto, 2008) can be considered as physiological protein stress requiring increased folding capacity and adaptations in the quality control network. The difference between these two types of protein stress is that the latter is enlisted and that substrate recognition and fate are pre-determined, for example cyclins are degraded upon orchestrated phosphorylation events (Murray, 2004). In contrast, sudden or accidental protein stress is unpredictable and requires a system that can recognize the client(s), which lack(s) a common motif that is usually present in the regular enlisted clients. Typically, these accidental clients become unfolded after stress or are intrinsically misfolded (i.e., genetically encoded).

Protein Stress and Its Impact on Human Pathology

Protein stress can accumulate over time as well, as is the case in many diseases that are associated with an accumulation of misfolded proteins. Often this type of slowly accumulating protein damage is—initially—not triggering a strong stress signaling, but remains undetected. Yet, this type of stress may also overwhelm the protein quality control network and cause a similar collapse, ultimately

resulting in the associated pathology. Diseases driven by protein stress range from cataract, type II diabetes, atrial amyloidosis to neurodegenerative diseases (Chiti and Dobson, 2006).

Moreover, it is known that hypomorphic mutations, a partial loss of gene function, can lead to an accelerated phenotype in the background of a compromised protein quality control network (Ben-Zvi et al., 2009). Suggesting that genome alterations can lead to metastable or aberrant polypeptides that stochastically accumulate in time. If true, the source of protein stress would expand to single nucleotide polymorphisms (SNPs) and somatic mutations that slowly accumulate as we age.

Molecular Chaperones

Heat Shock Proteins (HSPs) play a central role in protein homeostasis and are upregulated by the diverse protein stress signaling pathways in cells under many conditions, most prominently acute stress, that challenge protein homeostasis (Morimoto, 2008). For various HSPs, it has been established that they have so-called molecular chaperone activity. Usually, this is associated with folding nascent chains into their native state or refolding of stress-unfolded proteins. However, their activities are also needed for degradation of misfolded or mutant proteins and even for remodeling of active protein complexes. As a matter of fact, the most ancient members of the molecular chaperones, DnaJ, and DnaK (Hsp70/HSPA), were originally identified as essential components controlling (dis)assembly of phage lambda replication complexes (Konieczny and Zylicz, 1999).

A wide network of molecular chaperones exists that encompasses the chaperonins, the HSPAs (Hsp70s and Hsc70s, eleven in humans) with its co-chaperones: the DNAJs (50 members in humans) and the Nucleotide Exchange factors (NEF, 13 members in humans), the HSPBs (small HSPs, 10 members in humans) and the HSPCs (Hsp90s, five members in humans) (Kampinga and Craig, 2010). Here we will focus on the DNAJs in the HSPA system (**Figure 1A**), as these are involved in substrate recognition and seem to function at the crossroad of folding and degradation.

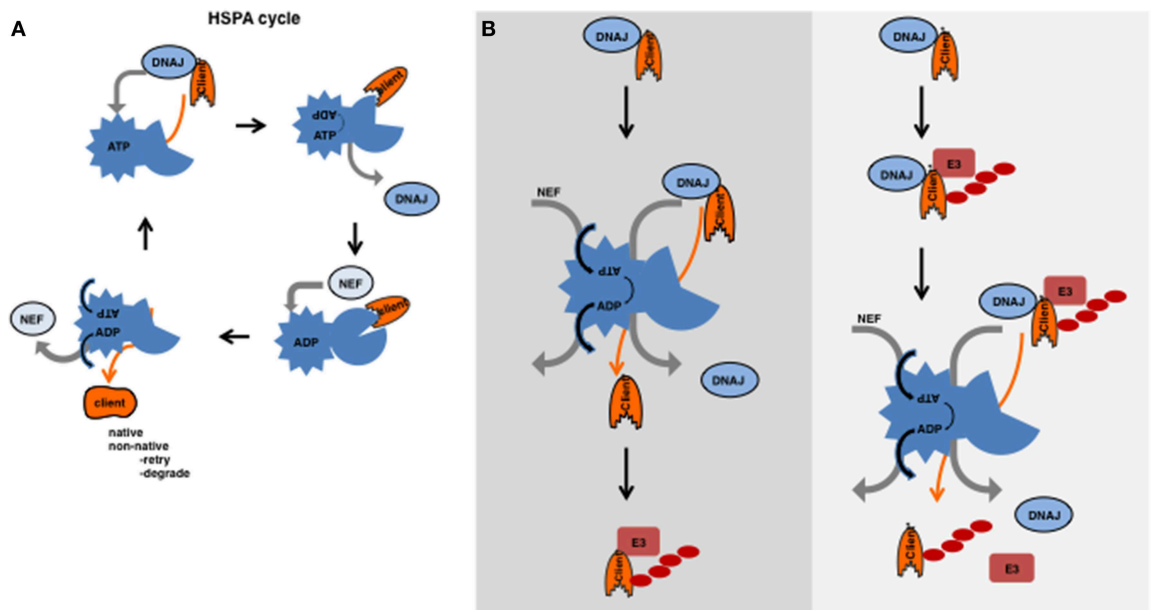


FIGURE 1 | (A) HSPA cycle. HSPAs (HSP70s and HSC70s) contain an N-terminal ATPase domain connected by a hydrophobic linker to a variable C-terminal peptide-binding domain (Hartl et al., 2011). This peptide-binding domain binds to a stretch of hydrophobic residues flanked by positive residues, which is predicted to occur every 40 amino acids (Frydman et al., 1999). In the folded state, these hydrophobic residues are buried inside, but are exposed in the unfolded or misfolded state. The energy obtained from the hydrolysis of ATP is required for assisted folding. However, the exact mechanism how an HSPA supports folding is still not yet completely understood. HSPA functions with the help of co-chaperones that orchestrate the cycle of ATP hydrolysis and substrate/client binding and release. DNAJs recognize clients and subsequently bind to the ATP-bound form of HSPA; upon binding of the DNAJ-client complex, ATP is hydrolysed by the HSPA and the DNAJ is released. Upon hydrolysis and DNAJ release, the peptide-binding-domain of HSPA undergoes a conformational change and clams around the polypeptide (the substrate) (Jiang et al., 2007; Swain et al., 2007; Bertelsen et al., 2009). The NEF has affinity to the ADP-bound form and mediates the exchange of ADP for ATP. The client has less affinity for the ATP-bound form of HSPA and releases together with the NEF. As a result, the client can fold or will re-enter the cycle if not completely folded, or somehow can be transferred to degradation machineries. **(B)** Substrates ubiquitylated before or after the action of HSPA. On the left, the canonical model is depicted in which an intrinsically unstable substrate is first recognized by a DNAJ then transferred to the HSPA cycle and after a futile folding event ubiquitylated and subsequently degraded. On the right, a model is presented in which an unfolded substrate is recognized by a DNAJ after which an E3 ligase can ubiquitylate it and the HSPA cycle acts on the ubiquitylated substrate. After the action of HSPA, the substrate is liberated from both the DNAJ and the E3 ligase (complex) and targeted for degradation.

The DNAJs: A Crucial Role in Protein Quality Control

The HSPAs function with the help of several co-chaperones and collaborate with the other chaperones. The most important co-chaperones are the DNAJs and the NEFs. DNAJs recognize substrates/clients and subsequently bind to the ATP-bound form of HSPA (for the HSPA cycle see **Figure 1A**).

DNAJs are defined by the presence of a J-domain, an approximately 70 amino acid domain that consist of four alpha helices and an accessible loop to which the (ATP-bound) ATPase domain of HSPA binds (Jiang et al., 2007). In this loop lies a Histidine Proline Aspartate (HPD) motif, which is crucial for binding to HSPAs. DNAJs are divided based on their domain structure into three classes, DNAJAs, DNAJBs and DNAJCs (Kampinga and Craig, 2010). Briefly, in both DNAJAs and DNAJBs, the N-terminal J domain is followed by a Glycine/Phenylalanine-rich stretch. In the DNAJA class proteins, two domains (CTDI and CTDII) are located at the C-terminus that are involved in substrate recognition by forming a hydrophobic pocket that binds to the substrate (Li et al., 2003). The first of these CTDs contains a zinc finger. The C terminus in the DNAJB lacks the organization into two CTDs or and more specifically contains no zinc finger and specific information of substrate binding regions is still lacking. The DNAJC class comprises any J-domain containing protein that does not fit in the A or B class.

A wide variety of domains can be found in the different J proteins. Many of these are known or thought to be involved in substrate interactions, thus a division based on substrate recognition has been proposed as well (Kampinga and Craig, 2010). Many J-proteins are believed to have a promiscuous client binding (mostly from class A and B and a few from class C) while others seem to display high substrate specificity (mostly class C).

Interplay between Degradation and Folding

The notion that there are around 50 DNAJs, 11 HSPAs, and 13 NEFs at least suggest a high potential of many possible combinations, theoretically resulting in flexibility and a broad substrate range. Some DNAJs and HSPAs are localized to specific organelles and seem to form combinations with the compartment specific Hsp70 (HSPA) members, but for other (cytosolic) HSPAs it has been shown that they can indeed operate with different DNAJs (Kampinga and Craig, 2010). The interplay between the HSPA cycle and the degradation machineries makes matters even more complex. The idea that the fate of a protein is determined by this interplay of the chaperone systems and the degradation systems is often referred to as the triage decision (Connell et al., 2001; Houck et al., 2012). The classical view is that the cell first attempts to (re)fold the damaged or metastable protein. If folding fails, the cell will then attempt to degrade it (**Figure 1B**). Many proteins that are part of the folding machinery interact with the cellular degradation machinery to make this transition smoothly. For example, DNAJB2 interacts with ubiquitylated proteins via its ubiquitin interacting motifs (UIM) (Westhoff et al., 2005) and Bag1 (a NEF) contains a ubiquitin-like (UBL) domain which are known to interact with subunits of the proteasome (Lüders et al., 2000). An alternative view would be that degradation and (re)folding act in parallel and that chaperones are an integral part of the degradation machinery and have a different function besides folding. These scenarios are not mutually exclusive and, depending on the substrate, both might happen.

How the HSPA machinery selects and recognizes the different types of substrates and which parameters determine the fate of the clients (folding, degradation or aggregation) are still open questions. Here we will give examples of clients that are dealt with by different combinations of DNAJs and HSPAs. Moreover, we will discuss the recent insights in the interplay between the HSPA cycle and the ubiquitin proteasome system (UPS) (**Box 1**).

De Novo Protein Folding

The folding of newly synthesized proteins is different from refolding reactions, partly due to the vectorial nature of translation. Indeed, early studies clearly demonstrated that nascent chain folding and refolding require different sets of chaperones (Frydman et al., 1994, 1999). Folding of newly synthesized proteins can occur co-translationally and sometimes also post-translationally. In prokaryotes, folding involves many different partners including trigger factor which prevents misfolding of nascent chains by delaying protein folding (Agashe et al., 2004). Although far from completely resolved, in co-translational folding, the different domains fold separately. As often, the fundamental studies in yeast have shed light on this process (Albanèse et al., 2006). Two complexes are associated with the ribosome and the emerging polypeptide. First, the nascent polypeptide associated complex (NAC), a heterodimer that consists of an alpha and a beta subunit, binds to all nascent chains. Second, a set of Hsp70s (Ssb1 and 2) is bound to translating ribosomes, which function together with the DNAJ Zuo1 and the NEF Sse1 and bind to the polypeptide that extrudes from the ribosomal exit tunnel. Zuo1 together with Ssz1 (another Hsp70) forms the Ribosome Associated Complex (RAC), and is thought to recruit Ssb1 and 2 to the ribosome. Global identification of Ssb1/2 substrates revealed that especially the more difficult substrates (e.g., longer, several domains, beta sheet enriched, and aggregation prone) are clients (Willmund et al., 2013). Beside Zuo1, two other DNAJ proteins have been identified as being important for nascent chain folding namely Ydj1 and Sis1. These DNAJs function with the "soluble" or cytoplasmic Hsp70s (the Ssa1-4 proteins) and the chaperonin system to guide the final (post-translational) folding of newly synthesized polypeptides (Kim et al., 2013).

Most of these yeast proteins have mammalian counterparts: Ssz1 is homologous to HSPA14, Zuo1 is homologous to DNAJC2 [also called, Mpp11 (M-phase phosphoprotein 11) (Hundley et al., 2005) or ZRF1 (zuotin related factor 1) (Richly et al., 2010)], Ydj1 is homologous to DNAJA1 and Sis1 is homologous to DNAJB1. HSPA8 is associated with the ribosome and homologous to Ssb2 (Hundley et al., 2005).

BOX 1 | Protein Degradation.

The various proteases in the cell are capable of degrading targeted substrates. As a general theme, access to proteases is restricted either due to a high specificity for specific substrates, a common but inducible recognition signal (e.g., the proteasome), or spacious confinement (e.g., the lysosome). The UPS and autophagy are the systems that degrade the bulk of proteins.

UPS

Recognition by the 26S proteasome is done by lysine (K) 48-linked polyubiquitin chains conjugated to substrates that require degradation. Ubiquitin is a polypeptide that is conjugated to target proteins by the subsequent action of three enzymes, an E1 (activating enzyme), an E2 (conjugating enzyme) and an E3 (ligating enzyme). The ligases are responsible for substrate recognition and thus determine the specificity, whereas the E2s usually mediate the conjugation of ubiquitin to the substrate. Occasionally an E4 ligase is required to extend already existing mono- or oligo-ubiquitylation events to polyubiquitin trees. The C-terminal glycine of ubiquitin either forms a peptide bond with the N-terminus of a substrate or an isopeptide bond with the epsilon-N in internal lysines of the substrate (although conjugation to cysteine and serine have been reported as well). Due to the internal lysines in ubiquitin itself a so-called polyubiquitin tree is build. Polyubiquitylation with a K48 linkage is considered canonical for degradation. Indeed, monoubiquitylation or other types of polyubiquitin trees with different linkage are not necessarily recognized as targeted for destruction. Receptors at the 26S proteasome recognize the polyubiquitin trees, which are cleaved off and recycled. The 26S proteasome is a multimeric complex that consists of a 20S and 19S component. After deubiquitylation, the substrate is unfolded by ATPases associated with various cellular activities (AAA-ATPases) of the 19S, and funneled into the proteolytic chamber (20S). Only when a protein resides in this chamber it is cleaved into peptides.

Autophagy

Autophagy is a process in which a double membrane-vesicle (the autophagosome) containing the cargo is targeted to, and fused with the lysosome in which the degradation (hydrolysis) takes place. The cargo consists of proteins, protein-complexes, aggregated proteins and even entire organelles. Autophagy is mediated by a set of proteins and includes two types of ubiquitin-like conjugation reactions; the most crucial one is the conjugation of LC3 or ATG8 (in yeast) to phosphoatidylethanolamine at the expanding autophagic membrane. Originally it was thought that autophagy is mostly nonspecific and just randomly engulfs cytoplasm. However, the current dogma is that most autophagy is in fact specific. Again, ubiquitylation can form the signal that is recognized by specific receptors that both bind polyubiquitylated substrates and LC3, the double membrane then encloses the cargo and translocates to the lysosome.

Quality Control at the Ribosome

Translational errors may occur due to aberrant mRNAs, misfolding due to intrinsic properties of the nascent chain itself or to other events that lead to stalling of the translational machinery. As a general theme, these errors are sensed during translation and the emerging polypeptide is co-translationally ubiquitylated, released and degraded. Depending on the type of translational problem and substrate, different chaperones and components of the UPS are involved. For example, long stretches of basic amino acids are recognized by the NAC complex and ubiquitylated by the E3 ligase Not4 (Dimitrova et al., 2009). Other types of translational difficulties, such as the translation of the polyA sequence that leads to pausing, require another E3 ligase, namely Ltn1. The ubiquitylated polypeptide requires $Cdc48^{Ufd1/Npl4}$ (p97/VCP in mammalians), a ubiquitin/ubiquitin-like-modifier-specific segregase (Jentsch and Rumpf, 2007), to remove it from the ribosome (Brandman et al., 2012; Defenouillère et al., 2013; Verma et al., 2013). Thus, translational errors usually result in immediate, co-translational, degradation (**Figure 2A**) in which chaperones act before ubiquitylation.

Degradation of the emerging polypeptide can in principle always occur at the ribosome, even in the absence of translational errors, as shown by elegant experiments using tandem expressed degradation-reporters (Turner and Varshavsky, 2000). This implies that folding or degradation is an intrinsic fate of the client rather than it is driven by different sets of chaperone machines. In other words, the different ribosome-associated chaperone complexes (in yeast Ssb1/2, RAC, and NAC) prevent aggregation during translation which -depending on the substrate- can result in folding or maintain the substrates/clients accessible to the various E3 ligases that are either present at, or have access to the ribosomes (examples in yeast: Ltn1, Not4, Ubr1).

Chaperones: the Prelude for Ubiquitylation of Proteasomal Clients?

The idea that unfolded or misfolded clients, similar to nascent chains, need to be kept accessible/soluble in order for a protein quality control E3 ligase to work seems appealing. The idea would be that a chaperone binds first to a potential substrate and keeps this substrate soluble or accessible for the E3 ligase to ubiquitylate this target. The requirement for a chaperone as a recognition or accessibility factor for ubiquitin E3 ligases would not be necessary for regular or enlisted clients (e.g., the cyclins), but only for those that become (partly) unfolded by a random stress event or for those clients that are intrinsically (genetically encoded) misfolded. Chaperones of the HSPB and DNAJ class are well known to be able to recognize these clients and thereby ensure their solubility. In the canonical view, these clients are next transferred to the HSPAs (Hsp70s). Release from the HSPAs occurs via NEFs and interactions with E3 ligases at which level client-fate is determined. However, as we will explain below, evidence is accumulating that other scenarios may also be operational (**Figures 1B**, **2**).

Ubiquitylation and DNAJs in ERAD

Clear examples where a DNAJ precedes the action of an E3 can be found within ER associated degradation (ERAD). Proteins that are targeted to the ER, but are unwanted because they are intrinsically unstable, aggregation prone or unfolded, are cleared via the ERAD pathway. For ER luminal and transmembrane clients, DNAJs (DNAJB11, DNAJB9, and DNAJC10 also known as Erdj3, -4, and -5), recognize misfolded clients that subsequently are ubiquitylated by the membrane embedded E3 ligases Hrd1 or RMA1 and the E4 ligase gp78 (Buchberger et al., 2010). For those clients that expose their unstable region to the cytosol, soluble-DNAJs play a role in

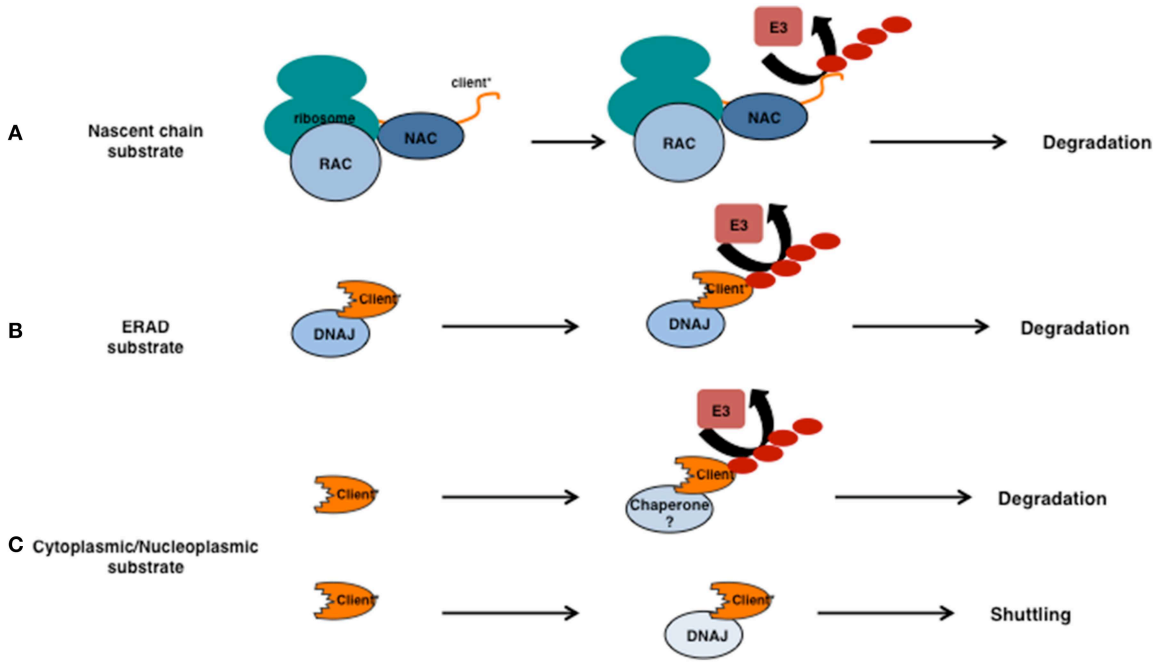


FIGURE 2 | Involvement for chaperones in substrate ubiquitylation. (A) The relationship between chaperones and ubiquitin E3 ligases for unstable nascent chains. Unstable nascent chains are bound by the chaperone systems present at the ribosome (here the NAC and RAC) and ubiquitylation of the emerging polypeptide happens co-translationally. **(B)** ERAD substrates that are intrinsically unstable (indicated by the *) are recognized by DNAJs prior their ubiquitylation and retrotranslocation. **(C)** If cytoplasmic or nucleoplasmic substrates require chaperones prior their ubiquitylation is currently unclear. Sis1/DNAJB1 is required for the nuclear relocalization of certain unstable substrates in an ubiquitin-independent manner.

conjunction with the E3 ligase Doa10 (Ravid et al., 2006). After or during ubiquitylation, all ERAD clients require retro-translocation over, or dislocation from the lipid bilayer, a process that is mediated by the p97/Cdc48$^{Ufd1/Npl4}$ complex (Tsai et al., 2002).

Also for integrated membrane proteins, DNAJs may act prior to E3 ligases in degradation. Several model substrates exist exposing their unstable region to the cytosol and for some of these the relationship between chaperone function and ubiquitylation status has been determined. For example degradation and ubiquitylation of the Doa10 substrates Ste6* and PMA1* depends on the cytosolic Hsp70s (Ssa1-4) and the (cytosolic) DNAJs Ydj1 and Hlj1 (Han et al., 2007; Nakatsukasa et al., 2008) (**Figure 2B**). Other DNAJs are also involved in Doa10-mediated substrate ubiquitylation as Sis1, but not the cytosolic Hsp70s (Ssa1-4), is required to ubiquitylate the degradation prone part from the kinetochore protein Ndc10. Instead, Hsp70s are required to prevent aggregation of the ubiquitylated client (Shiber et al., 2013) and clearly function after ubiquitylation. These results are in line with the idea that E3 ligases involved in clearing misfolded substrates require a molecular chaperone for their functioning (**Figure 2**).

However, for other substrates (e.g., soluble targets of the protein quality control E3 ligases Ubr1 and San1) Sis1 (or Ydj1) is not a prerequisite for ubiquitylation but is essential for efficient client degradation (Park et al., 2013; Mehnert et al., 2015; Miller et al., 2015) and thus seem to play a role downstream of ubiquitylation.

Ubiquitylation and DNAJs of Cytoplasmic and Nucleoplasmic Clients

Whereas, translation quality control and ERAD are vectorial by nature, the organization of quality control over soluble proteins is not. If the soluble (stress induced or accidentally misfolded) clients in the cytosol and nucleoplasm also require a chaperone prior ubiquitylation is currently unclear. The notion that overexpression of certain DNAJs accelerates degradation of substrates is suggestive of a direct impact of DNAJs on degradation/ubiquitylation (Westhoff et al., 2005). However, clear evidence that DNAJs or HSPBs are required for ubiquitylation of these accidental clients is currently lacking (**Figure 2C**). Deletion of individual DNAJs or other molecular chaperones typically does not result in accumulation of their unmodified clients. It is not unlikely that there is functional redundancy between the different and numerous chaperones (e.g., DNAJs) in these compartments making the fate of substrates/clients harder to study. Moreover, to distinguish this process from translational protein quality control (Wang et al., 2013), a substrate that becomes unstable after translation vs. a constitutively unstable substrate, is required. For example a substrate that after the addition of a specific small molecule triggers unfolding of that substrate would be a great asset. This type of substrate would enable the study of post-translational protein quality control under normal, non-stressed, conditions. This type of research is especially important as,

in many of the aforementioned diseases, protein stress occurs post-translationally.

Nuclear Transport of Cytosolic Substrates

As mentioned above, ERAD substrates are degraded in the cytoplasm and retro-translocation is necessary as the proteasome is not present in the ER. One might suspect that degradation of cytoplasmic clients is more straightforward as the proteasome is present in the same compartment. However, recent evidence suggests that, at least for some substrates, this is not the case. Instead, cytosolic model substrates were found to shuttle to the nucleus for degradation (**Figure 2C**) (Heck et al., 2010; Prasad et al., 2010; Park et al., 2013). This transfer across the nuclear envelope depends on the DNAJ Sis1 (DNAJB1 in humans) (Park et al., 2013). Increasing the proteotoxic stress hampers this process, probably due to a sequestration of Sis1 to other clients, and results in a reduction in degradation of these substrates (Park et al., 2013). Transport of un- or mis-folded substrates to a (juxta)nucleolar localization seems to be a more general feature for both nuclear (Nollen et al., 2001) and cytosolic proteins (Miller et al., 2015). Targeting of substrates to these structures depends on, again, Sis1 and the v-snare protein Btn2 (Malinovska et al., 2012; Miller et al., 2015). Whether the nucleolar accumulation *per se* determines the fate of the client is, however, unlikely as the accumulation in the nucleolus also occurs to nuclear clients that require refolding after heat stress (Nollen et al., 2001). Here, the accumulation of substrates in the nucleolus is reversible and accompanied by a strong sequestering of, normally cytosolic, HSPA1A (Hsp70) to the nucleolus. In line, ubiquitylation does not seem to play a role in the transfer across the nuclear envelope (Park et al., 2013; Miller et al., 2015).

What remains a question is how cytoplasmic proteins cross the nuclear barrier and whether or not posttranslational modifications play a role in this cytoplasmic-nuclear shuttling. Post-translational modifications as SUMOylation (Small Ubiquitin-like Modifier) and poly(ADP-ribosyl)ation have been implemented in protein stress responses (Haince et al., 2006; Golebiowski et al., 2009), the formation of stress bodies (Nagai et al., 2011) and also in nucleolar integrity (Finkbeiner et al., 2011; Boamah et al., 2012).

Why the nucleolus would serve as storage for proteins before folding or degradation has still remained enigmatic. Even more so, one may wonder why cytosolic substrates are translocated to the nucleus/nucleoli for degradation, given that in the cytosol proteasomes are fully active and autophagy is present. Notably, in all of these experiments the substrates used are engineered proteasomal clients. The transfer of these clients across the nuclear pore could therefore be an extension of the aforementioned ribosomal quality control mechanism, which indeed involves Sis1, and that shuttling for these substrates may occur co-translationally. However, adding an nuclear localization signal increases the degradation speed significantly (whereas adding a NES inhibits it) (Park et al., 2013), suggesting that the nuclear shuttling of cytoplasmic substrates is not that efficient and probably not coupled to translation.

One could hypothesize that substrates are normally already shuttling between the cytosol and nucleus and that degradation is simply faster in the nucleus in which the proteasomal concentration is higher (Russell et al., 1999). However, this would suggest that the capacity of the UPS in the cytoplasm is rate limiting, which seems not to be the case (Park et al., 2013). So, shuttling of un- or mis-folded clients to the nuclear/nucleolar compartment indeed seems an active, regulated process. Perhaps the cytosolic compartment, or the organelles herein, are more vulnerable to proteotoxic stress and the re-localization to the nucleus/nucleolus serves to avoid immediate cellular arrest or death. However, given the complex processes that take place in the nucleus, and the presence of the genetically vulnerable rDNA in the nucleolus, this might hamper nuclear processes such as transcription and replication.

CFTR; Escaping Initial Quality Control Is Not Enough

One substrate that illustrates the complexity of quality control is the disease-associated form of cystic fibrosis transmembrane conductance regulator (CFTR) (Younger et al., 2006; Okiyoneda et al., 2010). For some of the CFTR mutations, in particular the deletion of phenylalanine at position 508 ($CFTR^{delta508}$) (Lukacs and Verkman, 2012), premature degradation is thought to underlay CF-pathology. This deletion destabilizes or kinetically traps CFTR, which targets this protein to the ERAD pathway. Numerous chaperones, including DNAJs and HSPAs, and E3 ligases have been implicated in the clearance of mutant CFTR. $CFTR^{delta508}$ is ubiquitylated either by the Hrd1 E3 ligase complex or the Rma1/Derlin1 E3 ligase complex and the GP78 E4 ligase in a process that is enabled by the membrane embedded DNAJB12, after which retro-translocation by p97 and degradation occurs (Younger et al., 2006; Grove et al., 2011).

Skipping this initial ERAD quality control enables part of the protein to arrive at the plasma membrane to fulfill its function (Kälin et al., 1999). Unfortunately, this rescued mutant protein is still degraded, now by a different set of chaperones and E3 ligases, involving DNAJA1, DNAJB2, HSPA8, HSPA1A, Bag1, and the E3 ligase CHIP (C-terminal Hsc70/HSPA8 interacting protein) (Meacham et al., 2001; Okiyoneda et al., 2010). CHIP has a well-known function in cytoplasmic protein quality control, it binds directly to HSPA and is involved in ubiquitylating HSPA clients that need to be degraded. The work on $CFTR^{delta508}$ nicely illustrates that each cellular compartment has a different set of protein quality control components. Moreover, it points out that in the lifetime of a protein it encounters various and different quality checks.

Summary

The examples of substrates in the ERAD pathway and protein quality control during translation illustrated that for certain substrates a molecular chaperone (a DNAJ) is required for ubiquitylation while this is unclear for others.

The "holdase" function of DNAJs is well suited to assist E3 ligase function for those substrates that are intrinsically

unstable and thus would misfold, form aggregates or fibrils in the absence of a molecular chaperone. Intriguingly, for some of these substrates HSPAs are necessary for degradation but not for ubiquitylation, indicating that they act after the E3 ligase but before proteolysis. Perhaps to prevent aggregation in the brief moment a substrate is delivered to the proteasome. Instead, the energy provided by the ATP hydroslysis of Hsp70s could also facilitate the release of the DNAJ and the E3 ligase from the ubiquitylated substrate (**Figure 1B** right panel). This un-entanglement-function of HSPA would not be required for substrates that come from the ER lumen, as both E3 ligase and DNAJ are embedded in the membrane, and retro-translocation is sufficient to clear the substrate from these factors. The view that degradation substrates are immediately marked for destruction without attempts to fold the substrate is not ruling out that futile folding can lead to degradation as well (**Figure 1A** left panel), but indicates that the degradation pathways are even more intertwined with the folding machinery as was previously thought. In that regard, it is not unlikely that the HSPA cycle, besides its canonical role in protein folding, could function to untangle the substrate-E3/E2 ligase complexes for transfer of regular enlisted clients to the proteasome as well (Mehnert et al., 2015).

Acknowledgments

SD is supported by the Graduate School of Medical Science of the UMCG. HK is supported by a grants from Senter Novem (IOP-IGE7004), high Q foundation (project # 3.1.2), Hersenstichting (project 15F07(2)-58). SB is supported by a Hersenstichting grant (project no. F2013(1)-52).

References

Agashe, V. R., Guha, S., Chang, H. C., Genevaux, P., Hayer-Hartl, M., Stemp, M., et al. (2004). Function of trigger factor and DnaK in multidomain protein folding: increase in yield at the expense of folding speed. *Cell* 117, 199–209. doi: 10.1016/S0092-8674(04)00299-5

Albanèse, V., Yam, A. Y. W., Baughman, J., Parnot, C., and Frydman, J. (2006). Systems analyses reveal two chaperone networks with distinct functions in eukaryotic cells. *Cell* 124, 75–88. doi: 10.1016/j.cell.2005.11.039

Ben-Zvi, A., Miller, E. A., and Morimoto, R. I. (2009). Collapse of proteostasis represents an early molecular event in *Caenorhabditis elegans* aging. *Proc. Natl. Acad. Sci. U.S.A.* 106, 14914–14919. doi: 10.1073/pnas.0902882106

Bertelsen, E. B., Chang, L., Gestwicki, J. E., and Zuiderweg, E. R. P. (2009). Solution conformation of wild-type *E. coli* Hsp70 (DnaK) chaperone complexed with ADP and substrate. *Proc. Natl. Acad. Sci. U.S.A.* 106, 8471–8476. doi: 10.1073/pnas.0903503106

Boamah, E. K., Kotova, E., Garabedian, M., Jarnik, M., and Tulin, A. V. (2012). Poly(ADP-Ribose) polymerase 1 (PARP-1) regulates ribosomal biogenesis in drosophila nucleoli. *PLoS Genet.* 8:e1002442. doi: 10.1371/journal.pgen.1002442

Brandman, O., Stewart-Ornstein, J., Wong, D., Larson, A., Williams, C. C., Li, G.-W., et al. (2012). A ribosome-bound quality control complex triggers degradation of nascent peptides and signals translation stress. *Cell* 151, 1042–1054. doi: 10.1016/j.cell.2012.10.044

Buchberger, A., Bukau, B., and Sommer, T. (2010). Protein quality control in the cytosol and the endoplasmic reticulum: brothers in arms. *Mol. Cell* 40, 238–252. doi: 10.1016/j.molcel.2010.10.001

Chiti, F., and Dobson, C. M. (2006). Protein misfolding, functional amyloid, and human disease. *Annu. Rev. Biochem.* 75, 333–366. doi: 10.1146/annurev.biochem.75.101304.123901

Connell, P., Ballinger, C. A., Jiang, J., Wu, Y., Thompson, L. J., Höhfeld, J., et al. (2001). The co-chaperone CHIP regulates protein triage decisions mediated by heat-shock proteins. *Nat. Cell Biol.* 3, 93–96. doi: 10.1038/35050618

Defenouillère, Q., Yao, Y., Mouaikel, J., Namane, A., Galopier, A., Decourty, L., et al. (2013). Cdc48-associated complex bound to 60S particles is required for the clearance of aberrant translation products. *Proc. Natl. Acad. Sci. U.S.A.* 110, 5046–5051. doi: 10.1073/pnas.1221724110

Dimitrova, L. N., Kuroha, K., Tatematsu, T., and Inada, T. (2009). Nascent peptide-dependent translation arrest leads to Not4p-mediated protein degradation by the proteasome. *J. Biol. Chem.* 284, 10343–10352. doi: 10.1074/jbc.M808840200

Finkbeiner, E., Haindl, M., Raman, N., and Muller, S. (2011). SUMO routes ribosome maturation. *Nucleus* 2, 527–532. doi: 10.4161/nucl.2.6.17604

Frydman, J., Erdjument-Bromage, H., Tempst, P., and Hartl, F. U. (1999). Co-translational domain folding as the structural basis for the rapid *de novo* folding of firefly luciferase. *Nat. Struct. Biol.* 6, 697–705. doi: 10.1038/10754

Frydman, J., Nimmesgern, E., Ohtsuka, K., and Hartl, F. U. (1994). Folding of nascent polypeptide chains in a high molecular mass assembly with molecular chaperones. *Nature* 370, 111–117. doi: 10.1038/370111a0

Golebiowski, F., Matic, I., Tatham, M. H., Cole, C., Yin, Y., Nakamura, A., et al. (2009). System-wide changes to SUMO modifications in response to heat shock. *Sci. Signal.* 2, ra24. doi: 10.1126/scisignal.2000282

Grove, D. E., Fan, C.-Y., Ren, H. Y., and Cyr, D. M. (2011). The endoplasmic reticulum-associated Hsp40 DNAJB12 and Hsc70 cooperate to facilitate RMA1 E3-dependent degradation of nascent CFTRDeltaF508. *Mol. Biol. Cell* 22, 301–314. doi: 10.1091/mbc.E10-09-0760

Haince, J.-F., Rouleau, M., and Poirier, G. G. (2006). Transcription. Gene expression needs a break to unwind before carrying on. *Science* 312, 1752–1753. doi: 10.1126/science.1129808

Han, S., Liu, Y., and Chang, A. (2007). Cytoplasmic Hsp70 promotes ubiquitination for endoplasmic reticulum-associated degradation of a misfolded mutant of the yeast plasma membrane ATPase, PMA1. *J. Biol. Chem.* 282, 26140–26149. doi: 10.1074/jbc.M701969200

Hartl, F. U., Bracher, A., and Hayer-Hartl, M. (2011). Molecular chaperones in protein folding and proteostasis. *Nature* 475, 324–332. doi: 10.1038/nature10317

Heck, J. W., Cheung, S. K., and Hampton, R. Y. (2010). Cytoplasmic protein quality control degradation mediated by parallel actions of the E3 ubiquitin ligases Ubr1 and San1. *Proc. Natl. Acad. Sci. U.S.A.* 107, 1106–1111. doi: 10.1073/pnas.0910591107

Houck, S. A., Singh, S., and Cyr, D. M. (2012). Cellular responses to misfolded proteins and protein aggregates. *Methods Mol. Biol.* 832, 455–461. doi: 10.1007/978-1-61779-474-2_32

Hundley, H. A., Walter, W., Bairstow, S., and Craig, E. A. (2005). Human Mpp11 J protein: ribosome-tethered molecular chaperones are ubiquitous. *Science* 308, 1032–1034. doi: 10.1126/science.1109247

Jentsch, S., and Rumpf, S. (2007). Cdc48 (p97): a "molecular gearbox" in the ubiquitin pathway? *Trends Biochem. Sci.* 32, 6–11. doi: 10.1016/j.tibs.2006.11.005

Jiang, J., Maes, E. G., Taylor, A. B., Wang, L., Hinck, A. P., Lafer, E. M., et al. (2007). Structural basis of J cochaperone binding and regulation of Hsp70. *Mol. Cell* 28, 422–433. doi: 10.1016/j.molcel.2007.08.022

Kälin, N., Claass, A., Sommer, M., Puchelle, E., and Tümmler, B. (1999). DeltaF508 CFTR protein expression in tissues from patients with cystic fibrosis. *J. Clin. Invest.* 103, 1379–1389. doi: 10.1172/JCI5731

Kampinga, H. H., and Craig, E. A. (2010). The HSP70 chaperone machinery: J proteins as drivers of functional specificity. *Nat. Rev. Mol. Cell Biol.* 11, 579–592. doi: 10.1038/nrm2941

Kaufman, R. J., Scheuner, D., Schröder, M., Shen, X., Lee, K., Liu, C. Y., et al. (2002). The unfolded protein response in nutrient sensing and differentiation. *Nat. Rev. Mol. Cell Biol.* 3, 411–421. doi: 10.1038/nrm829

Kim, Y. E., Hipp, M. S., Bracher, A., Hayer-Hartl, M., and Hartl, F. U. (2013). Molecular chaperone functions in protein folding and proteostasis. *Annu. Rev. Biochem.* 82, 323–355. doi: 10.1146/annurev-biochem-060208-092442

Konieczny, I., and Zylicz, M. (1999). Role of bacterial chaperones in DNA replication. *Genet. Eng. (N.Y).* 21, 95–111. doi: 10.1007/978-1-4615-4707-5_6

Lee, A. S. (1992). Mammalian stress response: induction of the glucose-regulated protein family. *Curr. Opin. Cell Biol.* 4, 267–273. doi: 10.1016/0955-0674(92)90042-B

Li, J., Qian, X., and Sha, B. (2003). The crystal structure of the yeast Hsp40 Ydj1 complexed with its peptide substrate. *Structure* 11, 1475–1483. doi: 10.1016/j.str.2003.10.012

Lüders, J., Demand, J., and Höhfeld, J. (2000). The ubiquitin-related BAG-1 provides a link between the molecular chaperones Hsc70/Hsp70 and the proteasome. *J. Biol. Chem.* 275, 4613–4617. doi: 10.1074/jbc.275.7.4613

Lukacs, G. L., and Verkman, A. S. (2012). CFTR: folding, misfolding and correcting the?ΔF508 conformational defect. *Trends Mol. Med.* 18, 81–91. doi: 10.1016/j.molmed.2011.10.003

Malinovska, L., Kroschwald, S., Munder, M. C., Richter, D., and Alberti, S. (2012). Molecular chaperones and stress-inducible protein-sorting factors coordinate the spatiotemporal distribution of protein aggregates. *Mol. Biol. Cell* 23, 3041–3056. doi: 10.1091/mbc.E12-03-0194

Meacham, G. C., Patterson, C., Zhang, W., Younger, J. M., and Cyr, D. M. (2001). The Hsc70 co-chaperone CHIP targets immature CFTR for proteasomal degradation. *Nat. Cell Biol.* 3, 100–105. doi: 10.1038/35050509

Mehnert, M., Sommermeyer, F., Berger, M., Kumar Lakshmipathy, S., Gauss, R., Aebi, M., et al. (2015). The interplay of Hrd3 and the molecular chaperone system ensures efficient degradation of malfolded secretory proteins. *Mol. Biol. Cell* 26, 185–194. doi: 10.1091/mbc.E14-07-1202

Miller, S. B. M., Ho, C.-T., Winkler, J., Khokhrina, M., Neuner, A., Mohamed, M. Y. H., et al. (2015). Compartment-specific aggregases direct distinct nuclear and cytoplasmic aggregate deposition. *EMBO J.* 34, 778–797. doi: 10.15252/embj.201489524

Morimoto, R. I. (2008). Proteotoxic stress and inducible chaperone networks in neurodegenerative disease and aging. *Genes Dev.* 22, 1427–1438. doi: 10.1101/gad.1657108

Murray, A. W. (2004). Recycling the cell cycle: cyclins revisited. *Cell* 116, 221–234. doi: 10.1016/S0092-8674(03)01080-8

Nagai, S., Davoodi, N., and Gasser, S. M. (2011). Nuclear organization in genome stability: SUMO connections. *Cell Res.* 21, 474–485. doi: 10.1038/cr.2011.31

Nakatsukasa, K., Huyer, G., Michaelis, S., and Brodsky, J. L. (2008). Dissecting the ER-associated degradation of a misfolded polytopic membrane protein. *Cell* 132, 101–112. doi: 10.1016/j.cell.2007.11.023

Nollen, E. A., Salomons, F. A., Brunsting, J. F., van der Want, J. J., Sibon, O. C., and Kampinga, H. H. (2001). Dynamic changes in the localization of thermally unfolded nuclear proteins associated with chaperone-dependent protection. *Proc. Natl. Acad. Sci. U.S.A.* 98, 12038–12043. doi: 10.1073/pnas.201112398

Okiyoneda, T., Barrière, H., Bagdány, M., Rabeh, W. M., Du, K., Höhfeld, J., et al. (2010). Peripheral protein quality control removes unfolded CFTR from the plasma membrane. *Science* 329, 805–810. doi: 10.1126/science.1191542

Park, S.-H., Kukushkin, Y., Gupta, R., Chen, T., Konagai, A., Hipp, M. S., et al. (2013). PolyQ proteins interfere with nuclear degradation of cytosolic proteins by sequestering the Sis1p chaperone. *Cell* 154, 134–145. doi: 10.1016/j.cell.2013.06.003

Prasad, R., Kawaguchi, S., and Ng, D. T. W. (2010). A nucleus-based quality control mechanism for cytosolic proteins. *Mol. Biol. Cell* 21, 2117–2127. doi: 10.1091/mbc.E10-02-0111

Ravid, T., Kreft, S. G., and Hochstrasser, M. (2006). Membrane and soluble substrates of the Doa10 ubiquitin ligase are degraded by distinct pathways. *EMBO J.* 25, 533–543. doi: 10.1038/sj.emboj.7600946

Richly, H., Rocha-Viegas, L., Ribeiro, J. D., Demajo, S., Gundem, G., Lopez-Bigas, N., et al. (2010). Transcriptional activation of polycomb-repressed genes by ZRF1. *Nature* 468, 1124–1128. doi: 10.1038/nature 09574

Russell, S. J., Steger, K. A., and Johnston, S. A. (1999). Subcellular localization, stoichiometry, and protein levels of 26 s proteasome subunits in yeast. *J. Biol. Chem.* 274, 21943–21952. doi: 10.1074/jbc.274.31. 21943

Schelhaas, M., Malmström, J., Pelkmans, L., Haugstetter, J., Ellgaard, L., Grünewald, K., et al. (2007). Simian Virus 40 depends on ER protein folding and quality control factors for entry into host cells. *Cell* 131, 516–529. doi: 10.1016/j.cell.2007.09.038

Scheuner, D., Song, B., McEwen, E., Liu, C., Laybutt, R., Gillespie, P., et al. (2001). Translational control is required for the unfolded protein response and *in vivo* glucose homeostasis. *Mol. Cell* 7, 1165–1176. doi: 10.1016/S1097-2765(01)00265-9

Shiber, A., Breuer, W., Brandeis, M., and Ravid, T. (2013). Ubiquitin conjugation triggers misfolded protein sequestration into quality control foci when Hsp70 chaperone levels are limiting. *Mol. Biol. Cell* 24, 2076–2087. doi: 10.1091/mbc.E13-01-0010

Swain, J. F., Dinler, G., Sivendran, R., Montgomery, D. L., Stotz, M., and Gierasch, L. M. (2007). Hsp70 chaperone ligands control domain association via an allosteric mechanism mediated by the interdomain linker. *Mol. Cell* 26, 27–39. doi: 10.1016/j.molcel.2007.02.020

Tsai, B., Ye, Y., and Rapoport, T. A. (2002). Retro-translocation of proteins from the endoplasmic reticulum into the cytosol. *Nat. Rev. Mol. Cell Biol.* 3, 246–255. doi: 10.1038/nrm780

Turner, G. C., and Varshavsky, A. (2000). Detecting and measuring cotranslational protein degradation *in vivo*. *Science* 289, 2117–2120. doi: 10.1126/science.289.5487.2117

Verma, R., Oania, R. S., Kolawa, N. J., and Deshaies, R. J. (2013). Cdc48/p97 promotes degradation of aberrant nascent polypeptides bound to the ribosome. *Elife* 2:e00308. doi: 10.7554/eLife.00308

Wang, F., Durfee, L. A., and Huibregtse, J. M. (2013). A cotranslational ubiquitination pathway for quality control of misfolded proteins. *Mol. Cell* 50, 368–378. doi: 10.1016/j.molcel.2013.03.009

Westhoff, B., Chapple, J. P., van der Spuy, J., Höhfeld, J., and Cheetham, M. E. (2005). HSJ1 is a neuronal shuttling factor for the sorting of chaperone clients to the proteasome. *Curr. Biol.* 15, 1058–1064. doi: 10.1016/j.cub.2005. 04.058

Willmund, F., del Alamo, M., Pechmann, S., Chen, T., Albanèse, V., Dammer, E. B., et al. (2013). The cotranslational function of ribosome-associated Hsp70 in eukaryotic protein homeostasis. *Cell* 152, 196–209. doi: 10.1016/j.cell.2012.12.001

Younger, J. M., Chen, L., Ren, H.-Y., Rosser, M. F. N., Turnbull, E. L., Fan, C.-Y., et al. (2006). Sequential quality-control checkpoints triage misfolded cystic fibrosis transmembrane conductance regulator. *Cell* 126, 571–582. doi: 10.1016/j.cell.2006.06.041

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Molecular Biosciences

REVIEW
published: 09 October 2015
doi: 10.3389/fmolb.2015.00057

Metazoan Hsp70-based protein disaggregases: emergence and mechanisms

*Nadinath B. Nillegoda * and Bernd Bukau **

Center for Molecular Biology (ZMBH) of the University of Heidelberg and German Cancer Research Center (DKFZ), DKFZ-ZMBH Alliance, Heidelberg, Germany

Proteotoxic stresses and aging cause breakdown of cellular protein homeostasis, allowing misfolded proteins to form aggregates, which dedicated molecular machines have evolved to solubilize. In bacteria, fungi, protozoa and plants protein disaggregation involves an Hsp70•J-protein chaperone system, which loads and activates a powerful AAA+ ATPase (Hsp100) disaggregase onto protein aggregate substrates. Metazoans lack cytosolic and nuclear Hsp100 disaggregases but still eliminate protein aggregates. This longstanding puzzle of protein quality control is now resolved. Robust protein disaggregation activity recently shown for the metazoan Hsp70-based disaggregases relies instead on a crucial cooperation between two J-protein classes and interaction with the Hsp110 co-chaperone. An expanding multiplicity of Hsp70 and J-protein family members in metazoan cells facilitates different configurations of this Hsp70-based disaggregase allowing unprecedented versatility and specificity in protein disaggregation. Here we review the architecture, operation, and adaptability of the emerging metazoan disaggregation system and discuss how this evolved.

Keywords: Hsp70, J-protein, Hsp110, protein disaggregation, metazoan

OPEN ACCESS

Edited by:
Pierre Goloubinoff, University of Lausanne, Switzerland

Reviewed by:
Stefan G. D. Rüdiger, Utrecht University, Netherlands
Eileen M. Lafer, University of Texas Health Science Center at San Antonio, USA

****Correspondence:***
Nadinath B. Nillegoda and Bernd Bukau, Zentrum für Molekulare Biologie Heidelberg, University of Heidelberg, Im Neuenheimer Feld 282, 69120 Heidelberg, Germany
n.nillegoda@zmbh.uni-heidelberg.de; bukau@zmbh.uni-heidelberg.de

Specialty section:
This article was submitted to Protein Folding, Misfolding and Degradation, a section of the journal Frontiers in Molecular Biosciences

Received: *13 July 2015*
Accepted: *22 September 2015*
Published: *09 October 2015*

Citation:
Nillegoda NB and Bukau B (2015) Metazoan Hsp70-based protein disaggregases: emergence and mechanisms. Front. Mol. Biosci. 2:57. doi: 10.3389/fmolb.2015.00057

Introduction

In healthy cells, toxicities associated with protein misfolding are countered by regulated cellular processes that sequester damaged, sticky and potentially harmful proteins to intracellular protein deposit sites (Taylor et al., 2003; Arrasate et al., 2004; Miller et al., 2015) where protein quality control machineries operate to resolve aggregates (disaggregation) (Parsell et al., 1994; Mogk et al., 1999; Tyedmers et al., 2010; Doyle et al., 2013). Accumulation of protein aggregates however is a distinguishing feature of cellular stress and aging in all organisms (Morimoto, 2008; Hipp et al., 2014) and is associated with toxicities leading to pathology (Olzscha et al., 2011; Polymenidou and Cleveland, 2012; Park et al., 2013). Aggregates hallmark a plethora of human disorders ranging from neurodegeneration to diabetes and cancers (Knowles et al., 2014; de Oliveira et al., 2015; Mukherjee et al., 2015). Persistence of protein aggregates eventually also poses a threat to the integrity of the cytoskeleton and cellular signaling (Perutz et al., 1994; Kopito, 2000; Lee et al., 2004).

The ubiquitous presence of dedicated protein disaggregation machines (disaggregases) in all cells (Winkler et al., 2012a; Doyle et al., 2013) underlines the importance of aggregate solubilization activity. Polypeptides freed from solubilizing aggregates are sorted for either refolding (Glover and Lindquist, 1998) or degradation (Ravikumar et al., 2008; Douglas et al., 2009; Ciechanover and Kwon, 2015). Proteins essential for cellular processes must be rescued and refolded via protein

disaggregation activities for cell growth to resume after stress (Parsell et al., 1994; Weibezahn et al., 2004; Tessarz et al., 2008). Additionally, disaggregation and refolding activities greatly reduce resynthesis requirements (Sanchez and Lindquist, 1990; Mogk et al., 1999; Motohashi et al., 1999; Queitsch et al., 2000) which is arguably energetically favorable. Terminally damaged proteins that fail to refold are cleared from cells by proteolytic systems to prevent reaggregation and ensuing toxicities (Cuervo et al., 2004; Cohen et al., 2006). Protein disaggregation therefore, is central to the establishment of protein homeostasis and the promotion of cell survival.

The Non-metazoan Hsp100 and Hsp70•J-protein bi-chaperone Disaggregation System

The ability of cells to solubilize aggregated proteins is well established in prokaryotes and in non-metazoan eukaryotes (e.g., fungi, protozoa, and plants) (Parsell et al., 1994; Hübel et al., 1997; Mogk et al., 1999; Doyle et al., 2007; Lee et al., 2007). These disaggregase machineries involve cooperation between members of the Hsp70 and the Hsp100 chaperone families (Glover and Lindquist, 1998; Goloubinoff et al., 1999; Zietkiewicz et al., 2004; Doyle and Wickner, 2009). Hsp100s are powerful AAA+ ATPases that extract trapped polypeptides from aggregates via a threading mechanism. Briefly, a hexameric Hsp100 ring with a central pore interacts with the Hsp70 system (Seyffer et al., 2012; Rosenzweig et al., 2013) to load onto protein aggregates (Winkler et al., 2012b). Concomitantly, the Hsp70 system activates the Hsp100 disaggregase for ATP-dependent substrate threading (Seyffer et al., 2012; Lee et al., 2013; Lipinska et al., 2013; Carroni et al., 2014). Protruding polypeptide termini or surface loops of trapped polypeptides are drawn into the pore to interact with flexible aromatic loop residues internal to the pore (Schlieker et al., 2004; Weibezahn et al., 2004). Unfoldase activity of Hsp70 (Sharma et al., 2011) is thought to remodel the surface of a protein aggregate through J-protein (Hsp40) controlled substrate-binding cycles, to generate these surface loops (Zietkiewicz et al., 2006). It is generally accepted that ATP hydrolysis in Hsp100 powers movement of the aromatic residues with a ratchet-type mechanism, effectively pulling the polypeptide into the pore and disentangling it from the aggregate (Lum et al., 2004; Schlieker et al., 2004; Haslberger et al., 2008). Without the cooperation of the Hsp100 disaggregase, the bacterial and yeast Hsp70 systems show very limited protein disaggregation capability (Goloubinoff et al., 1999; Diamant et al., 2000; Ben-Zvi et al., 2004; Doyle et al., 2007; Rampelt et al., 2012) inadequate for survival after severe protein aggregation stresses (Sanchez and Lindquist, 1990; Squires et al., 1991; Hong and Vierling, 2000). The Hsp100 and Hsp70•J-protein bi-chaperone disaggregation system is powerful and efficient and supports rapid response to protein misfolding stresses, minimizing cytotoxicity associated with protein aggregation (Olzscha et al., 2011; Park et al., 2013).

The Hsp70•J-protein•Hsp110 System Forms a Potent Metazoan Disaggregase

Metazoan cells lack the core Hsp100 component of the bi-chaperone system (Doyle et al., 2013), and ambiguity in past results has made the very existence of robust protein disaggregation activity in metazoa contentious (Kampinga, 1993; Shorter, 2011; Murray et al., 2013). Recent work shows that efficient metazoan disaggregation activity requires the Hsp70 chaperone and a complex of J-proteins of two different classes (Nillegoda et al., 2015). Further cooperation with the Hsp110 co-chaperone, which acts as a nucleotide exchange factor (NEF) (Dragovic et al., 2006; Raviol et al., 2006b), boosts overall disaggregase capacity (Shorter, 2011; Rampelt et al., 2012; Nillegoda et al., 2015). This configuration provides metazoans with a potent Hsp70-based disaggregation activity that efficiently solubilizes a wide range of protein aggregates *in vitro*, comparable to the non-metazoan bi-chaperone disaggregation systems (Nillegoda et al., 2015).

Unraveling of aggregated proteins depends on substrate bind and release cycles of the metazoan Hsp70 in conjunction with J-protein and Hsp110 co-chaperones (**Figures 1A–C**). J-proteins target Hsp70 to substrates (Gamer et al., 1992; Laufen et al., 1999; Kampinga and Craig, 2010) and form the largest and the most structurally diverse chaperone family in metazoa (Cheetham and Caplan, 1998; Kampinga and Craig, 2010). Class A and B J-proteins (**Figure 1A**) contain conserved N-terminal J-domains (JDs) that interact with Hsp70 (Tsai and Douglas, 1996; Suh et al., 1999) and C-terminal domains (CTDs) involved in substrate binding (Lee et al., 2002; Li et al., 2003). Class A proteins contain a further zinc-finger-like region (ZFLR), also contributing to substrate recognition/binding (Lu and Cyr, 1998).

Concomitant interaction of the Hsp70 with a J-protein and substrate provides allosteric stimulation for ATP hydrolysis in Hsp70 (Bukau and Horwich, 1998; Laufen et al., 1999; Mayer and Bukau, 2005). This dual trigger traps aggregate substrate in the Hsp70 substrate-binding pocket. Substrate dissociation re-starts the Hsp70 chaperone cycle and requires release of the hydrolysis products, ADP+P_i, followed by binding of a new ATP molecule to Hsp70 (**Figure 1B**). ADP release from Hsp70 is triggered preferentially by the Hsp110 NEF during protein disaggregation (Rampelt et al., 2012). Here, we outline the newly defined architecture and function of efficient metazoan Hsp70•J-protein•Hsp110 disaggregation machineries and briefly discuss ensuing physiological implications and evolutionary considerations.

Hsp110 and Metazoan Protein Disaggregation

Hsp110 chaperones are a distinct eukaryotic branch of the conserved Hsp70 superfamily (Lee-Yoon et al., 1995; Yasuda et al., 1995; Easton et al., 2000) and share the Hsp70 bipartite domain architecture: an N-terminal nucleotide-binding domain (NBD) linked to a C-terminal substrate-binding domain (SBD) (**Figure 1C**). Hsp110 isoforms (three in humans,

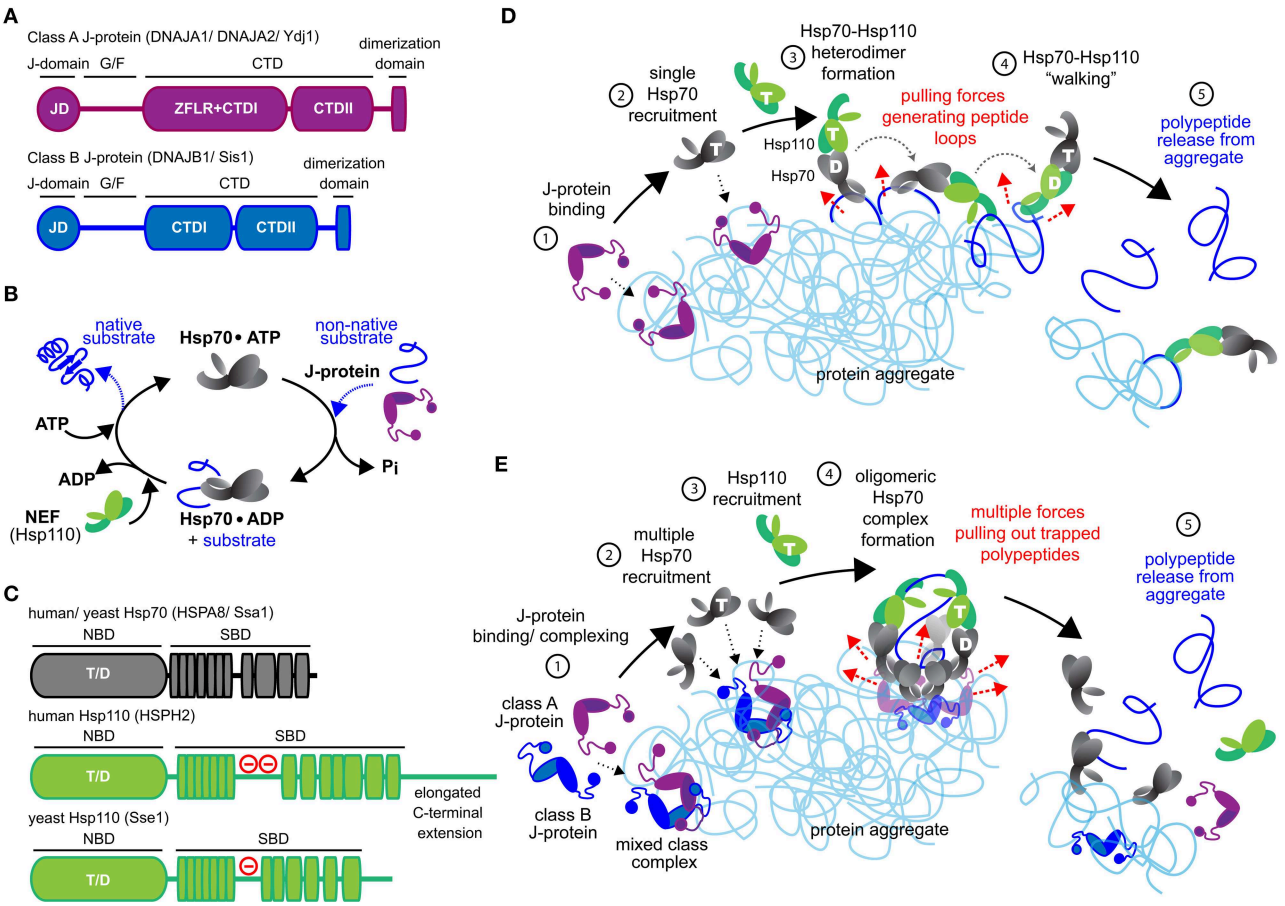


FIGURE 1 | Mechanistic models for Hsp70-based metazoan protein disaggregation. (A) Domain organization of class A and B J-proteins (as protomers). JD designates the conserved N-terminal J-domain. G/F denotes the glycine/phenylalanine rich flexible region; ZFLR, Zinc finger-like region; CTDI and CTDII, two homologous C-terminal β-sandwich substrate-binding domains. CTDs together with ZFLR provide substrate specificity. The dimerization domain forms functional J-protein homodimers. **(B)** Hsp70•J-protein•Hsp110 functional cycle. Concomitant interaction of Hsp70 with a J-protein and substrate results in allosteric stimulation of ATP hydrolysis trapping the substrate in Hsp70. Subsequent Hsp110 mediated ADP release from Hsp70 allows ATP rebinding, which triggers substrate release to complete Hsp70 cycle. **(C)** Schematic representation of the domain organization of yeast and human Hsp110 and Hsp70. (–) in red indicates the acidic region inserted between the terminal strands of the predicted β-sheet structure. The acidic loop determines the nuclear/cytoplasmic localization of human HSPH1 (Saito et al., 2009). The extended C-terminal domain is noted in HSPH2. ATP and ADP nucleotides bound to the NBD of Hsp70 and Hsp110s are denoted as "T" and "D," respectively. **(D)** "Clamp and walk" model for Hsp70 and Hsp110 mediated protein disaggregation. Hsp70, J-protein and Hsp110 indicated in gray, purple and green, respectively. Nucleotide state at the NBDs of Hsp70 and Hsp110 indicated by T and D. Sequential reaction steps (encircled numbers): 1, J-protein targets aggregate; 2, J-protein recruits Hsp70; 3, Hsp110 recruitment and formation of Hsp70•Hsp110 heterodimer; 4, Hsp70•Hsp110 heterodimer "walking" on aggregate by alternating scanning (ATP state) and clamping (ADP state) substrate-interaction modes that generate pulling forces (dashed red arrows) on trapped polypeptides. Pulling forces result in forming peptide loops (dark blue) that fold to native-like conformations; 5, Releasing of polypeptides from aggregate due to accumulation of native-like folding events in trapped substrates. **(E)** Metazoan "nucleation" model for efficient Hsp70-based protein disaggregation. 1, J-protein targets and nucleates on aggregate; 2, Localized, multiple Hsp70 recruitment by J-protein assemblies on aggregates; 3, Hsp110 recruitment; 4, formation of oligomeric chaperone complex containing J-protein, Hsp110 and multiple Hsp70 molecules and buildup of entropic pulling forces (dashed red arrows) leading to extraction of trapped polypeptides (dark blue). 5, Hsp110 NEF activity triggered releasing of polypeptides from aggregate.

Hsp105α/HSPH1, Apg-2/HSPH2, and Apg-1/HSPH3) form one of the three distinct classes of NEFs (along with Bag-type and HspBP1-type), which interact with Hsp70 molecules (Dragovic et al., 2006; Raviol et al., 2006b; Shaner et al., 2006). *In vitro,* all three cytosolic human Hsp110-type NEFs support protein disaggregation equally (Rampelt et al., 2012). However, Hsp105α knockout mouse cells show severe defects in reactivating aggregated proteins after heat stress (Yamagishi et al., 2011) despite the presence of the two other cytosolic Hsp110 members (Apg-1 and Apg-2). This apparent hierarchy among Hsp110 members *in vivo* may reflect differences in cellular localization (Saito et al., 2007) and/or tissue specific abundance (Kaneko et al., 1997; Okui et al., 2000). RNAi depletion of the single *C. elegans* cytosolic Hsp110 also shows defects in aggregate clearance after heat-stress (Rampelt et al., 2012). These *in vivo* defects most likely reflect Hsp110's role in boosting metazoan protein disaggregation identified *in vitro* (Shorter, 2011; Rampelt et al., 2012; Gao et al., 2015; Nillegoda et al., 2015). However, lack of aggregate

clearance *in vivo* may also arise partly from Hsp110 involvement in holdase-type functions preventing aggregation (Ishihara et al., 2003; Yamagishi et al., 2003; Yamashita et al., 2007), and/or involvement in other protein quality control processes such as protein degradation (Heck et al., 2010; Saxena et al., 2012).

Knockdown of Hsp110, but not the Bag-1 NEF, abolishes aggregate clearance in *C. elegans* (Rampelt et al., 2012). Accordingly, substitution of Hsp110 by Bag-1 does not support efficient protein disaggregation with human Hsp70•single J-protein configuration *in vitro* (Rampelt et al., 2012; Gao et al., 2015), implying Hsp110 specialization for protein disaggregation.

The precise nature of Hsp110 specialization/function during metazoan protein disaggregation however, is currently under debate. The basic question revolves around the primary function of Hsp110 in protein disaggregation: Is Hsp110 function limited to nucleotide exchange (as a specialized NEF) or does Hsp110 function extend beyond NEF activity and act as a vital substrate-binding chaperone within the composite disaggregase machinery? The answer to this question is central to the mechanism of disaggregation.

Evidence for Hsp110 Function Beyond NEF Activity

Hsp110 and Bag-1 are NEFs that trigger similar structural changes in the NBD of Hsp70 inducing release of nucleotides (Sondermann et al., 2001; Andréasson et al., 2008; Schuermann et al., 2008). Why in general Bag-1 can neither substitute for Hsp110 in protein disaggregation *in vitro* nor *in vivo* is therefore puzzling. Existence of unique structural features such as an SBD (Oh et al., 1999; Goeckeler et al., 2008; Polier et al., 2010), which is absent in other types of NEFs, may support a role for Hsp110 beyond NEF activity in metazoan Hsp70-based disaggregases.

The ability of Hsp110 to directly bind aberrant protein substrates (via the SBD) is reflected in holdase activity, where Hsp110 binds to misfolding proteins and prevents thermally induced aggregation (Oh et al., 1997, 1999). Hsp110 has distinct peptide binding specificity to that of Hsp70 (Goeckeler et al., 2008; Xu et al., 2012), arising from sequence differences in the SBD (Raviol et al., 2006a). Hsp110 proteins preferentially bind aromatic residue-rich peptides, whereas canonical Hsp70s prefer aliphatic-rich peptides. Yeast Hsp110 (Sse1) exhibits reduced affinity for peptide substrate in the presence of ATP, indicating nucleotide binding induces substrate release (Xu et al., 2012). This suggests allosteric coupling between the NBD and SBD of Hsp110 proteins prompting the idea that the NEF could function as a substrate binding/unbinding Hsp70-like chaperone in protein disaggregation. However, such nucleotide dependent substrate release activity was not observed with other Sse1 specific peptide substrates (Goeckeler et al., 2008). Further, the ATP-induced peptide release activity observed by Xu and coworkers is restricted to yeast Hsp110s and is residual only, in human Hsp110 (Xu et al., 2012).

A study in fruit flies however suggests suppression of aggregation of polyQ containing proteins requires ATPase driven allosteric coupling of NBD-SBD in the fly Hsp110, since unlike wild-type fly-Hsp110, overexpression of an ATPase deficient mutant of fly-Hsp110 is unable to suppress the toxicities associated with aggregation. Suppression however, also requires co-overexpression of a J-protein (Kuo et al., 2013). The authors propose an Hsp70•J-protein-like cooperation between Hsp110 and J-proteins, beyond NEF activity. Hsp110•J-protein combinations however, are incapable of solubilizing aggregates *in vitro*. Adding Hsp70 drives solubilization (Shorter, 2011; Rampelt et al., 2012). Also, this study is *in vivo,* and therefore an Hsp70•J-protein•Hsp110 requirement is not excluded.

More compelling support for Hsp110 function beyond NEF activity comes from a study that shows human Hsp110 (Hsp105α) is an ATP-dependent foldase capable of refolding preformed misfolded polypeptides into native proteins (Mattoo et al., 2013). This study further shows a bi-directional communication linking Hsp110 and Hsp70, which allows Hsp110 to induce substrate release from Hsp70 in the absence of ATP binding. Similarly, Hsp70 induces substrate release from Hsp110. Under the conditions used, Mattoo and coworkers find optimal Hsp70•J-protein•Hsp110 disaggregase activity at a 1:1 stoichiometry for Hsp70:Hsp110 (Mattoo et al., 2013). Based on 1:1 optimal activity stoichiometry and the foldase capacity of Hsp110 (both of which activities require a J-protein to be present) these authors propose an Hsp70•Hsp110 core functional unit for metazoan disaggregases and the first mechanistic model for the metazoan Hsp70-based disaggregase.

The Hsp70•Hsp110 "Clamp and Walk" Model

The "*clamp and walk*" model proposed by Mattoo et al. (2013) (**Figure 1D**) postulates an Hsp70•Hsp110 heterodimer as the functional core unit of the Hsp70-based disaggregase. The spatial arrangement of the proposed heterodimer depicted in **Figure 1D** derives from the crystal structure of bovine Hsc70 NBD and yeast Hsp110 Sse1 (Schuermann et al., 2008). Hsp70 (black) and Hsp110 (green) toggle between ATP (T) and ADP (D) bound states, triggering alternately coordinated substrate binding and release for each. The ATP bound chaperone state is engaged in scanning for new proximal substrate contacts, while the other ADP-bound chaperone is anchored to the protein aggregate. Capture of a new aggregated polypeptide segment by the first molecule triggers substrate release in the anchored molecule, locally unwinding the released polypeptide segment to form an unfolded polypeptide loop. Such sequential bind-and-release events, or "walking" of the Hsp70•Hsp110 heterodimer, are predicted to constitute a power-stroke action (Sousa and Lafer, 2006), which pulls out and unfolds a series of polypeptide loops from trapped substrates on the surface of an aggregate (dark blue, **Figure 1D**). Polypeptide segments in these loops would then spontaneously refold to native-like conformations. Accumulation of these small refolding events along an aggregated polypeptide would promote polypeptide dissociation from the aggregate (Mattoo et al., 2013).

Irreconcilable Data

Though the "*clamp and walk*" model is attractive, it is inconsistent with accumulating and emerging data. The foldase activity of Hsp110 is debatable, as is the concerted action of

Hsp70 and Hsp110 in protein disaggregation proposed by Mattoo and coworkers (Mattoo et al., 2013). Studies previous to the Mattoo report do not see any foldase activity by an Hsp110•J-protein pair (Oh et al., 1997; Yamagishi et al., 2000; Dragovic et al., 2006), although the kind of substrates used in the Mattoo analysis may have given rise to different results. In addition, mechanistic studies on yeast and human Hsp110s consistently fail to detect any hallmark features of canonical Hsp70s (Shaner et al., 2004; Raviol et al., 2006a; Liu and Hendrickson, 2007; Andréasson et al., 2008). Direct analysis shows that characteristic ATP and J-protein triggered conformational rearrangements of Hsp70 are absent in yeast and human Hsp110s (Raviol et al., 2006a; Andréasson et al., 2008; Goeckeler et al., 2008) although ATP hydrolysis occurs in Hsp110 (Raviol et al., 2006a; Goeckeler et al., 2008; Mattoo et al., 2013). Taken together, the majority of studies shows a lack of Hsp70-like allosteric coupling between NBD and SBD and foldase activity for Hsp110s (Oh et al., 1997; Yamagishi et al., 2000; Dragovic et al., 2006; Raviol et al., 2006a; Andréasson et al., 2008; Goeckeler et al., 2008). Further, a NEF activity-deficient mutant of human Hsp110, but not an ATPase deficient mutant, is defective in Hsp70•J-protein mediated protein disaggregation (Rampelt et al., 2012). This strongly suggests the primary function of Hsp110 in protein disaggregation is nucleotide exchange and not an activity requiring ATP-dependent structural rearrangements in Hsp110.

In general, NEFs act at substoichiometric levels to Hsp70 to avoid futile nucleotide exchange cycles in Hsp70 leading to inhibitory effects during non-metazoan protein disaggregation and/or refolding assays *in vitro* (Goloubinoff et al., 1999; Yamagishi et al., 2000; Zietkiewicz et al., 2006; Genest et al., 2011). Human Hsp110 displays characteristics of a typical NEF during metazoan protein disaggregation/refolding and works optimally at substoichiometric levels relative to Hsp70. Accordingly, higher Hsp110 to Hsp70 ratios inhibit protein solubilization by the human Hsp70-based disaggregation system (Rampelt et al., 2012; Gao et al., 2015; Nillegoda et al., 2015). However, under the conditions Mattoo and colleagues use, highest protein disaggregation activity is found at equimolar concentrations of Hsp70 and Hsp110 (Mattoo et al., 2013). This contradiction in Hsp70 to Hsp110 stoichiometry perhaps arises from differences in experimental conditions. The rationale for an Hsp70•Hsp110 heterodimer based disaggregase model however, depends heavily on this observation. Furthermore, structurally, the elongated C-terminal extension (**Figure 1C**) unique to human Hsp110s and important for substrate binding (shown for holdase activity) (Oh et al., 1999; Raviol et al., 2006a) is superfluous for protein disaggregation. The yeast Sse1 NEF which has a stunted C-terminal extension functionally dispensable *in vivo* (Shaner et al., 2004; Liu and Hendrickson, 2007) and *in vitro* for NEF activity (Andréasson et al., 2008), is capable of fully substituting for the human Hsp110 during protein disaggregation (Rampelt et al., 2012). This strongly suggests, but does not formally show, that substrate-binding features of human Hsp110 are dispensable for disaggregation. Altogether, these observations further consolidate a primarily nucleotide exchange function for metazoan Hsp110 in protein disaggregation. Finally and most tellingly, Hsp110 is not strictly essential for activity in some disaggregase configurations (Nillegoda et al., 2015), though not others (Gao et al., 2015). The *in vitro* activity on amorphous aggregates by human Hsp70-based disaggregases containing heterocomplexed J-proteins is ∼33% less efficient without Hsp110 (Nillegoda et al., 2015). This is reminiscent of the yeast bi-chaperone-based disaggregase system where Hsp110 acts as a NEF, which boosts, but is dispensable for, disaggregation (Glover and Lindquist, 1998; Rampelt et al., 2012).

The "*clamp and walk*" model is unclear as to how J-proteins, an essential component of metazoan disaggregase machinery (Nillegoda et al., 2015), participate in the "walking" dynamics of the proposed Hsp70•Hsp110 heterodimer on the aggregate (**Figure 1D**, beyond step 1). Cumulatively, these points suggest a central role for an Hsp70•Hsp110 heterodimer in metazoan protein disaggregation is unlikely, and point instead to a central involvement of J-proteins.

In short, both the analytical discrepancies outlined regarding NEF independent function and the latest developments in Hsp70-based disaggregase biology involving J-protein requirements (Nillegoda et al., 2015) suggest the primary role of Hsp110 in disaggregation is nucleotide exchange. These considerations argue against a central architectural role for Hsp110 as a key substrate-binding chaperone in the Hsp70-based disaggregases.

Metazoan NEF Specialization in Protein Disaggregation

One explanation for the specialization of Hsp110 in protein disaggregation may lie in the kinetics of NEF driven Hsp70 cycling through ATP/ADP states. A further consideration is the overall architecture of the assembled Hsp70 disaggregase, which may limit steric accessibility of one NEF over another in some configurations. Hsp110 and Bag1 NEFs utilize a similar mechanism to induce nucleotide release from Hsp70, but have discrete binding interfaces on the Hsp70 NBD (Sondermann et al., 2001; Andréasson et al., 2008). The assembled disaggregase core architecture (J-proteins and Hsp70) probably favors accessibility of one binding interface (Hsp110) over the other (Bag1), depending on the specific J-protein combinations incorporated into the core architecture. This accounts well for the inability of the Bag-1 NEF to promote efficient nucleotide exchange in Hsp70-based protein disaggregation in some combinations but not others. Hsp70•DNAJB1•Bag-1 combinations show poor disaggregation activity compared with Hsp70•DNAJB1•Hsp110 combination. In contrast, Hsp70•DNAJA2 combinations, which specifically target smaller aggregates (Nillegoda et al., 2015), function equally well with Hsp110 and Bag-1 *in vitro* (Rampelt et al., 2012). Bag-1 displays similarly differential cooperative efficacy in refolding proteins, with Hsp70•DNAJA2 providing highest activity (Terada and Mori, 2000). Although not directly shown, these observations are entirely consistent with steric exclusion due to architectural constraints leading to lack of function, and suggest Hsp110 has evolved to provide a specialized NEF used by all Hsp70-based disaggregases.

On balance, we predict that Hsp110 co-chaperones play a dual role in protein disaggregation. Hsp110 primarily provides NEF activity facilitating efficient substrate release from Hsp70 molecules, which resets the disaggregase machine for another round of polypeptide extraction. The contribution of Hsp110 NEF activity to protein disaggregation is dispensable and varies with J-protein class (Rampelt et al., 2012), J-protein class cooperation (Nillegoda et al., 2015), and substrate-type (Gao et al., 2015; Nillegoda et al., 2015). Hsp110 however may also perform an extra, but non-essential holdase function in disaggregation by interacting directly with polypeptides during extraction from the aggregate, as invoked by the earlier "*clamp and walk*" model (see **Figure 1E** for a newly proposed model for Hsp70-based disaggregases).

The Metazoan "Nucleation" Model for Efficient Hsp70-based Protein Disaggregation

A different mechanism for metazoan Hsp70-based disaggregation incorporates the latest data (**Figure 1E**) and resolves the analytical discrepancies outlined. This mechanism involves initial formation of oligomeric, higher order chaperone structures containing multiple Hsp70 molecules on the aggregate surface. Clustered binding of Hsp70 molecules, potentially to the same trapped substrate polypeptide, will increase the extracting force on the polypeptide due to decreasing entropy (De Los Rios et al., 2006; Goloubinoff and De Los Rios, 2007), facilitating local disaggregation. Repulsive forces generated by steric exclusion of bulky clustered Hsp70 molecules are also proposed to disrupt strong peptide-peptide interactions (Kellner et al., 2014). Together, such forces are thought to help release trapped polypeptides from aggregates. Unfolding is a prerequisite for subsequent correct protein refolding of extracted polypeptides. Unlike non-metazoan Hsp100 AAA+ ATPases where extracting polypeptides are unfolded by threading through a molecular tunnel (Weibezahn et al., 2004; Hinnerwisch et al., 2005; Haslberger et al., 2008; Doyle et al., 2012), the metazoan Hsp70-based disaggregase probably relies instead on the unfoldase power of Hsp70 chaperones to directly unravel the disaggregating polypeptides (Sharma et al., 2011). The multi-component disaggregase complex may also form a channel-like or cavity-like structure to stabilize the disaggregating, unfolded polypeptide (**Figure 1E**, step 4). However, how are multiple Hsp70 molecules efficiently attracted to one site on the surface of an aggregate? This is the crucial first step for this model.

A Central Role for J-proteins in Disaggregase Structure

Recent work reveals the formation of transient heterocomplexes between class A and class B homodimer J-proteins via intermolecular JD•CTD interactions. These mixed class J-protein complexes formed on the surface of amorphous aggregates boost the efficacy of metazoan Hsp70-based disaggregases (Nillegoda et al., 2015). Canonical J-protein homodimers present two J-domains for potential interaction with two independent Hsp70 molecules (Morgner et al., 2015). On this basis, a minimal mixed-class dimer-dimer J-protein complex would present four J-domains and could therefore recruit up to four Hsp70 molecules after binding to an aggregate (**Figure 1E**, step 2). Conglomeration of Hsp70 molecules would further increase if recruited Hsp70 molecules themselves further formed homodimers, as recently seen in bacteria (Malinverni et al., 2015; Sarbeng et al., 2015). J-protein nucleation on the surface of protein aggregates therefore would provide a foundation upon which multiple Hsp70 molecules are recruited to form oligomeric Hsp70-based efficient disaggregation machines. The precise basis for J-proteins nucleation on aggregates has not been defined but presumably J-proteins nucleate where looped out polypeptide stretches are available for binding.

Summarizing the Support for the Two Models

The crucial difference between the two models lies in the molecular architecture of the core disaggregase, which dictates mechanism of aggregate solubilization. In the earlier model an Hsp70•Hsp110 heterodimer core enables a ratcheted bind-and-release of aggregate substrate, in a "walking" disaggregation action to create successive disaggregated domains on a polypeptide, eventually leading to full disaggregation. This presumes ATP-hydrolysis coordinates substrate capture and release by Hsp70, which is well established, but also for Hsp110, which is experimentally unsupported. Further, recent data show Hsp110 is not strictly essential in some metazoan disaggregase configurations. J-proteins on the other hand, are indispensable. The clamp and walk mechanism, based on an Hsp70•Hsp110 core architecture, strictly requires Hsp110, and makes no provision for J-protein function other than the initial targeting of Hsp70 or Hsp110 to the aggregate. Together, these points make an Hsp70•Hsp110 heterodimer architecture and the ensuing ratchet mechanism less plausible.

In contrast, the new model requires initial nucleation by J-proteins for Hsp70-based disaggregation to proceed. In this model J-proteins target amorphous aggregate surfaces, recruiting multiple Hsp70 molecules via established interaction interfaces, to foci on the aggregate, nucleating higher order Hsp70-J-protein core disaggregase structures. The NEF activity of Hsp110 is beneficial and often essential for enhancing disaggregation function, but for some disaggregase configurations dispensable. There is also evolutionary precedence suggesting a bacterial J-protein-mediated Hsp70 clustering mechanism driving Hsp100 dependent disaggregation (Seyffer et al., 2012). In the metazoan context, J-protein nucleation on aggregate surfaces is therefore very plausible.

A J-protein Gearbox Regulates Metazoan Protein Disaggregation Efficacy

Both efficacy and substrate (protein aggregate) specificity of Hsp70-based disaggregases are determined by J-proteins.

Hsp70-based disaggregases containing class A vs. class B J-proteins specifically target different amorphous aggregates. Human Hsp70 and Hsp110 combined with class A J-protein (DNAJA1, DNAJA2) targets only small aggregates (Mattoo et al., 2013; Nillegoda et al., 2015). In contrast, the Hsp70•class B J-protein (DNAJB1)•Hsp110 system solubilizes only large aggregates (Nillegoda et al., 2015). This explains the superior disaggregation activity of the Hsp70•DNAJB1•Hsp110 combination in previous work which used substrates consisting predominantly of large aggregates (i.e., aggregated luciferase formed under high luciferase concentration) (Rampelt et al., 2012). Selection is based on aggregate size/structure rather than substrate type, possibly arising from differences in class A vs. B J-protein mode of binding (Terada and Oike, 2010) and/or peptide binding characteristics (Fan et al., 2004). Unlike single class J-proteins, mixed class J-protein complexes provide broad substrate specificity, allowing Hsp70-based disaggregases to target aggregates over a wide size range (Nillegoda et al., 2015). This is most likely due to combined presence of different substrate binding CTDs in the complex. Different aggregate types (amorphous vs. amyloid) are resolved by markedly different configurations of Hsp70-based disaggregases. For example, the Hsp70•DNAJB1•Hsp110 single J-protein configuration, which specifically targets large amorphous aggregates is also sufficient for efficient disintegration of α-synuclein amyloid fibrils and does not require mixed class J-protein complexing (Gao et al., 2015).

Overall, it is clear that during protein disaggregation, J-proteins can function both independently in a class-dependent manner, and as mixed-class complexes with markedly distinct properties, dependent on specific constituent J-proteins. Humans have over 50 members in the J-protein family (**Figure 2A**) (Kampinga and Craig, 2010), as do other metazoans like *C. elegans* (∼30 members) (Yook et al., 2012). A wide range of complexed J-protein combinations is therefore available to metazoa, essentially providing a metazoan gearbox for fine-tuning target selectivity and efficacy of protein disaggregation.

The Emergence of Hsp70-based Protein Disaggregases During Evolution

Gene losses occur in all major lineage transitions of life. Such losses are usually reflected as deficiencies in specific biological activities (Danchin et al., 2006). The abrupt loss of cytosolic/nuclear Hsp100 class members in the transition to metazoa has no immediately obvious basis, since protein disaggregation activity is preserved and is essential in the metazoa.

Loss of Hsp100 during metazoan evolution coincides with gain-of-disaggregation function in Hsp70 machines during metazoan evolution (**Figure 2B**). Three major changes in cellular protein quality control could account for reduction of the disaggregation machine from an Hsp100-Hsp70 dual system to the single Hsp70 system: (1) The appearance of vacuolar/lysosomal-based autophagic protein degradation in eukaryotes diversifies and augments mechanisms of aggregate clearance in metazoa (Lu et al., 2014; Rogov et al., 2014). Presence of an alternative pathway could reduce selection pressure for the relatively energy-expensive Hsp100. (2) Habitat wise, free living bacteria, fungi and plants are exposed to constantly changing harsh environmental stresses, unlike metazoans, and rely heavily on Hsp100-based disaggregases for survival after extreme heat stress (Sanchez and Lindquist, 1990; Squires et al., 1991; Hong and Vierling, 2000). Hsp100 however is dispensable for central biological processes (Hong and Vierling, 2001) and under unstressed growth conditions is actually detrimental to fitness (Escusa-Toret et al., 2013). The fitness cost associated with maintaining a powerful Hsp100-based disaggregase system therefore, may have driven better stress-buffering in metazoan cells (Durieux et al., 2011; Gidalevitz et al., 2011; Van Oosten-Hawle et al., 2013) and loss of Hsp100. (3) The emergence of enhanced disaggregation versatility, via J-protein and NEF configurations providing a highly tunable Hsp70-based protein disaggregation system, may have also contributed to loss of Hsp100. A substrate-tailored versatile disaggregation system is better suited to the needs of multicellular organisms than the potent, but inflexible and less specialized Hsp100-based bi-chaperone disaggregase system found in non-metazoan life-forms.

Metazoan Hsp70, particularly the constitutive Hsc70 (HSPA8) also harbor critical evolutionary changes that support protein disaggregation. Appearance of Hsp110 in non-metazoan eukaryotes may have triggered concomitant development of accessorizing features of the partner protein Hsp70. Yeast Sse1 boosts the activity of human Hsp70•J-protein (HSPA8•DNAJB1) disaggregation system, but is unable to do so to the same level for yeast counterparts (Ssa1•Ydj1 or Ssa1•Sis1) (Rampelt et al., 2012). This points clearly to specialization of metazoan Hsc70 in protein disaggregation and this remains to be dissected. What also remains unclear is the evolution of mixed class J-protein complexing in protein disaggregation, especially since both class A and class B J-proteins exist in non-metazoans (**Figure 2B**).

Concluding Remarks

In metazoans, the expanded number of Hsp70, J-protein and NEF class members enables greater flexibility of disaggregase machinery configuration, suggesting a natural selection in favor of versatility of function. However, increased system diversification, versatility and components also increases the scope for defects arising in protein quality control processes, with the potential to translate into disease.

The substrate spectrum of the metazoan Hsp70-based disaggregase is currently poorly understood *in vivo*. It is of particular interest to examine how disease-linked amyloid-type aggregates that form stable fibrils can be disassembled by Hsp70-based disaggregases. Components of the human Hsp70-based disaggregase have been isolated from a variety of amyloid-type aggregates (Olzscha et al., 2011; Kirstein-Miles et al., 2013; Song et al., 2013) indicating that Hsp70 machinery may play a role in amyloid related neuropathies.

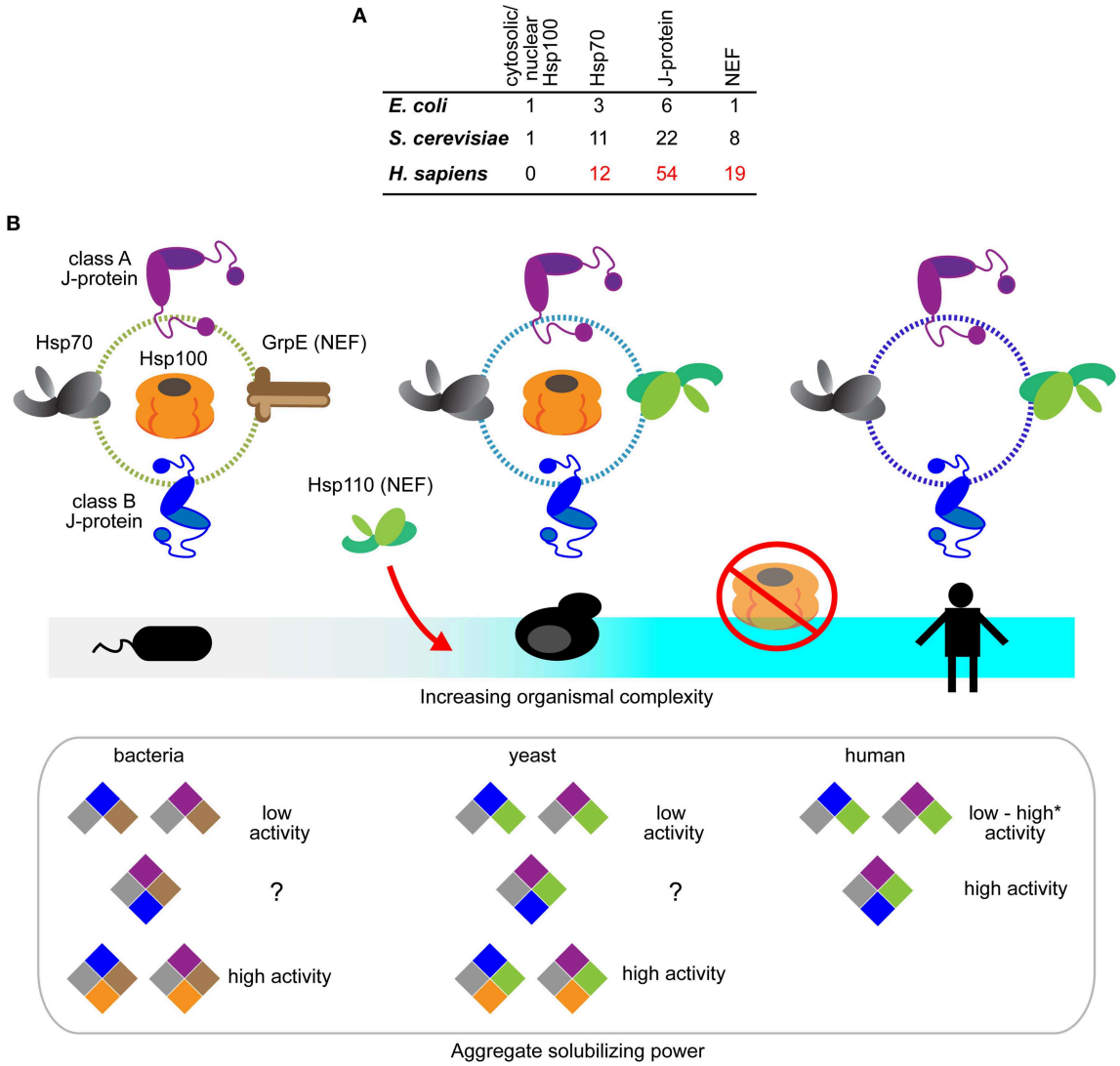


FIGURE 2 | Evolution of the Hsp70-based protein disaggregases. (A) Tabulation of Hsp100, Hsp70, J-protein and nucleotide exchange factors in prokaryotes (*E. coli*), non-metazoan eukaryotes (*S. cerevisiae*), and metazoa (*H. sapiens*). The listings include experimentally established isoforms of the respective chaperones and co-chaperones (Genevaux et al., 2001; Lu et al., 2006; Saito et al., 2009; Kampinga and Craig, 2010). **(B)** Schematic diagram depicting the emergence of Hsp70-based protein disaggregases and loss of Hsp100 and Hsp70 bi-chaperone machines during evolution. Hsp100, GrpE (bacterial NEF), Hsp70, J-proteins and Hsp110 indicated in orange, brown, gray, purple (class A J-protein), blue (class B J-protein) and green, respectively. Hsp110 NEFs only appear in eukaryotes. Hsp100 is absent in metazoan cytosol and nucleus. Aggregate solubilization power of bacterial, yeast and human disaggregation systems indicated bellow. Color-coding depicts different components of the disaggregation systems. *Denotes the high protein disaggregation activity by Hsp70•JB1•Hsp110 configuration that rapidly disassemble α-synuclein fibrils (Gao et al., 2015).

A recent *in vitro* study now shows a specific architecture of the Hsp70•Hsp110•J-protein configuration rapidly disassembles α-synuclein fibrils, via a fibril-specific mechanism, involving both fragmentation and depolymerization (Gao et al., 2015). This is particularly exciting, as the timeframe of disassembly is physiologically relevant. The full physiological impact, interplay and function of metazoan disaggregase machines *in vivo* however, remains largely unexplored and the most immediate challenge is to dissect the molecular composition, dynamics, and regulation of the basic disaggregation process in human cells.

Acknowledgments

We thank D. L. Guilbride for critically reading and editing the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft (SFB1036) to BB and a postdoctoral fellowship of the Alexander von Humboldt Foundation to NBN.

References

Andréasson, C., Fiaux, J., Rampelt, H., Druffel-Augustin, S., and Bukau, B. (2008). Insights into the structural dynamics of the Hsp110-Hsp70 interaction reveal the mechanism for nucleotide exchange activity. *Proc. Natl. Acad. Sci. U.S.A.* 105, 16519–16524. doi: 10.1073/pnas.0804187105

Arrasate, M., Mitra, S., Schweitzer, E. S., Segal, M. R., and Finkbeiner, S. (2004). Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. *Nature* 431, 805–810. doi: 10.1038/nature02998

Ben-Zvi, A., De Los Rios, P., Dietler, G., and Goloubinoff, P. (2004). Active solubilization and refolding of stable protein aggregates by cooperative unfolding action of individual hsp70 chaperones. *J. Biol. Chem.* 279, 37298–37303. doi: 10.1074/jbc.M405627200

Bukau, B., and Horwich, A. L. (1998). The Hsp70 and Hsp60 chaperone machines. *Cell* 92, 351–366. doi: 10.1016/S0092-8674(00)80928-9

Carroni, M., Kummer, E., Oguchi, Y., Wendler, P., Clare, D. K., Sinning, I., et al. (2014). Head-to-tail interactions of the coiled-coil domains regulate ClpB activity and cooperation with Hsp70 in protein disaggregation. *Elife* 3:e02481. doi: 10.7554/eLife.02481

Cheetham, M. E., and Caplan, A. J. (1998). Structure, function and evolution of DnaJ: conservation and adaptation of chaperone function. *Cell Stress Chaperones* 3, 28–36.

Ciechanover, A., and Kwon, Y. T. (2015). Degradation of misfolded proteins in neurodegenerative diseases: therapeutic targets and strategies. *Exp. Mol. Med.* 47:e147. doi: 10.1038/emm.2014.117

Cohen, E., Bieschke, J., Perciavalle, R. M., Kelly, J. W., and Dillin, A. (2006). Opposing activities protect against age-onset proteotoxicity. *Science* 313, 1604–1610. doi: 10.1126/science.1124646

Cuervo, A. M., Stefanis, L., Fredenburg, R., Lansbury, P. T., and Sulzer, D. (2004). Impaired degradation of mutant alpha-synuclein by chaperone-mediated autophagy. *Science* 305, 1292–1295. doi: 10.1126/science.1101738

Danchin, E. G., Gouret, P., and Pontarotti, P. (2006). Eleven ancestral gene families lost in mammals and vertebrates while otherwise universally conserved in animals. *BMC Evol. Biol.* 6:5. doi: 10.1186/1471-2148-6-5

De Los Rios, P., Ben-Zvi, A., Slutsky, O., Azem, A., and Goloubinoff, P. (2006). Hsp70 chaperones accelerate protein translocation and the unfolding of stable protein aggregates by entropic pulling. *Proc. Natl. Acad. Sci. U.S.A.* 103, 6166–6171. doi: 10.1073/pnas.0510496103

de Oliveira, G. A., Rangel, L. P., Costa, D. C., and Silva, J. L. (2015). Misfolding, Aggregation, and Disordered Segments in c-Abl and p53 in Human Cancer. *Front. Oncol.* 5:97. doi: 10.3389/fonc.2015.00097

Diamant, S., Ben-Zvi, A. P., Bukau, B., and Goloubinoff, P. (2000). Size-dependent disaggregation of stable protein aggregates by the DnaK chaperone machinery. *J. Biol. Chem.* 275, 21107–21113. doi: 10.1074/jbc.M001293200

Douglas, P. M., Summers, D. W., and Cyr, D. M. (2009). Molecular chaperones antagonize proteotoxicity by differentially modulating protein aggregation pathways. *Prion* 3, 51–58. doi: 10.4161/pri.3.2.8587

Doyle, S. M., Genest, O., and Wickner, S. (2013). Protein rescue from aggregates by powerful molecular chaperone machines. *Nat. Rev. Mol. Cell Biol.* 14, 617–629. doi: 10.1038/nrm3660

Doyle, S. M., Hoskins, J. R., and Wickner, S. (2007). Collaboration between the ClpB AAA+ remodeling protein and the DnaK chaperone system. *Proc. Natl. Acad. Sci. U.S.A.* 104, 11138–11144. doi: 10.1073/pnas.0703980104

Doyle, S. M., Hoskins, J. R., and Wickner, S. (2012). DnaK chaperone-dependent disaggregation by caseinolytic peptidase B (ClpB) mutants reveals functional overlap in the N-terminal domain and nucleotide-binding domain-1 pore tyrosine. *J. Biol. Chem.* 287, 28470–28479. doi: 10.1074/jbc.M112.383091

Doyle, S. M., and Wickner, S. (2009). Hsp104 and ClpB: protein disaggregating machines. *Trends Biochem. Sci.* 34, 40–48. doi: 10.1016/j.tibs.2008.09.010

Dragovic, Z., Broadley, S. A., Shomura, Y., Bracher, A., and Hartl, F. U. (2006). Molecular chaperones of the Hsp110 family act as nucleotide exchange factors of Hsp70s. *EMBO J.* 25, 2519–2528. doi: 10.1038/sj.emboj.7601138

Durieux, J., Wolff, S., and Dillin, A. (2011). The cell-non-autonomous nature of electron transport chain-mediated longevity. *Cell* 144, 79–91. doi: 10.1016/j.cell.2010.12.016

Easton, D. P., Kaneko, Y., and Subjeck, J. R. (2000). The hsp110 and Grp1 70 stress proteins: newly recognized relatives of the Hsp70s. *Cell Stress Chaper.* 5, 276–290. doi: 10.1379/1466-1268(2000)005<0276:THAGSP>2.0.CO;2

Escusa-Toret, S., Vonk, W. I. M., and Frydman, J. (2013). Spatial sequestration of misfolded proteins by a dynamic chaperone pathway enhances cellular fitness during stress. *Nat. Cell Biol.* 15, 1231–U1253. doi: 10.1038/ncb2838

Fan, C. Y., Lee, S., Ren, H. Y., and Cyr, D. M. (2004). Exchangeable chaperone modules contribute to specification of type I and type II Hsp40 cellular function. *Mol. Biol. Cell* 15, 761–773. doi: 10.1091/mbc.E03-03-0146

Gamer, J., Bujard, H., and Bukau, B. (1992). Physical Interaction between Heat-Shock Proteins Dnak, Dnaj, and Grpe and the Bacterial Heat-Shock Transcription Factor-Sigma(32). *Cell* 69, 833–842. doi: 10.1016/0092-8674(92)90294-M

Gao, X., Carroni, M., Nussbaum-Krammer, C., Mogk, A., Nillegoda, N. B., Szlachcic, A., et al. (2015). Human Hsp70 disaggregase reverses parkinson's-linked alpha-synuclein amyloid fibrils. *Mol. Cell.* 59, 781–793. doi: 10.1016/j.molcel.2015.07.012

Genest, O., Hoskins, J. R., Camberg, J. L., Doyle, S. M., and Wickner, S. (2011). Heat shock protein 90 from Escherichia coli collaborates with the DnaK chaperone system in client protein remodeling. *Proc. Natl. Acad. Sci. U.S.A.* 108, 8206–8211. doi: 10.1073/pnas.1104703108

Genevaux, P., Schwager, F., Georgopoulos, C., and Kelley, W. L. (2001). The djlA gene acts synergistically with dnaJ in promoting Escherichia coli growth. *J. Bacteriol.* 183, 5747–5750. doi: 10.1128/JB.183.19.5747-5750.2001

Gidalevitz, T., Prahlad, V., and Morimoto, R. I. (2011). The stress of protein misfolding: from single cells to multicellular organisms. *Cold Spring Harb. Perspect. Biol.* 3:a009704. doi: 10.1101/cshperspect.a009704

Glover, J. R., and Lindquist, S. (1998). Hsp104, Hsp70, and Hsp40: a novel chaperone system that rescues previously aggregated proteins. *Cell* 94, 73–82. doi: 10.1016/S0092-8674(00)81223-4

Goeckeler, J. L., Petruso, A. P., Aguirre, J., Clement, C. C., Chiosis, G., and Brodsky, J. L. (2008). The yeast Hsp110, Sse1p, exhibits high-affinity peptide binding. *FEBS Lett.* 582, 2393–2396. doi: 10.1016/j.febslet.2008.05.047

Goloubinoff, P., and De Los Rios, P. (2007). The mechanism of Hsp70 chaperones: (entropic) pulling the models together. *Trends Biochem. Sci.* 32, 372–380. doi: 10.1016/j.tibs.2007.06.008

Goloubinoff, P., Mogk, A., Zvi, A. P., Tomoyasu, T., and Bukau, B. (1999). Sequential mechanism of solubilization and refolding of stable protein aggregates by a bichaperone network. *Proc. Natl. Acad. Sci. U.S.A.* 96, 13732–13737. doi: 10.1073/pnas.96.24.13732

Haslberger, T., Zdanowicz, A., Brand, I., Kirstein, J., Turgay, K., Mogk, A., et al. (2008). Protein disaggregation by the AAA+ chaperone ClpB involves partial threading of looped polypeptide segments. *Nat. Struct. Mol. Biol.* 15, 641–650. doi: 10.1038/nsmb.1425

Heck, J. W., Cheung, S. K., and Hampton, R. Y. (2010). Cytoplasmic protein quality control degradation mediated by parallel actions of the E3 ubiquitin ligases Ubr1 and San1. *Proc. Natl. Acad. Sci. U.S.A.* 107, 1106–1111. doi: 10.1073/pnas.0910591107

Hinnerwisch, J., Fenton, W. A., Furtak, K. J., Farr, G. W., and Horwich, A. L. (2005). Loops in the central channel of ClpA chaperone mediate protein binding, unfolding, and translocation. *Cell* 121, 1029–1041. doi: 10.1016/j.cell.2005.04.012

Hipp, M. S., Park, S. H., and Hartl, F. U. (2014). Proteostasis impairment in protein-misfolding and -aggregation diseases. *Trends Cell Biol.* 24, 506–514. doi: 10.1016/j.tcb.2014.05.003

Hong, S. W., and Vierling, E. (2000). Mutants of Arabidopsis thaliana defective in the acquisition of tolerance to high temperature stress. *Proc. Natl. Acad. Sci. U.S.A.* 97, 4392–4397. doi: 10.1073/pnas.97.8.4392

Hong, S. W., and Vierling, E. (2001). Hsp101 is necessary for heat tolerance but dispensable for development and germination in the absence of stress. *Plant J.* 27, 25–35. doi: 10.1046/j.1365-313x.2001.01066.x

Hübel, A., Krobitsch, S., Horauf, A., and Clos, J. (1997). Leishmania major Hsp100 is required chiefly in the mammalian stage of the parasite. *Mol. Cell. Biol.* 17, 5987–5995.

Ishihara, K., Yamagishi, N., Saito, Y., Adachi, H., Kobayashi, Y., Sobue, G., et al. (2003). Hsp105alpha suppresses the aggregation of truncated androgen receptor with expanded CAG repeats and cell toxicity. *J. Biol. Chem.* 278, 25143–25150. doi: 10.1074/jbc.M302975200

Kampinga, H. H. (1993). Thermotolerance in mammalian cells. Protein denaturation and aggregation, and stress proteins. *J. Cell Sci.* 104 (Pt 1), 11–17.

Kampinga, H. H., and Craig, E. A. (2010). The HSP70 chaperone machinery: J proteins as drivers of functional specificity. *Nat. Rev. Mol. Cell Biol.* 11, 579–592. doi: 10.1038/nrm2941

Kaneko, Y., Nishiyama, H., Nonoguchi, K., Higashitsuji, H., Kishishita, M., and Fujita, J. (1997). A novel hsp110-related gene, apg-1, that is abundantly expressed in the testis responds to a low temperature heat shock rather than the traditional elevated temperatures. *J. Biol. Chem.* 272, 2640–2645. doi: 10.1074/jbc.272.5.2640

Kellner, R., Hofmann, H., Barducci, A., Wunderlich, B., Nettels, D., and Schuler, B. (2014). Single-molecule spectroscopy reveals chaperone-mediated expansion of substrate protein. *Proc. Natl. Acad. Sci. U.S.A.* 111, 13355–13360. doi: 10.1073/pnas.1407086111

Kirstein-Miles, J., Scior, A., Deuerling, E., and Morimoto, R. I. (2013). The nascent polypeptide-associated complex is a key regulator of proteostasis. *EMBO J.* 32, 1451–1468. doi: 10.1038/emboj.2013.87

Knowles, T. P., Vendruscolo, M., and Dobson, C. M. (2014). The amyloid state and its association with protein misfolding diseases. *Nat. Rev. Mol. Cell Biol.* 15, 384–396. doi: 10.1038/nrm3810

Kopito, R. R. (2000). Aggresomes, inclusion bodies and protein aggregation. *Trends Cell Biol.* 10, 524–530. doi: 10.1016/S0962-8924(00)01852-3

Kuo, Y., Ren, S., Lao, U., Edgar, B. A., and Wang, T. (2013). Suppression of polyglutamine protein toxicity by co-expression of a heat-shock protein 40 and a heat-shock protein 110. *Cell Death Dis.* 4, e833. doi: 10.1038/cddis.2013.351

Laufen, T., Mayer, M. P., Beisel, C., Klostermeier, D., Mogk, A., Reinstein, J., et al. (1999). Mechanism of regulation of hsp70 chaperones by DnaJ cochaperones. *Proc. Natl. Acad. Sci. U.S.A.* 96, 5452–5457. doi: 10.1073/pnas.96.10.5452

Lee, J., Kim, J. H., Biter, A. B., Sielaff, B., Lee, S., and Tsai, F. T. F. (2013). Heat shock protein (Hsp) 70 is an activator of the Hsp104 motor. *Proc. Natl. Acad. Sci. U.S.A.* 110, 8513–8518. doi: 10.1073/pnas.1217988110

Lee, S., Fan, C. Y., Younger, J. M., Ren, H., and Cyr, D. M. (2002). Identification of essential residues in the type II Hsp40 Sis1 that function in polypeptide binding. *J. Biol. Chem.* 277, 21675–21682. doi: 10.1074/jbc.M111075200

Lee, U., Rioflorido, I., Hong, S. W., Larkindale, J., Waters, E. R., and Vierling, E. (2007). The Arabidopsis ClpB/Hsp100 family of proteins: chaperones for stress and chloroplast development. *Plant J.* 49, 115–127. doi: 10.1111/j.1365-313x.2006.02940.x

Lee, W. C., Yoshihara, M., and Littleton, J. T. (2004). Cytoplasmic aggregates trap polyglutamine-containing proteins and block axonal transport in a Drosophila model of Huntington's disease. *Proc. Natl. Acad. Sci. U.S.A.* 101, 3224–3229. doi: 10.1073/pnas.0400243101

Lee-Yoon, D., Easton, D., Murawski, M., Burd, R., and Subjeck, J. R. (1995). Identification of a major subfamily of large hsp70-like proteins through the cloning of the mammalian 110-kDa heat shock protein. *J. Biol. Chem.* 270, 15725–15733. doi: 10.1074/jbc.270.26.15725

Li, J., Qian, X., and Sha, B. (2003). The crystal structure of the yeast Hsp40 Ydj1 complexed with its peptide substrate. *Structure* 11, 1475–1483. doi: 10.1016/j.str.2003.10.012

Lipinska, N., Zietkiewicz, S., Sobczak, A., Jurczyk, A., Potocki, W., Morawiec, E., et al. (2013). Disruption of ionic interactions between the nucleotide binding domain 1 (NBD1) and middle (M) domain in Hsp100 disaggregase unleashes toxic hyperactivity and partial independence from Hsp70. *J. Biol. Chem.* 288, 2857–2869. doi: 10.1074/jbc.M112.387589

Liu, Q., and Hendrickson, W. A. (2007). Insights into Hsp70 chaperone activity from a crystal structure of the yeast Hsp110 Sse1. *Cell* 131, 106–120. doi: 10.1016/j.cell.2007.08.039

Lu, B., Garrido, N., Spelbrink, J. N., and Suzuki, C. K. (2006). Tid1 isoforms are mitochondrial DnaJ-like chaperones with unique carboxyl termini that determine cytosolic fate. *J. Biol. Chem.* 281, 13150–13158. doi: 10.1074/jbc.M509179200

Lu, K., Psakhye, I., and Jentsch, S. (2014). Autophagic clearance of polyQ proteins mediated by ubiquitin-Atg8 adaptors of the conserved CUET protein family. *Cell* 158, 549–563. doi: 10.1016/j.cell.2014.05.048

Lu, Z., and Cyr, D. M. (1998). Protein folding activity of Hsp70 is modified differentially by the hsp40 co-chaperones Sis1 and Ydj1. *J. Biol. Chem.* 273, 27824–27830. doi: 10.1074/jbc.273.43.27824

Lum, R., Tkach, J. M., Vierling, E., and Glover, J. R. (2004). Evidence for an unfolding/threading mechanism for protein disaggregation by Saccharomyces cerevisiae Hsp104. *J. Biol. Chem.* 279, 29139–29146. doi: 10.1074/jbc.M403777200

Malinverni, D., Marsili, S., Barducci, A., and De Los Rios, P. (2015). Large-Scale Conformational Transitions and Dimerization Are Encoded in the Amino-Acid Sequences of Hsp70 Chaperones. *PLoS Comput. Biol.* 11:e1004262. doi: 10.1371/journal.pcbi.1004262

Mattoo, R. U., Sharma, S. K., Priya, S., Finka, A., and Goloubinoff, P. (2013). Hsp110 is a bona fide chaperone using ATP to unfold stable misfolded polypeptides and reciprocally collaborate with Hsp70 to solubilize protein aggregates. *J. Biol. Chem.* 288, 21399–21411. doi: 10.1074/jbc.M113.479253

Mayer, M. P., and Bukau, B. (2005). Hsp70 chaperones: cellular functions and molecular mechanism. *Cell. Mol. Life Sci.* 62, 670–684. doi: 10.1007/s00018-004-4464-6

Miller, S. B., Mogk, A., and Bukau, B. (2015). Spatially organized aggregation of misfolded proteins as cellular stress defense strategy. *J. Mol. Biol.* 427, 1564–1574. doi: 10.1016/j.jmb.2015.02.006

Mogk, A., Tomoyasu, T., Goloubinoff, P., Rudiger, S., Röder, D., Langen, H., et al. (1999). Identification of thermolabile *Escherichia coli* proteins: prevention and reversion of aggregation by DnaK and ClpB. *EMBO J.* 18, 6934–6949. doi: 10.1093/emboj/18.24.6934

Morgner, N., Schmidt, C., Beilsten-Edmands, V., Ebong, I. O., Patel, N. A., Clerico, E. M., et al. (2015). Hsp70 forms antiparallel dimers stabilized by post-translational modifications to position clients for transfer to Hsp90. *Cell Rep.* 11, 759–769. doi: 10.1016/j.celrep.2015.03.063

Morimoto, R. I. (2008). Proteotoxic stress and inducible chaperone networks in neurodegenerative disease and aging. *Genes Dev.* 22, 1427–1438. doi: 10.1101/gad.1657108

Motohashi, K., Watanabe, Y., Yohda, M., and Yoshida, M. (1999). Heat-inactivated proteins are rescued by the DnaK.J-GrpE set and ClpB chaperones. *Proc. Natl. Acad. Sci. U.S.A.* 96, 7184–7189.

Mukherjee, A., Morales-Scheihing, D., Butler, P. C., and Soto, C. (2015). Type 2 diabetes as a protein misfolding disease. *Trends Mol. Med.* 21, 439–449. doi: 10.1016/j.molmed.2015.04.005

Murray, A. N., Palhano, F. L., Bieschke, J., and Kelly, J. W. (2013). Surface adsorption considerations when working with amyloid fibrils in multiwell plates and Eppendorf tubes. *Prot. Sci.* 22, 1531–1541. doi: 10.1002/pro.2339

Nillegoda, N. B., Kirstein, J., Szlachcic, A., Berynskyy, M., Stank, A., Stengel, F., et al. (2015). Crucial HSP70 co-chaperone complex unlocks metazoan protein disaggregation. *Nature* 524, 247–251. doi: 10.1038/nature14884

Oh, H. J., Chen, X., and Subjeck, J. R. (1997). Hsp110 protects heat-denatured proteins and confers cellular thermoresistance. *J. Biol. Chem.* 272, 31636–31640. doi: 10.1074/jbc.272.50.31636

Oh, H. J., Easton, D., Murawski, M., Kaneko, Y., and Subjeck, J. R. (1999). The chaperoning activity of hsp110. Identification of functional domains by use of targeted deletions. *J. Biol. Chem.* 274, 15712–15718. doi: 10.1074/jbc.274.22.15712

Okui, M., Ito, F., Ogita, K., Kuramoto, N., Kudoh, J., Shimizu, N., et al. (2000). Expression of APG-2 protein, a member of the heat shock protein 110 family, in developing rat brain. *Neurochem. Int.* 36, 35–43. doi: 10.1016/S0197-0186(99)00095-9

Olzscha, H., Schermann, S. M., Woerner, A. C., Pinkert, S., Hecht, M. H., Tartaglia, G. G., et al. (2011). Amyloid-like aggregates sequester numerous metastable proteins with essential cellular functions. *Cell* 144, 67–78. doi: 10.1016/j.cell.2010.11.050

Park, S. H., Kukushkin, Y., Gupta, R., Chen, T., Konagai, A., Hipp, M. S., et al. (2013). PolyQ proteins interfere with nuclear degradation of cytosolic proteins by sequestering the Sis1p chaperone. *Cell* 154, 134–145. doi: 10.1016/j.cell.2013.06.003

Parsell, D. A., Kowal, A. S., Singer, M. A., and Lindquist, S. (1994). Protein disaggregation mediated by heat-shock protein Hsp104. *Nature* 372, 475–478. doi: 10.1038/372475a0

Perutz, M. F., Johnson, T., Suzuki, M., and Finch, J. T. (1994). Glutamine repeats as polar zippers: their possible role in inherited neurodegenerative diseases. *Proc. Natl. Acad. Sci. U.S.A.* 91, 5355–5358. doi: 10.1073/pnas.91.12.5355

Polier, S., Hartl, F. U., and Bracher, A. (2010). Interaction of the Hsp110 molecular chaperones from S. cerevisiae with substrate protein. *J. Mol. Biol.* 401, 696–707. doi: 10.1016/j.jmb.2010.07.004

Polymenidou, M., and Cleveland, D. W. (2012). Prion-like spread of protein aggregates in neurodegeneration. *J. Exp. Med.* 209, 889–893. doi: 10.1084/jem.20120741

Queitsch, C., Hong, S. W., Vierling, E., and Lindquist, S. (2000). Heat shock protein 101 plays a crucial role in thermotolerance in Arabidopsis. *Plant Cell* 12, 479–492. doi: 10.1105/tpc.12.4.479

Rampelt, H., Kirstein-Miles, J., Nillegoda, N. B., Chi, K., Scholz, S. R., Morimoto, R. I., et al. (2012). Metazoan Hsp70 machines use Hsp110 to power protein disaggregation. *EMBO J.* 31, 4221–4235. doi: 10.1038/emboj.2012.264

Ravikumar, B., Sarkar, S., and Rubinsztein, D. C. (2008). Clearance of mutant aggregate-prone proteins by autophagy. *Methods Mol. Biol.* 445, 195–211. doi: 10.1007/978-1-59745-157-4_13

Raviol, H., Bukau, B., and Mayer, M. P. (2006a). Human and yeast Hsp110 chaperones exhibit functional differences. *FEBS Lett.* 580, 168–174. doi: 10.1016/j.febslet.2005.11.069

Raviol, H., Sadlish, H., Rodriguez, F., Mayer, M. P., and Bukau, B. (2006b). Chaperone network in the yeast cytosol: Hsp110 is revealed as an Hsp70 nucleotide exchange factor. *EMBO J.* 25, 2510–2518. doi: 10.1038/sj.emboj.7601139

Rogov, V., Dötsch, V., Johansen, T., and Kirkin, V. (2014). Interactions between autophagy receptors and ubiquitin-like proteins form the molecular basis for selective autophagy. *Mol. Cell* 53, 167–178. doi: 10.1016/j.molcel.2013.12.014

Rosenzweig, R., Moradi, S., Zarrine-Afsar, A., Glover, J. R., and Kay, L. E. (2013). Unraveling the mechanism of protein disaggregation through a ClpB-DnaK interaction. *Science* 339, 1080–1083. doi: 10.1126/science.1233066

Saito, Y., Yamagishi, N., and Hatayama, T. (2007). Different localization of Hsp105 family proteins in mammalian cells. *Exp. Cell Res.* 313, 3707–3717. doi: 10.1016/j.yexcr.2007.06.009

Saito, Y., Yamagishi, N., and Hatayama, T. (2009). Nuclear localization mechanism of Hsp105beta and its possible function in mammalian cells. *J. Biochem.* 145, 185–191. doi: 10.1093/jb/mvn155

Sanchez, Y., and Lindquist, S. L. (1990). HSP104 required for induced thermotolerance. *Science* 248, 1112–1115. doi: 10.1126/science.2188365

Sarbeng, E. B., Liu, Q., Tian, X., Yang, J., Li, H., Wong, J. L., et al. (2015). A functional DnaK dimer is essential for the efficient interaction with Hsp40 heat shock protein. *J. Biol. Chem.* 290, 8849–8862. doi: 10.1074/jbc.M114.596288

Saxena, A., Banasavadi-Siddegowda, Y. K., Fan, Y., Bhattacharya, S., Roy, G., Giovannucci, D. R., et al. (2012). Human heat shock protein 105/110 kDa (Hsp105/110) regulates biogenesis and quality control of misfolded cystic fibrosis transmembrane conductance regulator at multiple levels. *J. Biol. Chem.* 287, 19158–19170. doi: 10.1074/jbc.M111.297580

Schlieker, C., Weibezahn, J., Patzelt, H., Tessarz, P., Strub, C., Zeth, K., et al. (2004). Substrate recognition by the AAA+ chaperone ClpB. *Nat. Struct. Mol. Biol.* 11, 607–615. doi: 10.1038/nsmb787

Schuermann, J. P., Jiang, J., Cuellar, J., Llorca, O., Wang, L., Gimenez, L. E., et al. (2008). Structure of the Hsp110:Hsc70 nucleotide exchange machine. *Mol. Cell* 31, 232–243. doi: 10.1016/j.molcel.2008.05.006

Seyffer, F., Kummer, E., Oguchi, Y., Winkler, J., Kumar, M., Zahn, R., et al. (2012). Hsp70 proteins bind Hsp100 regulatory M domains to activate AAA+ disaggregase at aggregate surfaces. *Nat. Struct. Mol. Biol.* 19, 1347–1355. doi: 10.1038/nsmb.2442

Shaner, L., Sousa, R., and Morano, K. A. (2006). Characterization of Hsp70 binding and nucleotide exchange by the yeast Hsp110 chaperone Sse1. *Biochemistry* 45, 15075–15084. doi: 10.1021/bi061279k

Shaner, L., Trott, A., Goeckeler, J. L., Brodsky, J. L., and Morano, K. A. (2004). The function of the yeast molecular chaperone Sse1 is mechanistically distinct from the closely related hsp70 family. *J. Biol. Chem.* 279, 21992–22001. doi: 10.1074/jbc.M313739200

Sharma, S. K., De Los Rios, P., and Goloubinoff, P. (2011). Probing the different chaperone activities of the bacterial HSP70-HSP40 system using a thermolabile luciferase substrate. *Proteins* 79, 1991–1998. doi: 10.1002/prot.23024

Shorter, J. (2011). The mammalian disaggregase machinery: Hsp110 synergizes with Hsp70 and Hsp40 to catalyze protein disaggregation and reactivation in a cell-free system. *PLoS ONE* 6:e26319. doi: 10.1371/journal.pone.0026319

Sondermann, H., Scheufler, C., Schneider, C., Hohfeld, J., Hartl, F. U., and Moarefi, I. (2001). Structure of a Bag/Hsc70 complex: convergent functional evolution of Hsp70 nucleotide exchange factors. *Science* 291, 1553–1557. doi: 10.1126/science.1057268

Song, Y., Nagy, M., Ni, W., Tyagi, N. K., Fenton, W. A., Lopez-Giráldez, F., et al. (2013). Molecular chaperone Hsp110 rescues a vesicle transport defect produced by an ALS-associated mutant SOD1 protein in squid axoplasm. *Proc. Natl. Acad. Sci. U.S.A.* 110, 5428–5433. doi: 10.1073/pnas.1303279110

Sousa, R., and Lafer, E. M. (2006). Keep the traffic moving: mechanism of the Hsp70 motor. *Traffic* 7, 1596–1603. doi: 10.1111/j.1600-0854.2006.00497.x

Squires, C. L., Pedersen, S., Ross, B. M., and Squires, C. (1991). ClpB is the Escherichia coli heat shock protein F84.1. *J. Bacteriol.* 173, 4254–4262.

Suh, W. C., Lu, C. Z., and Gross, C. A. (1999). Structural features required for the interaction of the Hsp70 molecular chaperone DnaK with its cochaperone DnaJ. *J. Biol. Chem.* 274, 30534–30539. doi: 10.1074/jbc.274.43.30534

Taylor, J. P., Tanaka, F., Robitschek, J., Sandoval, C. M., Taye, A., Markovic-Plese, S., et al. (2003). Aggresomes protect cells by enhancing the degradation of toxic polyglutamine-containing protein. *Hum. Mol. Genet.* 12, 749–757. doi: 10.1093/hmg/ddg074

Terada, K., and Mori, M. (2000). Human DnaJ homologs dj2 and dj3, and bag-1 are positive cochaperones of hsc70. *J. Biol. Chem.* 275, 24728–24734. doi: 10.1074/jbc.M002021200

Terada, K., and Oike, Y. (2010). Multiple molecules of Hsc70 and a dimer of DjA1 independently bind to an unfolded protein. *J. Biol. Chem.* 285, 16789–16797. doi: 10.1074/jbc.M110.101501

Tessarz, P., Mogk, A., and Bukau, B. (2008). Substrate threading through the central pore of the Hsp104 chaperone as a common mechanism for protein disaggregation and prion propagation. *Mol. Microbiol.* 68, 87–97. doi: 10.1111/j.1365-2958.2008.06135.x

Tsai, J., and Douglas, M. G. (1996). A conserved HPD sequence of the J-domain is necessary for YDJ1 stimulation of Hsp70 ATPase activity at a site distinct from substrate binding. *J. Biol. Chem.* 271, 9347–9354. doi: 10.1074/jbc.271.16.9347

Tyedmers, J., Mogk, A., and Bukau, B. (2010). Cellular strategies for controlling protein aggregation. *Nat. Rev. Mol. Cell Biol.* 11, 777–788. doi: 10.1038/nrm2993

Van Oosten-Hawle, P., Porter, R. S., and Morimoto, R. I. (2013). Regulation of organismal proteostasis by transcellular chaperone signaling. *Cell* 153, 1366–1378. doi: 10.1016/j.cell.2013.05.015

Weibezahn, J., Tessarz, P., Schlieker, C., Zahn, R., Maglica, Z., Lee, S., et al. (2004). Thermotolerance requires refolding of aggregated proteins by substrate translocation through the central pore of ClpB. *Cell* 119, 653–665. doi: 10.1016/j.cell.2004.11.027

Winkler, J., Tyedmers, J., Bukau, B., and Mogk, A. (2012a). Chaperone networks in protein disaggregation and prion propagation. *J. Struct. Biol.* 179, 152–160. doi: 10.1016/j.jsb.2012.05.002

Winkler, J., Tyedmers, J., Bukau, B., and Mogk, A. (2012b). Hsp70 targets Hsp100 chaperones to substrates for protein disaggregation and prion fragmentation. *J. Cell Biol.* 198, 387–404. doi: 10.1083/jcb.201201074

Xu, X., Sarbeng, E. B., Vorvis, C., Kumar, D. P., Zhou, L., and Liu, Q. (2012). Unique peptide substrate binding properties of 110-kDa heat-shock protein (Hsp110) determine its distinct chaperone activity. *J. Biol. Chem.* 287, 5661–5672. doi: 10.1074/jbc.M111.275057

Yamagishi, N., Ishihara, K., Saito, Y., and Hatayama, T. (2003). Hsp105 but not Hsp70 family proteins suppress the aggregation of heat-denatured protein in the presence of ADP. *FEBS Lett.* 555, 390–396. doi: 10.1016/S0014-5793(03)01292-4

Yamagishi, N., Nishihori, H., Ishihara, K., Ohtsuka, K., and Hatayama, T. (2000). Modulation of the chaperone activities of Hsc70/Hsp40 by Hsp105alpha and Hsp105beta. *Biochem. Biophys. Res. Commun.* 272, 850–855. doi: 10.1006/bbrc.2000.2864

Yamagishi, N., Yokota, M., Yasuda, K., Saito, Y., Nagata, K., and Hatayama, T. (2011). Characterization of stress sensitivity and chaperone activity of Hsp105 in mammalian cells. *Biochem. Biophys. Res. Commun.* 409, 90–95. doi: 10.1016/j.bbrc.2011.04.114

Yamashita, H., Kawamata, J., Okawa, K., Kanki, R., Nakamizo, T., Hatayama, T., et al. (2007). Heat-shock protein 105 interacts with and suppresses aggregation of mutant Cu/Zn superoxide dismutase: clues to a possible strategy for treating ALS. *J. Neurochem.* 102, 1497–1505. doi: 10.1111/j.1471-4159.2007.04534.x

Yasuda, K., Nakai, A., Hatayama, T., and Nagata, K. (1995). Cloning and expression of murine high molecular mass heat shock proteins, HSP105. *J. Biol. Chem.* 270, 29718–29723. doi: 10.1074/jbc.270.50.29718

Yook, K., Harris, T. W., Bieri, T., Cabunoc, A., Chan, J., Chen, W. J., et al. (2012). WormBase 2012: more genomes, more data, new website. *Nucleic Acids Res.* 40, D735–741. doi: 10.1093/nar/gkr954

Zietkiewicz, S., Krzewska, J., and Liberek, K. (2004). Successive and synergistic action of the Hsp70 and Hsp100 chaperones in protein disaggregation. *J. Biol. Chem.* 279, 44376–44383. doi: 10.1074/jbc.M402405200

Zietkiewicz, S., Lewandowska, A., Stocki, P., and Liberek, K. (2006). Hsp70 chaperone machine remodels protein aggregates at the initial step of Hsp70-Hsp100-dependent disaggregation. *J. Biol. Chem.* 281, 7022–7029. doi: 10.1074/jbc.M507893200

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Molecular Biosciences

MINI REVIEW
published: 07 April 2015
doi: 10.3389/fmolb.2015.00010

The nucleotide exchange factors of Hsp70 molecular chaperones

Andreas Bracher and Jacob Verghese*

Department of Cellular Biochemistry, Max-Planck-Institute of Biochemistry, Martinsried, Germany

Molecular chaperones of the Hsp70 family form an important hub in the cellular protein folding networks in bacteria and eukaryotes, connecting translation with the downstream machineries of protein folding and degradation. The Hsp70 folding cycle is driven by two types of cochaperones: J-domain proteins stimulate ATP hydrolysis by Hsp70, while nucleotide exchange factors (NEFs) promote replacement of Hsp70-bound ADP with ATP. Bacteria and organelles of bacterial origin have only one known NEF type for Hsp70, GrpE. In contrast, a large diversity of Hsp70 NEFs has been discovered in the eukaryotic cell. These NEFs belong to the Hsp110/Grp170, HspBP1/Sil1, and BAG domain protein families. In this short review we compare the structures and molecular mechanisms of nucleotide exchange factors for Hsp70 and discuss how these cochaperones contribute to protein folding and quality control in the cell.

Keywords: BAG domain, cochaperone, GrpE, HspBP1/Sil1, Hsp110/Grp170, protein folding, proteostasis

OPEN ACCESS

Edited by:
Matthias Peter Mayer, Ruprecht-Karls-Universität Heidelberg, Germany

Reviewed by:
Jason C. Young, McGill University, Canada
Rui Joaquim Sousa, University of Texas Health Science Center, San Antonio, USA

****Correspondence:***
Andreas Bracher, Department of Cellular Biochemistry, Max-Planck-Institute of Biochemistry, Am Klopferspitz 18, 82152 Martinsried, Germany
bracher@biochem.mpg.de

Specialty section:
This article was submitted to Protein Folding, Misfolding and Degradation, a section of the journal Frontiers in Molecular Biosciences

Received: *13 February 2015*
Accepted: *18 March 2015*
Published: *07 April 2015*

Citation:
Bracher A and Verghese J (2015) The nucleotide exchange factors of Hsp70 molecular chaperones. Front. Mol. Biosci. 2:10. doi: 10.3389/fmolb.2015.00010

Introduction

Molecular chaperones of the Hsp70 family use a nucleotide-dependent conformational cycle to support protein folding. Hsp70 proteins comprise an N-terminal nucleotide binding domain (NBD) and a substrate binding domain (SBD), which communicate by allosteric signals (reviewed in Mayer, 2013). The nucleotide is bound at the center of a two-lobed structure, commonly divided into subdomains IA, IB, IIA, and IIB. ATP binding favors a twist between the NBD lobes which allows extensive contacts with the SBD. In this conformation interactions with misfolded proteins are dynamic. In contrast, the ADP-bound state exhibits an extended structure with tight interactions between the SBD of Hsp70 and exposed hydrophobic peptide segments. ATP hydrolysis by Hsp70 thus induces stable substrate complex formation, and ATP re-binding triggers substrate release from Hsp70. Hsp70 has however high affinity for ADP and its intrinsic ATP hydrolase activity is low. Hence Hsp70 function critically depends on cochaperones, specifically J-domain proteins (JDP) and nucleotide exchange factors (NEFs), which accelerate ATP hydrolysis and ADP-ATP exchange, respectively. The great diversity of JDPs, especially in eukaryotes, suggests that Hsp70 is recruited for specific tasks by forming ternary complexes with substrates and these cochaperones. Eubacterial genomes encode only one NEF for Hsp70, GrpE. While in mitochondria and chloroplasts GrpE homologs are preserved, the cytosol and endoplasmic reticulum (ER) of eukaryotes contain three divergent families of NEFs: Hsp110/Grp170, HspBP1/Sil1 homologs and BAG-domain proteins (Höhfeld and Jentsch, 1997; Kabani et al., 2002; Steel et al., 2004; Dragovic et al., 2006; Raviol et al., 2006). The human genome encodes four Hsp110/Grp170 and two HspBP1/Sil1 homologs in addition to five BAG-domain proteins and two mitochondrial GrpE species (**Figure 1**). In addition, isoforms of NEFs resulting from alternative initiation sites and splicing exist. This NEF diversity may contribute to the appropriate allocation of Hsp70 folding capacity within the proteostasis network. Interestingly, metazoans, plants and some protists also

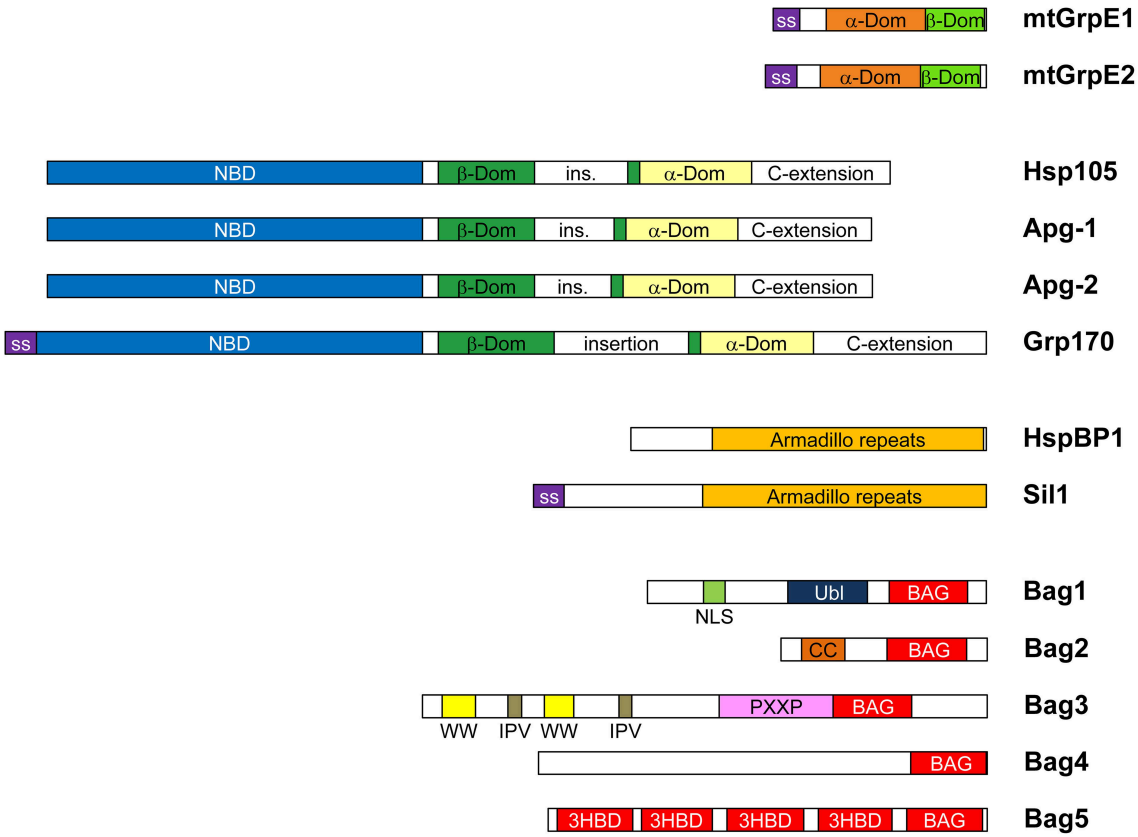


FIGURE 1 | Domain compositions of human NEF homologs. Human cells express two mitochondrial GrpE isoforms (mtGrpE1/GRPEL1 and mtGrpE2/GRPEL2), three Hsp110 homologs (Hsp105/HSPH1, Apg-1/HSPA4L, and Apg-2/HSPA4—the codes designate the gene names) and five BAG-domain proteins. There is only one form of Grp170/HYOU1, HspBP1, and Sil1, respectively. Isoforms arising from alternative initiation sites and splicing were described for Hsp105, HspBP1, and Bag1 (not shown). mtGrpE isoforms contain mitochondrial signal sequences (ss). The α- and β-domains (orange and green, respectively) are conserved with GrpE from *E. coli*. The Hsp110/Grp170 family proteins consist of an N-terminal nucleotide binding domain (NBD, blue), a β-sandwich (β-Dom, green) and a α-helix bundle domain (α-Dom, pale yellow). All isoforms contain long variable insertions in the β-sandwich and at the C-terminus. SS indicates signal sequences for ER import of Grp170 and Sil1. HspBP1 and Sil have characteristic Armadillo repeat folds (orange). All members of the BAG family in humans, Bag1-5, contain C-terminal Hsp70-binding BAG domains (red), but have otherwise divergent domain composition. Bag1 contains an Ubiquitin-like domain (Ubl, dark blue), which might associate with the regulatory particle of the 26S proteasome, and a NLS sequences (purple) for nuclear targeting. Bag2 contains a coiled-coil dimerization domain (CC, orange) (Page et al., 2012). Bag3 comprises multiple N-terminal sequence motifs including WW domains (WW, yellow), IPV sequence motifs (brown) and PXXP repeats (pink), which mediate interactions with proline-rich motifs, HspB8 and SH3 domains, respectively (Doong et al., 2000; Fuchs et al., 2010; Iwasaki et al., 2010; Ulbricht et al., 2013). Bag5 has four additional 3-helix bundle domains of unknown function (Arakawa et al., 2010). Bag6 is not shown because the original assignment as an NEF of Hsp70 was incorrect (Mock et al., 2015).

harbor the additional cochaperone Hip (gene ST13), which antagonizes NEF function by stalling Hsp70 cycling and stabilizing Hsp70 complexes with specific substrates.

In this review we compare the structures and molecular mechanisms of different NEF families and discuss how these cochaperones contribute to protein remodeling, folding and quality control in the cell. For a more comprehensive overview, please see our earlier work (Bracher and Verghese, 2015).

GrpE, the Bacterial NEF

Protein folding by the eubacterial Hsp70 homolog DnaK in *Escherichia coli* depends on GrpE, which is encoded by an essential gene (Ang and Georgopoulos, 1989). Together with the JDP DnaJ, GrpE greatly accelerates ATP hydrolysis and thus conformational cycling of DnaK (Liberek et al., 1991; Laufen et al., 1999). However, it should be noted that *E. coli* also expresses two specialized Hsp70 isoforms, HscA and HscC, which do not depend on GrpE (Brehmer et al., 2001).

Structurally, GrpE is composed of an α-helical dimerization domain and a β-domain that mediates most of the interactions with DnaK (Harrison et al., 1997). The α-helical domains form a stalk-like, coiled-coil structure with a four-helix bundle at the C-terminal end; the β-domains protrude like wings from the helix bundle (**Figure 2**). In the complex with the NBD of DnaK, one β-domain inserts into the nucleotide binding cleft, forcing the nucleotide binding pocket open by rotation of subdomain IIB (**Figure 2**). This NBD conformation has greatly

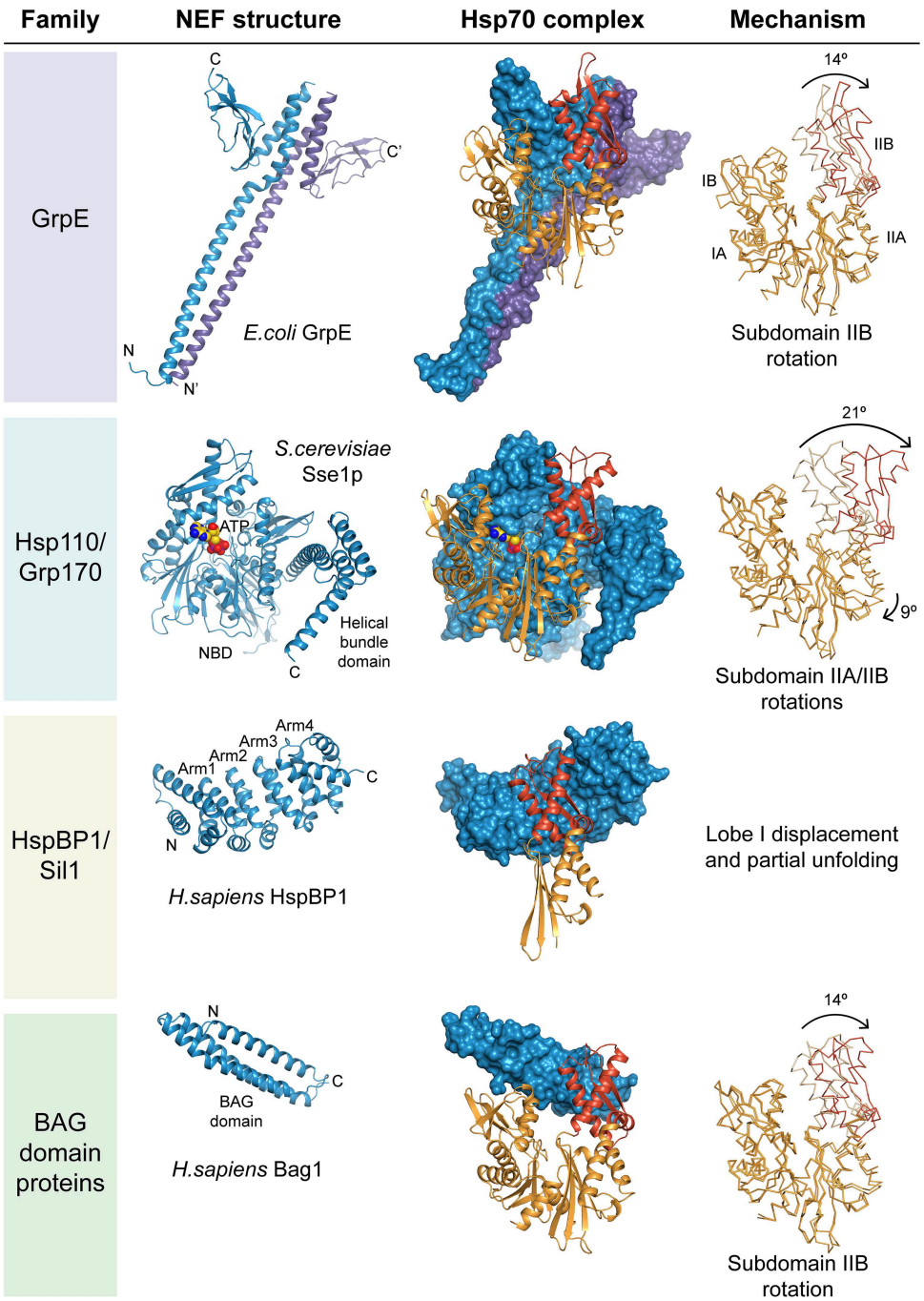


FIGURE 2 | Structure and mechanism of nucleotide exchange factors. Exemplary structures of the four NEF families are shown together with respective Hsp70 complexes. The NEF is always shown in blue; the Hsp70 NBD in orange with subdomain IIB highlighted in red. On the right the structure of the NBD in the complex is superposed with the ADP-bound conformation, and the putative nucleotide exchange mechanism indicated. The drawings are based on the PDB coordinate sets 1DKG (GrpE•DnaK, Harrison et al., 1997), 2V7Y (DnaK•ADP, Chang et al., 2008), 3D2F (Sse1p•Hsp70, Polier et al., 2008), 1HPM (Hsc70•ADP, Wilbanks and Mckay, 1995), 1XQS (HspBP1•Hsp70-lobeII, Shomura et al., 2005) and 1HX1 (Bag1•Hsc70, Sondermann et al., 2001).

diminished affinity for nucleotide. Simulations of the highly conserved NBD suggest that subdomain IIB motion is facilitated by a flexible hinge connection (Ung et al., 2013). GrpE thus utilizes an in-built feature of Hsp70 for its function. In addition to the β-domain, parts of the stalk and flexible N-terminus of GrpE contribute to DnaK binding. The latter segment appears

to compete with substrate binding to DnaK (Harrison et al., 1997).

GrpE function in *E. coli* appears to be regulated by temperature (Grimshaw et al., 2001). At the optimal growth temperature, GrpE is dimeric and active, enabling rapid DnaK cycling needed for folding newly synthesized proteins. Under thermal stress, the stalk of GrpE appears to unfold, which prevents the cochaperone from binding to DnaK•substrate complexes and thus limits futile cycling and ATP expenditure. During thermal stress, interactions with DnaK stabilize substrates against aggregation. The transition in GrpE is fully reversible and the DnaK-DnaJ-GrpE system begins to refold DnaK-stabilized substrates upon recovery.

Eukaryotic GrpE Homologs

Mitochondria and chloroplasts, the eukaryotic organelles of eubacterial origin preserve the bacterial Hsp70 system including GrpE homologs. The most conspicuous function of this system is the import of organellar proteins from the cytosol across membranes (see accompanying reviews). This is essential because most of the matrix/stroma proteins are encoded in the nucleus. Moreover, protein folding within these organelles also depends on GrpE function. The thermosensory function of GrpE appears to be preserved in eukaryotes (Moro and Muga, 2006; Willmund et al., 2007).

Evolution of Eukaryotic NEFs

In contrast to DnaK, authentic eukaryotic Hsp70 homologs have weakened contacts across the nucleotide binding cleft. Probably the function of the eukaryotic Hsp70 ancestor did not require NEFs. Dependence on NEFs apparently evolved only later, and thus modern GrpE and the mammalian NEF Bag1 function only with their cognate Hsp70 partners (Brehmer et al., 2001). In the presence of physiological phosphate concentrations, the ADP dissociation rates of eukaryotic Hsp70 diminish to the levels similar to DnaK in *E. coli*, and thus NEFs are required for efficient cycling *in vivo* (Gässler et al., 2001; Arakawa et al., 2011). The most ancient and universal type of eukaryotic NEF is Hsp110/Grp170, which shares its molecular architecture with canonical Hsp70 proteins (**Figure 2**). The other two NEF families, HspBP1/Sil1 homologs and BAG-domain proteins, have generic structural scaffolds, Armadillo repeats and three-helix bundles, respectively, which are also found in other functional contexts such as nuclear transport and vesicle fusion (**Figure 2**). Interestingly, despite their considerable structural diversity, all NEFs target subdomain IIB in the NBD of Hsp70. This suggests convergent evolution toward activating the molecular switch built into the Hsp70 NBD. It appears that the structurally homologous NEFs HspBP1 and Sil1p evolved independently, since their contact regions with the cognate Hsp70 homolog differ markedly (Shomura et al., 2005; Yan et al., 2011). Convergent evolution might also explain the perplexing diversity in BAG-domain architecture, which became only apparent when the individual structures were determined.

Nucleotide Exchange Mechanism

All eukaryotic NEFs seem to capture open conformations of the NBD. GrpE literally drives a molecular wedge into the nucleotide binding cleft of DnaK, whereas the Hsp110 homolog in *Saccharomyces cerevisiae*, Sse1p, attaches to the flank of subdomain IIB while anchoring itself onto the remainder of the NBD (Polier et al., 2008). HspBP1/Sil1 homologs and BAG-domain proteins use their bulk mass to fix Hsp70 in an open conformation. HspBP1 binding even induces partial unfolding of the NBD. Because their shapes vary considerably, the NBD subdomains are displaced in different ways, as evidenced by cocrystal structures with Hsp70 NBDs (**Figure 2**). ATP but not ADP binding subsequently displaces NEFs from eukaryotic Hsp70 for a new round of substrate binding.

Hsp110/Grp170

Hsp110 and its ER-lumenal homolog Grp170 share their domain architecture with canonical Hsp70 proteins, consisting of an N-terminal NBD, followed by a β-sandwich and an α-helix bundle domain, but have long insertions and C-terminal extensions compared to Hsp70 (**Figure 1**). Their sequence conservation is much lower than in canonical Hsp70. Crystal structures of the Hsp110 homolog Sse1p in complex with Hsp70 showed that the two NBDs face each other (**Figure 2**) (Polier et al., 2008; Schuermann et al., 2008). The NBD of Hsp70 is fixed in an open conformation by additional contacts with the α-helix bundle domain of Sse1p. These contacts are highly conserved in the Hsp110/Grp170 family, and presumably all members employ the same binding mode (Andreasson et al., 2010; Hale et al., 2010). To function, Sse1p requires ATP binding, which induces a compact conformation, but not ATP hydrolysis (Shaner et al., 2004; Raviol et al., 2006). Indeed, expression of ATP-binding competent, but ATPase-deficient Sse1p mutants rescues the lethal phenotype of the deletion of *SSE1*/*SSE2*. Because of low sequence conservation in the β-sandwich domain, it is unclear whether the substrate binding mode seen in DnaK·ADP is also employed by Hsp110/Grp170. Hsp110 homologs seem to prefer aromatic residues in target sequences, while canonical Hsp70 has a bias toward aliphatic hydrophobic and proline residues (Xu et al., 2012). Mammalian and yeast Hsp110 homologs are potent holdases for misfolded luciferase, preventing its aggregation until refolding with Hsp70/Hsp40 commences (Oh et al., 1997, 1999). Sse1p requires heat activation for this activity (Polier et al., 2010). Whether nucleotide-dependent cycling of Hsp110/Grp170 is required for holdase activity is controversial.

HspBP1/Sil1

HspBP1 and Sil1 represent the cytosolic and ER-lumenal forms of a NEF family with Armadillo repeat architecture, respectively. Sil1 homologs can be identified in most eukaryotes; *Caenorhabditis elegans* apparently lacks a cytosolic HspBP1 homolog. The C-terminal 260-residue NEF domain of human HspBP1 consists of four Armadillo repeats capped at each end with α-helix pairs. The short N-terminal regions are highly

divergent and their function is unknown. The structure of the human HspBP1 NEF domain in complex with a fragment of Hsp70 showed that the concave face of the Armadillo repeat structure wraps around subdomain IIB of the NBD (**Figure 2**) (Shomura et al., 2005). Comparison with other structures of the Hsp70 NBD suggests that the bulk of HspBP1 would clash with subdomain IB. Protease sensitivity and tryptophan fluorescence indicates that Hsp70 evades this through local unfolding in the complex. Surprisingly, the yeast ER paralog Sil1p employs different contacts and stops at mere opening of the nucleotide binding cleft (Yan et al., 2011). Interestingly, the interactions of metazoan Sil1 with the ER-Hsp70, BiP, seem more similar to HspBP1 than to the putative yeast ortholog Sil1p (Hale et al., 2010; Howes et al., 2012). Whether ATP binding preempts NBD unfolding under physiological conditions remains unknown.

BAG Domain Proteins

The BAG domain-containing proteins represent the most divergent group among eukaryotic NEFs. Human Bag1 was the first eukaryotic NEF to be identified, after its initial characterization as the binding partner of anti-apoptotic protein Bcl-2 (Höhfeld and Jentsch, 1997). It contains a 3-helix bundle domain of 110 residues having NEF activity (**Figure 2**) (Sondermann et al., 2001). Subsequently four more homologs with putative Bag domains were identified in humans, Bag2-Bag5 (Takayama and Reed, 2001). All have distinct domain compositions (**Figure 1**). Structural analysis of the respective Bag domains revealed a surprising diversity of α-helix bundle architectures, but all share a conserved sequence signature that targets subdomain IIB of Hsp70. The Hsp70-binding BAG domains of Bag3, Bag4, and Bag5 form shorter 3-helix bundles than Bag1 (Briknarova et al., 2002; Brockmann et al., 2004; Arakawa et al., 2010). The "Brand New Bag" (BNB) domain of Bag2 has a dimeric four-helix bundle structure that can accommodate two Hsp70s (Xu et al., 2008). This could help target complexes of multiple Hsp70 molecules attached to one substrate molecule.

The diverse domains found together with the NEF domain in BAG proteins probably allow recruitment of Hsp70 for specific purposes. However, only the domain compositions of Bag1 and Bag3 appear conserved among metazoans. Bag1 homologs also seem to occur in plants (Kabbage and Dickman, 2008). Bag1 contains an Ubiquitin-like domain (Ubl) in addition to the NEF domain, suggesting a role in targeting substrates for proteasomal degradation. In murine Bag1, the Ubl domain also mediates interactions with EGF-like growth factor (Hung et al., 2014). Bag3 has multiple interaction motifs and connects Hsc70 with the small heat shock protein HspB8 and the dynein adaptor protein 14-3-3γ in targeting protein aggregates for degradation by autophagy via the microtubule network (Arndt et al., 2010; Fuchs et al., 2010; Xu et al., 2013). Bag2 forms stable ternary complexes with Hsc70 and the Hsp70-associated dimeric ubiquitin ligase CHIP, inhibiting proteasome targeting of inducible Hsp70 and substrate proteins (Arndt et al., 2005; Dai et al., 2005). The BNB domain has also been implicated in binding to substrate directly (Xu et al., 2008).

Antagonism between Hip and NEF Function

The dimeric multi-domain protein Hip antagonizes the function of Bag1 and probably other NEFs (Kanelakis et al., 2000). Hip slows the dissociation of ADP from Hsp70, thus stabilizing substrate protein association in presence of ATP (Höhfeld et al., 1995; Li et al., 2013). The crystal structure of the core complex of Hip with Hsp70·ADP revealed that the binding interfaces of Hip and NEFs overlap, resulting in mutually exclusive binding (Li et al., 2013). The binding affinity of NEFs — with the exception of Bag2 — is however ∼100 times higher, and stable binding of Hip thus requires additional interactions. Simultaneous interactions with two Hsp70 molecules attached to a slow-folding substrate or an aggregate would boost affinity toward the Hip dimer through avidity. Hip might also directly recognize specific substrates such as the Hsp90-client protein glucocorticoid receptor via its DP domains (Nelson et al., 2004). Interaction of Hsp70-substrate complexes with Hip might thus enable slow cycling and limit futile energy expenditure by Hsp70 on ill-fated substrate proteins and downstream chaperone clients. Concomitantly, Hip binding might also prolong the time window for proteasomal targeting, consistent with increased disposal of mutant androgen receptor upon Hip overexpression (Wang et al., 2013).

Involvement of NEFs in Protein Folding and Import

In the model organism *S. cerevisiae*, Sse1p is by far the most abundant cytosolic NEF, followed by its inducible isoform Sse2p and the HspBP1 ortholog Fes1p. Together they constitute about 1/10 of the total concentration of cellular Hsp70 (Ghaemmaghami et al., 2003; Kulak et al., 2014). The Bag protein Snl1p is only present at low concentration and probably highly specialized (Verghese and Morano, 2012). Sse1p collaborates with ribosome-bound and cytosolic Hsp70 isoforms in folding and processing a large proportion of newly made proteins (Yam et al., 2005). Deletion of *SSE1* causes a growth defect, which is only partially rescued by Fes1p overexpression (Raviol et al., 2006). Overexpression of Sse1p impairs growth as well, suggesting that Sse1p competes with ATP binding to Hsp70 under these conditions (Liu et al., 1999). Well-balanced concentration ratios between Hsp70 and NEFs seem essential for proper protein folding. Sse1p, Sse2p, and Fes1p are upregulated together with Hsp70 under heat stress. Deletion of *FES1* causes a massive heat shock response in the absence of thermal stress (Gowda et al., 2013; Abrams et al., 2014), a folding defect of the reporter protein firefly luciferase (FLuc) (Shomura et al., 2005) and a thermosensitivity phenotype (Ahner et al., 2005). Besides their function in *de novo* protein folding, Sse1p and Fes1p also contribute in distributing substrates to downstream chaperones Hsp90 and TRiC and to clear misfolded species (Goeckeler et al., 2002; Mcclellan et al., 2005; Gowda et al., 2013).

The diversity of eukaryotic NEFs might enable adaptation of the Hsp70 cycling rate to the folding needs of specific substrate proteins. Consistent with this hypothesis, comparative *in vitro* studies with mammalian homologs showed that certain Hsp70/JDP/NEF combinations work much better in FLuc folding

than others and some not at all (Tzankov et al., 2008; Rauch and Gestwicki, 2014). How specific combinations select suitable clients is unknown. NEF diversity might also allow differential distribution of substrates to downstream chaperones via adaptor proteins like HOP (Knapp et al., 2014). Functional redundancy of mammalian NEFs is, however, considerable. While knockout of the Hsp110 isoform Hsp105 causes no obvious defect (Nakamura et al., 2008), individual deletions of HspBP1 and Apg-2, another Hsp110 isoform, severely affect spermatogenesis (Held et al., 2011; Rogon et al., 2014). Only simultaneous knockout of Apg-1 and Apg-2 is lethal (Mohamed et al., 2014).

The ER-lumenal NEFs Sil1 and Grp170 contribute substantially to protein folding in the secretory pathway (Behnke et al., 2015). In addition they function together with BiP and the pore-associated JDP Sec63 in protein import, possibly by preventing substrate backsliding through BiP binding and release cycles (Zimmermann et al., 2011). Deletions of the respective NEF homologs in yeast, Sil1p and Lhs1p, activate the Unfolded Protein Response and cause import defects (Tyson and Stirling, 2000). Both Sil1 and Grp170 are upregulated under ER stress. In mice, knockout of Grp170 is lethal (Kitao et al., 2004). Mutations that inactivate Sil1 cause Marinesco-Sjögren syndrome in humans (Anttonen et al., 2005; Senderek et al., 2005) and the Woozy phenotype in mice (Zhao et al., 2005), respectively, conditions characterized by neurodegeneration and myopathy, likely because persistent ER stress induces apoptosis.

Role in Protein Quality Control

The folding of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) may serve as a paradigm for the role of NEFs in protein quality control involving proteasomal degradation. CFTR, a transmembrane protein, has inefficiently folding cytoplasmic domains, which may explain why a large proportion undergoes CHIP-mediated degradation before reaching the epithelial membrane (Meacham et al., 2001). Hsp105 appears to have a prominent role in CFTR folding at the ER and later at the epithelial membrane, employing its holdase activity (Saxena et al., 2012). Binding of HspBP1 to Hsc70 stimulates CFTR maturation (Alberti et al., 2004), whereas Bag1 collaborates with CHIP in CFTR degradation (Demand et al., 2001).

The alternative to proteasomal degradation is disposal of faulty proteins by the lysosome via autophagy. This route seems especially important for muscle structure maintenance, which requires the adaptor function of Bag3 (Arndt et al., 2010; Ulbricht et al., 2013). Bag3 deletion in mice results in severe myopathy (Homma et al., 2006). Similar phenotypes were reported for deletions of the probable *Drosophila melanogaster* and *C. elegans* orthologs, Starvin, and unc23, respectively (Arndt et al., 2010; Papsdorf et al., 2014). Bag3 mutations in humans are associated with autosomal dominant forms of myofibrillar myopathy and dilated cardiomyopathy (Selcen et al., 2009; Norton et al., 2011). Interestingly, Bag3 is the only stress-inducible BAG-domain protein (Franceschelli et al., 2008), and its increased abundance might tip the balance from proteasomal to lysosomal degradation (Gamerdinger et al., 2009).

Hsp110 proteins were found associated with aggregates of misfolding-prone proteins that cause neurodegenerative disease, including mutant SOD in Amyotrophic Lateral Sclerosis (Wang et al., 2009) and poly-Q androgen receptor in Spinal and Bulbar Muscular Atrophy (Ishihara et al., 2003). Hsp105-knockout mice accumulate hyper-phosphorylated tau similar to neurofibrillary tangles in Alzheimer's disease (Eroglu et al., 2010). Hsp110, together with Hsp70 and Hsp40, has been implicated in a metazoan disaggregase activity analogous to ClpB/Hsp104 in bacteria and fungi, which might resolubilize such aggregates for new folding attempts or proteasomal degradation (Shorter, 2011; Rampelt et al., 2012). Hsp110 thus may be considered the ultimate profolding NEF in eukaryotes, consistent with increased vulnerability of fast-growing cancer cells with a dominant-negative allele of this cochaperone (Dorard et al., 2011).

Outlook

How the different components in the Hsp70 system intersect with other branches of the proteostasis network is only beginning to emerge. Different expression levels of competing NEFs and opposing factors like Hip may change the fate of specific substrate proteins in individual cell types. The intracellular distribution of proteostasis components might furthermore alter the dynamics of protein folding and degradation. Thus, information on cellular dose and distribution in healthy and diseased cells will be needed for an integrated picture of NEF roles in Hsp70-dependent cellular processes.

Author Contributions

Both authors contributed to drafting and critically revising the work.

Acknowledgments

We thank F. Ulrich Hartl and the Max Planck Society for continuous support, and Courtney L. Klaips for critical reading of the manuscript. JV is supported by the Rudolf Haas Fellowship from the Jung Foundation for Science and Research.

References

Abrams, J. L., Verghese, J., Gibney, P. A., and Morano, K. A. (2014). Hierarchical functional specificity of cytosolic heat shock protein 70 (Hsp70) nucleotide exchange factors in yeast. *J. Biol. Chem.* 289, 13155–13167. doi: 10.1074/jbc.M113.530014

Ahner, A., Whyte, F. M., and Brodsky, J. L. (2005). Distinct but overlapping functions of Hsp70, Hsp90, and an Hsp70 nucleotide exchange factor during protein biogenesis in yeast. *Arch. Biochem. Biophys.* 435, 32–41. doi: 10.1016/j.abb.2004.11.005

Alberti, S., Bohse, K., Arndt, V., Schmitz, A., and Höhfeld, J. (2004). The Co-Chaperone HspBP1 Inhibits the CHIP ubiquitin ligase and stimulates the

maturation of the cystic fibrosis transmembrane conductance regulator. *Mol. Biol. Cell* 15, 4003–4010. doi: 10.1091/mbc.E04-04-0293

Andreasson, C., Rampelt, H., Fiaux, J., Druffel-Augustin, S., and Bukau, B. (2010). The endoplasmic reticulum Grp170 acts as a nucleotide exchange factor of Hsp70 via a mechanism similar to that of the cytosolic Hsp110. *J. Biol. Chem.* 285, 12445–12453. doi: 10.1074/jbc.M109.096735

Ang, D., and Georgopoulos, C. (1989). The heat-shock-regulated grpE gene of Escherichia coli is required for bacterial growth at all temperatures but is dispensable in certain mutant backgrounds. *J. Bacteriol.* 171, 2748–2755.

Anttonen, A. K., Mahjneh, I., Hamalainen, R. H., Lagier-Tourenne, C., Kopra, O., Waris, L., et al. (2005). The gene disrupted in Marinesco-Sjogren syndrome encodes SIL1, an HSPA5 cochaperone. *Nat. Genet.* 37, 1309–1311. doi: 10.1038/ng1677

Arakawa, A., Handa, N., Ohsawa, N., Shida, M., Kigawa, T., Hayashi, F., et al. (2010). The C-terminal BAG domain of BAG5 induces conformational changes of the Hsp70 nucleotide-binding domain for ADP-ATP exchange. *Structure* 18, 309–319. doi: 10.1016/j.str.2010.01.004

Arakawa, A., Handa, N., Shirouzu, M., and Yokoyama, S. (2011). Biochemical and structural studies on the high affinity of Hsp70 for ADP. *Protein Sci.* 20, 1367–1379. doi: 10.1002/pro.663

Arndt, V., Daniel, C., Nastainczyk, W., Alberti, S., and Höhfeld, J. (2005). BAG-2 acts as an inhibitor of the chaperone-associated ubiquitin ligase CHIP. *Mol. Biol. Cell* 16, 5891–5900. doi: 10.1091/mbc.E05-07-0660

Arndt, V., Dick, N., Tawo, R., Dreiseidler, M., Wenzel, D., Hesse, M., et al. (2010). Chaperone-assisted selective autophagy is essential for muscle maintenance. *Curr. Biol.* 20, 143–148. doi: 10.1016/j.cub.2009.11.022

Behnke, J., Feige, M. J., and Hendershot, L. M. (2015). BiP and its nucleotide exchange factors Grp170 and Sil1: mechanisms of action and biological functions. *J. Mol. Biol.* 427, 1589–1608. doi: 10.1016/j.jmb.2015.02.011

Bracher, A., and Verghese, J. (2015). GrpE, Hsp110/Grp170, HspBP1/Sil1 and BAG Domain Proteins: Nucleotide Exchange Factors for Hsp70 Molecular Chaperones. *Subcell. Biochem.* 78, 1–33. doi: 10.1007/978-3-319-11731-7_1

Brehmer, D., Rüdiger, S., Gässler, C. S., Klostermeier, D., Packschies, L., Reinstein, J., et al. (2001). Tuning of chaperone activity of Hsp70 proteins by modulation of nucleotide exchange. *Nat. Struct. Biol.* 8, 427–432. doi: 10.1038/87588

Briknarova, K., Takayama, S., Homma, S., Baker, K., Cabezas, E., Hoyt, D. W., et al. (2002). BAG4/SODD protein contains a short BAG domain. *J. Biol. Chem.* 277, 31172–31178. doi: 10.1074/jbc.M202792200

Brockmann, C., Leitner, D., Labudde, D., Diehl, A., Sievert, V., Büssow, K., et al. (2004). The solution structure of the SODD BAG domain reveals additional electrostatic interactions in the HSP70 complexes of SODD subfamily BAG domains. *FEBS Lett.* 558, 101–106. doi: 10.1016/S0014-5793(03)01490-X

Chang, Y. W., Sun, Y. J., Wang, C., and Hsiao, C. D. (2008). Crystal structures of the 70-kDa heat shock proteins in domain disjoining conformation. *J. Biol. Chem.* 283, 15502–15511. doi: 10.1074/jbc.M708992200

Dai, Q., Qian, S. B., Li, H. H., Mcdonough, H., Borchers, C., Huang, D., et al. (2005). Regulation of the cytoplasmic quality control protein degradation pathway by BAG2. *J. Biol. Chem.* 280, 38673–38681. doi: 10.1074/jbc.M507986200

Demand, J., Alberti, S., Patterson, C., and Höhfeld, J. (2001). Cooperation of a ubiquitin domain protein and an E3 ubiquitin ligase during chaperone/proteasome coupling. *Curr. Biol.* 11, 1569–1577. doi: 10.1016/S0960-9822(01)00487-0

Doong, H., Price, J., Kim, Y. S., Gasbarre, C., Probst, J., Liotta, L. A., et al. (2000). CAIR-1/BAG-3 forms an EGF-regulated ternary complex with phospholipase C-gamma and Hsp70/Hsc70. *Oncogene* 19, 4385–4395. doi: 10.1038/sj.onc.1203797

Dorard, C., De Thonel, A., Collura, A., Marisa, L., Svrcek, M., Lagrange, A., et al. (2011). Expression of a mutant HSP110 sensitizes colorectal cancer cells to chemotherapy and improves disease prognosis. *Nat. Med.* 17, 1283–1289. doi: 10.1038/nm.2457

Dragovic, Z., Broadley, S. A., Shomura, Y., Bracher, A., and Hartl, F. U. (2006). Molecular chaperones of the Hsp110 family act as nucleotide exchange factors of Hsp70s. *EMBO J.* 25, 2519–2528. doi: 10.1038/sj.emboj.7601138

Eroglu, B., Moskophidis, D., and Mivechi, N. F. (2010). Loss of Hsp110 leads to age-dependent tau hyperphosphorylation and early accumulation of insoluble amyloid beta. *Mol. Cell. Biol.* 30, 4626–4643. doi: 10.1128/MCB.01493-09

Franceschelli, S., Rosati, A., Lerose, R., De Nicola, S., Turco, M. C., and Pascale, M. (2008). Bag3 gene expression is regulated by heat shock factor 1. *J. Cell Physiol.* 215, 575–577. doi: 10.1002/jcp.21397

Fuchs, M., Poirier, D. J., Seguin, S. J., Lambert, H., Carra, S., Charette, S. J., et al. (2010). Identification of the key structural motifs involved in HspB8/HspB6-Bag3 interaction. *Biochem. J.* 425, 245–255. doi: 10.1042/BJ20090907

Gamerdinger, M., Hajieva, P., Kaya, A. M., Wolfrum, U., Hartl, F. U., and Behl, C. (2009). Protein quality control during aging involves recruitment of the macroautophagy pathway by BAG3. *EMBO J.* 28, 889–901. doi: 10.1038/emboj.2009.29

Gässler, C. S., Wiederkehr, T., Brehmer, D., Bukau, B., and Mayer, M. P. (2001). Bag-1M accelerates nucleotide release for human Hsc70 and Hsp70 and can act concentration-dependent as positive and negative cofactor. *J. Biol. Chem.* 276, 32538–32544. doi: 10.1074/jbc.M105328200

Ghaemmaghami, S., Huh, W. K., Bower, K., Howson, R. W., Belle, A., Dephoure, N., et al. (2003). Global analysis of protein expression in yeast. *Nature* 425, 737–741. doi: 10.1038/nature02046

Goeckeler, J. L., Stephens, A., Lee, P., Caplan, A. J., and Brodsky, J. L. (2002). Overexpression of yeast Hsp110 homolog Sse1p suppresses ydj1-151 thermosensitivity and restores Hsp90-dependent activity. *Mol. Biol. Cell* 13, 2760–2770. doi: 10.1091/mbc.02-04-0051

Gowda, N. K., Kandasamy, G., Froehlich, M. S., Dohmen, R. J., and Andreasson, C. (2013). Hsp70 nucleotide exchange factor Fes1 is essential for ubiquitin-dependent degradation of misfolded cytosolic proteins. *Proc. Natl. Acad. Sci. U.S.A.* 110, 5975–5980. doi: 10.1073/pnas.1216778110

Grimshaw, J. P., Jelesarov, I., Schonfeld, H. J., and Christen, P. (2001). Reversible thermal transition in GrpE, the nucleotide exchange factor of the DnaK heat-shock system. *J. Biol. Chem.* 276, 6098–6104. doi: 10.1074/jbc.M009290200

Hale, S. J., Lovell, S. C., De Keyzer, J., and Stirling, C. J. (2010). Interactions between Kar2p and its nucleotide exchange factors Sil1p and Lhs1p are mechanistically distinct. *J. Biol. Chem.* 285, 21600–21606. doi: 10.1074/jbc.M110.111211

Harrison, C. J., Hayer-Hartl, M., Di Liberto, M., Hartl, F., and Kuriyan, J. (1997). Crystal structure of the nucleotide exchange factor GrpE bound to the ATPase domain of the molecular chaperone DnaK. *Science* 276, 431–435. doi: 10.1126/science.276.5311.431

Held, T., Barakat, A. Z., Mohamed, B. A., Paprotta, I., Meinhardt, A., Engel, W., et al. (2011). Heat-shock protein HSPA4 is required for progression of spermatogenesis. *Reproduction* 142, 133–144. doi: 10.1530/REP-11-0023

Höhfeld, J., and Jentsch, S. (1997). GrpE-like regulation of the hsc70 chaperone by the anti-apoptotic protein BAG-1. *EMBO J.* 16, 6209–6216. doi: 10.1093/emboj/16.20.6209

Höhfeld, J., Minami, Y., and Hartl, F. U. (1995). Hip, a novel cochaperone involved in the eukaryotic Hsc70/Hsp40 reaction cycle. *Cell* 83, 589–598. doi: 10.1016/0092-8674(95)90099-3

Homma, S., Iwasaki, M., Shelton, G. D., Engvall, E., Reed, J. C., and Takayama, S. (2006). BAG3 deficiency results in fulminant myopathy and early lethality. *Am. J. Pathol.* 169, 761–773. doi: 10.2353/ajpath.2006.060250

Howes, J., Shimizu, Y., Feige, M. J., and Hendershot, L. M. (2012). C-terminal mutations destabilize SIL1/BAP and can cause Marinesco-Sjogren syndrome. *J. Biol. Chem.* 287, 8552–8560. doi: 10.1074/jbc.M111.333286

Hung, K. W., Huang, H. W., Cho, C. C., Chang, S. C., and Yu, C. (2014). Nuclear magnetic resonance structure of the cytoplasmic tail of heparin binding EGF-like growth factor (proHB-EGF-CT) complexed with the ubiquitin homology domain of Bcl-2-associated athanogene 1 from Mus musculus (mBAG-1-UBH). *Biochemistry* 53, 1935–1946. doi: 10.1021/bi5003019

Ishihara, K., Yamagishi, N., Saito, Y., Adachi, H., Kobayashi, Y., Sobue, G., et al. (2003). Hsp105alpha suppresses the aggregation of truncated androgen receptor with expanded CAG repeats and cell toxicity. *J. Biol. Chem.* 278, 25143–25150. doi: 10.1074/jbc.M302975200

Iwasaki, M., Tanaka, R., Hishiya, A., Homma, S., Reed, J. C., and Takayama, S. (2010). BAG3 directly associates with guanine nucleotide exchange factor of Rap1, PDZGEF2, and regulates cell adhesion. *Biochem. Biophys. Res. Commun.* 400, 413–418. doi: 10.1016/j.bbrc.2010.08.092

Kabani, M., Beckerich, J. M., and Brodsky, J. L. (2002). Nucleotide exchange factor for the yeast Hsp70 molecular chaperone Ssa1p. *Mol. Cell. Biol.* 22, 4677–4689. doi: 10.1128/MCB.22.13.4677-4689.2002

Kabbage, M., and Dickman, M. B. (2008). The BAG proteins: a ubiquitous family of chaperone regulators. *Cell. Mol. Life Sci.* 65, 1390–1402. doi: 10.1007/s00018-008-7535-2

Kanelakis, K. C., Murphy, P. J., Galigniana, M. D., Morishima, Y., Takayama, S., Reed, J. C., et al. (2000). hsp70 interacting protein Hip does not affect

glucocorticoid receptor folding by the hsp90-based chaperone machinery except to oppose the effect of BAG-1. *Biochemistry* 39, 14314–14321. doi: 10.1021/bi001671c

Kitao, Y., Hashimoto, K., Matsuyama, T., Iso, H., Tamatani, T., Hori, O., et al. (2004). ORP150/HSP12A regulates Purkinje cell survival: a role for endoplasmic reticulum stress in cerebellar development. *J. Neurosci.* 24, 1486–1496. doi: 10.1523/JNEUROSCI.4029-03.2004

Knapp, R. T., Wong, M. J., Kollmannsberger, L. K., Gassen, N. C., Kretzschmar, A., Zschocke, J., et al. (2014). Hsp70 cochaperones HspBP1 and BAG-1M differentially regulate steroid hormone receptor function. *PLoS ONE* 9:e85415. doi: 10.1371/journal.pone.0085415

Kulak, N. A., Pichler, G., Paron, I., Nagaraj, N., and Mann, M. (2014). Minimal, encapsulated proteomic-sample processing applied to copy-number estimation in eukaryotic cells. *Nat. Methods* 11, 319–324. doi: 10.1038/nmeth.2834

Laufen, T., Mayer, M. P., Beisel, C., Klostermeier, D., Mogk, A., Reinstein, J., et al. (1999). Mechanism of regulation of hsp70 chaperones by DnaJ cochaperones. *Proc. Natl. Acad. Sci. U.S.A.* 96, 5452–5457. doi: 10.1073/pnas.96.10.5452

Li, Z., Hartl, F. U., and Bracher, A. (2013). Structure and function of Hip, an attenuator of the Hsp70 chaperone cycle. *Nat. Struct. Mol. Biol.* 20, 929–935. doi: 10.1038/nsmb.2608

Liberek, K., Marszalek, J., Ang, D., Georgopoulos, C., and Zylicz, M. (1991). Escherichia coli DnaJ and GrpE heat shock proteins jointly stimulate ATPase activity of DnaK. *Proc. Natl. Acad. Sci. U.S.A.* 88, 2874–2878. doi: 10.1073/pnas.88.7.2874

Liu, X. D., Morano, K. A., and Thiele, D. J. (1999). The yeast Hsp110 family member, Sse1, is an Hsp90 cochaperone. *J. Biol. Chem.* 274, 26654–26660. doi: 10.1074/jbc.274.38.26654

Mayer, M. P. (2013). Hsp70 chaperone dynamics and molecular mechanism. *Trends Biochem. Sci.* 38, 507–514. doi: 10.1016/j.tibs.2013.08.001

Mcclellan, A. J., Scott, M. D., and Frydman, J. (2005). Folding and quality control of the VHL tumor suppressor proceed through distinct chaperone pathways. *Cell* 121, 739–748. doi: 10.1016/j.cell.2005.03.024

Meacham, G. C., Patterson, C., Zhang, W., Younger, J. M., and Cyr, D. M. (2001). The Hsc70 co-chaperone CHIP targets immature CFTR for proteasomal degradation. *Nat. Cell Biol.* 3, 100–105. doi: 10.1038/35050509

Mock, J. Y., Chartron, J. W., Zaslaver, M., Xu, Y., Ye, Y., and Clemons, W. M. Jr. (2015). Bag6 complex contains a minimal tail-anchor-targeting module and a mock BAG domain. *Proc. Natl. Acad. Sci. U.S.A.* 112, 106–111. doi: 10.1073/pnas.1402745112

Mohamed, B. A., Barakat, A. Z., Held, T., Elkenani, M., Mühlfeld, C., Männer, J., et al. (2014). Respiratory distress and early neonatal lethality in hspa4l/hspa4 double-mutant mice. *Am. J. Respir. Cell. Mol. Biol.* 50, 817–824. doi: 10.1165/rcmb.2013-0132OC

Moro, F., and Muga, A. (2006). Thermal adaptation of the yeast mitochondrial Hsp70 system is regulated by the reversible unfolding of its nucleotide exchange factor. *J. Mol. Biol.* 358, 1367–1377. doi: 10.1016/j.jmb.2006.03.027

Nakamura, J., Fujimoto, M., Yasuda, K., Takeda, K., Akira, S., Hatayama, T., et al. (2008). Targeted disruption of Hsp110/105 gene protects against ischemic stress. *Stroke* 39, 2853–2859. doi: 10.1161/STROKEAHA.107.506188

Nelson, G. M., Prapapanich, V., Carrigan, P. E., Roberts, P. J., Riggs, D. L., and Smith, D. F. (2004). The heat shock protein 70 cochaperone hip enhances functional maturation of glucocorticoid receptor. *Mol. Endocrinol.* 18, 1620–1630. doi: 10.1210/me.2004-0054

Norton, N., Li, D., Rieder, M. J., Siegfried, J. D., Rampersaud, E., Züchner, S., et al. (2011). Genome-wide studies of copy number variation and exome sequencing identify rare variants in BAG3 as a cause of dilated cardiomyopathy. *Am. J. Hum. Genet.* 88, 273–282. doi: 10.1016/j.ajhg.2011.01.016

Oh, H. J., Chen, X., and Subjeck, J. R. (1997). Hsp110 protects heat-denatured proteins and confers cellular thermoresistance. *J. Biol. Chem.* 272, 31636–31640. doi: 10.1074/jbc.272.50.31636

Oh, H. J., Easton, D., Murawski, M., Kaneko, Y., and Subjeck, J. R. (1999). The chaperoning activity of hsp110. Identification of functional domains by use of targeted deletions. *J. Biol. Chem.* 274, 15712–15718. doi: 10.1074/jbc.274.22.15712

Page, R. C., Xu, Z., Amick, J., Nix, J. C., and Misra, S. (2012). Crystallization and preliminary X-ray crystallographic analysis of the Bag2 amino-terminal domain from Mus musculus. *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.* 68, 647–651. doi: 10.1107/S1744309112013267

Papsdorf, K., Sacherl, J., and Richter, K. (2014). The balanced regulation of Hsc70 by DNJ-13 and UNC-23 is required for muscle functionality. *J. Biol. Chem.* 289, 25250–25261. doi: 10.1074/jbc.M114.565234

Polier, S., Dragovic, Z., Hartl, F. U., and Brache, A. (2008). Structural basis for the cooperation of Hsp70 and Hsp110 chaperones in protein folding. *Cell* 133, 1068–1079. doi: 10.1016/j.cell.2008.05.022

Polier, S., Hartl, F. U., and Bracher, A. (2010). Interaction of the Hsp110 molecular chaperones from S. cerevisiae with substrate protein. *J. Mol. Biol.* 401, 696–707. doi: 10.1016/j.jmb.2010.07.004

Rampelt, H., Kirstein-Miles, J., Nillegoda, N. B., Chi, K., Scholz, S. R., Morimoto, R. I., et al. (2012). Metazoan Hsp70 machines use Hsp110 to power protein disaggregation. *EMBO J.* 31, 4221–4235. doi: 10.1038/emboj.2012.264

Rauch, J. N., and Gestwicki, J. E. (2014). Binding of human nucleotide exchange factors to heat shock protein 70 (Hsp70) generates functionally distinct complexes *in vitro*. *J. Biol. Chem.* 289, 1402–1414. doi: 10.1074/jbc.M113.521997

Raviol, H., Sadlish, H., Rodriguez, F., Mayer, M. P., and Bukau, B. (2006). Chaperone network in the yeast cytosol: Hsp110 is revealed as an Hsp70 nucleotide exchange factor. *EMBO J.* 25, 2510–2518. doi: 10.1038/sj.emboj.7601139

Rogon, C., Ulbricht, A., Hesse, M., Alberti, S., Vijayaraj, P., Best, D., et al. (2014). HSP70-binding protein HSPBP1 regulates chaperone expression at a posttranslational level and is essential for spermatogenesis. *Mol. Biol. Cell* 25, 2260–2271. doi: 10.1091/mbc.E14-02-0742

Saxena, A., Banasavadi-Siddegowda, Y. K., Fan, Y., Bhattacharya, S., Roy, G., Giovannucci, D. R., et al. (2012). Human heat shock protein 105/110 kDa (Hsp105/110) regulates biogenesis and quality control of misfolded cystic fibrosis transmembrane conductance regulator at multiple levels. *J. Biol. Chem.* 287, 19158–19170. doi: 10.1074/jbc.M111.297580

Schuermann, J. P., Jiang, J., Cuellar, J., Llorca, O., Wang, L., Gimenez, L. E., et al. (2008). Structure of the Hsp110:Hsc70 nucleotide exchange machine. *Mol. Cell* 31, 232–243. doi: 10.1016/j.molcel.2008.05.006

Selcen, D., Muntoni, F., Burton, B. K., Pegoraro, E., Sewry, C., Bite, A. V., et al. (2009). Mutation in BAG3 causes severe dominant childhood muscular dystrophy. *Ann. Neurol.* 65, 83–89. doi: 10.1002/ana.21553. doi: 10.1002/ana.21553

Senderek, J., Krieger, M., Stendel, C., Bergmann, C., Moser, M., Breitbach-Faller, N., et al. (2005). Mutations in SIL1 cause Marinesco-Sjogren syndrome, a cerebellar ataxia with cataract and myopathy. *Nat. Genet.* 37, 1312–1314. doi: 10.1038/ng1678

Shaner, L., Trott, A., Goeckeler, J. L., Brodsky, J. L., and Morano, K. A. (2004). The function of the yeast molecular chaperone Sse1 is mechanistically distinct from the closely related hsp70 family. *J. Biol. Chem.* 279, 21992–22001. doi: 10.1074/jbc.M313739200

Shomura, Y., Dragovic, Z., Chang, H. C., Tzvetkov, N., Young, J. C., Brodsky, J. L., et al. (2005). Regulation of Hsp70 function by HspBP1: Structural analysis reveals an alternate mechanism for Hsp70 nucleotide exchange. *Mol. Cell* 17, 367–379. doi: 10.1016/j.molcel.2004.12.023

Shorter, J. (2011). The mammalian disaggregase machinery: Hsp110 synergizes with Hsp70 and Hsp40 to catalyze protein disaggregation and reactivation in a cell-free system. *PLoS ONE* 6:e26319. doi: 10.1371/journal.pone.0026319

Sondermann, H., Scheufler, C., Schneider, C., Höhfeld, J., Hartl, F. U., and Moarefi, I. (2001). Structure of a Bag/Hsc70 complex: convergent functional evolution of Hsp70 nucleotide exchange factors. *Science* 291, 1553–1557. doi: 10.1126/science.1057268

Steel, G. J., Fullerton, D. M., Tyson, J. R., and Stirling, C. J. (2004). Coordinated activation of Hsp70 chaperones. *Science* 303, 98–101. doi: 10.1126/science.1092287

Takayama, S., and Reed, J. C. (2001). Molecular chaperone targeting and regulation by BAG family proteins. *Nat. Cell Biol.* 3, E237–E241. doi: 10.1038/35082637

Tyson, J. R., and Stirling, C. J. (2000). LHS1 and SIL1 provide a lumenal function that is essential for protein translocation into the endoplasmic reticulum. *EMBO J.* 19, 6440–6452. doi: 10.1093/emboj/19.23.6440

Tzankov, S., Wong, M. J., Shi, K., Nassif, C., and Young, J. C. (2008). Functional divergence between co-chaperones of Hsc70. *J. Biol. Chem.* 283, 27100–27109. doi: 10.1074/jbc.M803923200

Ulbricht, A., Eppler, F. J., Tapia, V. E., Van Der Ven, P. F., Hampe, N., Hersch, N., et al. (2013). Cellular mechanotransduction relies on tension-induced and chaperone-assisted autophagy. *Curr. Biol.* 23, 430–435. doi: 10.1016/j.cub.2013.01.064

Ung, P. M., Thompson, A. D., Chang, L., Gestwicki, J. E., and Carlson, H. A. (2013). Identification of key hinge residues important for nucleotide-dependent allostery in E. coli Hsp70/DnaK. *PLoS Comput. Biol.* 9:e1003279. doi: 10.1371/journal.pcbi.1003279

Verghese, J., and Morano, K. A. (2012). A lysine-rich region within fungal BAG domain-containing proteins mediates a novel association with ribosomes. *Eukaryot. Cell* 11, 1003–1011. doi: 10.1128/EC.00146-12

Wang, A. M., Miyata, Y., Klinedinst, S., Peng, H. M., Chua, J. P., Komiyama, T., et al. (2013). Activation of Hsp70 reduces neurotoxicity by promoting polyglutamine protein degradation. *Nat. Chem. Biol.* 9, 112–118. doi: 10.1038/nchembio.1140

Wang, J., Farr, G. W., Zeiss, C. J., Rodriguez-Gil, D. J., Wilson, J. H., Furtak, K., et al. (2009). Progressive aggregation despite chaperone associations of a mutant SOD1-YFP in transgenic mice that develop ALS. *Proc. Natl. Acad. Sci. U.S.A.* 106, 1392–1397. doi: 10.1073/pnas.0813045106

Wilbanks, S. M., and Mckay, D. B. (1995). How potassium affects the activity of the molecular chaperone Hsc70. II. Potassium binds specifically in the ATPase active site. *J. Biol. Chem.* 270, 2251–2257. doi: 10.1074/jbc.270.5.2251

Willmund, F., Mühlhaus, T., Wojciechowska, M., and Schroda, M. (2007). The NH2-terminal domain of the chloroplast GrpE homolog CGE1 is required for dimerization and cochaperone function *in vivo*. *J. Biol. Chem.* 282, 11317–11328. doi: 10.1074/jbc.M608854200

Xu, X., Sarbeng, E. B., Vorvis, C., Kumar, D. P., Zhou, L., and Liu, Q. (2012). Unique peptide substrate binding properties of 110-kDa heat-shock protein (Hsp110) determine its distinct chaperone activity. *J. Biol. Chem.* 287, 5661–5672. doi: 10.1074/jbc.M111.275057

Xu, Z., Graham, K., Foote, M., Liang, F., Rizkallah, R., Hurt, M., et al. (2013). 14-3-3 protein targets misfolded chaperone-associated proteins to aggresomes. *J. Cell. Sci.* 126, 4173–4186. doi: 10.1242/jcs.126102

Xu, Z., Page, R. C., Gomes, M. M., Kohli, E., Nix, J. C., Herr, A. B., et al. (2008). Structural basis of nucleotide exchange and client binding by the Hsp70 cochaperone Bag2. *Nat. Struct. Mol. Biol.* 15, 1309–1317. doi: 10.1038/nsmb.1518

Yam, A. Y., Albanese, V., Lin, H. T., and Frydman, J. (2005). HSP110 cooperates with different cytosolic HSP70 systems in a pathway for de novo folding. *J. Biol. Chem.* 280, 41252–41261. doi: 10.1074/jbc.M503615200

Yan, M., Li, J., and Sha, B. (2011). Structural analysis of the Sil1-Bip complex reveals the mechanism for Sil1 to function as a nucleotide-exchange factor. *Biochem. J.* 438, 447–455. doi: 10.1042/BJ20110500

Zhao, L., Longo-Guess, C., Harris, B. S., Lee, J. W., and Ackerman, S. L. (2005). Protein accumulation and neurodegeneration in the woozy mutant mouse is caused by disruption of SIL1, a cochaperone of BiP. *Nat. Genet.* 37, 974–979. doi: 10.1038/ng1620

Zimmermann, R., Eyrisch, S., Ahmad, M., and Helms, V. (2011). Protein translocation across the ER membrane. *Biochim. Biophys. Acta* 1808, 912–924. doi: 10.1016/j.bbamem.2010.06.015

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Molecular Biosciences

MINI REVIEW
published: 19 May 2015
doi: 10.3389/fmolb.2015.00026

The role of molecular chaperones in clathrin mediated vesicular trafficking

*Rui Sousa and Eileen M. Lafer**

Department of Biochemistry and Center for Biomedical Neuroscience, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

The discovery that the 70 kD "uncoating ATPase," which removes clathrin coats from vesicles after endocytosis, is the constitutively expressed Hsc70 chaperone was a surprise. Subsequent work, however, revealed that uncoating is an archetypal Hsp70 reaction: the cochaperone auxilin, which contains a clathrin binding domain and an Hsc70 binding J domain, recruits Hsc70∗ATP to the coat and, concomitant with ATP hydrolysis, transfers it to a hydrophobic Hsc70-binding element found on a flexible tail at the C-terminus of the clathrin heavy chain. Release of clathrin in association with Hsc70∗ADP follows, and the subsequent, persistent association of clathrin with Hsc70 is important to prevent aberrant clathrin polymerization. Thus, the two canonical functions of Hsp70—dissociation of existing protein complexes or aggregates, and binding to a protein to inhibit its inappropriate aggregation—are recapitulated in uncoating. Association of clathrin with Hsc70 *in vivo* is regulated by Hsp110, an Hsp70 NEF that is itself a member of the Hsp70 family. How Hsp110 activity is itself regulated to make Hsc70-free clathrin available for endocytosis is unclear, though at synapses it's possible that the influx of calcium that accompanies depolarization activates the Ca^{++}/calmodulin dependent calcineurin phosphatase which then dephosphorylates and activates Hsp110 to stimulate ADP/ATP exchange and release clathrin from Hsc70∗ADP:clathrin complexes.

Keywords: clathrin, endocytosis, chaperone, NEF, Hsp70, Hsc70, Hsp110, auxilin

OPEN ACCESS

Edited by:
Matthias Peter Mayer, Ruprecht-Karls-Universität Heidelberg, Germany

Reviewed by:
Rafael Fernández-Chacón, The Neuroscience Institute at Seville, Spain
Dana Reichmann, The Hebrew University of Jerusalem, Israel

****Correspondence:***
Eileen M. Lafer, Department of Biochemistry, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, Room 415B, San Antonio, TX 78229, USA
lafer@uthscsa.edu

Specialty section:
This article was submitted to Protein Folding, Misfolding and Degradation, a section of the journal Frontiers in Molecular Biosciences

Received: *09 April 2015*
Accepted: *05 May 2015*
Published: *19 May 2015*

Citation:
Sousa R and Lafer EM (2015) The role of molecular chaperones in clathrin mediated vesicular trafficking. Front. Mol. Biosci. 2:26. doi: 10.3389/fmolb.2015.00026

The Uncoating ATPase

In 1984 the Rothman lab reported identification of an "uncoating ATPase" (Braell et al., 1984; Schlossman et al., 1984), an abundant 70 kD protein required to uncoat the clathrin coated vesicles that are the transient products of clathrin-mediated endocytosis at plasma membranes and clathrin-mediated intracellular traffic involving Golgi, endosomes, and lysosomes (**Figure 1**). The ATPase activity of the 70 kD protein was reported to be stimulated by clathrin and, by stabilizing coats with low pH or high Mg^{++} concentrations so that uncoating was blocked, it could be shown that clathrin binding by the uncoating enzyme (and stimulation of its ATPase activity) preceded the uncoating step. They showed further that the uncoating enzyme dissociated the coats into clathrin triskelia and remained associated with the triskelia after uncoating, thereby sequestering and blocking the

Abbreviations: Hsc70, constitutive heat-shock protein 70; Hsp70, heat-shock protein 70; NEF, nucleotide exchange factor; Hsp110, heat-shock protein 110 (aka Hsp105, Hsp105α, Hsp105β).

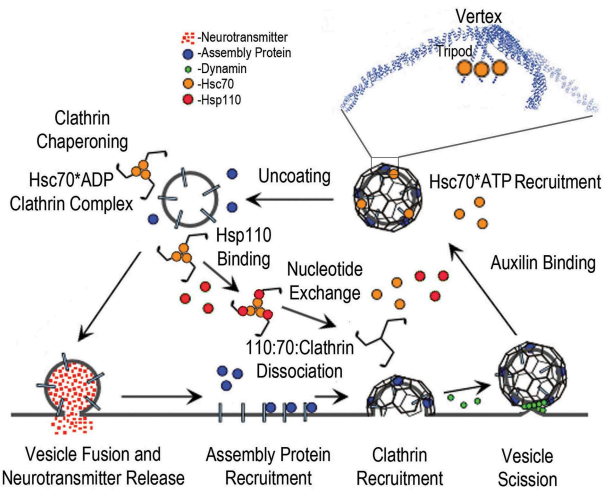


FIGURE 1 | The synaptic vesicle cycle and the roles of Hsc70, Auxilin, and Hsp110. The cycle begins with synaptic vesicle fusion and release of neurotransmitter. Assembly/adaptor proteins then bind to the synaptic vesicle membrane proteins and phosphoinositides, and recruit clathrin; leading to membrane invagination and subsequent vesicle scission by dynamin (green spheres), followed by binding of auxilin, which recruits Hsc70*ATP (orange spheres) to the vesicle coat. The boxed region of the coated vesicle is expanded to show how Hsc70 binds to the flexible C-terminal tails that emerge from the helical tripod that is positioned under each vertex of the clathrin coat. Hsc70-driven disassembly (uncoating) of the coat follows, resulting in stable Hsc70*ADP:Clathrin complexes. Hsp110 binding then induces ADP to ATP exchange and dissociation of the Hsp110:Hsc70:Clathrin complex.

reassembly of the triskelia into coats. Shortly thereafter, Ungewickell reported in 1985 that the uncoating ATPase appeared to be a member of the Hsp70 stress-protein family (Ungewickell, 1985).

Subsequent work by the Ungewickell group led to identification of another protein, dubbed auxilin, that associated with and promoted the assembly of clathrin coats (Ahle and Ungewickell, 1990). Auxilin was shown to be identical to a protein identified by the Eisenberg lab to be required for the Hsc70 mediated disassembly of clathrin coats (Prasad et al., 1993). Auxilin was shown to be a J cochaperone which—like DnaJ, the founding member of the J cochaperone family—contains a J domain which binds Hsc70∗ATP (Schroder et al., 1995; Ungewickell et al., 1995; Holstein et al., 1996). The presence of both J- and clathrin-binding domains in auxilin provides the mechanism by which Hsc70 is recruited to the clathrin coat to subsequently drive its disassembly, with auxilin acting catalytically as each auxilin molecule is able to recruit multiple Hsc70 molecules to the coated vesicle (Barouch et al., 1997). The Eisenberg group showed further that uncoating followed burst kinetics: addition of limiting amounts of Hsc70 to clathrin coats resulted in an initial, rapid burst of uncoating, followed by a slow steady-state rate (Barouch et al., 1994). These kinetics were attributed to Hsc70∗ADP becoming stably associated with the released triskelia, so that it only becomes available to carry out further rounds of uncoating upon the slow release of ADP. ADP release then allows ATP to bind and stimulate release of Hsc70 from the clathrin.

Auxilin homologs were subsequently identified in several species including yeast, *C. elegans*, and mammals (Gall et al., 2000; Umeda et al., 2000; Greener et al., 2001). Mammalian forms include the neuronal-specific auxilin 1 and the ubiquitously expressed auxilin 2 (also called cyclin G-associated protein kinase, or GAK) (Ahle and Ungewickell, 1990; Ungewickell et al., 1995; Kanaoka et al., 1997; Kimura et al., 1997; Greener et al., 2000; Umeda et al., 2000). While the amino acid sequences of auxilins are somewhat divergent between species, they all contain both clathrin binding and J domains (Gall et al., 2000; Greener et al., 2000, 2001; Pishvaee et al., 2000; Lemmon, 2001). Interestingly however, structural studies of auxilin led to the discovery that the structural diversity amongst J domains is greater than was initially anticipated: the ∼100 amino acid auxilin J domain is, by comparison to the canonical ∼70 residue domain exemplified by DnaJ, extended by insertion of a long but structured loop which contributes to the interface with Hsc70 (Jiang et al., 2003). The roles of J-domain structural diversity in the specificity and regulation of Hsp70 function are still poorly understood (Hennessy et al., 2005).

A series of genetic and acute perturbation studies subsequently extended these biochemical studies *in vivo* (Pishvaee et al., 2000; Greener et al., 2001; Morgan et al., 2001; Newmyer and Schmid, 2001; Lee et al., 2005, 2006; Massol et al., 2006; Yim et al., 2010) and demonstrated that, despite the initial surprise at attributing the specialized function of vesicle uncoating to the generalist Hsc70 chaperone, vesicle uncoating both *in vitro* and *in vivo* follows what we would now describe as a canonical Hsp70 mechanism (Sousa, 2014) in which a J cochaperone binds Hsp70*ATP and delivers it to a substrate protein concomitant with ATP hydrolysis, formation of a stable Hsp70∗ADP:protein substrate complex and release of the J cochaperone:Hsp70 interaction. More recent studies have shown that recapitulation of this canonical mechanism extends even to the structural details of the clathrin:Hsc70 interaction. The Hsc70 binding site on the clathrin heavy chain has been mapped to a typical Hsc70 binding sequence ("QLMLT") which is present in an extended, flexible tail that emerges from the C-termini of each of the three helices that associate in each triskelion to form a helical tripod on the inner surface of each vertex of the clathrin coat (**Figure 1**) (Fotin et al., 2004; Rapoport et al., 2008; Xing et al., 2010).

Models for Hsc70 Mediated Clathrin Coat Disassembly

While the process of auxilin-mediated Hsc70 recruitment to the clathrin coat is now well understood, the mechanism by which Hsc70, once delivered to the coat, drives its disassembly is less clear and somewhat controversial. The Smith group has proposed a sequential disassembly mechanism in which auxilin first recruits an Hsc70 molecule to each of the three clathrin heavy chains that comprise each triskelion in a coat structure (Rothnie et al., 2011). Only after 3 Hsc70s have been loaded is the triskelion released from the coat assembly. In contrast, the Kirchhausen group has obtained evidence for a mechanism in which coat disassembly can be initiated with a substoichiometric

number of Hsc70s, with as few as 1 Hsc70s for every ~6 clathrin heavy chains being sufficient to initiate uncoating (Bocking et al., 2011). Moreover, this number itself is variable: clathrin coats become more stable at lower pH and it was seen that, under such conditions, more Hsc70s had to bind to initiate disassembly. Conversely, coats destabilized by higher pH or mutations that disrupted clathrin:clathrin interactions required fewer Hsc70s to bind before disassembly was observed (Bocking et al., 2014). The mechanism proposed by the Kirchhausen group also differed from that of the Smith group in being non-sequential: coat disassembly was observed to begin when a certain number of Hsc70s (determined by pH and ionic conditions which modulate coat stability) had bound the coat, but Hsc70s continued to be recruited to the coat and further accelerated its disassembly even after disassembly had begun (Bocking et al., 2011). Though the Kirchhausen group developed their mechanism based on single-molecule experiments, while the Smith lab used data from ensemble experiments which can obscure mechanistic details, the latter group's conclusion are more consistent with early data (Schmid and Rothman, 1985) indicating a 1:1 stoichiometric association of Hsc70 with clathrin heavy chain during uncoating. However, it appears likely that the Kirchhausen group is correct in their conclusions that coats can be disassembled with fewer than stoichiometric numbers of Hsc70s bound. The observation of stoichiometric binding may well be a consequence of the fact that, at high Hsc70 concentrations, auxilin recruits the chaperone to the coats faster than the coats themselves disassemble (Bocking et al., 2011). As a consequence, at high Hsc70 concentrations the end state of the disassembly reaction is triskelia with Hsc70s bound 1:1 to clathrin heavy chain (3 Hsc70s per triskelion), but experiments carried out at lower Hsc70 concentrations reveal that coats can be disassembled with substoichiometric numbers of Hsc70.

Based on their structural studies of Hsc70s bound to clathrin coats, the Harrison and Kirchhausen groups proposed further that the bound Hsc70s drive disassembly by a "Brownian/Steric Wedge" mechanism. They suggested that, even in the absence of Hsc70, coats are always experiencing spontaneous fluctuations that loosen interactions between triskelia, but that such fluctuations never accumulate to a point that leads to coat disassembly (Xing et al., 2010). However, when Hsc70s are bound to the C-terminal tails under each coat vertex, they sterically block reversal of these loosening fluctuations, which then accumulate to a point where they result in coat disassembly. A different model, based on the excluded volume/entropic pulling mechanism proposed by De Los Rios and Goloubinoff (De Los Rios et al., 2006; Goloubinoff and De Los Rios, 2007) to explain how Hsp70s move proteins through channels or dissociate protein aggregates, has also been advanced. This model suggests that it is not the ability of Hsc70s to act as a passive, steric wedge that causes disassembly, but the fact that they are bound under each coat vertex by association with flexible polypeptide tethers. Such flexible tethering allows the Hsc70s to generate a disassembling force through intermolecular collisions with the walls of the coat (Lafer et al., 2014). In contrast to the "steric wedge" model, this might be described as a "wrecking ball" model for coat disassembly. Determination of which of these mechanistic models is correct awaits further experimentation.

Control of Hsc70:Clathrin Chaperoning by Hsp110

The highly specialized uncoating reaction may provide insight into the mechanism of the more general proteostatic functions of the Hsp70 chaperones. Indeed, Hsc70 plays at least two roles in uncoating that may be considered analogous to its functions in supporting native protein folding. First, the disassembly of the clathrin coat may be considered analogous to reactions in which Hsp70s dissociate aggregates of misfolded proteins (Goloubinoff et al., 1999; Diamant et al., 2000; Ben-Zvi and Goloubinoff, 2001; Ben-Zvi et al., 2004; Shorter, 2011; Rampelt et al., 2012). Second, once disassembled, Hsc70 remains associated with triskelia (Schuermann et al., 2008) and inhibits their aberrant polymerization; i.e., Hsc70 not only disassembles coats, it also chaperones triskelia (**Figure 1**) via a mechanism that may be considered analogous to that by which Hsp70s sequester and inhibit aggregation of misfolded proteins (Mogk et al., 1999). Indeed, just as mutations in Hsp70 can accelerate accumulation of protein aggregates *in vivo* (Hesterkamp and Bukau, 1998), mutations in Hsc70 lead to aberrant clathrin polymerization and defects in endocytosis (Newmyer and Schmid, 2001).

The conclusion that Hsc70 not only disassembles clathrin coats, but also sequesters depolymerized clathrin implies that this chaperoning activity must be regulated so that clathrin can be released and made available as required for endocytosis. The most likely candidates for such regulators are the Hsp70 nucleotide exchange factors (NEFs), which control the association of Hsp70s with their protein substrates by stimulating the release of ADP from the Hsp70 (Packschies et al., 1997). This allows ATP to bind, which stimulates release of the substrate from the otherwise very stable Hsp70*ADP:protein substrate complex (Mccarty et al., 1995; Takeda and Mckay, 1996; Theyssen et al., 1996). *In vitro*, NEFs have been shown to release Hsc70 from triskelia and to accelerate the slow steady-state rate of uncoating that follows addition of limiting amounts of Hsc70 to reactions with clathrin coats and ATP (Schuermann et al., 2008), consistent with the Eisenberg group's conclusion that these burst kinetics reflect formation of stable Hsc70*ADP:clathrin complexes (Barouch et al., 1994). *In vitro*, both the Bag1 and Hsp110 NEFs could accelerate steady-state uncoating and Hsc70:clathrin dissociation, leaving it unclear which of these NEFs regulates clathrin:Hsc70 association *in vivo*. Recent experiments indicate that the relevant *in vivo* NEF is likely to be Hsp110, a protein that is itself a member of the Hsp70 family and the most abundant Hsp70 NEF in vertebrate brain (Morgan et al., 2013). Acute inhibition of Hsp110 at lamprey giant reticulospinal synapses was shown to inhibit endocytosis (specifically, synaptic vesicle recycling), presumably because inhibition of Hsp110 blocked its ability to stimulate nucleotide exchange and thereby blocked release of clathrin from Hsc70 (Morgan et al., 2013).

The question of how Hsc70:clathrin association is controlled so as to coordinate clathrin availability with endocytic activity may therefore reduce, at least in part, to the question of how Hsp110 activity is regulated. Currently, answers to this question must remain largely speculative, but one possible mechanism involves the dephosphin hypothesis (Cousin and Robinson, 2001) that has been developed to explain how the depolarization

induced calcium influx that occurs at nerve terminals during synaptic transmission stimulates endocytosis. This hypothesis proposes that a coordinated wave of dephosphorylation of a number of proteins required for endocytosis (the "dephosphins" dynamin I, amphiphysins I and II, synaptojanin, epsin, eps15 and AP180) (Cousin et al., 2001) is driven by Ca^{++} activation of the calcium/calmodulin dependent protein phophatase calcineurin. Dephosphorylation of these proteins is proposed to stimulate their activity and thereby drive endocytosis.

Could such a mechanism regulate availability of Hsc70-free clathrin for endocytosis? Hsp110 is known to be phosphorylated, primarily at serine residues, both *in vivo* and *in vitro* (Ishihara et al., 2000). Evidence suggests that the relevant *in vivo* kinase(s) may be casein kinase II (CKII) or a kinase that has CKII-like specificity. *In vitro*, CKII phosphorylates mouse Hsp110 at S509, and this residue is also phosphorylated *in vivo* (Ishihara et al., 2003). Phosphorylation at S509 relieves the inhibitory effect of Hsp110 on Hsp70-mediated luciferase refolding *in vitro*, and an Hsp110 S509A mutant suppresses Hsp70-mediated luciferase refolding *in vivo*.

These observations require further comment as Hsp110 is usually observed, and is expected to, stimulate, rather than inhibit, Hsp70 functions. However, the net effect of stimulation of Hsp70 nucleotide exchange by Hsp110 is to shorten the lifetime of the Hsp70:protein substrate complex. If we consider an Hsp70-mediated reaction, such as refolding of misfolded proteins, it is easy to imagine that there will be an optimal lifetime for this complex that balances the reduction in second order processes like aggregation due to sequestration of misfolded proteins with Hsp70, and the rate of first order processes like refolding, which require that the misfolded protein be released from the Hsp70. Indeed, it is observed that when Hsp110 is titrated into Hsp70-mediated protein refolding reactions, there is an Hsp110 concentration that drives maximal refolding, and that Hsp110 concentrations in excess of this can slow refolding (Dragovic et al., 2006; Tzankov et al., 2008; Rampelt et al., 2012).

Since, in the experiments described above, phosphorylation of Hsp110 was observed to relieve inhibition of Hsp70-mediated luciferase refolding, this indicates that these experiments were carried out under conditions where Hsp110 was inducing Hsp70 nucleotide exchange and protein substrate release to a degree that inhibited protein refolding. This implies that phosphorylation of Hsp110 reduces its ability to act as an Hsp70 NEF, and suggests the following mechanism for how Ca^{++}/calmodulin activation of the calcineurin phosphatase causes Hsc70 to release clathrin and make it available for endocytosis: in a resting synapse, clathrin may be mostly sequestered by Hsc70, which is in excess of clathrin (Morgan et al., 2013). Hsp110 will be phosphorylated (the brain has been shown to be exceptionally rich in the phosphorylated form of Hsp110 Ishihara et al., 2000), and its NEF activity will be correspondingly repressed. Upon depolarization, the influx of Ca^{++} into the pre-synaptic terminal will activate calmodulin and calcineurin, leading to a wave of dephosphorylation of multiple proteins, including Hsp110. Dephosphorylation of Hsp110 will stimulate its NEF activity and ultimately lead to increased release of clathrin from Hsc70, thus driving the clathrin-mediated endocytic retrieval of the synaptic vesicle proteins that follows the fusion of the synaptic vesicle with the plasma membrane and release of neurotransmitter (**Figure 1**).

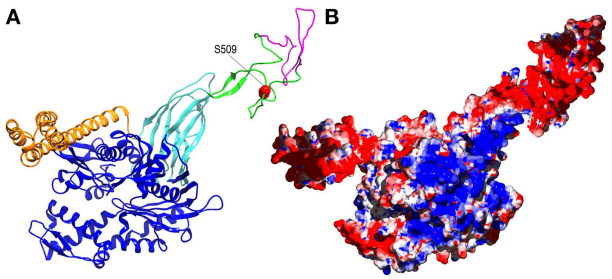


FIGURE 2 | Alternative splicing and phosphorylation of the acidic insertion loop may regulate Hsp110 activity. (A) Ribbon model of human Hsp110 based on the crystal structure of yeast Hsp110 (pbd 3C7N Schuermann et al., 2008) with nucleotide binding domain in blue, the β-sandwich and α-helical portions of the protein binding domain in, respectively, cyan and orange, and the acidic insertion loop in green with the element that is removed by alternative splicing in magenta and the phosphorylated S509 side-chain in red. **(B)** The same model as in A but in surface representation with positively and negatively charged regions in blue and red, respectively.

How might phosphorylation regulate Hsp110 NEF activity? Again, any answer must be speculative, but it is striking that the phosphorylated S509 residue sits within the flexible ∼95 residue acidic insertion loop that is positioned near the end of the β-sandwich segment of the Hsp110 protein binding domain (**Figure 2A**). The acidic insertion loop uniquely distinguishes the Hsp110 proteins from the more distantly related members of the Hsp70 family. There is some evidence that this loop may regulate Hsp110: the Hendershot lab observed that deletions in the insertion loop of ER Hsp110 (Grp170) affect its ability to interact with protein substrates (Pereira et al., 2014), and it has been found that the RNA encoding this loop undergoes alternative splicing (Yasuda et al., 1995). The full-length version of mouse Hsp110 (also called Hsp105α as Hsp110 has also been designated Hsp105) is apparently the predominant, constitutively expressed version of the protein, while the alternatively spliced Hsp105β, which may be expressed upon heat shock (Hatayama et al., 1994), is missing 44 residues from the loop (**Figure 2A**). There is no information on how this loop might regulate Hsp110 activity, but the loop's flexibility and negative charge could allow it to interact with multiple electropositive regions on the Hsp110 (or Hsp70) surface (**Figure 2B**), and phosphorylation might modulate these ionic interactions. All of these highly speculative mechanisms await testing by future experimentation.

Summary

Hsc70 disassembly of clathrin coats follows a canonical Hsp70 mechanism in which a J cochaperone binds Hsp70∗ATP and transfers it to a substrate, concomitant with ATP hydrolysis to form a stable Hsp70*ADP:substrate complex. After disassembling the coat, Hsc70 remains associated with

clathrin to prevent its aberrant polymerization. When required for endocytosis clathrin is released from Hsc70 by a nucleotide exchange factor, whose activity may be regulated by Ca^{++} dependent dephosphorylation.

Acknowledgments

Work in the EL and RS laboratories is supported by NIH-NINDS grant NS029051.

References

Ahle, S., and Ungewickell, E. (1990). Auxilin, a newly identified clathrin-associated protein in coated vesicles from bovine brain. *J. Cell Biol.* 111, 19–29. doi: 10.1083/jcb.111.1.19

Barouch, W., Prasad, K., Greene, L. E., and Eisenberg, E. (1994). ATPase activity associated with the uncoating of clathrin baskets by Hsp70. *J. Biol. Chem.* 269, 28563–28568.

Barouch, W., Prasad, K., Greene, L., and Eisenberg, E. (1997). Auxilin-induced interaction of the molecular chaperone Hsc70 with clathrin baskets. *Biochemistry* 36, 4303–4308. doi: 10.1021/bi962727z

Ben-Zvi, A., De Los Rios, P., Dietler, G., and Goloubinoff, P. (2004). Active solubilization and refolding of stable protein aggregates by cooperative unfolding action of individual hsp70 chaperones. *J. Biol. Chem.* 279, 37298–37303. doi: 10.1074/jbc.M405627200

Ben-Zvi, A. P., and Goloubinoff, P. (2001). Review: mechanisms of disaggregation and refolding of stable protein aggregates by molecular chaperones. *J. Struct. Biol.* 135, 84–93. doi: 10.1006/jsbi.2001.4352

Bocking, T., Aguet, F., Harrison, S. C., and Kirchhausen, T. (2011). Single-molecule analysis of a molecular disassemblase reveals the mechanism of Hsc70-driven clathrin uncoating. *Nat. Struct. Mol. Biol.* 18, 295–301. doi: 10.1038/nsmb.1985

Bocking, T., Aguet, F., Rapoport, I., Banzhaf, M., Yu, A., Zeeh, J. C., et al. (2014). Key interactions for clathrin coat stability. *Structure* 22, 819–829. doi: 10.1016/j.str.2014.04.002

Braell, W. A., Schlossman, D. M., Schmid, S. L., and Rothman, J. E. (1984). Dissociation of clathrin coats coupled to the hydrolysis of ATP: role of an uncoating ATPase. *J. Cell Biol.* 99, 734–741. doi: 10.1083/jcb.99.2.734

Cousin, M. A., and Robinson, P. J. (2001). The dephosphins: dephosphorylation by calcineurin triggers synaptic vesicle endocytosis. *Trends Neurosci.* 24, 659–665. doi: 10.1016/S0166-2236(00)01930-5

Cousin, M. A., Tan, T. C., and Robinson, P. J. (2001). Protein phosphorylation is required for endocytosis in nerve terminals: potential role for the dephosphins dynamin I and synaptojanin, but not AP180 or amphiphysin. *J. Neurochem.* 76, 105–116. doi: 10.1046/j.1471-4159.2001.00049.x

De Los Rios, P., Ben-Zvi, A., Slutsky, O., Azem, A., and Goloubinoff, P. (2006). Hsp70 chaperones accelerate protein translocation and the unfolding of stable protein aggregates by entropic pulling. *Proc. Natl. Acad. Sci. U.S.A.* 103, 6166–6171. doi: 10.1073/pnas.0510496103

Diamant, S., Ben-Zvi, A. P., Bukau, B., and Goloubinoff, P. (2000). Size-dependent disaggregation of stable protein aggregates by the DnaK chaperone machinery. *J. Biol. Chem.* 275, 21107–21113. doi: 10.1074/jbc.M001293200

Dragovic, Z., Broadley, S. A., Shomura, Y., Bracher, A., and Hartl, F. U. (2006). Molecular chaperones of the Hsp110 family act as nucleotide exchange factors of Hsp70s. *EMBO J.* 25, 2519–2528. doi: 10.1038/sj.emboj.7601138

Fotin, A., Cheng, Y., Sliz, P., Grigorieff, N., Harrison, S. C., Kirchhausen, T., et al. (2004). Molecular model for a complete clathrin lattice from electron cryomicroscopy. *Nature* 432, 573–579. doi: 10.1038/nature03079

Gall, W. E., Higginbotham, M. A., Chen, C., Ingram, M. F., Cyr, D. M., and Graham, T. R. (2000). The auxilin-like phosphoprotein Swa2p is required for clathrin function in yeast. *Curr. Biol.* 10, 1349–1358. doi: 10.1016/S0960-9822(00)00771-5

Goloubinoff, P., and De Los Rios, P. (2007). The mechanism of Hsp70 chaperones: (entropic) pulling the models together. *Trends Biochem. Sci.* 32, 372–380. doi: 10.1016/j.tibs.2007.06.008

Goloubinoff, P., Mogk, A., Zvi, A. P., Tomoyasu, T., and Bukau, B. (1999). Sequential mechanism of solubilization and refolding of stable protein aggregates by a bichaperone network. *Proc. Natl. Acad. Sci. U.S.A.* 96, 13732–13737. doi: 10.1073/pnas.96.24.13732

Greener, T., Grant, B., Zhang, Y., Wu, X., Greene, L. E., Hirsh, D., et al. (2001). Caenorhabditis elegans auxilin: a J-domain protein essential for clathrin-mediated endocytosis *in vivo*. *Nat. Cell Biol.* 3, 215–219. doi: 10.1038/35055137

Greener, T., Zhao, X., Nojima, H., Eisenberg, E., and Greene, L. E. (2000). Role of cyclin G-associated kinase in uncoating clathrin-coated vesicles from non-neuronal cells. *J. Biol. Chem.* 275, 1365–1370. doi: 10.1074/jbc.275.2.1365

Hatayama, T., Yasuda, K., and Nishiyama, E. (1994). Characterization of high-molecular-mass heat shock proteins and 42 degrees C-specific heat shock proteins of murine cells. *Biochem. Biophys. Res. Commun.* 204, 357–365. doi: 10.1006/bbrc.1994.2467

Hennessy, F., Nicoll, W. S., Zimmermann, R., Cheetham, M. E., and Blatch, G. L. (2005). Not all J domains are created equal: implications for the specificity of Hsp40-Hsp70 interactions. *Protein Sci.* 14, 1697–1709. doi: 10.1110/ps.051406805

Hesterkamp, T., and Bukau, B. (1998). Role of the DnaK and HscA homologs of Hsp70 chaperones in protein folding in *E. coli*. *EMBO J.* 17, 4818–4828. doi: 10.1093/emboj/17.16.4818

Holstein, S. E., Ungewickell, H., and Ungewickell, E. (1996). Mechanism of clathrin basket dissociation: separate functions of protein domains of the DnaJ homologue auxilin. *J. Cell Biol.* 135, 925–937. doi: 10.1083/jcb.135.4.925

Ishihara, K., Yamagishi, N., and Hatayama, T. (2003). Protein kinase CK2 phosphorylates Hsp105 alpha at Ser509 and modulates its function. *Biochem. J.* 371, 917–925. doi: 10.1042/BJ20021331

Ishihara, K., Yasuda, K., and Hatayama, T. (2000). Phosphorylation of the 105-kDa heat shock proteins, HSP105alpha and HSP105beta, by casein kinase II. *Biochem. Biophys. Res. Commun.* 270, 927–931. doi: 10.1006/bbrc.2000.2541

Jiang, J., Taylor, A. B., Prasad, K., Ishikawa-Brush, Y., Hart, P. J., Lafer, E. M., et al. (2003). Structure-function analysis of the auxilin J-domain reveals an extended Hsc70 interaction interface. *Biochemistry* 42, 5748–5753. doi: 10.1021/bi034270g

Kanaoka, Y., Kimura, S. H., Okazaki, I., Ikeda, M., and Nojima, H. (1997). GAK: a cyclin G associated kinase contains a tensin/auxilin-like domain. *FEBS Lett.* 402, 73–80. doi: 10.1016/S0014-5793(96)01484-6

Kimura, S. H., Tsuruga, H., Yabuta, N., Endo, Y., and Nojima, H. (1997). Structure, expression, and chromosomal localization of human GAK. *Genomics* 44, 179–187. doi: 10.1006/geno.1997.4873

Lafer, E. M., Jin, S., and Sousa, R. (2014). "Hsc70 disassembly of clathrin cages driven by osmotic force amplified by chaperone self-association," in *Society for Neuroscience Annual Meeting 2014* (Washington, DC). Program No. 403.09.

Lee, D. W., Wu, X., Eisenberg, E., and Greene, L. E. (2006). Recruitment dynamics of GAK and auxilin to clathrin-coated pits during endocytosis. *J. Cell Sci.* 119, 3502–3512. doi: 10.1242/jcs.03092

Lee, D. W., Zhao, X., Zhang, F., Eisenberg, E., and Greene, L. E. (2005). Depletion of GAK/auxilin 2 inhibits receptor-mediated endocytosis and recruitment of both clathrin and clathrin adaptors. *J. Cell Sci.* 118, 4311–4321. doi: 10.1242/jcs.02548

Lemmon, S. K. (2001). Clathrin uncoating: auxilin comes to life. *Curr. Biol.* 11, R49–R52. doi: 10.1016/S0960-9822(01)00010-0

Massol, R. H., Boll, W., Griffin, A. M., and Kirchhausen, T. (2006). A burst of auxilin recruitment determines the onset of clathrin-coated vesicle uncoating. *Proc. Natl. Acad. Sci. U.S.A.* 103, 10265–10270. doi: 10.1073/pnas.0603369103

Mccarty, J. S., Buchberger, A., Reinstein, J., and Bukau, B. (1995). The role of ATP in the functional cycle of the DnaK chaperone system. *J. Mol. Biol.* 249, 126–137. doi: 10.1006/jmbi.1995.0284

Mogk, A., Tomoyasu, T., Goloubinoff, P., Rudiger, S., Roder, D., Langen, H., et al. (1999). Identification of thermolabile *Escherichia coli* proteins: prevention and reversion of aggregation by DnaK and ClpB. *EMBO J.* 18, 6934–6949. doi: 10.1093/emboj/18.24.6934

Morgan, J. R., Jiang, J., Oliphint, P. A., Jin, S., Gimenez, L. E., Busch, D. J., et al. (2013). A role for an hsp70 nucleotide exchange factor in the regulation of synaptic vesicle endocytosis. *J. Neurosci.* 33, 8009–8021. doi: 10.1523/JNEUROSCI.4505-12.2013

Morgan, J. R., Prasad, K., Jin, S., Augustine, G. J., and Lafer, E. M. (2001). Uncoating of clathrin-coated vesicles in presynaptic terminals: roles for Hsc70 and auxilin. *Neuron* 32, 289–300. doi: 10.1016/S0896-6273(01)00467-6

Newmyer, S. L., and Schmid, S. L. (2001). Dominant-interfering Hsc70 mutants disrupt multiple stages of the clathrin-coated vesicle cycle *in vivo*. *J. Cell Biol.* 152, 607–620. doi: 10.1083/jcb.152.3.607

Packschies, L., Theyssen, H., Buchberger, A., Bukau, B., Goody, R. S., and Reinstein, J. (1997). GrpE accelerates nucleotide exchange of the molecular chaperone DnaK with an associative displacement mechanism. *Biochemistry* 36, 3417–3422. doi: 10.1021/bi962835l

Pereira, E. R., Frudd, K., Awad, W., and Hendershot, L. M. (2014). Endoplasmic reticulum (ER) stress and hypoxia response pathways interact to potentiate hypoxia-inducible factor 1 (HIF-1) transcriptional activity on targets like vascular endothelial growth factor (VEGF). *J. Biol. Chem.* 289, 3352–3364. doi: 10.1074/jbc.M113.507194

Pishvaee, B., Costaguta, G., Yeung, B. G., Ryazantsev, S., Greener, T., Greene, L. E., et al. (2000). A yeast DNA J protein required for uncoating of clathrin-coated vesicles *in vivo*. *Nat. Cell Biol.* 2, 958–963. doi: 10.1038/35046619

Prasad, K., Barouch, W., Greene, L., and Eisenberg, E. (1993). A protein cofactor is required for uncoating of clathrin baskets by uncoating ATPase. *J. Biol. Chem.* 268, 23758–23761.

Rampelt, H., Kirstein-Miles, J., Nillegoda, N. B., Chi, K., Scholz, S. R., Morimoto, R. I., et al. (2012). Metazoan Hsp70 machines use Hsp110 to power protein disaggregation. *EMBO J.* 31, 4221–4235. doi: 10.1038/emboj.2012.264

Rapoport, I., Boll, W., Yu, A., Bocking, T., and Kirchhausen, T. (2008). A motif in the clathrin heavy chain required for the hsc70/auxilin uncoating reaction. *Mol. Biol. Cell* 19, 405–413. doi: 10.1091/mbc.E07-09-0870

Rothnie, A., Clarke, A. R., Kuzmic, P., Cameron, A., and Smith, C. J. (2011). A sequential mechanism for clathrin cage disassembly by 70-kDa heat-shock cognate protein (Hsc70) and auxilin. *Proc. Natl. Acad. Sci. U.S.A.* 108, 6927–6932. doi: 10.1073/pnas.1018845108

Schlossman, D. M., Schmid, S. L., Braell, W. A., and Rothman, J. E. (1984). An enzyme that removes clathrin coats: purification of an uncoating ATPase. *J. Cell Biol.* 99, 723–733. doi: 10.1083/jcb.99.2.723

Schmid, S. L., and Rothman, J. E. (1985). Enzymatic dissociation of clathrin cages in a two-stage process. *J. Biol. Chem.* 260, 10044–10049.

Schroder, S., Morris, S. A., Knorr, R., Plessmann, U., Weber, K., Nguyen, G. V., et al. (1995). Primary structure of the neuronal clathrin-associated protein auxilin and its expression in bacteria. *Eur. J. Biochem.* 228, 297–304. doi: 10.1111/j.1432-1033.1995.tb20263.x

Schuermann, J. P., Jiang, J., Cuellar, J., Llorca, O., Wang, L., Gimenez, L. E., et al. (2008). Structure of the Hsp110:Hsc70 nucleotide exchange machine. *Mol. Cell* 31, 232–243. doi: 10.1016/j.molcel.2008.05.006

Shorter, J. (2011). The mammalian disaggregase machinery: Hsp110 synergizes with Hsp70 and Hsp40 to catalyze protein disaggregation and reactivation in a cell-free system. *PLoS ONE* 6:e26319. doi: 10.1371/journal.pone.0026319

Sousa, R. J. (2014). Structural mechanisms of chaperone mediated protein disaggregation. *Front. Mol. Biosci.* 1:12. doi: 10.3389/fmolb.2014.00012

Takeda, S., and Mckay, D. B. (1996). Kinetics of peptide binding to the bovine 70 kDa heat shock cognate protein, a molecular chaperone. *Biochemistry* 35, 4636–4644. doi: 10.1021/bi952903o

Theyssen, H., Schuster, H. P., Packschies, L., Bukau, B., and Reinstein, J. (1996). The second step of ATP binding to DnaK induces peptide release. *J. Mol. Biol.* 263, 657–670. doi: 10.1006/jmbi.1996.0606

Tzankov, S., Wong, M. J., Shi, K., Nassif, C., and Young, J. C. (2008). Functional divergence between co-chaperones of Hsc70. *J. Biol. Chem.* 283, 27100–27109. doi: 10.1074/jbc.M803923200

Umeda, A., Meyerholz, A., and Ungewickell, E. (2000). Identification of the universal cofactor (auxilin 2) in clathrin coat dissociation. *Eur. J. Cell Biol.* 79, 336–342. doi: 10.1078/S0171-9335(04)70037-0

Ungewickell, E. (1985). The 70-kd mammalian heat shock proteins are structurally and functionally related to the uncoating protein that releases clathrin triskelia from coated vesicles. *EMBO J.* 4, 3385–3391.

Ungewickell, E., Ungewickell, H., Holstein, S. E., Lindner, R., Prasad, K., Barouch, W., et al. (1995). Role of auxilin in uncoating clathrin-coated vesicles. *Nature* 378, 632–635. doi: 10.1038/378632a0

Xing, Y., Bocking, T., Wolf, M., Grigorieff, N., Kirchhausen, T., and Harrison, S. C. (2010). Structure of clathrin coat with bound Hsc70 and auxilin: mechanism of Hsc70-facilitated disassembly. *EMBO J.* 29, 655–665. doi: 10.1038/emboj.2009.383

Yasuda, K., Nakai, A., Hatayama, T., and Nagata, K. (1995). Cloning and expression of murine high molecular mass heat shock proteins, HSP105. *J. Biol. Chem.* 270, 29718–29723. doi: 10.1074/jbc.270.50.29718

Yim, Y. I., Sun, T., Wu, L. G., Raimondi, A., De Camilli, P., Eisenberg, E., et al. (2010). Endocytosis and clathrin-uncoating defects at synapses of auxilin knockout mice. *Proc. Natl. Acad. Sci. U.S.A.* 107, 4412–4417. doi: 10.1073/pnas.1000738107

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Molecular Biosciences

MINI REVIEW
published: 26 June 2015
doi: 10.3389/fmolb.2015.00034

Plasmodial HSP70s are functionally adapted to the malaria parasite life cycle

Jude M. Przyborski[1], *Mathias Diehl*[1] *and Gregory L. Blatch*[2,3]*

[1] *Parasitology, Philipps University Marburg, Marburg, Germany,* [2] *Centre for Chronic Disease Prevention and Management, College of Health and Biomedicine, Victoria University, Melbourne, VIC, Australia,* [3] *Biomedical Biotechnology Research Unit, Department of Biochemistry and Microbiology, Rhodes University, Grahamstown, South Africa*

The human malaria parasite, *Plasmodium falciparum*, encodes a minimal complement of six heat shock protein 70s (PfHSP70s), some of which are highly expressed and are thought to play an important role in the survival and pathology of the parasite. In addition to canonical features of molecular chaperones, these HSP70s possess properties that reflect functional adaptation to a parasitic life style, including resistance to thermal insult during fever periods and host–parasite interactions. The parasite even exports an HSP70 to the host cell where it is likely to be involved in host cell modification. This review focuses on the features of the PfHSP70s, particularly with respect to their adaptation to the malaria parasite life cycle.

Keywords: HSP70, HSP40, molecular chaperone, malaria, *Plasmodium falciparum*

OPEN ACCESS

Edited by:
Pierre Goloubinoff, University of Lausanne, Switzerland

Reviewed by:
Ehud Cohen, The Hebrew University of Jerusalem, Israel
Johnathan Paul Labbadia, Northwestern University, USA

***Correspondence:**
Gregory L. Blatch, College of Health and Biomedicine, Victoria University, PO Box 14428, Melbourne, VIC 8001, Australia
gregory.blatch@vu.edu.au

Specialty section:
This article was submitted to Protein Folding, Misfolding and Degradation, a section of the journal Frontiers in Molecular Biosciences

Received: *30 April 2015*
Accepted: *12 June 2015*
Published: *26 June 2015*

Citation:
Przyborski JM, Diehl M and Blatch GL (2015) Plasmodial HSP70s are functionally adapted to the malaria parasite life cycle. Front. Mol. Biosci. 2:34. doi: 10.3389/fmolb.2015.00034

Introduction

Malaria rates amongst one of the major global health challenges facing developing countries, and over half a million people die from malaria annually, 90% of which are in Africa. *Plasmodium falciparum* causes the most lethal form of human malaria, and nearly all malaria deaths result from infection by this species of *Plasmodium* (World Health Organisation, 2014).

HSP70 chaperones and their HSP40 co-chaperones are critical to the maintenance of cellular proteostasis through their role in the folding, refolding, aggregation suppression, translocation, and degradation of proteins. *P. falciparum* heat shock protein 70 (PfHSP70) proteins are proposed to play a major role in parasite development and survival, particularly within the human host (Shonhai et al., 2007, 2011; Njunge et al., 2013; Pesce and Blatch, 2014). Malaria parasites invade human hepatocytes and erythrocytes, and have adapted to their intracellular host environment, particularly to stresses such as the temperature fluctuations associated with febrile episodes. Part of this adaptation has been to evolve an optimal arsenal of six PfHSP70s (**Table 1**), half the number found in humans and other eukaryotic system, including other parasites (Shonhai et al., 2011). To nevertheless allow interaction with a large array of potential substrates, it appears that the parasite has increased its HSP40 co-chaperone complement to 49 homologs (Botha et al., 2007; Pesce and Blatch, 2014). This suggests that the plasmodial PfHSP70–PfHSP40 chaperone system is an extreme example of HSP70 general chaperone capacity that is highly specified through its HSP40 chaperone partners. The PfHSP70s have been more extensively studied than the PfHSP40s, and although progress has been made in recent years, we still know relatively little about PfHSP70–PfHSP40 partnerships (Pesce and Blatch, 2014). Five of the PfHSP70s are located, or predicted to be located, within the parasite (PfHsp70-1, PfHsp70-2, PfHsp70-3, PfHsp70-y, and PfHsp70-z) and one is secreted to the parasitophorous vacuole (PV) and further exported to the host cell (PfHsp70-x; **Table 1**; **Figure 1**). Orthologs of the five

TABLE 1 | Accession numbers, localization, and properties of *P. falciparum* HSP70 homologs.

Protein	PlasmoDB accession	Localization	Properties
PfHsp70-1	PF3D7_0818900	Cytosol, nucleus	Protein folding and protein aggregation suppression. C-terminal EEVD sequence for interaction with PfHOP and possibly other partners
PfHsp70-2	PF3D7_0917900	ER	BiP, grp. N-terminal signal sequence and C-terminal ER retrieval sequence. Translocation of proteins into the ER and retrograde translocation of proteins for degradation
PfHsp70-3	PF3D7_1134000	Mitochondria?	Predicted N-terminal mitochondrial transit peptide. Translocation of proteins into the mitochondrion?
PfHsp70-x	PF3D7_0831700	PV, host cell	N-terminal signal sequence and non-PEXEL export sequence. C-terminal EEVN. Associated with J-dots. Trafficking of specialized virulence proteins
PfHsp70-y	PF3D7_1344200	ER?	HSP110, likely NEF for PfHsp70-2. N-terminal signal sequence and C-terminal ER retrieval sequence
PfHsp70-z	PF3D7_0708800	Cytosol?	HSP110, likely NEF for PfHsp70-1. Suppression of the aggregation of asparagine repeat-rich proteins

BiP, immunoglobulin binding protein; ER, endoplasmic reticulum; grp, glucose regulated protein; Hop, HSP70/HSP90 organizing protein; NEF, nucleotide exchange factor; PEXEL, Plasmodium export element; PV, parasitophorous vacuole.

parasite-resident PfHSP70s are found in all *Plasmodium* species, and very likely play a fundamental role in proteostasis of the subcellular compartments that they occupy. In contrast, PfHsp70-x is only found in those *Plasmodium* species with highly expanded exportomes (*P. falciparum* and *P. reichenowi*), suggesting a specialized function in protein trafficking or folding (Külzer et al., 2012). This review focuses on the chaperone properties of the six PfHSP70s, particularly with respect to their adaptation to the malaria parasite life cycle.

Proteostasis of the Cytoplasm and the Nucleus: PfHp70-1 and PfHsp70-z

The parasite resident PfHsp70-1 exhibits key features that suggest that it is uniquely adapted to provide cytoprotection under stressful conditions such as febrile episodes. In particular, PfHsp70-1 has a thermostable C-terminal domain that is proposed to stabilize the overall conformation of the protein (Misra and Ramachandran, 2009), making it more thermostable than the human HSP70. Furthermore, it has the properties of a typical molecular chaperone (Matambo et al., 2004; Ramya et al., 2006) and has been shown using *in vitro* assays (Shonhai et al., 2008; Cockburn et al., 2011) and *in vivo* assays in bacteria (Shonhai et al., 2005) and yeast (Bell et al., 2011) to be efficient at suppressing protein aggregation. There are high levels of this molecular chaperone throughout the erythrocytic stages of the parasite life cycle (Acharya et al., 2009), with increased levels at febrile temperatures (Kumar et al., 1991; Joshi et al., 1992; Pesce et al., 2008). The localization of PfHsp70-1 to the parasite nucleus and cytosol (**Table 1**; **Figure 1**; Kumar et al., 1991; Pesce et al., 2008) suggests that it is an agent of proteostasis within these subcellular compartments. In addition, PfHsp70-1 could be important in keeping certain proteins in an unfolded translocation-competent state; such as proteins destined for the mitochondria. There are eight cytoplasmic PfHSP40s (Njunge et al., 2013), and there is experimental evidence for an interaction of PfHsp70-1 with Pfj4 (PF3D7_1211400; Pesce et al., 2008), PfHsp40 (PF3D7_1437900; Botha et al., 2011), and PFB0595w (PF3D7_0213100; Njunge et al., 2015). The other cytoplasmic PfHSP40s include proteins predicted to be ribosome-associated, membrane-associated and involved in diphthamide biosynthesis (Njunge et al., 2013). Therefore, a range of different unrelated protein substrates could potentially be delivered to PfHsp70-1 through these cytoplasmic co-chaperones.

PfHsp70-z belongs to the HSP110 family and is likely to act as a nucleotide exchange factor for PfHsp70-1. The protein has recently been shown to be essential for parasite viability, and there is evidence that it is able suppress the aggregation of asparagine repeat-rich proteins more efficiently than its eukaryotic orthologs (Muralidharan et al., 2012). Proteins containing asparagine repeats are prone to aggregation, and the proteome of the malaria parasite is rich in these proteins. PfHsp70-z may have evolved to protect the malaria parasite against the harmful effects of these proteins during febrile episodes, and this is supported by the recent finding that, similarly to PfHsp70-1, PfHsp70-z is also a thermostable molecular chaperone (Zininga et al., 2015). Therefore, PfHsp70-1 and PfHsp70-z together ensure that proteostasis is maintained in the parasite cytoplasm (**Table 1**; **Figure 1**).

Protein Secretion and Degradation: PfHsp70-2/PfBiP and PfHsp70-y

Proteins bearing a hydrophobic N-terminal signal sequence are routed via the endoplasmic reticulum (ER) to the PV (Adisa et al., 2003), unless they possess further targeting signals (Deponte et al., 2012). The ER-based HSP70, PfHsp70-2 (also called *P. falciparum* immunoglobulin binding protein, PfBiP; and *P. falciparum* glucose regulated protein, Pfgrp; Kumar et al., 1988, 1991; Kumar and Zheng, 1992) has not been extensively studied, but is very likely to be involved in protein secretion

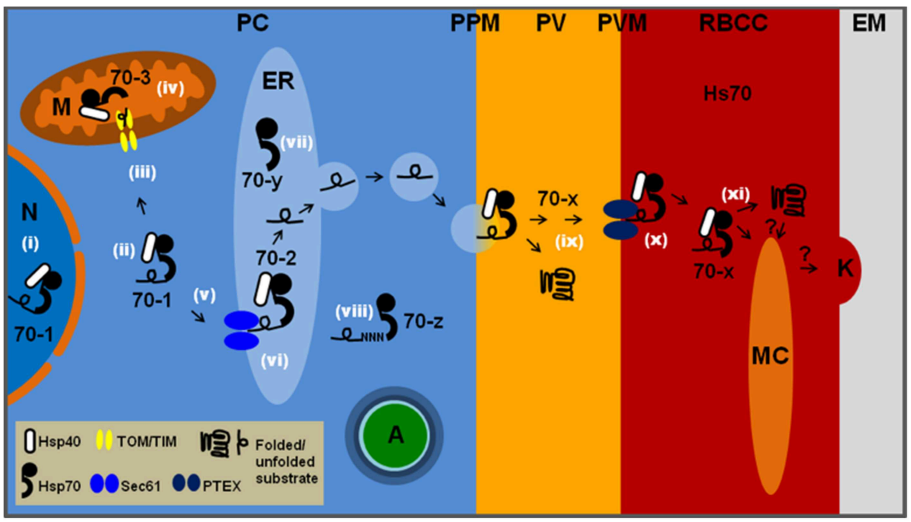


FIGURE 1 | The location and function of the six PfHSP70s in *P. falciparum*-infected erythrocytes. PC, parasite cytosol; PPM, parasite plasma membrane; PV, parasitophorous vacuole; PVM, parasitophorous vacuolar membrane; RBCC, red blood cell cytosol; EM, external milieu; N, nucleus; M, mitochondrion; ER, endoplasmic reticulum; MC, Maurer's cleft; K, knob; A, apicoplast; Hs70, human HSP70. All other HSP70s are parasite encoded and labeled as in **Table 1**. Likely functions at specific cellular localizations are noted by white roman numerals: (i) general proteostasis in the nucleus by 70-1; (ii) general proteostasis in the parasite cytosol by 70-1; (iii) involvement of 70-1 as part of a guidance complex for mitochondrial proteins; (iv) involvement of 70-3 in translocation across mitochondrial membranes followed by protein folding; (v) potential involvement of 70-1 in post-translational ER targeting of secretory proteins; (vi) involvement of 70-2 in protein translocation across PfSec61, followed by general protein quality control via the unfolded protein response; (vii) 70-y is likely to function as a NEF for 70-2; (viii) 70-z is likely to function as a NEF for 70-1 and has been shown to be involved in stabilization of asparagine-rich proteins; (ix) 70-x may be involved in maintaining proteins in an unfolded state prior to passage across PTEX in combination with PfHsp101, and also folding of PV resident proteins; (x) both 70-x and Hs70 may be involved in translocation through PTEX followed by protein folding and; (xi) insertion into the Maurer's clefts, knobs and erythrocyte plasma membrane. Many of the above processes are likely to require co-chaperone activity provided by various PfHSP40s.

and degradation processes associated with the ER (**Table 1**; **Figure 1**). Similarly to PfHsp70-1, PfHsp70-2 has been shown to exhibit chaperone properties. Using *in vitro* assays PfHsp70-2 was found to protect alcohol dehydrogenase and glutamate dehydrogenase from thermally-induced unfolding (Ramya et al., 2006). PfHsp70-2 and associated co-chaperones (e.g., the membrane bound PfHSP40, PfSec63/PF3D7_1318800) have been proposed to be involved in the secretory pathway, working closely with the translocon machinery (PfSec61 complex; Tuteja, 2007). Analysis of the components of the PfSec61 complex suggests the existence of a signal recognition particle-based co-translational ER translocation mechanism, similar to the mammalian system (Zimmermann and Blatch, 2009); however, a post-translational mechanism cannot be excluded. ER proteins misfolded beyond repair are probably removed by retrograde translocation, possibly using components of the ER-associated protein degradation pathway (ERAD), which have previously been identified in the parasite (Spork et al., 2009). PfHsp70-2 may work together with an ER-luminal PfHSP40 (e.g., Pfj2/PF3D7_1108700; Botha et al., 2007; Pesce et al., 2010) in such a quality control process, similar to the human BiP-ERdj5 system (Hagiwara and Nagata, 2012). PfHsp70-y, similar to PfHsp70-z, belongs to the HSP110 family, and is thus likely to function as a NEF for PfHsp70-2. Although this protein contains a predicted apicoplast targeting signal, it also contains a C-terminal ER retrieval sequence which seems to be dominant (Heiny et al., 2012). Thus, it appears unlikely that this protein is actually targeted to the apicoplast, but is rathermore likely to be ER localized.

Import into Organelles: PfHsp70-3

Plasmodium parasites contain both a mitochondrion and a secondary plastid, referred to as the apicoplast. Protein import to these compartments is post-translational, and thus proteins must be kept in an unfolded state prior to membrane translocation. In other systems, organellar HSP70s provide assistance in this process. Indeed, *P. falciparum* is predicted to encode a mitochondrial HSP70, PfHsp70-3 (Njunge et al., 2013; **Table 1**; **Figure 1**). Although not yet experimentally addressed, it is likely that cytosolic PfHsp70-1 also contributes to mitochondrial protein import, possibly as part of a chaperoning guidance complex which keeps proteins in a translocation competent state prior to passage across the mitochondrial membranes. Based on other systems, it is likely that PfHsp90 and Pf14-3-3 are also part of this complex.

Several studies have suggested that PfHSP70s may also play a role in transport of proteins to the apicoplast, a secondary endosymbiotic organelle which is related to chloroplasts (Foth et al., 2003; Misra and Ramachandran, 2009). However, so far no evidence has verified the presence of a HSP70 homolog within the apicoplast. As all other PfHSP70 homologs have been localized to other cellular compartments, it seems unlikely that PfHSP70s play a direct role in translocation across apicoplast membranes.

Nevertheless, the observation that inhibitors of HSP70 cause a delay in apicoplast protein transport (Ramya et al., 2006) suggests that possibly PfHsp70-2 (PfBiP) may be involved in chaperoning client proteins prior to passage across the SELMA (symbiont-derived ERAD-like machinery) translocon which is believed to be the gateway to the apicoplast (Hempel et al., 2007; Spork et al., 2009). This interpretation is supported by studies in the related apicomplexan, *Toxoplasma gondii,* which identified TgBiP as an important *trans* factor in protein targeting to the apicoplast (Yung et al., 2003). Although as yet unproven, it has been suggested that apicoplast targeted HSP60 (cpn60; GroEL) or HSP100 (ClpC) may have taken over the function of HSP70 in protein translocation across the multiple apicoplast membranes (Sato, 2011). If this is true, it represents a fascinating evolutionary example of a parasite "losing" one chaperone and co-opting another to take over its function.

Protein Export: PfHsp70-x

P. falciparum is predicted to export over 450 proteins (8% of the entire proteome) into the human erythrocyte where they are involved in modifications of the host cell which are essential for parasite survival (Hiller et al., 2004; Marti et al., 2004; Sargeant et al., 2006; Maier et al., 2008). The protein export pathway involves: (i) entry into and transit through the ER; (ii) secretion into the PV; (iii) translocation across the parasitophorous vacuolar membrane (PVM) into the erythrocyte; (iv) trafficking of soluble proteins to their final destination in the cytosol; or (v) trafficking of membrane proteins in complexes or through vesicular networks to the Maurer's Clefts and to the plasma membrane of the erythrocyte (Deponte et al., 2012; **Figure 1**). Therefore, any exported parasite protein must cross a number of different membranes in an unfolded translocation-competent conformation by a process that will very likely require molecular chaperones at all stages. Two classes of exported proteins have been identified. One class contains a so-called *Plasmodium* export element or host targeting (PEXEL/HT) motif downstream of an N-terminal ER-type hydrophobic signal sequence. The PEXEL/HT motif is cleaved in the ER (Chang et al., 2008; Boddey et al., 2010; Russo et al., 2010) and (in a process not yet understood) directs proteins into an export pathway to the host cell. Another class of proteins, so called PEXEL Negative Exported Proteins (PNEPs) do not contain any recognizable conserved export motif, and indeed often do not contain an N-terminal ER-type signal sequence (Spielmann and Gilberger, 2010; Heiber et al., 2013). A notable member of the PNEPs is *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) which is transported to the host cell plasma membrane, and that is directly implicated in malaria pathology by a process called cytoadherance. Despite divergence in the signals mediating export, the export pathway of PEXEL and PNE proteins appears to converge at a protein translocon within the PVM, referred to as PTEX (*Plasmodium* translocon of exported proteins; de Koning-Ward et al., 2009; Gruring et al., 2012). Thus, down-regulation or functional interruption of a PTEX component leads to a transport block of both PEXEL and PNE proteins (Beck et al., 2014; Elsworth et al., 2014). Additionally, inhibition of protein unfolding also leads to a transport block of both protein classes in the PV (Gehde et al., 2009; Gruring et al., 2012). Recently, PfHsp70-x was shown to be localized to both the PV and host cell cytosol (**Table 1**; **Figure 1**). Within the host cell cytosol, PfHsp70-x could be localized to small punctate structures known as J-dots (Külzer et al., 2012). These structures are motile within the host cytosol, and have also previously been shown to contain PfEMP1 (Külzer et al., 2010). Thus, the possibility exists that PfHsp70-x is involved in protein traffic both (a) across the PV, possibly as an associated factor of the PTEX translocon, and (b) within the host cell cytosol, possibly as part of a chaperoning complex to carry proteins to their final sub-cellular localization. A role for PfHsp70-x within the PV would make sense, as the PTEX translocon is partially composed of PfHsp101, a molecular chaperone known (in other systems) to often functionally associate with HSP70s. Alternatively, in the PV PfHsp70-x may be involved in keeping exported proteins unfolded prior to passage across the PVM via PTEX. Once proteins reach the *trans* side of the PVM, they need to be folded and transported to their final destination. PfHsp70-x may be involved in this process, as may residual human HSP70s. Additionally, PfHsp70-x may be required to chaperone larger proteins complexes through the erythrocyte cytosol, and assist in their insertion into either the Maurer's clefts, or host cell plasma membrane. Of note, PfHsp70-x is only encoded by parasite species which export EMP1-like proteins, suggesting that it may play a special role in export of this specialized (but highly important) protein family (Külzer et al., 2012).

PfHSP40s

Although this review concentrates on PfHsp70s, it would be unwise not to comment on the HSP40s. HSP40s function as co-chaperones for HSP70s and are involved in specificity of substrate recognition and stimulation of HSP70 ATP hydrolysis activity. Of all the parasite-resident PfHSP40s, so far only Pfj4 has been captured in a common complex with PfHsp70-1 using immunoprecipitation assays (Pesce et al., 2008). Interestingly, there is evidence from gel filtration studies that PfHsp70-1 may occur in two distinct complexes, one with Pfj4 and another with PfHsp90 (Pesce et al., 2008). This PfHsp90–PfHsp70-1 complex may include and be functionalized by *P. falciparum* HSP70/HSP90 organizing protein (PfHOP) (Gitau et al., 2012).

Amazingly, in addition to exporting PfHsp70-x, *P. falciparum* may export almost half (23) of its expanded complement of 49 HSP40s (Sargeant et al., 2006; Botha et al., 2007). Although the exact nature of PfHSP70–PfHSP40 pairings has yet to be investigated in detail, localization data suggests that at least two of the exported PfHSP40s colocalize and are found in a complex with PfHsp70-x and thus may by involved in regulating PfHsp70-x function (Külzer et al., 2012). Several other exported HSP40s have been implicated in host cell modification, including biogenesis of knob structures under the surface of the erythrocyte, insertion of PfEMP1 into these structures and alteration of erythrocyte stiffness, all features which are likely to have a direct effect on pathogenicity of parasites in the human host (Maier et al., 2008). It is however not yet known

whether these proteins carry out their function mainly in conjunction with PfHsp70-x, or possibly together with residual human HSP70 which is found in high concentrations within the host cell, a fascinating area of research which deserves further study. A recent publication suggests that exported HSP40s may be able to functionally associate with both PfHsp70-x and human HSP70, further increasing the flexibility of the potential chaperone-cochaperone pairing (Hatherley et al., 2014). So far, *in silico* data suggest that *P. falciparum* has evolved to use a minimal complement of PfHSP70s, supported by an expanded HSP40 complement to confer substrate specificity and diversity. Deciphering the role and functional interactions of the expanded PfHSP40 complement will allow us a window into understanding this evolutionary flexibility.

PfHSP70s as Drug Targets

Although counter-intuitive due to the high sequence conservation amongst members of the HSP70 family from highly divergent organisms, they may actually be specially suited as drug targets. Such highly conserved proteins are often essential for cell survival, a key factor for any potential drug target. Furthermore, these proteins evolve considerably slower than non-conserved protein families, leading to less variation under drug selection pressure, and hence less chance of the emergence of drug resistance (Edkins and Blatch, 2012). However, there is sufficient structural and functional variation among HSP70s of different origins, for them to be specifically inhibited (Shonhai et al., 2007; Pesce et al., 2010; Shonhai, 2010). Due to interest in their potential as anti-cancer medication, a growing number of HSP70 inhibitors are being made available. Indeed, certain features of HSP70s make them amenable to functional inhibition (and hence druggable): such as their ability to bind small molecules that can be readily detected in simple assays (e.g., ATP); and their association with regulatory factors (e.g., ATP hydrolysis stimulation by HSP40s) whose inhibition has detectable functional consequences. A number of different compound classes have been identified as modulators of HSP70 activity, including ATP mimics, spergualins, pyrimidinones, and peptides (Zininga and Shonhai, 2014). PfHsp70-1 has also been found to be modulated by a number of different classes of small molecules. Pyrimidinones have been shown to significantly inhibit the ATPase activity of PfHsp70-1 as well as the *in vitro* growth of the malaria parasite (Chiang et al., 2009). Further studies on these pyrimidinones have shown that they are able to modulate both the basal and HSP40-stimulated *in vitro* ATPase activities of PfHsp70-1 (Botha et al., 2011). PfHsp70-1 and PfHsp70-x are able to efficiently suppress the aggregation of an aggregation-prone model substrate (malate dehydrogenase) in a thermal denaturation-based assay (Cockburn et al., 2011, 2014). A number of natural product compounds showing anti-plasmodial activity were found to selectively modulate the *in vitro* protein aggregation suppression activity of PfHsp70-1 (1,4-naphthoquinones and marine prenylated alkaloids or malonganenones; Cockburn et al., 2011). Some of these compounds were also found to selectively modulate the *in vitro* protein aggregation suppression activity of PfHsp70-x, as well as the basal and HSP40-stimulated ATPase activities of both PfHsp70-1 and PfHsp70-x, compared with human HSP70 (Cockburn et al., 2014). Therefore, PfHsp70-1 and PfHsp70-x appear to be druggable; but a much more concerted effort in drug discovery is needed before the first drug hits the market.

Conclusion

Although detailed functional research into the HSP70 chaperone complement of *P. falciparum* is still in its infancy, we have already begun to notice small but important differences between this parasite and other model systems. This knowledge has already been applied to identify small molecules which differentially inhibit PfHSP70s compared to their human counterparts. A deeper knowledge of the mechanistic peculiarities of PfHSP70s, especially with regard to their HSP40 co-chaperones, is likely to reveal further unusual features of this chaperone system which may be amenable to drug-design strategies. To this end, important progress is being made by recombinant expression and purification of all the PfHSP70s and potential PfHSP40s for functional assays. The reduced HSP70 complement of the parasite suggests that certain members have evolved to carry out a larger number of functions than their orthologs in other species. This may represent a "chaperone bottleneck" which can be targeted by selective inhibitor design.

Author Contributions

GB and JP contributed by drafting and critically revising the manuscript. MD contributed by conceptualizing and rendering **Figure 1** and critically revising the manuscript.

Acknowledgments

This work was supported by funding from the National Research Foundation of South Africa (GB) and the German Research Council Projects in Infectology (JP).

References

Acharya, P., Pallavi, R., Chandran, S., Chakravarti, H., Middha, S., Acharya, J., et al. (2009). A glimpse into the clinical proteome of human malaria parasites *Plasmodium falciparum* and *Plasmodium vivax*. *Proteomics Clin. Appl.* 3, 1314–1325. doi: 10.1002/prca.200900090

Adisa, A., Rug, M., Klonis, N., Foley, M., Cowman, A. F., and Tilley, L. (2003). The signal sequence of exported protein-1 directs the green fluorescent protein to the parasitophorous vacuole of transfected malaria parasites. *J. Biol. Chem.* 278, 6532–6542. doi: 10.1074/jbc.M207039200

Beck, J. R., Muralidharan, V., Oksman, A., and Goldberg, D. E. (2014). PTEX component HSP101 mediates export of diverse malaria effectors into host erythrocytes. *Nature* 511, 592–595. doi: 10.1038/nature13574

Bell, S. L., Chiang, A. N., and Brodsky, J. L. (2011). Expression of a malarial Hsp70 improves defects in chaperone-dependent activities in ssa1 mutant yeast. *PLoS ONE* 6:e20047. doi: 10.1371/journal.pone.0020047

Boddey, J. A., Hodder, A. N., Gunther, S., Gilson, P. R., Patsiouras, H., Kapp, E. A., et al. (2010). An aspartyl protease directs malaria effector proteins to the host cell. *Nature* 463, 627–631. doi: 10.1038/nature08728

Botha, M., Chiang, A. N., Needham, P. G., Stephens, L. L., Hoppe, H. C., Kulzer, S., et al. (2011). *Plasmodium falciparum* encodes a single cytosolic type I Hsp40 that functionally interacts with Hsp70 and is upregulated by heat shock. *Cell Stress Chaperones* 16, 389–401. doi: 10.1007/s12192-010-0250-6

Botha, M., Pesce, E. R., and Blatch, G. L. (2007). The Hsp40 proteins of *Plasmodium falciparum* and other apicomplexa: regulating chaperone power in the parasite and the host. *Int. J. Biochem. Cell Biol.* 39, 1781–1803. doi: 10.1016/j.biocel.2007.02.011

Chang, H. H., Falick, A. M., Carlton, P. M., Sedat, J. W., DeRisi, J. L., and Marletta, M. A. (2008). N-terminal processing of proteins exported by malaria parasites. *Mol. Biochem. Parasitol.* 160, 107–115. doi: 10.1016/j.molbiopara.2008.04.011

Chiang, A. N., Valderramos, J. C., Balachandran, R., Chovatiya, R. J., Mead, B. P., Schneider, C., et al. (2009). Select pyrimidinones inhibit the propagation of the malarial parasite, *Plasmodium falciparum*. *Bioorg. Med. Chem.* 17, 1527–1533. doi: 10.1016/j.bmc.2009.01.024

Cockburn, I. L., Boshoff, A., Pesce, E. R., and Blatch, G. L. (2014). Selective modulation of plasmodial Hsp70s by small molecules with antimalarial activity. *Biol. Chem.* 395, 1353–1362. doi: 10.1515/hsz-2014-0138

Cockburn, I. L., Pesce, E. R., Pryzborski, J. M., Davies-Coleman, M. T., Clark, P. G., Keyzers, R. A., et al. (2011). Screening for small molecule modulators of Hsp70 chaperone activity using protein aggregation suppression assays: inhibition of the plasmodial chaperone PfHsp70-1. *Biol. Chem.* 392, 431–438. doi: 10.1515/bc.2011.040

de Koning-Ward, T. F., Gilson, P. R., Boddey, J. A., Rug, M., Smith, B. J., Papenfuss, A. T., et al. (2009). A newly discovered protein export machine in malaria parasites. *Nature* 459, 945–949. doi: 10.1038/nature08104

Deponte, M., Hoppe, H. C., Lee, M. C., Maier, A. G., Richard, D., Rug, M., et al. (2012). Wherever I may roam: protein and membrane trafficking in *P. falciparum*-infected red blood cells. *Mol. Biochem. Parasitol.* 186, 95–116. doi: 10.1016/j.molbiopara.2012.09.007

Edkins, A. L., and Blatch, G. L. (2012). "Targeting conserved pathways as a strategy for novel drug development: disabling the cellular stress response," in *Drug Discovery in Africa,* eds K. Chibale, M. Davies-Coleman, and C. Masimirembwa (Berlin; Heidelberg: Springer), 85–99. doi: 10.1007/978-3-642-28175-4_4

Elsworth, B., Matthews, K., Nie, C. Q., Kalanon, M., Charnaud, S. C., Sanders, P. R., et al. (2014). PTEX is an essential nexus for protein export in malaria parasites. *Nature* 511, 587–591. doi: 10.1038/nature13555

Foth, B. J., Ralph, S. A., Tonkin, C. J., Struck, N. S., Fraunholz, M., Roos, D. S., et al. (2003). Dissecting apicoplast targeting in the malaria parasite *Plasmodium falciparum*. *Science* 299, 705–708. doi: 10.1126/science.1078599

Gehde, N., Hinrichs, C., Montilla, I., Charpian, S., Lingelbach, K., and Przyborski, J. M. (2009). Protein unfolding is an essential requirement for transport across the parasitophorous vacuolar membrane of *Plasmodium falciparum*. *Mol. Microbiol.* 71, 613–628. doi: 10.1111/j.1365-2958.2008.06552.x

Gitau, G. W., Mandal, P., Blatch, G. L., Przyborski, J., and Shonhai, A. (2012). Characterisation of the Plasmodium falciparum Hsp70-Hsp90 organising protein (PfHop). *Cell Stress Chaperones* 17, 191–202. doi: 10.1007/s12192-011-0299-x

Gruring, C., Heiber, A., Kruse, F., Flemming, S., Franci, G., Colombo, S. F., et al. (2012). Uncovering common principles in protein export of malaria parasites. *Cell Host Microbe* 12, 717–729. doi: 10.1016/j.chom.2012.09.010

Hagiwara, M., and Nagata, K. (2012). Redox-dependent protein quality control in the endoplasmic reticulum: folding to degradation. *Antioxid. Redox. Signal.* 16, 1119–1128. doi: 10.1089/ars.2011.4495

Hatherley, R., Blatch, G. L., and Bishop, O. T. (2014). *Plasmodium falciparum* Hsp70-x: a heat shock protein at the host-parasite interface. *J. Biomol. Struct. Dyn.* 32, 1766–1779. doi: 10.1080/07391102.2013.834849

Heiber, A., Kruse, F., Pick, C., Gruring, C., Flemming, S., Oberli, A., et al. (2013). Identification of new PNEPs indicates a substantial non-PEXEL exportome and underpins common features in *Plasmodium falciparum* protein export. *PLoS Pathog.* 9:e1003546. doi: 10.1371/journal.ppat.1003546

Heiny, S., Spork, S., and Przyborski, J. M. (2012). The apicoplast of the human malaria parasite *P. falciparum*. *J. Endocytobiosis Cell Res.* 23, 91–95.

Hempel, F., Bozarth, A., Sommer, M. S., Zauner, S., Przyborski, J. M., and Maier, U. G. (2007). Transport of nuclear-encoded proteins into secondarily evolved plastids. *Biol. Chem.* 388, 899–906. doi: 10.1515/BC.2007.119

Hiller, N. L., Bhattacharjee, S., van Ooij, C., Liolios, K., Harrison, T., Lopez-Estrano, C., et al. (2004). A host-targeting signal in virulence proteins reveals a secretome in malarial infection. *Science* 306, 1934–1937. doi: 10.1126/science.1102737

Joshi, B., Biswas, S., and Sharma, Y. D. (1992). Effect of heat-shock on *Plasmodium falciparum* viability, growth and expression of the heat-shock protein 'PFHSP70-I' gene. *FEBS Lett.* 312, 91–94. doi: 10.1016/0014-5793(92)81417-K

Külzer, S., Charnaud, S., Dagan, T., Riedel, J., Mandal, P., Pesce, E. R., et al. (2012). *Plasmodium falciparum*-encoded exported hsp70/hsp40 chaperone/co-chaperone complexes within the host erythrocyte. *Cell. Microbiol.* 14, 1784–1795. doi: 10.1111/j.1462-5822.2012.01840.x

Külzer, S., Rug, M., Brinkmann, K., Cannon, P., Cowman, A., Lingelbach, K., et al. (2010). Parasite-encoded Hsp40 proteins define novel mobile structures in the cytosol of the *P. falciparum*-infected erythrocyte. *Cell. Microbiol.* 12, 1398–1420. doi: 10.1111/j.1462-5822.2010.01477.x

Kumar, N., Koski, G., Harada, M., Aikawa, M., and Zheng, H. (1991). Induction and localization of *Plasmodium falciparum* stress proteins related to the heat shock protein 70 family. *Mol. Biochem. Parasitol.* 48, 47–58. doi: 10.1016/0166-6851(91)90163-Z

Kumar, N., Syin, C. A., Carter, R., Quakyi, I., and Miller, L. H. (1988). *Plasmodium falciparum* gene encoding a protein similar to the 78-kDa rat glucose-regulated stress protein. *Proc. Natl. Acad. Sci. U.S.A.* 85, 6277–6281. doi: 10.1073/pnas.85.17.6277

Kumar, N., and Zheng, H. (1992). Nucleotide sequence of a *Plasmodium falciparum* stress protein with similarity to mammalian 78-kDa glucose-regulated protein. *Mol. Biochem. Parasitol.* 56, 353–356. doi: 10.1016/0166-6851(92)90187-O

Maier, A. G., Rug, M., O'Neill, M. T., Brown, M., Chakravorty, S., Szestak, T., et al. (2008). Exported proteins required for virulence and rigidity of *Plasmodium falciparum*-infected human erythrocytes. *Cell* 134, 48–61. doi: 10.1016/j.cell.2008.04.051

Marti, M., Good, R. T., Rug, M., Knuepfer, E., and Cowman, A. F. (2004). Targeting malaria virulence and remodeling proteins to the host erythrocyte. *Science* 306, 1930–1933. doi: 10.1126/science.1102452

Matambo, T. S., Odunuga, O. O., Boshoff, A., and Blatch, G. L. (2004). Overproduction, purification, and characterization of the *Plasmodium falciparum* heat shock protein 70. *Protein Expr. Purif.* 33, 214–222. doi: 10.1016/j.pep.2003.09.010

Misra, G., and Ramachandran, R. (2009). Hsp70-1 from *Plasmodium falciparum*: protein stability, domain analysis and chaperone activity. *Biophys. Chem.* 142, 55–64. doi: 10.1016/j.bpc.2009.03.006

Muralidharan, V., Oksman, A., Pal, P., Lindquist, S., and Goldberg, D. E. (2012). *Plasmodium falciparum* heat shock protein 110 stabilizes the asparagine repeat-rich parasite proteome during malarial fevers. *Nat. Commun.* 3, 1310. doi: 10.1038/ncomms2306

Njunge, J. M., Ludewig, M. H., Boshoff, A., Pesce, E. R., and Blatch, G. L. (2013). Hsp70s and J proteins of *Plasmodium* parasites infecting rodents and primates: structure, function, clinical relevance, and drug targets. *Curr. Pharm. Des.* 19, 387–403. doi: 10.2174/138161213804143734

Njunge, J. M., Mandal, P., Przyborski, J. M., Boshoff, A., Pesce, E. R., and Blatch, G. L. (2015). PFB0595w is a *Plasmodium falciparum* J protein that co-localizes with PfHsp70-1 and can stimulate its *in vitro* ATP hydrolysis activity. *Int. J. Biochem. Cell Biol.* 62, 47–53. doi: 10.1016/j.biocel.2015.02.008

Pesce, E. R., Acharya, P., Tatu, U., Nicoll, W. S., Shonhai, A., Hoppe, H. C., et al. (2008). The *Plasmodium falciparum* heat shock protein 40, Pfj4, associates with heat shock protein 70 and shows similar heat induction and localisation patterns. *Int. J. Biochem. Cell Biol.* 40, 2914–2926. doi: 10.1016/j.biocel.2008.06.011

Pesce, E. R., and Blatch, G. L. (2014). Plasmodial Hsp40 and Hsp70 chaperones: current and future perspectives. *Parasitology* 141, 1167–1176. doi: 10.1017/S003118201300228X

Pesce, E. R., Cockburn, I. L., Goble, J. L., Stephens, L. L., and Blatch, G. L. (2010). Malaria heat shock proteins: drug targets that chaperone other drug targets. *Infect. Disord. Drug Targets* 10, 147–157. doi: 10.2174/187152610791163417

Ramya, T. N., Surolia, N., and Surolia, A. (2006). 15-Deoxyspergualin modulates *Plasmodium falciparum* heat shock protein function. *Biochem. Biophys. Res. Commun.* 348, 585–592. doi: 10.1016/j.bbrc.2006.07.082

Russo, I., Babbitt, S., Muralidharan, V., Butler, T., Oksman, A., and Goldberg, D. E. (2010). Plasmepsin V licenses *Plasmodium* proteins for export into the host erythrocyte. *Nature* 463, 632–636. doi: 10.1038/nature08726

Sargeant, T. J., Marti, M., Caler, E., Carlton, J. M., Simpson, K., Speed, T. P., et al. (2006). Lineage-specific expansion of proteins exported to erythrocytes in malaria parasites. *Genome Biol.* 7:R12. doi: 10.1186/gb-2006-7-2-r12

Sato, S. (2011). The apicomplexan plastid and its evolution. *Cell. Mol. Life Sci.* 68, 1285–1296. doi: 10.1007/s00018-011-0646-1

Shonhai, A. (2010). Plasmodial heat shock proteins: targets for chemotherapy. *FEMS Immunol. Med. Microbiol.* 58, 61–74. doi: 10.1111/j.1574-695X.2009.00639.x

Shonhai, A., Boshoff, A., and Blatch, G. L. (2005). *Plasmodium falciparum* heat shock protein 70 is able to suppress the thermosensitivity of an *Escherichia coli* DnaK mutant strain. *Mol Genet. Genomics* 274, 70–78. doi: 10.1007/s00438-005-1150-9

Shonhai, A., Boshoff, A., and Blatch, G. L. (2007). The structural and functional diversity of Hsp70 proteins from *Plasmodium falciparum*. *Protein Sci.* 16, 1803–1818. doi: 10.1110/ps.072918107

Shonhai, A., Botha, M., de Beer, T. A., Boshoff, A., and Blatch, G. L. (2008). Structure-function study of a *Plasmodium falciparum* Hsp70 using three dimensional modelling and *in vitro* analyses. *Protein Pept. Lett.* 15, 1117–1125. doi: 10.2174/092986608786071067

Shonhai, A., Maier, A. G., Przyborski, J. M., and Blatch, G. L. (2011). Intracellular protozoan parasites of humans: the role of molecular chaperones in development and pathogenesis. *Protein Pept. Lett.* 18, 143–157. doi: 10.2174/092986611794475002

Spielmann, T., and Gilberger, T. W. (2010). Protein export in malaria parasites: do multiple export motifs add up to multiple export pathways? *Trends Parasitol.* 26, 6–10. doi: 10.1016/j.pt.2009.10.001

Spork, S., Hiss, J. A., Mandel, K., Sommer, M., Kooij, T. W., Chu, T., et al. (2009). An unusual ERAD-like complex is targeted to the apicoplast of *Plasmodium falciparum*. *Eukaryot. Cell* 8, 1134–1145. doi: 10.1128/EC.00083-09

Tuteja, R. (2007). Unraveling the components of protein translocation pathway in human malaria parasite *Plasmodium falciparum*. *Arch. Biochem. Biophys.* 467, 249–260. doi: 10.1016/j.abb.2007.08.031

World Health Organisation. (2014). *World Malaria Report 2014*. Geneva: World Health Organisation.

Yung, S. C., Unnasch, T. R., and Lang-Unnasch, N. (2003). Cis and trans factors involved in apicoplast targeting in *Toxoplasma gondii*. *J. Parasitol.* 89, 767–776. doi: 10.1645/GE-88R

Zimmermann, R., and Blatch, G. L. (2009). A novel twist to protein secretion in eukaryotes. *Trends Parasitol.* 25, 147–150. doi: 10.1016/j.pt.2009.01.002

Zininga, T., Achilonu, I., Hoppe, H., Prinsloo, E., Dirr, H. W., and Shonhai, A. (2015). Overexpression, purification and characterisation of the *Plasmodium falciparum* Hsp70-z (PfHsp70-z). *PLoS ONE* 10:e0129445. doi: 10.1371/journal.pone.0129445

Zininga, T., and Shonhai, A. (2014). Are heat shock proteins druggable candidates. *Am. J. Biochem. Biotechnol.* 10, 209–210. doi: 10.3844/ajbbsp.2014.208.210

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read, for greatest visibility

COLLABORATIVE PEER-REVIEW
Designed to be rigorous – yet also collaborative, fair and constructive

FAST PUBLICATION
Average 85 days from submission to publication (across all journals)

COPYRIGHT TO AUTHORS
No limit to article distribution and re-use

TRANSPARENT
Editors and reviewers acknowledged by name on published articles

SUPPORT
By our Swiss-based editorial team

IMPACT METRICS
Advanced metrics track your article's impact

GLOBAL SPREAD
5'100'000+ monthly article views and downloads

LOOP RESEARCH NETWORK
Our network increases readership for your article

Frontiers
EPFL Innovation Park, Building I • 1015 Lausanne • Switzerland
Tel +41 21 510 17 00 • Fax +41 21 510 17 01 • info@frontiersin.org
www.frontiersin.org

Find us on