GOLGI PATHOLOGY IN NEURODEGENERATIVE DISEASES

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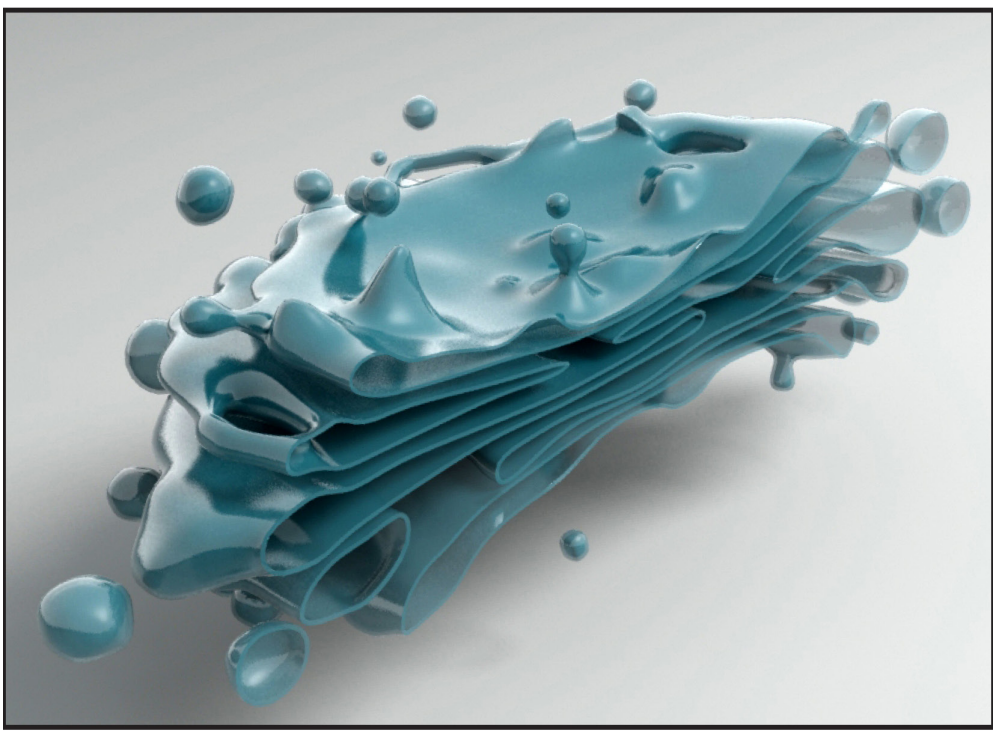

"Memory Foam Pullover" (top and cover) by textil designer Nadine Goepfert (collection "The Garments May Vary", 2013) shows resemblance to the Golgi apparatus. Website http://nadinegoepfert.com

3D modeling of the Golgi apparatus (bottom) by biomed expert Dr. Vicente Díaz-Martínez (Web4Bio company) depicts stacked membrane compartments and vesicles associated with the Golgi.
Website http://web4bio.com/en/3d-scientific.html

GOLGI PATHOLOGY IN NEURODEGENERATIVE DISEASES

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The Golgi apparatus is a central organelle that lies at the heart of the secretory pathway. It ensures post-translational protein modifications such as glycosylation and cleavage as well as protein sorting to neuronal axons and dendrites.

Structural and functional alterations of the Golgi apparatus (fragmentation and atrophy), which are collectively termed Golgi pathology, are now recognized as a constant feature of many neurodegenerative diseases. However, the molecular mechanisms underlying these changes and their precise relevance to neurodegeneration have not yet been completely elucidated.

This eBook contains 13 reviews that address the molecular mechanisms of Golgi pathology in Parkinson and Alzheimer diseases, amyotrophic lateral sclerosis (ALS) and spinal muscular atrophies, and discuss their potential relevance to the pathological loss of neuronal cell bodies, axons and synapses.

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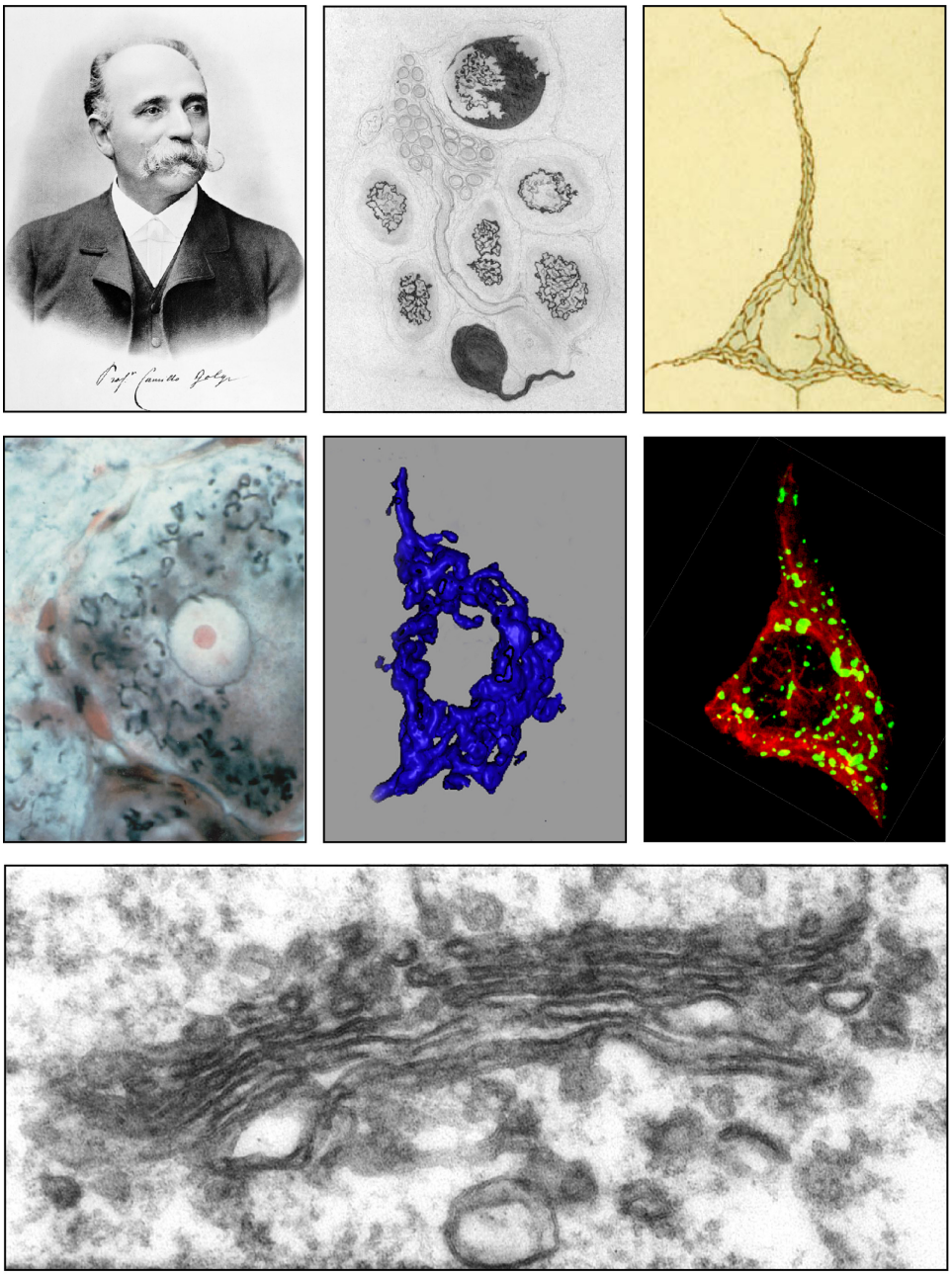

The Golgi apparatus in neurons (panels from upper left to lower right).

Portrait of Camillo Golgi around 1905.

Internal reticular apparatus in ganglion cells partially stained with the black reaction. Original drawing by Camillo Golgi.

Golgi apparatus in nerve cells of mouse cerebral cortex. Original drawing preserved with Golgi's papers.

Original preparation from Golgi's lab showing dorsal root ganglion neurons stained by the black reaction.

3D surface modeling of the Golgi apparatus in a mouse spinal cord motor neuron after immunolabeling for the MG160 glycoprotein (in blue).

Fragmented Golgi apparatus in a cultured NSC-34 motor neuron several minutes after washout of the microtubule-disrupting drug Nocodazole. Golgi elements are labeled by GFP-tagged Mannosidase II (in green) and regrowing microtubules by antibodies against α-tubulin (in red).

Electron microscopy of stacked Golgi membranes and vesicles in a mouse spinal cord motor neuron.

The original Golgi drawings are from the Sistema Museale di Ateneo (Museum for the History of the University of Pavia, Pavia, Italy) and reproduced with the kind permission of Paolo Mazzarello. The immunolabeling and 3D modeling images are from Sarah Bellouze and Georg Haase at Institut de Neurosciences de la Timone, Marseille, France. The electron microscopy image is from Catherine Rabouille at Hubrecht Institute, Utrecht, Netherlands.

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EDITORIAL
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Editorial: Golgi Pathology in Neurodegenerative Diseases

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Keywords: Golgi apparatus, neurodegenerative disease, microtubules, vesicle trafficking, cell death, signaling, axon degeneration, cellular stress

The Editorial on the Research Topic

Golgi Pathology in Neurodegenerative Diseases

The Golgi apparatus is a central organelle that lies at the heart of the secretory pathway sustaining the delivery of proteins from their site of synthesis in the endoplasmic reticulum to their final destination, the extracellular medium, the plasma membrane, and the endo-lysosomal system. It ensures post-translational protein modifications such as glycosylation and proteolytic cleavage and processing and acts as a sorting device including to neuronal axons and dendrites (Horton and Ehlers, 2003; Ye et al., 2007).

The mammalian Golgi apparatus was first described by Camillo Golgi in 1998 as "apparato reticolare interno," "a fine and elegant network within the cell body …completely internal in the nerve cells" (Golgi, 1898a,b). This large reticulum comprises stacks of flattened membrane bound compartments called cisternae which are laterally linked to form the so-called Golgi ribbon.

Structural and functional alterations of the Golgi apparatus, which are here collectively termed Golgi pathology, are now recognized as a constant pathological hallmark of various neurodegenerative diseases including amyotrophic lateral sclerosis (ALS), Parkinson, Alzheimer, Huntington, and prion diseases (Fan et al., 2008). In ALS, structural Golgi alterations have been revealed by the pioneering work of Gonatas and colleagues (Mourelatos et al., 1990; Gonatas et al., 1992; Fujita et al., 2002). They manifest as fragmentation—transformation of the Golgi ribbon into disconnected stacks, cisternae, tubules and vesicles, and as atrophy—loss of Golgi membrane material.

These morphological changes are often accompanied by functional Golgi alterations, such as those affecting the anterograde and retrograde transport in the early secretory pathway, both in cellular models of Parkinson (Cooper et al., 2006; Cho et al., 2014), Huntington (Caviston et al., 2007; Pardo et al., 2010), and Alzheimer (Annaert et al., 1999; Joshi et al., 2014) diseases as well as in ALS (Stieber et al., 2004; Soo et al., 2015).

At least in ALS, Golgi pathology manifests as an early pre-clinical feature in degenerating neurons both in affected patients and in animal models (Mourelatos et al., 1996), suggesting that it may be relevant to the disease process instead of just representing an epiphenomenon. Yet, neither the molecular mechanisms underlying the changes in the functional organization of the Golgi apparatus nor their precise relevance to neurodegeneration have yet been completely elucidated.

These important questions got a new boost by the discovery of mutations in genes encoding Golgi-related proteins as direct causes of neurodegeneration. For instance, mutations in Optineurin (Maruyama et al., 2010), VPS54/wobbler (Schmitt-John et al., 2005), and TBCE/pmn (Martin et al., 2002) have been identified in ALS and related motor neuron diseases. Furthermore, mutations in the Parkinson disease-associated proteins α-Synuclein (Cooper et al., 2006;

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Thayanidhi et al., 2010), LRRK2 (Lin et al., 2009; Cho et al., 2014), Parkin (Shimura et al., 1999; Kubo et al., 2001), and VPS35 (McGough et al., 2014; Zavodszky et al., 2014; Malik et al., 2015) have been shown to affect Golgi structure or transport processes to and from the Golgi.

Furthermore, the recognition of Golgi-derived microtubules and their specific functions, the better understanding of Golgi transport processes, the recognition of the Golgi apparatus as a sensor of cellular stress and as trigger of Golgi-specific cell death pathways provide new hints to the molecular mechanisms underlying Golgi pathology.

To cover these emerging themes, this Frontiers Research Topic is organized as follows. The issue starts with a summary on Golgi functional organization in neurons (Valenzuela and Perez) and the relation of this organelle with microtubules (Sanders and Kaverina).

This is followed by pathological, genetic, and mechanistic descriptions of the major neurodegenerative diseases including Parkinson disease (Wang and Hay), Alzheimer disease by Wang and colleagues (Joshi et al.) and ALS by Atkin and colleagues (Sundaramoorthy et al.). The Research topic then focuses on Golgi fragmentation brought about by defects in vesicle biogenesis and dynamics to and through the Golgi by Lupashin and colleagues (Climer et al.) and by Schmitt-John, including those caused by defects in Golgi-derived microtubules in ALS (Haase and Rabouille) and microtubule-dependent motors in proximal SMA (Jaarsma and Hoogenraad; Wirth and Martinez-Carrera).

The third part of this issue starts by posing the hypothesis of Farhan and colleagues that cellular stress can be the cause of Golgi fragmentation, which in turn amplifies cellular stress and leads to neurodegeneration (Alvarez-Miranda et al.). This is argued by reviews on the effect of DNA damage on the Golgi by Field and colleagues (Buschman et al.) and on the role of the Golgi as a cell death trigger (Machamer).

Future studies on Golgi pathology in neurodegenerative diseases will continue to benefit not only from conceptual advances but also from new technical developements that have been gigantic since Golgi's original description of the black reaction (tissue hardening with potassium dichromate and cell staining by silver impregnation) (Mazzarello et al., 2009).

Electron microscopy has been used to unravel Golgi fragmentation (Mourelatos et al., 1996) and in particular the Golgi fragmentation into tubules and vesicles observed in degenerating motor neurons (Bellouze et al., 2014), and its resolution may be further improved in tissues prepared by high pressure freezing (Walther et al., 2013). 3D reconstructions of the Golgi and its microtubules (Marsh et al., 2001; Efimov et al., 2007) may illustrate pathological changes in their intricate connections.

Golgi fragmentation can also be monitored by live imaging (Altan-Bonnet et al., 2006), and super resolution microscopy (Betzig et al., 2006; Lippincott-Schwartz and Manley, 2009) may help refining the process. Last, system biology approches may shed light on new pathways connecting Golgi fragmentation to neurodegeneration by identifying novel gene networks (Alvarez-Miranda et al.).

However, this field faces further challenges. It will be crucial to determine whether Golgi pathology is contributory, causative or homeostatic in neurodegeneration. In particular, it is important to understand whether Golgi alterations are linked to axonal degeneration and synapse loss or dysfunction.

It will also be crucial to analyze whether Golgi pathology in each neurodegenerative disease is restricted to the neuron types that are specifically affected, i.e., motor neurons in ALS, dopaminergic neurons in PD, striatal neurons in Huntington. If so, what may be the corresponding mechanisms of vulnerability and resistance?

Furthermore, we will need to determine whether Golgi alterations in degenerating neurons impact on the function of their non-neuronal cellular neighbors, including astrocytes, microglia and Schwann cells. Can this provide a potential explanation for the non-cell autonomous disease spread observed in numerous neurodegenerative diseases?

Finally and most importantly, can our burgeoning knowledge on the molecular mechanisms of Golgi pathology in neurodegenerative diseases be translated into earlier disease diagnosis and new therapies for these severe and hitherto untreatable disorders?

AUTHOR CONTRIBUTIONS

CR and GH prepared and wrote the manuscript.

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MINI REVIEW
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Diversifying the secretory routes in neurons

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Nervous system homeostasis and synaptic function need dedicated mechanisms to locally regulate the molecular composition of the neuronal plasma membrane and allow the development, maintenance and plastic modification of the neuronal morphology. The cytoskeleton and intracellular trafficking lies at the core of all these processes. In most mammalian cells, the Golgi apparatus (GA) is at the center of the biosynthetic pathway, located in the proximity of the microtubule-organizing center. In addition to this central localization, the somatic GA in neurons is complemented by satellite Golgi outposts (GOPs) in dendrites, which are essential for dendritic morphogenesis and are emerging like local stations of membranes trafficking to synapses. Largely, GOPs participation in post-ER trafficking has been determined by imaging the transport of the exogenous protein VSVG. Here we review the diversity of neuronal cargoes that traffic through GOPs and the assortment of different biosynthetic routes to synapses. We also analyze the recent advances in understanding the role of cytoskeleton and Golgi matrix proteins in the biogenesis of GOPs and how the diversity of secretory routes can be generated.

Keywords: secretory route, endoplasmic reticulum, Golgi apparatus, Golgi outposts, protein trafficking, neuronal trafficking

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Introduction

The directional relay of information in the nervous system requires morphologically asymmetric synaptic contacts. Neurons are highly polarized cells composed of two major functional domains: the somatodendritic compartment, responsible for receiving signals, and the axonal compartment, responsible of transmitting information. In addition, neurons develop a diversity of functional and morphologic subdomains (e.g., dendritic spines, synaptic buttons, Ranvier nodes, etc.), which implies that, throughout their lifetime, neurons need to precisely control their local molecular composition. Neurons mature from small symmetrical progenitors and increase by more than 200 folds their plasma membrane area to reach cellular surfaces more than 10,000 times bigger than a typical epithelial cell (Horton and Ehlers, 2004). The intracellular machinery involved in the acquisition, maintenance and plastic modification of neuronal morphology depends on the proper coordination of the cytoskeleton and the intracellular membrane trafficking.

During neuronal development, fundamental processes such as axonal specification and differential outgrowth of dendrites and axons critically depend on the supply of proteins and lipids through the secretory route. It is composed of well-organized compartments that include the endoplasmic reticulum (ER), the ER-to-Golgi intermediate compartment (ERGIC), the Golgi apparatus (GA) and the trans-Golgi network (TGN), in addition to membrane-bound intermediates that allow transport between compartments in a sequential and finely controlled way. In morphologically non-differentiated neurons, the selective supply of post-Golgi

vesicles to one particular neurite precedes its specification as the future axon (Bradke and Dotti, 1997). Perturbing the secretory pathway modulates axonal growth. For example, inhibiting the activity of the Sar1 small GTPase, a primary component for the COPII-mediated vesicular export from ER, generates neurons with smaller axons while its overexpression produces neurons with longer axons (Aridor and Fish, 2009). After axon specification, inhibition of the secretory pathway by expressing a GTPase deficient mutant of the Arf1 small GTPase, or by blocking the post-Golgi trafficking using a kinase-dead mutant of protein kinase D1 (PKD1), results in a decrease of dendrites outgrowth (Horton et al., 2005). Interestingly, a genetic screen carried out in *Drosophila* identified the homologs of *Sec23*, *Sar1*, and *Rab1* as essentials for dendritic arbors outgrowth, but not for axons (Ye et al., 2007). These proteins are important for the ER-to-Golgi transport mediated by COPII vesicles, hence revealing a differential susceptibility of dendrites and axons to perturbations of membrane traffic during development. Inhibition of the secretory pathway in mature neurons still decreases the average total dendritic length, indicating it is required to maintain the dendritic arbor (Horton et al., 2005).

The number and density of neurotransmitter receptors control the potency of synapses. Neurotransmitter receptors supply thus needs to be regulated with a high spatiotemporal precision (Kennedy and Ehlers, 2006). Although endocytosis and recycling of synaptic receptors has been extensively studied, little is known about their site of synthesis and secretory transport. In this context, two neighbor synapses, separated by a few micrometers, may present a very different protein landscape at steady state. For example, one single spine contains between several tens to several hundreds of glutamate receptors. Thus, addition or removal of just a few receptors from the synaptic surface may be enough to elicit changes in the neurotransmission (Newpher and Ehlers, 2008) indicating that a tight control of secretion and endocytosis has to be established. Indeed, the long-term potentiation and the NMDA-induced increase of AMPA receptors (AMPARs) expressed at the plasma membrane directly depend on the secretory transport of AMPARs (Broutman and Baudry, 2001), highlighting the relevance of intracellular trafficking in neuronal physiology.

Here, we analyze the particular organization of the secretory pathway in neurons, the different possibilities of cargo trafficking that it offers and review recent evidences that help to understand how this diversity is generated.

Organization of Secretory Routes in Dendrites

In neurons, the general principles underlying the control of the secretory pathway applies, but the arrangement of secretory organelles presents unique particularities in terms of the enormous distances involved and the distribution of these organelles, particularly the GA (Horton and Ehlers, 2004; Ramírez and Couve, 2011).

As in any eukaryotic cell, the starting point of the secretory route is the ER, where the synthesis of most of membrane and secreted proteins occurs. Electron microscopy (EM) studies have reported the presence of a continuous endomembrane network of ER that spans the neuronal arborescence including soma, dendrites, axons, and in some cases reaching the inner of dendritic spines (Tsukita and Ishikawa, 1976; Broadwell and Cataldo, 1983; Spacek and Harris, 1997; Gardiol et al., 1999). Regions with a higher complexity of ER network have been described at dendritic branch points and near dendritic spines (Cui-Wang et al., 2012). The ER found in the soma is mainly composed of sheets of ribosome-decorated rough ER, while in dendrites the ER is constituted mostly by tubules of smooth ER running in parallel to the dendritic shaft with only few ribosomes attached (Broadwell and Cataldo, 1983; Martone et al., 1993; Krijnse-Locker et al., 1995; Spacek and Harris, 1997; Cooney et al., 2002). mRNAs translation of transmembrane proteins have been observed in dendrites and specialized compartments such as ERES, have been shown to be functional in the dendritic arbor (Gardiol et al., 1999; Aridor et al., 2004; Holt and Schuman, 2013).

The ERGIC is composed of long-lived structures that constitute sorting stations of anterograde and retrograde cargoes interconnected by highly mobile short-lived elements (Ben-Tekaya et al., 2005; Appenzeller-Herzog and Hauri, 2006). Several ERGIC markers are present in dendrites (Krijnse-Locker et al., 1995; Torre and Steward, 1996; Gardiol et al., 1999) forming stationary and mobile tubulo-vesicular structures whose distribution reaches territories distant from the soma (Hanus et al., 2014).

The GA is the main station of posttranslational modification, maturation and sorting. It consists of a polarized arrangement of stacked cisternae where occurs transport of proteins in lipids emanating from the ER and destined to post-Golgi station and the simultaneous retrograde flow of cargoes toward the ER (Farquhar and Palade, 1998; Johannes and Popoff, 2008). In neurons, as in many animal cells, the GA is found in a perinuclear area. In addition, a set of satellite Golgi outposts (GOPs) is present in around 18% of dendrites of matures neurons (Horton et al., 2005). 70–80% of mature hippocampal neurons display GOPs, which are preferentially localized to first-order segment of the apical dendrite in the cortex or to its corresponding major dendrite in hippocampal neurons in culture (Horton and Ehlers, 2003; Horton et al., 2005; Quassollo et al., 2015). They are discrete structures, discontinuous with the somatic Golgi, and some of them are composed of stacked cisternae (Horton and Ehlers, 2004; Pierce et al., 2001). Markers of *cis*, *medial*, and *trans* Golgi compartments have been detected in dendrites by immunofluorescence and EM (Pierce et al., 2001; Horton et al., 2005). Additionally, ER- and Golgi-dependent protein glycosylation can be carried out in isolated dendrites (Torre and Steward, 1996). Recent evidences point to the existence of punctate single-compartment GOPs (scGOPs) in dendritic shafts of *Drosophila* neurons *in vivo* in which *cis, medial, and trans* cisternae are thought to be disconnected from each other. This contrasts to multi-compartment GOPs (mcGOPs), localized in both shafts and dendritic branch points, which are organized as stacks in a GM130-dependent manner (Zhou et al., 2014). Further research will be needed to decipher whether

the proposed scGOPs are transport carriers cycling within the secretory pathway or effectively correspond to *bona fide* GOPs.

The overall organization of secretory organelles in neurons allows the co-existence of two major routes for the secretory trafficking of proteins and membranes in dendrites, namely canonical and local routes that differ in the alternative usage of somatic GA or GOPs, respectively (**Figure 1**; Horton and Ehlers, 2003; Ramírez and Couve, 2011). The canonical trafficking is similar to the trafficking occurring in non-neuronal cells: newly synthesized secretory proteins are transported from the ER to the perinuclear GA where they mature to be sorted in post-Golgi intermediates to their insertion/secretion sites in later compartments (the plasma membrane, endosomes, lysosomes, etc.). In the local route, nascent secretory proteins are locally translated into dendritic ER (dER) structures, or transported inside the dER to peripheral sites. There, they are transported from the dER to satellite GOPs, hence establishing a local trafficking route for these cargoes in the dendritic compartment.

Diversity of Secretory Routes and Cargoes in Neurons

ER-to-Golgi transport has been classically studied using the thermosensitive mutant of VSVG (VSVG^{ts045}), a viral glycoprotein which is retained within the ER at the restrictive temperature of 39.5°C. Switching to the permissive temperature of 32°C enables its rapid export from the ER and synchronous

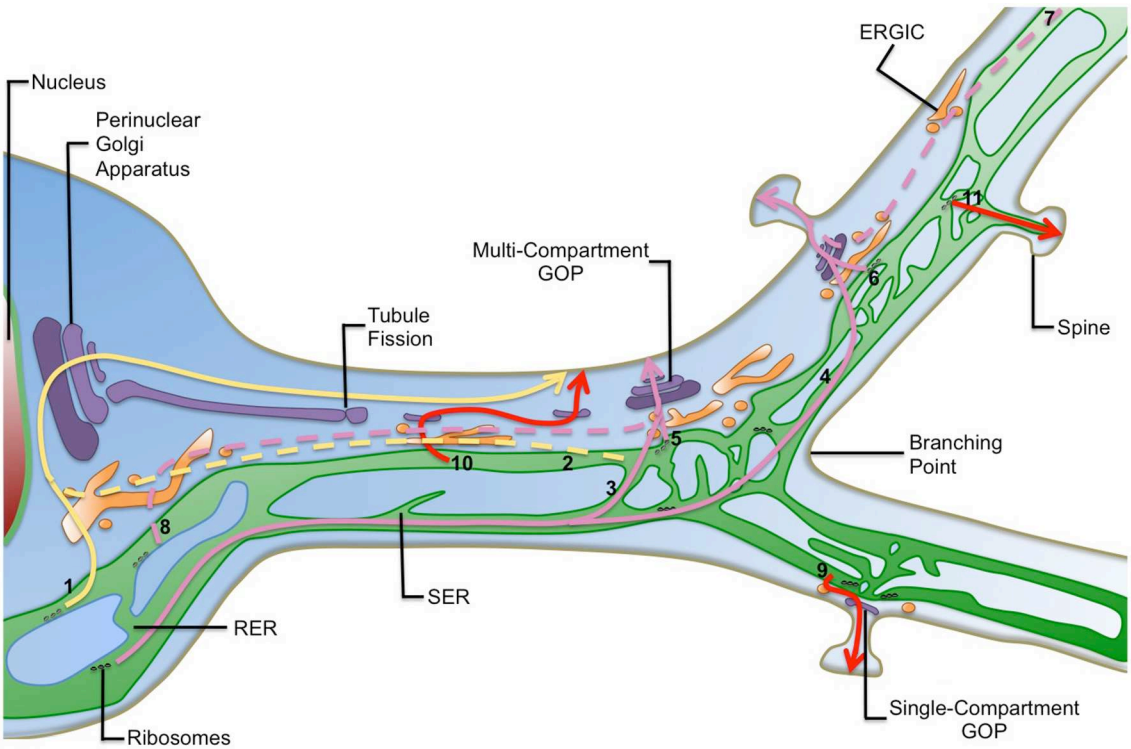

FIGURE 1 | Diversity of the secretory routes in dendrites. Known and suspected routes to the plasma membrane in dendrites are shown. For simplicity post-Golgi carriers are not shown. In the canonical secretory route (yellow arrows) cargoes can be synthetized and exported from the somatic endoplasmic reticulum (ER) and transported through the ER-to-Golgi intermediate compartment (ERGIC) in the cell body to the perinuclear Golgi apparatus (1). Alternatively they can be exported distally in the dendritic ER and use long-haul ER-to-Golgi transport (2, dashed lines) to reach the somatic Golgi apparatus. Several possibilities of local secretory trafficking coexist in neurons (pink arrows): protein cargoes can be synthetized within the rough endoplasmic reticulum (RER) in somas and be transported over long-distances through the dendritic smooth endoplasmic reticulum (SER). They can then be preferentially exported from the ER at dendritic branching points (3) or synapses (4) where local zones of ER complexity confine cargo mobility and would favor secretion through GOPs. Cargo proteins can be also synthetized in ER-associated ribosomes in dendrites and transported locally using satellite GOPs. Export to GOPs will occur mainly at dendritic branching points (5) and synaptic contacts (6) where they are enriched and seems to support the dynamics of dendritic arborization and the synaptic delivery of neurotransmitter receptors respectively. Additionally, cargoes can use long-haul ER-to-Golgi transport before reaching GOPs (pink dashed lines). Subsequently, synaptic activity may restrict the scale of post-ER trafficking. Therefore, cargoes can be exported from the dendritic ER far away from GOPs (7) or from the somatic ER and travel in post-ER carriers to GOPs (8). Some more hypothetical secretory routes are also depicted (red arrows): the presence of single-compartment GOPs raises the possibility that particular cargoes may use only one Golgi compartment (*cis*, *medial*, or *trans*) in their way to the plasma membrane (9). Alternatively, they may sequentially use separated Golgi compartments before reaching their final destination (10). These options may increase the diversity of posttranslational modifications patterns of cargoes. Additionally, the ER dynamically explores the neck of dendritic spines, suggesting that cargoes may be transferred directly or indirectly from the ER to synapses within spines (11). Indeed, a specialization of the ER, called spine apparatus, has been reported in spines. Finally, GOPs formation may be driven by fission from the perinuclear Golgi, which would then be transported to remote areas, allowing the transport of large cargo loads within fissioned Golgi stacks to distal dendrites.

transport to the cell surface (Presley et al., 1997; Scales et al., 1997). In neurons, live-cell imaging have shown that GFP-tagged $VSVG^{ts045}$ is exported from the dER and a fraction of post-ER carriers are fused with GOPs (Horton and Ehlers, 2003), suggesting that protein processing and secretion can be carried out far from the soma. Importantly, blocking Golgi export at 20°C accumulates $VSVG^{ts045}$ in dendritic branching points in primary neurons (Horton et al., 2005; Cui-Wang et al., 2012). Mannosidase II-positive GOPs are enriched in dendritic branching points of *Drosophila* neurons *in vivo* and their photo-ablation inhibits the dynamics of extension and retraction of dendritic branches. This suggests that GOPs locally support the membrane turnover required for dendritic remodeling (Ye et al., 2007), although this may also be explained by an indirect effect on microtubules polymerization in dendritic branching points (Ori-McKenney et al., 2012; Nguyen et al., 2014; Zhou et al., 2014). Nevertheless, in mammalian neurons, Golgi-dependent trafficking supports dendrites outgrowth and the complete biosynthetic machinery is enriched in dendritic branch points (Cui-Wang et al., 2012; Horton et al., 2005). Indeed, ER and Golgi export of $VSVG^{ts045}$ occurs preferentially in dendritic branching points (Cui-Wang et al., 2012; Horton et al., 2005) and local ER-release of a light-controlled VSVG leads to its accumulation within branch points, presumably in GOPs (Chen et al., 2013). Moreover, dendritic branching points represent hot spots of exocytosis for VAMP2 (Cui-Wang et al., 2012).

$VSVG^{ts045}$ has been essential to unravel dendritic secretory routes, but it is an exogenous protein that represents only one category of cargo, limiting further physiological interpretations. Diverse cargo proteins needs dedicated routes to specifically manage their transport (Boncompain and Perez, 2013). For example, in contrast to $VSVG^{ts045}$, the voltage-gated potassium channel Kv4.2, which is crucial for the repolarization phase of action potentials, binds to its auxiliary subunit KChIP1 and traffic in COPI-dependent and COPII-independent post-ER carriers (Hasdemir et al., 2005). In hippocampal neurons, KChIP1 accumulates in GOPs (Hasdemir et al., 2005), highlighting the relevance of studying not only model cargos but also neuronal relevant proteins. Another neuronal cargo is the brain-derived neurotrophic factor (BDNF), which has major roles in neuron survival, differentiation, dendritic outgrowth, synaptic formation, and memory. At 20°C BDNF localizes in both the somatic Golgi and GOPs (Horton and Ehlers, 2003) suggesting that remote BDNF secretion could locally regulate synaptic remodeling in nearby dendritic territories.

Biosynthesis at- and transport through- the dER is closely linked to local trafficking, constituting an early stage of protein sorting and synaptic regulation of membrane trafficking (Aridor et al., 2004; Jeyifous et al., 2009; Ramírez and Couve, 2011; Cui-Wang et al., 2012; Valenzuela et al., 2014). An example is the subunit α7 of nicotinic acetylcholine receptor, the trafficking of which can be locally coordinated in dendrites ER: at high levels, the Ric3 chaperone promotes ER retention and transport of α7-receptors within the dER, while at low levels it stimulates the assembly, ER release and surface expression of α7-receptors (Alexander et al., 2010). Another example is the $GABA_B$ receptor ($GABA_BR$), an heterodimeric G protein-coupled receptor (GPCRs) that regulates the slow and tonic inhibition in the brain. The $GABA_{B1}$ subunit has an ER retention signal (Margeta-Mitrovic et al., 2000), which allows its long-range transport through the dER by a mechanism that, in addition to diffusion, involves microtubule-dependent transport (Ramírez et al., 2009; Valenzuela et al., 2014). The $GABA_BR$ traffics through both somatic and dendritic Golgi to the somatodendritic membrane (Valenzuela et al., 2014). Visualization of synchronized ER-to-Golgi trafficking of a $GABA_{B1}$ mutant that lacks of the ER retention signals, revealed carriers traveling over a long distance prior to fusion with GOPs, suggesting that local dER export does not necessarily determine a local trafficking through GOPs and that different spatial ranges of ERGIC transport can regulate the local protein supply (Hanus et al., 2014; Valenzuela et al., 2014).

It has been proposed that bidirectional ER-to-Golgi transport constitutes a checkpoint to modulate the balance between local vs. long-range trafficking in dendrites (Hanus et al., 2014). The serotonin receptor $5\text{-}HT_{1A}R$, which is a main target of psychotropic and antidepressant molecules, is transported to dendrites in a complex with Yip1A, Rab6 and molecular motors Kif5B and dynein (Carrel et al., 2008; Al Awabdh et al., 2012) suggesting that ER-to-GOPs trafficking is necessary for the transport of $5\text{-}HT_{1A}R$ to distal dendrites. It interacts with Yif1B that regulates specifically its anterograde ER-to-Golgi transport (Alterio et al., 2015). Interestingly, the amyotrophic lateral sclerosis related protein VAPB interacts with Yif1A. Although knockdown of either VAPB or Yif1A does not affects $5\text{-}HT_{1A}R$ transport, both proteins are required for the trafficking to dendrites of the transmembrane protein GFP-CD8 and for normal dendrite morphology (Kuijpers et al., 2013).

A fundamental issue is whether canonical and local routes present selectivity for a particular subset of cargoes. For example, AMPARs seems to be sorted apart from NMDA receptors (NMDARs) within the somatic ER. NMDARs are then transported by the kinesin Kif17 in a dER subcompartment as a complex with scaffold proteins CASK and SAP97, which leads to the exclusive fusion of pre-Golgi NMDARs carriers with GOPs (Jeyifous et al., 2009). Consistent with this local route, NMDARs accumulate in dendritic ERES far away from the soma after group I metabotropic glutamate receptors (mGluRs) stimulation (Aridor et al., 2004). Activity-dependent control of mRNA splicing or interaction with the chaperone BIP (for NR1 or GluN2A subunits respectively) regulates the supply of NMDARs from the dER to synapses, which is relevant for memory formation (Mu et al., 2003; Zhang et al., 2015). In contrast, AMPARs seem to use specifically the canonical secretory route (Jeyifous et al., 2009). This specific sorting seems to depend on the conformation of the MAGUK-family protein SAP97, which associates with AMPARs early in the secretory pathway (Sans et al., 2001). Upon CASK binding, the conformation of SAP97 is altered, allowing its association to NMDARs and their sorting to GOPs (Jeyifous et al., 2009; Lin et al., 2013). ADAM10, the enzyme responsible for the α-secretase cleavage that prevents the formation of β–amyloid in neurons, associates with SAP97 in a protein kinase C (PKC) dependent fashion, which is essential for ADAM10 trafficking from GOPs to synapses, but not from the ER to GOPs (Saraceno et al., 2014). Importantly, regulatory proteins

seem to be involved in the local transport of several cargos. For example, Kif17 participates to the transport of NMDARs, Kv4.2 and VSVG^{ts045} (Setou et al., 2000; Chu et al., 2006; Hanus et al., 2014), raising the question of whether Kif17 is a main microtubule-dependent motor for pre-Golgi carriers during local trafficking. It will be interesting to examine if other Kif17 cargoes such as kainate receptors, also use local trafficking (Kayadjanian et al., 2007).

An outstanding question is whether the usage of GOPs is a wide mechanism for dendritic membrane transport. Notably, BDNF, VSVG^{ts045}, and $GABA_BRs$ have been shown to be able to use both canonical and local trafficking paths (Horton and Ehlers, 2003; Valenzuela et al., 2014). An attractive possibility is that synaptic activity modulates the alternative usage of these pathways. In this context, mGluRs signaling locally confines the cargo mobility within the dER, increasing secretion by a mechanism involving PKC and the ER protein CLIMP63 (Cui-Wang et al., 2012). Similarly, ionotropic glutamate receptor activation restricts the spatial range of post-ER carriers transport in a CaMKII and Kif17 dependent fashion. This increases VSVG^{ts045} delivery to the plasma membrane, with a minor effect on cargo diffusion within the dER (Cui-Wang et al., 2012; Hanus et al., 2014), revealing a functional link between synaptic activity and the early secretory pathway in dendrites.

Generating the Trafficking Diversity: GOPs Biogenesis

GOPs formation is developmentally regulated in hippocampal neurons, meaning that it steadily increases during neuronal growth and differentiation (Horton and Ehlers, 2003; Horton et al., 2005). Two main options could explain the biogenesis of GOPs: (i) a local GOPs production from the dER or (ii) a somatic Golgi fragmentation and subsequent dispersion into dendrites. Recently, it has been shown that GOPs destined to the major dendrite are generated by a sequential process that involves polarized deployment and fission of tubules derived from the somatic GA, which are then transported and condensed in dendrites (Quassollo et al., 2015). In *Drosophila* neurons GOPs are transported by dynein through their interaction with the golgin Lava lamp (Lva; Ye et al., 2007; Zheng et al., 2008). Parkinson's disease-associated kinase Lrrk binds to- and phosphorylates Lva, inhibiting GOPs movement due to Lva/dynein dissociation (Lin et al., 2015). GOPs formation is controlled by the RhoA-Rock pathway through key components of the Golgi fission machinery, including the kinases LIMK1 and PKD1 which regulate the activity of the actin dynamizing factor Cofilin, its activating phosphatase slingshot-1, and dynamin-2 (Quassollo et al., 2015). Activation or inactivation of this pathway within the GA regulates GOPs biogenesis.

In *Drosophila* neurons, GM130 is both necessary and sufficient to generate mcGOPs (Zhou et al., 2014). Interestingly, scGOPs move faster than mcGOPs raising the question of whether GM130 is needed for GOPs transport or for condensation in dendrites (Zhou et al., 2014). During GOPs generation, some Golgi-emanating tubules contain markers of proximal and distal cisternae, implying that mcGOPs could be directly derived from the somatic GA (Quassollo et al., 2015). Interestingly, the RhoA-Rock pathway is not involved in Golgi-derived tubule formation, elongation or polarized distribution, suggesting that additional factors regulate these processes (Quassollo et al., 2015). Reelin stimulates Cdc42- or α-pix- mediated GA translocation into the apical dendrite (Matsuki et al., 2010; Meseke et al., 2013). This seems to be essential for apical dendrite orientation and dendritic development, suggesting that the reelin pathway may be involved in polarized Golgi-derived tubule deployment and GOPs generation during development (Meseke et al., 2013; Quassollo et al., 2015). Currently, the mechanisms of GOPs biogenesis in minor dendrites remain unknown. In this context, the overexpression of the Golgi structural protein GRASP65 causes Golgi dispersal into multiple dendrites, without inhibiting secretory trafficking, generating a marked reduction in the polarity of the dendritic arbor (Horton et al., 2005). Thus, Golgi stacking and ribbon linking may modulate GOPs formation in minor dendrites.

Projections

The multiplicity of GOPs compositions confers a range of possibilities for routing different cargoes, contributing to diversify their output in terms of dynamics, localization, and post-translational modifications patterns. How to balance the usage of canonical and local routes and what determines the specific use of local routes, or the trafficking through a scGOP, are outstanding questions. The emerging possibility of *in situ* detection of *de novo* synthesis of any interest protein (tom Dieck et al., 2015), in combination with systems that allow trafficking synchronization of specific cargoes (Rivera et al., 2000; Boncompain et al., 2012; Chen et al., 2013) coupled to super-resolution microscopy (Maglione and Sigrist, 2013) will give us an unprecedented opportunity to decipher the diversity of secretory routes in highly differentiated structures like dendrites and axons. A fundamental issue is how trafficking routes contribute to the synaptic function and neuronal physiology, and conversely, how synapses control these secretory routes. Combination of trafficking synchronization assays with optogenetic tools for the local manipulation of synaptic activity arise as suitable approaches to gain a better understanding of how changes in intracellular transport events could lead to changes in cognition, emotions, memory, and learning.

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Nucleation and Dynamics of Golgi-derived Microtubules

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Integrity of the Golgi apparatus requires the microtubule (MT) network. A subset of MTs originates at the Golgi itself, which in this case functions as a MT-organizing center (MTOC). Golgi-derived MTs serve important roles in post-Golgi trafficking, maintenance of Golgi integrity, cell polarity and motility, as well as cell type-specific functions, including neurite outgrowth/branching. Here, we discuss possible models describing the formation and dynamics of Golgi-derived MTs. How Golgi-derived MTs are formed is not fully understood. A widely discussed model implicates that the critical step of the process is recruitment of molecular factors, which drive MT nucleation (γ-tubulin ring complex, or γ-TuRC), to the Golgi membrane via specific scaffolding interactions. Based on recent findings, we propose to introduce an additional level of regulation, whereby MT-binding proteins and/or local tubulin dimer concentration at the Golgi helps to overcome kinetic barriers at the initial nucleation step. According to our model, emerging MTs are subsequently stabilized by Golgi-associated MT-stabilizing proteins. We discuss molecular factors potentially involved in all three steps of MT formation. To preserve proper cell functioning, a balance must be maintained between MT subsets at the centrosome and the Golgi. Recent work has shown that certain centrosomal factors are important in maintaining this balance, suggesting a close connection between regulation of centrosomal and Golgi-derived MTs. Finally, we will discuss potential functions of Golgi-derived MTs based on their nucleation site location within a Golgi stack.

Keywords: microtubule dynamics, Golgi-derived microtubules, γ-TuRC, CLASP, microtubule nucleation, Golgi apparatus

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INTRODUCTION

The intracellular microtubule (MT) network consisting of polarized alpha/beta tubulin polymer tubes plays important roles in intracellular trafficking, membrane dynamics, and organelle positioning. The textbook view of interphase microtubule organization is a radial array extending from a single juxtanuclear centrosome. Such organization is clearly dominant in proliferating cells where centrosome-based MTOCs are used to build mitotic spindles. Interphase cells, however, often develop non-centrosomal MT arrays. It is especially characteristic for differentiated cells that have specific function and morphology. In most specialized cell types, radial MT geometry is not ideal for precise delivery of cargos to specific cellular locations. Differentiation-related non-centrosomal microtubule networks have been described for more than 25 years; yet they remain understudied. In the last few years, the research field has started to understand the mechanisms that rearrange MTs in specialized cells and their functional significance. Non-centrosomal MT populations arise when MT-nucleating and/or stabilizing factors are

concentrated at cellular scaffolds alternative to the traditional scaffold (pericentrosomal material). In many cell types the role of scaffold can be acquired by the Golgi apparatus membrane, and non-centrosomal MTs are derived from the Golgi (Chabin-Brion et al., 2001; Efimov et al., 2007; Ori-Mckenney et al., 2012; Oddoux et al., 2013). Evolutionally, it can be explained by the convenience of direct association of MT tracks with the major cellular sorting and trafficking facility. Indeed, Golgi-derived MTs were shown to support both Golgi integrity and directionality of post-Golgi trafficking (Miller et al., 2009; Hurtado et al., 2011; Vinogradova et al., 2012).

To date, Golgi-derived MTs have been characterized in hepatocytes (Chabin-Brion et al., 2001), epithelial cells (Efimov et al., 2007; Rivero et al., 2009), neurons with their strikingly diverse axonal and dendritic MT bundles (Ori-Mckenney et al., 2012; Yalgin et al., 2015), skeletal muscle (Zaal et al., 2011; Oddoux et al., 2013), and pancreatic beta cells where this MT subpopulation fine-tunes insulin secretion (Zhu et al., 2015); it is very likely that abundance of this MT sub-population will expand as we learn more about cellular architecture in differentiated tissues. Thus, the functional significance of Golgi-derived MTs is undoubted. At the same time, much remains to be elucidated about the mechanisms underlying Golgi-derived MT nucleation, stabilization, dynamics, and regulation. This review aims to give an updated view on Golgi-derived MTs, including several possible mechanisms through which Golgi-derived MTs could be formed and regulated, and how they are important for proper cell function and behavior.

RECRUITMENT OF γ-TuRC TO THE GOLGI MEMBRANES

The first requirement for MT formation in a cell, where tubulin concentrations are relatively low, is availability of MT nucleation templates (Oakley et al., 2015; Petry and Vale, 2015). Solid evidence indicates that MT nucleation at the Golgi starts off γ-tubulin ring complexes (γ-TuRC; (Chabin-Brion et al., 2001; Efimov et al., 2007; Ori-Mckenney et al., 2012), similar to classic centrosomal MTOC. γ-Tubulin was found associated with isolated Golgi membranes (Chabin-Brion et al., 2001; Ori-Mckenney et al., 2012; Wang et al., 2014), and only γ-tubulin-associated Golgi elements were capable of MT nucleation during *in vitro* reconstitution assays (Chabin-Brion et al., 2001; Ori-Mckenney et al., 2012).In line with this, siRNA-driven depletion of γ-tubulin eliminates Golgi-derived MT nucleation in cells (Efimov et al., 2007). To understand regulation of MT nucleation at the Golgi, multiple investigators addressed a decisive question of γ-tubulin (or, rather, γ-tubulin ring complexes (γ-TuRC)) recruitment to the Golgi membrane. Several proteins with γ-TuRC-scaffolding capacity were identified in association with the Golgi membrane, including AKAP450 (also known as AKAP9, AKAP350, CG-NAP, Yotiao; Rivero et al., 2009), several isoforms of myomegalin (Roubin et al., 2013; Wang et al., 2014), CDK5Rap2 (Rios, 2014) or its homolog centrosomin in *Drosophila* neurons (Yalgin et al., 2015), and pericentrin in skeletal muscle (Oddoux et al., 2013). Also, PTTG/securin was identified as associated with this scaffolding complex (Moreno-Mateos et al., 2011). Depletion of each of these listed molecules has been demonstrated to attenuate Golgi-derived MT formation. Most significantly, AKAP450 has proven to be essential for Golgi-derived MT formation in multiple experimental systems and organisms (Rivero et al., 2009; Hurtado et al., 2011; Ori-Mckenney et al., 2012; Maia et al., 2013), likely because it can recruit γ-TuRC either directly, or indirectly via CDK5Rap2 or myomegalin interactions. However, AKAP450 function is not essential for MT nucleation at the Golgi in myotubes, likely because γ-TuRC is recruited to the Golgi by pericentrin in this cell type (Oddoux et al., 2013). So far, γ-TuRC-scaffolding to the Golgi has been attributed to proteins known as components of pericentrosomal matrix (see also Rios, 2014, for detailed review). The exception is a myomegalin splice-variant, MMG8 (Wang et al., 2014), which therefore might be important for specific regulation of MT nucleation at the Golgi. In any case, the pool of MT-nucleating factors in cells is likely restricted and has to be redistributed between the centrosome and the Golgi via regulated scaffolding. For example, release of centrosomal nucleation machinery by Cep192 depletion facilitates Golgi-derived nucleation (O'Rourke et al., 2014).This does not occur if the centrosomal nucleating complex is destroyed/denatured by laser ablation (Efimov et al., 2007).

This collected data prompted a widely accepted view that anchoring of γ-TuRCs at Golgi membranes is essential for Golgi-derived MT formation. The nucleation event might occur at the already Golgi-bound γ-TuRC; alternatively, MTs that are randomly nucleated at cytosolic γ-TuRCs in the vicinity of the Golgi membrane might be recruited to the Golgi thereafter. However, it cannot be overlooked that the concentration of γ-TuRCs at the Golgi is only slightly, if at all, higher than in the surrounding cytosol; it can only be detected on isolated Golgi membranes (Chabin-Brion et al., 2001; Ori-Mckenney et al., 2012), or after pre-extraction of cytosolic γ-tubulin (Wang et al., 2014), which is in sharp contrast to high γ-TuRC concentration in the pericentrosomal material. Since molecular anchoring does not concentrate γ-tubulin at the Golgi, it cannot be the reason for preferential nucleation of MTs at the Golgi as compared to other cytoplasmic sites, and the mechanism that allows the Golgi membrane to serve as a MTOC remains unclear. An attractive, though unexplored, possibility is that γ-TuRC structure and efficiency as a template requires specific stabilization/optimization of its structure by Golgi membrane-associated factors. Other mechanisms favoring MT nucleation at the Golgi might enhance MT formation at available templates. For example, Golgi environment could promote tubulin polymerization off the γ-TuRC templates and/or facilitate stabilization of MTs that already started to polymerize, preventing their immediate catastrophe. Such support of MT formation can be provided by MT plus tip tracking proteins (+TIPs) and stabilizing factors, if such factors are enriched at the MTOC. Indeed, similar to the centrosome-based MTOC, formation of Golgi-derived MT involves both types of molecular components. Below, we will briefly discuss the current knowledge on these two types of molecular components.

REGULATION OF MT POLYMERIZATION AS A FACTOR IN GOLGI-DERIVED MT NUCLEATION

While providing a template for MT nucleation is the initial condition for MT outgrowth, it has been recently proven to be insufficient for MT formation: additional factors are required to overcome the kinetic barrier and make MT nucleation kinetically favorable (Wieczorek et al., 2015). The process of "approval" of MT outgrowth from provided templates is referred to as "templated nucleation" and is tuned by MT +TIPs with variable activities.

One of the first MT-binding proteins implicated in MT nucleation at the Golgi is CLASP (Efimov et al., 2007), a known multi-functional MT stabilizer. CLASP functions include promotion of MT rescues, stabilization of MT seeds in fission yeast, capture of MTs at the cell cortex and kinetochores, and modification of polymerizing MT lattice (Galjart, 2005; Bratman and Chang, 2007; Kumar et al., 2009; Al-Bassam et al., 2010; Maia et al., 2012; Grimaldi et al., 2014). Both CLASP paralogs (CLASP1 and CLASP2 in mammals) strongly accumulate at the Trans-Golgi Network (TGN) membranes via scaffolding golgin GCC185, and are essential for efficient MT nucleation (Efimov et al., 2007). To date, the mechanistic role of CLASP in MT nucleation at the Golgi is not clear. We and others have proposed that CLASP stabilizes MTs as they start to form, based on the finding that newly-nucleated Golgi-derived MTs are coated with CLASP, which likely relocalized from the Golgi membrane. Now that CLASP is known to modify polymerizing MTs (Grimaldi et al., 2014), an alternative hypothesis arises that CLASP is essential for the initial polymerization steps of Golgi-derived MTs (templated nucleation), rather than simply stabilizing already assembled polymers. This function could be mediated through its TOG (tumor overexpressed gene) domains, which are MT-binding domains required for CLASP's MT-interaction (Slep, 2009; Maki et al., 2015). It is important in this regard that another TOG-domain containing protein, XMAP215, was recently shown to directly assist MT nucleation off a variety of templates (Wieczorek et al., 2015). Interestingly, another recent study indicates that XMAP215 synergizes in MT nucleation activity with TPX2 (Roostalu et al., 2015), a major stimulator of non-centrosomal MT formation in mitotic cells (Neumayer et al., 2014).

Furthermore, both Golgi-specific isoforms of myomegalin bind MT End Binding proteins (EB1 and/or EB3). These isoforms include MMG8, with both γ-TuRC and EB-binding motifs (Wang et al., 2014) and EB-MMG, with EB-binding motif only (Roubin et al., 2013). EBs are multi-functional + TIPs (Morrison, 2007; Slep, 2010), which cooperate with XMAP215 to promote MT polymerization (Zanic et al., 2013), actively regulate MT polymerization by modulating MT structure (Zhang et al., 2015), and are mutually regulated by CLASPs (Grimaldi et al., 2014). EB-binding myomegalins have recently emerged as critical regulators of MT nucleation at the Golgi (Roubin et al., 2013; Wang et al., 2014); it is possible that myomegalin interaction creates a local pool of EB molecules that can readily relocalize to MT-nucleating sites to support templated nucleation.

We suggest that regulation of Golgi-derived MTs at the level of templated nucleation at already available γ-TuRCs serves for fine modulation of this MT subpopulation because MT +TIPs are known as highly regulated by cell cycle and signaling cues. For example, both CLASP2 and EB1 are phosphorylated by cell cycle kinases (Maia et al., 2012; Banerjee et al., 2014). Also, the amount of CLASP2 at the Golgi membrane is tightly regulated by aPKC-driven phosphorylation (Matsui et al., 2015), indicating a potential link between polarity signaling and Golgi-derived MTs. Moreover, it was recently shown that during mitosis, one of the major Golgi components GM130 (among other molecules) is capable to promote TPX2-dependent MT nucleation, indicating a likely alternative mechanism of MT nucleation in the vicinity of Golgi membrane (Wei et al., 2015).

Besides MT + TIPs, an important factor that likely restricts MT nucleation efficiency of the Golgi is availability of functional alpha/beta tubulin dimers. Templated nucleation is kinetically favored at higher tubulin concentration than MT polymerization *per se* (Wieczorek et al., 2015). It has become clear recently that tubulin folding "quality control" performed by tubulin chaperones is important not only at the protein synthesis stage but also for tubulin recycling during dynamic MT reorganization (Nithianantham et al., 2015). One significant alpha-tubulin chaperone, TBCE, is concentrated at the Golgi membrane in an Arf1-regulated manner (Schaefer et al., 2007; Bellouze et al., 2014), and facilitates both nucleation rates and polymerization speed of Golgi-derived MTs (Bellouze et al., 2014). Because of low γ-TuRC abundance at the Golgi, nucleation of MTs off these γ-TuRCs templates may require high local concentration of functional tubulin dimers, which could be achieved by TBCE concentrating and reviving tubulin in the vicinity of nucleation sites. Regulation of TBCE via Arf1 activity (Bellouze et al., 2014) adds another level to potential signaling pathways that fine-tune the Golgi-derived MT population.

STABILIZATION OF GOLGI-DERIVED MICROTUBULES

MT function, in general, depends strongly on their lifespan, which can be extremely variable within a single cell and between different cell types. MTs associated with the Golgi are known to be more stable compared to the majority of cellular MTs, as has been detected both directly, in depolymerization resistance assays, and indirectly, via accumulated post-translational modifications of tubulin (see references below). A number of MT stabilizing factors have been identified as specifically active in the Golgi region. First of all, major Golgi-derived MT-promoting proteins AKAP450 and CLASP, which have been discussed above in conjunction with MT nucleation steps, are both capable of MT stabilization (Akhmanova et al., 2001; Larocca et al., 2006; Hurtado et al., 2011). Other factors, which specifically stabilize MTs in the Golgi region include: (1) recently identified microtubule cross-linking protein MTCL1, which interestingly can be recruited to the Golgi in both CLASP-dependent and AKAP450-dependent manner (Sato et al., 2013, 2014), (2) MT-stabilizing tumor suppressor RASSF1A (Arnette et al., 2014), (3) Golgi-anchored Cap-Gly-domain containing CAP350

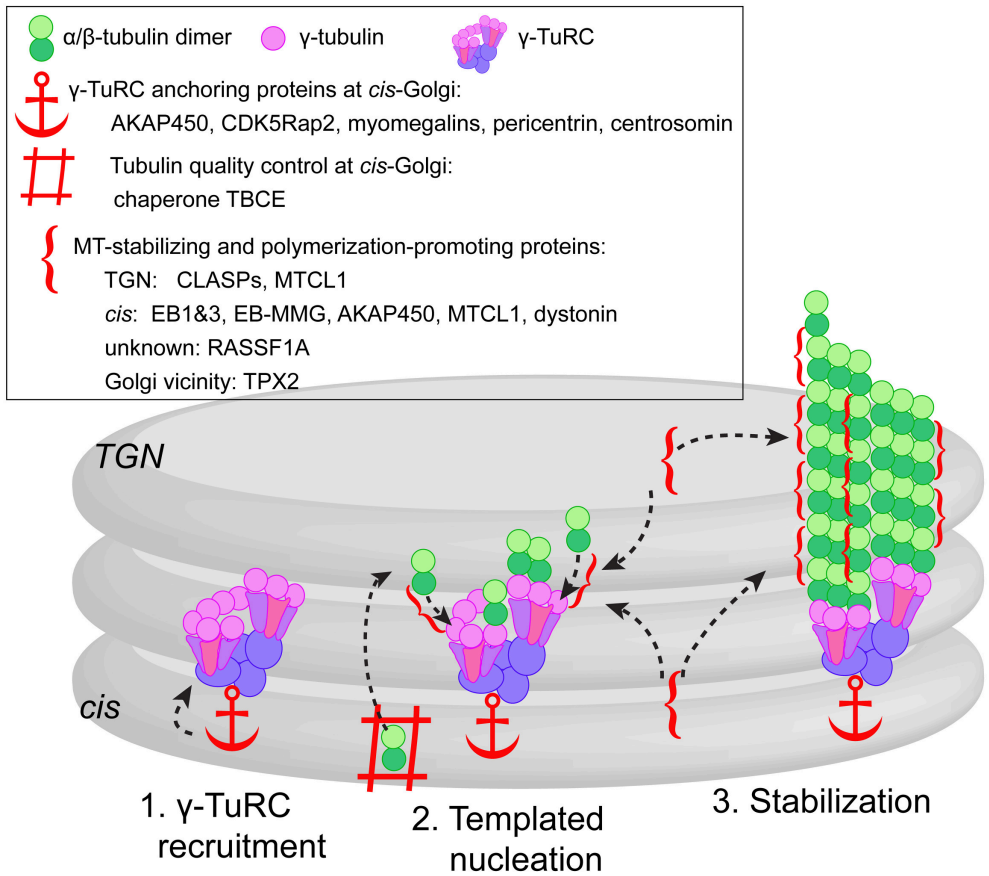

FIGURE 1 | Model of three-step MT formation at the Golgi. (1) γ-TuRCs are recruited to the *cis*-Golgi membranes by scaffolding proteins, some of which might function as a complex (e.g., AKAP450, CDK5Rap2, and myomegalins) or as alternative cell type-specific mechanism (e.g., pericentrin). (2) Actual MT nucleation at γ-TuRC template is supported by tubulin pool provided by chaperone TBCE from the *cis*-face, and promoted by +TIPs (CLASPs, EBs concentrated by EB-MMG interaction) and MT stabilizing proteins (MTCL1, AKAP450, RASSF1A) localized at both *cis* and TGN faces of the Golgi. In mitosis, MT nucleation in the Golgi vicinity is facilitated by TPX2 (not depicted). (3) After successful nucleation event, the same set of +TIPs and MAPs promotes persistent growth and stabilizes emerging MTs.

(Hoppeler-Lebel et al., 2007), and (4) cytoskeletal linker dystonin, which localizes to the Golgi and nearby ER (Ryan et al., 2012) and stabilizes MTs in the Golgi area via interaction with MAP1b (Ryan et al., 2012).

In most studies, the origin of MTs, stabilized by specific proteins in the Golgi vicinity, has not been tested, and it would not be correct to imply that only MTs nucleated at the Golgi can be stabilized via these mechanisms. However, Golgi-derived MTs obviously fall into the category of MTs in the Golgi vicinity, and are stabilized by the described factors. Importantly, the pioneering study of the Pous group described rapid acetylation of newly-nucleated Golgi-derived MTs (Chabin-Brion et al., 2001), which suggests that these MTs can indeed be stabilized as soon as they form. Moreover, depletion of most MT stabilizers mentioned above, disturbs Golgi complex integrity (Hoppeler-Lebel et al., 2007; Oddoux et al., 2013; Arnette et al., 2014; Sato et al., 2014), a well-known function of Golgi-derived MTs (Miller et al., 2009; Vinogradova et al., 2012). Along the same lines, Golgi integrity also requires CAMSAP2 and 3 proteins (Tanaka et al., 2012), which specifically stabilize minus ends of MTs (Akhmanova and Hoogenraad, 2015) and have been recently implicated in stabilization and long lifespan of non-centrosomal MTs in neurons (Yau et al., 2014).

Mechanisms of MT stabilization at the Golgi, which are obviously numerous and probably redundant, likely serve to extend Golgi-derived MT lifetime, allowing for their robust function. Thus, we consider MT stabilization as an important step in MT formation at the Golgi (**Figure 1**). That being said, while we have grouped known molecular factors in two groups based on existing evidence, it is possible that many MT stabilizers are capable of templated MT nucleation support, and vice versa. It is also possible that increased stability of these MTs is essential for their specific functions because MT-dependent molecular motors often sense post-translational modifications at stable MT lattice, which influences motor affinity and/or activity (Reed et al., 2006; Verhey and Hammond, 2009).

FUNCTIONAL SIGNIFICANCE OF MT ANCHORING AT THE GOLGI

Evidence that factors localized both at the *cis*-Golgi (γ-TuRC scaffolding complex; Rivero et al., 2009) and at the TGN (CLASPs; Efimov et al., 2007) are required for Golgi-derived MT nucleation, present an interesting, as of yet, not fully answered question: Where exactly are Golgi-derived MTs nucleated? As mentioned above, localization of MT minus ends at the Golgi is likely important for specialization of MT function in trafficking, and association with a specific compartment would predict Golgi-derived MT application in trafficking to/from this Golgi domain. Live-cell imaging of new MT formation during nocodazole washout reveals existence of nucleation "hot spots" within a single Golgi stack (Efimov et al., 2007). It is possible that Golgi-derived MTs are nucleated at extensions of the *cis*-Golgi and TGN membranes, which bring two protein pools into close proximity. It is plausible to hypothesize that MTCL1, which interacts with both *cis*-localized AKAP450 and TGN-bound CLASP (Sato et al., 2014), plays a role in organization of nucleation "hot spots"; however, the nature of the exact Golgi-derived MT nucleation/trapping sites is yet to be determined.

From the functional point of view, the exact localization of MT-nucleating sites is important because, in many cases, it defines localization of MT minus ends, and thus directionality of MT-dependent transport. It is known that when γ-TuRC scaffolding factor ninein is absent from the centrosome, MT anchoring is compromised despite remaining γ-TuRC abundance. This scenario is utilized at the daughter centriole in G2 cells (Delgehyr et al., 2005), where MTs are nucleated but rapidly released. Thus, tight γ-TuRC anchoring at the *cis*-Golgi could serve as a mechanism for retaining MT minus ends at the *cis*-Golgi membrane and maintaining perfect positioning of MT minus ends in regard to ER-to-Golgi and Golgi-to-ER transport. Similarly, because MT nucleation requires proximity of the TGN-concentrated factors, MTs are likely readily positioned to the sites of TGN carrier formation (Luini et al., 2008), or late endosome-TGN recycling (Itin et al., 1999).

CONCLUDING REMARKS

As mentioned above, the vast majority of molecules involved in Golgi-derived MT formation are also accumulated in the pericentrosomal material and facilitate MT nucleation at the centrosome. Thus, an important factor of Golgi-derived MT nucleation is the balance between the two pools: depletion of centrosomal scaffolding factors results in the boost of Golgi MTOC activity (O'Rourke et al., 2014). Similarly, in differentiating myotubes, pericentrosomal protein complexes relocate to the Golgi simultaneously with the centrosome silencing (Zaal et al., 2011; Oddoux et al., 2013).

Another implication of the knowledge discussed above is that while anchoring of γ-TuRCs to the Golgi membrane is essential for the Golgi-derived MT array, MT nucleation at this location must be triggered at the level of subsequent steps of MT formation: tubulin polymerization off the template and/or MT lattice stabilization, as it was described for other non-centrosomal MT nucleation sites (Petry and Vale, 2015). We favor these views simply because MT +TIPs and MT stabilizers are strongly concentrated in the Golgi region, in contrast to γ-TuRCs.

Finally, it is plausible to suggest that specific anchoring sites of MT minus ends at the Golgi are important for their function as cellular roadways, and involvement of both *cis*- and TGN membranes in organization of the nucleation sites might serve to provide routes for both *cis*- and *trans*-Golgi associated trafficking.

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frontiers in Neuroscience

MINI REVIEW
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Alpha-synuclein Toxicity in the Early Secretory Pathway: How It Drives Neurodegeneration in Parkinsons Disease

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Alpha-synuclein is a predominant player in the pathogenesis of Parkinson's Disease. However, despite extensive study for two decades, its physiological and pathological mechanisms remain poorly understood. Alpha-synuclein forms a perplexing web of interactions with lipids, trafficking machinery, and other regulatory factors. One emerging consensus is that synaptic vesicles are likely the functional site for alpha-synuclein, where it appears to facilitate vesicle docking and fusion. On the other hand, the dysfunctions of alpha-synuclein are more dispersed and numerous; when mutated or over-expressed, alpha-synuclein affects several membrane trafficking and stress pathways, including exocytosis, ER-to-Golgi transport, ER stress, Golgi homeostasis, endocytosis, autophagy, oxidative stress, and others. Here we examine recent developments in alpha-synuclein's toxicity in the early secretory pathway placed in the context of emerging themes from other affected pathways to help illuminate its underlying pathogenic mechanisms in neurodegeneration.

Keywords: alpha-synuclein, vesicle trafficking, golgi, ER stress response, neurodegenerative diseases, Parkinson disease, LRRK2, ER to golgi transport

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INTRODUCTION

Alpha-synuclein (a-syn), a 14 kD cytosolic neuronal protein, has been a major focus of Parkinson's Disease (PD) pathological studies. As shown in **Figure 1**, a-syn consists of an N-terminal domain that adopts an alpha-helical conformation upon binding to membranes, and a charged C-terminus with multiple phosphorylation sites (Bendor et al., 2013; Snead and Eliezer, 2014). The N-terminal domain of alpha-syn contains seven membrane-interacting repeats, and can either bind the membrane as a long helix, or in a "broken helix" conformation with two helices connected by a linker (Bartels et al., 2010). Alpha-syn monomers can either assemble into tetramers (Bartels et al., 2011) or beta-sheet fibrils that aggregate to form Lewy bodies, a primary indicator of Parkinson's disease (PD) accompanied by neurodegeneration in the Substantia nigra, locus coeruleus, and dorsal vagal nucleus (Kingsbury et al., 2010). Using a prion-like mechanism, normal a-syn is converted to the aggregated form by a-syn molecules that have already been converted (Deleersnijder et al., 2013), a process mediated by the non-amyloid-β component (NAC) region of a-syn, comprising residues 61–95. Alpha-syn gene duplication, triplication, and several dominant mutations, including A30P, E46K, H50Q, and A53T cause familial PD (Polymeropoulos et al., 1997; Zarranz et al., 2004; Khalaf et al., 2014).

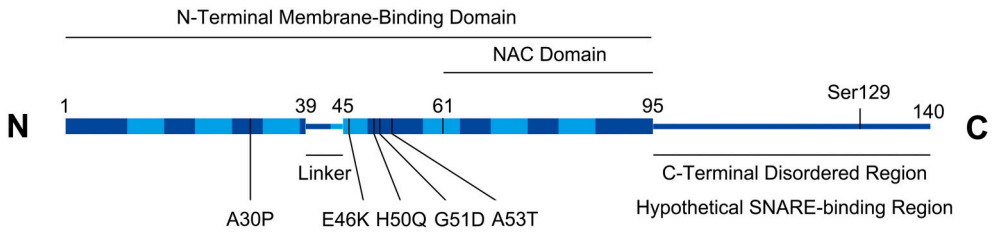

FIGURE 1 | Alpha-synuclein structural schematic. Boxed regions represent alpha-helices. Seven repeats (residues 10–15, 21–26, 32–37, 43–48, 58–63, 69–74, and 80–85) in the helical region are depicted with lighter shading. Numbering on figure refers to amino acids. PD-associated missense mutations are indicated. Serine129 is a functionally implicated phosphorylation site. The C-terminal region contains a high density of charged residues (not shown).

The alpha-, beta-, and gamma-synuclein genes are somewhat functionally redundant (Greten-Harrison et al., 2010; Ninkina et al., 2012; Vargas et al., 2014), which probably accounts for the lack of a significant phenotype from a-syn single knockout (Abeliovich et al., 2000; Cabin et al., 2002). Synuclein triple knockout, however, alters synapse structure and leads to age-dependent neuronal dysfunction in mice (Greten-Harrison et al., 2010). Alpha-syn participates in the maintenance of the presynaptic vesicle pool as well as the release of neurotransmitter (Murphy et al., 2000; Cabin et al., 2002; Ben Gedalya et al., 2009; Nemani et al., 2010; Cheng et al., 2011).

The neuronal toxicity of alpha-synuclein over-expression or mutation appears to involve a wide range of pathways and cellular functions including ER-to-Golgi transport, Golgi homeostasis, presynaptic trafficking, endocytosis, autophagy, the ubiquitin-proteasome system, ER and oxidative stress (Snead and Eliezer, 2014). This review focuses primarily on the dysfunction of alpha-synuclein and pathogenically related proteins in the early secretory pathway.

PHYSIOLOGICAL FUNCTIONS

Although present in various compartments of the cell, alpha-synuclein accumulates at the synapse (Maroteaux et al., 1988; Kahle et al., 2000), and is recruited to synaptic vesicles through interactions with specific lipids and trafficking machinery (Fortin et al., 2004; Burré et al., 2010; Diao et al., 2013). In addition, a-syn is capable of sensing membrane curvature and lipid composition, with a preference for synaptic vesicle's small shape, high curvature, and charged lipid head groups (Pranke et al., 2011; Bendor et al., 2013). The Rab family of small GTPases appear to facilitate the recruitment process (Lee et al., 2011; Chen et al., 2013) as does C-terminal phosphorylation (Oueslati et al., 2010; Paleologou et al., 2010).

One emerging consensus is that a-syn, perhaps in cooperation with cysteine string protein (CSP) (Chandra et al., 2005), acts as a SNARE chaperone to potentiate SNARE assembly for fusion (Burré et al., 2010). Interactions between a-syn and synaptic SNAREs are established, and a-syn can promote SNARE-mediated vesicle fusion in a manner that requires both lipid and SNARE interactions. The precise step modulated by a-syn seems to lie slightly upstream of final SNARE assembly during clustering, docking, and/or SNARE priming for assembly (Diao et al., 2013; Burré et al., 2014).

DYSFUNCTIONS I: THE SYNAPSE

Alpha-syn's potential dysfunctions at its native site, the synapse, have been intensely researched but will be briefly treated here. Confusingly and despite the positive functions in docking and fusion described above, a-syn has been shown to inhibit these steps as well. Large alpha-syn oligomers potently inhibit vesicle fusion (Choi et al., 2013, 2015) and soluble, non-aggregated a-syn also inhibits at higher concentrations (Lai et al., 2014). In some conditions, a-syn's interactions with lipids alone may inhibit SNARE-mediated fusion (DeWitt and Rhoades, 2013; Lai et al., 2014). Alpha-syn synaptic function vs. dysfunction is likely very sensitive to concentration and the aggregation state of a-syn species, keeping in mind that conversion to pathogenic forms will also deplete cells of the functional form. However, it remains to be established whether a-syn synaptic dysfunction is a major driver of neurodegeneration in PD.

DYSFUNCTIONS II: THE ER-GOLGI AXIS

The mechanism of a-syn toxicity leading to neurodegeneration is very complex, multi-pronged, and highly integrated with cellular stress pathways. Most likely, a-syn oligomers directly intoxicate several cellular membrane-dependent pathways, each of which causes damage and activation of unsuccessful stress responses leading to apoptosis.

Evidence has mounted that one of the initiating insults results from a-syn's disruption of ER-to-Golgi transport, the first and rate-limiting step in the biosynthetic secretory pathway. This effect was first identified in a yeast screen for genetic suppressors of a-syn toxicity (Cooper et al., 2006). Surprisingly, the most efficient group of suppressors were membrane trafficking proteins, including Ypt1p/rab1, which coordinates ER/Golgi vesicle tethering; and Ykt6p, a SNARE that mediates ER/Golgi-related fusions. That the most successful suppressors were ER/Golgi trafficking machinery suggested that this pathway is deeply involved in a-syn toxicity. Follow-up studies by multiple groups have demonstrated potent inhibition of ER-to-Golgi transport by physiologically relevant over-expression levels of a-syn in normal rat kidney cells, HeLa cells, neuroendocrine PC12 cells, and dopaminergic SH-SY5Y cells (Thayanidhi et al., 2010; Winslow et al., 2010; Oaks et al., 2013). The evidence supports a potent inhibition of the secretory pathway by mild over-expression of wildtype a-syn and at even lower

concentrations by PD-associated mutants. The locus of a-syn inhibition appears to be post- ER budding, perhaps through interference with rab- and SNARE-dependent COPII vesicle tethering and/or fusion (Gitler et al., 2008; Thayanidhi et al., 2010). Interestingly, superoxide dismutase-1 (SOD1) mutants that cause neurodegeneration in amyotrophic lateral sclerosis (ALS) have also been shown to inhibit ER-to-Golgi transport in neurons resulting in ER stress that can be ameliorated by over-expression of ER/Golgi vesicle machinery (Atkin et al., 2014). Is there a common feature of aggregated cytosolic proteins that is fundamentally linked to ER export?

Normal neurons may thus be treading a delicate balance in which they must express a-syn at a sufficient level to promote synaptic trafficking yet below a threshold where it inhibits biosynthetic trafficking and causes stress in the cell body. How can a-syn be on the one hand a chaperone for facilitating SNARE-dependent synaptic vesicle docking/fusion, and on the other hand a disruptor of mechanistically similar membrane docking/fusion events in the early secretory pathway? One key could be the membrane binding properties of a-syn, which cause it to preferentially interact with post-Golgi, highly curved, charged membranes (Pranke et al., 2011) and lead the N-terminal region to adopt helical structure and self-aggregate (Burré et al., 2014). If these membrane interactions affect a-syn interactions with SNAREs and rabs, then perhaps only post-Golgi lipid compositions support positive effects. Conversely, a-syn molecules that are not properly primed by lipid interactions would fail to exert positive effects, or even negatively affect SNARE assembly on pre-Golgi membranes. Both SNAREs and a-syn undergo binding-induced helix formation, which could make their coordinated assembly extremely context dependent. It would be very useful to know whether a-syn can have different effects at distinct membrane trafficking steps under the same expression conditions in the same cell.

Recently, the link between ER/Golgi trafficking and PD became even stronger. Mutations in Leucine-rich repeat kinase 2 (LRRK2) are the most frequent genetic lesions associated with PD (Kumari and Tan, 2009). Strong genetic and possible physical interactions between a-syn and LRRK2 suggest that they may intoxicate a common pathway (Lin et al., 2009; Qing et al., 2009; Guerreiro et al., 2013). While the precise functions of LRRK2 are still emerging, they appear to be converging on ER export and secretory trafficking. Interactions between LRRK2 and the ER exit site (ERES) scaffold sec16 led to the discovery that LRRK2 is required for ERES assembly and efficient ER cargo exit (Cho et al., 2014). Sec16 is believed to work in concert with Sar1 GTPase to facilitate the assembly of COPII coat components (Watson et al., 2006). One of the common PD-associated LRRK2 mutants, R1441C, disrupts targeting of sec16 to ERES. In parallel, another mechanism by which LRRK2 may impact secretion has emerged. Screens for LRRK2 interactions identified BCL2-associated athanogene 5 (BAG5), Rab7L1, and cyclin-G–associated kinase (GAK) as binding partners. These proteins appeared to form a single complex targeted to the TGN that positively regulates TGN turnover (Beilina et al., 2014). Over-expression of any of these proteins alone, or expression of PD-associated mutations of LRRK2, led to ablation of TGN markers, and presumably the organelle itself, via an autophagy mechanism (Beilina et al., 2014). Severe down-regulation of the TGN would disrupt secretory trafficking and ultimately lead to intracellular accumulation of secretory cargo at multiple steps. Elucidating the multiple cellular functions of LRRK2 is essential for understanding the secretory pathology of PD. Another development was that rab39b lesions are associated with early-onset Parkinsonism (Wilson et al., 2014). Although still very early, it appears that rab39b and protein interacting with C-kinase 1 (PICK1) regulate ER-to-Golgi transport of synaptic machinery (Mignogna et al., 2015). To summarize, genetic lesions in a-syn, LRRK2 and other genes–including the most potent and frequent lesions causing PD–may initiate neuronal stress by disrupting early steps in secretion. It is urgent to elucidate the complex relationship between ER/Golgi trafficking, neuronal stress, and PD.

Alpha-syn probably disrupts intra-Golgi and post-Golgi secretory trafficking in addition to ER-to-Golgi trafficking. Alpha-syn interacts with Rab8 (Golgi), Rab3a (post-Golgi), and Rab5 (early endosomal) in a-syn A30P transgenic mice (Dalfó et al., 2004); in addition, Rab8 (Golgi), Rab3a (post-Golgi), and rab11 (recycling endosomal) overexpression rescue alpha-syn's toxicity (Cooper et al., 2006; Gitler et al., 2008; Yin et al., 2014; Breda et al., 2015). That a-syn toxicity involves simultaneous block of multiple steps in the biosynthetic secretory pathway is also supported by the potent suppression of a-syn transport blocks by ykt6 (Cooper et al., 2006; Thayanidhi et al., 2010), an R-SNARE that can participate in multiple fusogenic SNARE complexes. One secretory cargo of particular importance to dopaminergic neurons is the dopamine transporter (DAT); indeed DAT accumulates in microsomal fractions upon a-syn over-expression and dopamine uptake is consequently inhibited (Oaks et al., 2013). However, as outlined below, ER accumulation, as opposed to lack of proper delivery of secretory and membrane proteins may drive neurodegeneration even more substantially.

DYSFUNCTIONS III: ER STRESS

Alpha-syn over-expression causes ER stress (ERS) and activation of the unfolded protein response (UPR) in cellular models (Smith et al., 2005), which causes apoptosis if unresolved. Accumulating evidence from many systems and approaches indicates that UPR plays a rate-limiting role in the progression of neurodegeneration and a-syn pathology (Mercado et al., 2013). UPR is activated in PD neurons and UPR modulation protects or potentiates PD progression. Low levels of UPR protects neurons from a-syn toxicity, presumably by inducing chaperones and secretory machinery to counteract ERS (Fouillet et al., 2012; Valdés et al., 2014). Similarly, prevention of the adaptive response or inducing acute ERS accelerates a-syn-associated neuronal death (Colla et al., 2012a; Salganik et al., 2015). Phosphorylation of Ser129 on alpha-syn appears to be important for triggering UPR (Colla et al., 2012a). Interestingly, a-syn intoxication does not activate all three classical branches of the UPR equally, which could help explain why this particular ERS cannot be adapted to. Work in PD models found that induction of chaperones and CHOP occurs without phosphorylation of eIF2α by PERK

(Colla et al., 2012a), perhaps indicating that adaptive and pro-apoptotic responses are out of balance. Other studies indicate that activation of IRE1/XBP1 is key to neuronal survival in PD, and that this particular UPR branch may be selectively important in neurons of the substantia nigra (Valdés et al., 2014). Still other work finds that a-syn toxicity involves the imbalanced outcomes of increased chaperones accompanied by decreased activation of the ATF6 pathway (Credle et al., 2015). A compelling correlate of this hypothesis is that activation of the ATF6 pathway requires ER/Golgi transport, and ATF6 export from the ER is potently inhibited by a-syn (Credle et al., 2015).

Precisely how does a-syn cause ERS in the first place? The most obvious mechanism would be inhibition of ER/Golgi transport and/or later steps in the biosynthetic secretory pathway, leading to ER overload. The finding that over-expression of secretory rabs and other machinery alleviates a-syn toxicity is consistent with ERS originating from a trafficking defect (Gitler et al., 2008; Yin et al., 2014). However, ER/Golgi transport could also be viewed as a relief valve for ERS caused by perturbation of other ER functions. In addition, some of the secretory machinery whose expression rescues stress, rab1a for example, may also more directly regulate UPR events or autophagy induction (Winslow et al., 2010; Chua and Tang, 2013).

Evidence exists for several modes by which a-syn could initiate ERS through mechanisms distinct from ER/Golgi trafficking. For example, it has been suggested that a-syn may find its way inside the lumen of the ER, where it could aggregate with chaperones to cause ERS (Colla et al., 2012b). A mechanism for a-syn to cross the ER membrane has not been demonstrated. One speculation could be uptake of extracellular a-syn aggregates by endocytosis followed by retrograde vesicular transport to the ER lumen, as in the case of certain bacterial toxins (Spooner and Lord, 2012). A completely distinct mechanism by which a-syn may trigger ERS is by inhibition of ER associated degradation (ERAD), which would cause buildup of excess unfolded proteins. Homocysteine-induced ER protein (Herp) is overexpressed in PD and is a component of Lewy bodies (Slodzinski et al., 2009) whose knockdown sensitizes PC12 cells to ERS-induced apoptosis and a-syn toxicity (Belal et al., 2012). Herp plays an essential role in ERAD (Schulze et al., 2005), particularly ERAD-dependent turnover of ER calcium channels (Belal et al., 2012). Herp inhibition or aggregation in PD could lead to general ERAD inhibition or ER calcium depletion, either of which could cause ERS. ERAD relies upon the ubiquitin-proteasome system (UPS) for the disposal of ER-dislocated substrates, and there are many links between the UPS and PD, only discussed here as it relates to ERS. Several E3 ubiquitin ligases, for example parkin, HRD1 and Rsp5/Nedd4, can protect cells from a-syn intoxication and ERS (Tsai et al., 2003; Omura et al., 2013; Tardiff et al., 2013). The mechanism by which ubiquitin ligases protect neurons from ERS is unclear. One possibility is that the UPS contributes to the removal of specific stress-causing membrane proteins such as parkin-associated endothelin receptor-like receptor (Pael-R) that have been implicated in PD pathology (Kitao et al., 2007; Omura et al., 2013). Parkin also triggers mitophagy when recruited to defective mitochondria by the voltage sensor PINK (Kroemer et al., 2010). But there could also be more fundamental link(s) whereby the UPS promotes ER homeostasis, for example through a direct regulatory role in the initiation of UPR, ERAD, or ER-phagy. This could help explain why multiple neurodegenerative diseases involving aggregated cytosolic proteins result in UPR–a process conventionally viewed as initiated in the ER lumen.

DYSFUNCTIONS IV: GOLGI HOMEOSTASIS

Golgi fragmentation is commonly observed in PD as well as other neurodegenerative diseases (Gonatas et al., 2006; Fan et al., 2008; Bexiga and Simpson, 2013). Overexpression of a-syn in cultured mammalian cell lines and neurons can lead to Golgi fragmentation; formation of a-syn prefibrillar aggregates correlates tightly with Golgi and microtubule disruption (Gosavi et al., 2002; Lee et al., 2006). Alpha-syn accumulation in yeast results in mislocalization of Golgi markers and aggregation of secretory vesicles (Soper et al., 2008, 2011). However, the functional and pathological significance of Golgi disruption remains unclear. In some cases, Golgi disruption seems to precede microtubule disruption and inhibition of ER/Golgi transport (Rendón et al., 2013), implying that Golgi disruption is not a result of the trafficking block, and could represent an independent branch of the pathology. In other cases the Golgi disruption seems to accompany and be due to the ER/Golgi transport inhibition (Coune et al., 2011) or be absent during transport inhibition (Thayanidhi et al., 2010).

The lack of a consistent correlation between ER/Golgi transport inhibition and Golgi disruption makes the role of Golgi homeostasis in PD hard to evaluate. However, an intriguing idea is that Golgi dispersal may be a means to propagate an a-syn-caused Golgi-based stress signal. Increased neuronal firing activity causes reversible Golgi fragmentation, suggesting it may be part of a homeostatic stress signaling mechanism (Thayer et al., 2013). Furthermore, inhibition of Golgi dispersal through over-expression of Golgi tethering proteins can reduce the toxicity of chemically-induced ER or oxidative stress (Nakagomi et al., 2008). How Golgi fragmentation is initiated in response to a-syn remains unclear, but signaling mechanisms mediating other stress-related Golgi fragmentation have been outlined. For example, the small GTPase ADP-ribosylation factor 4 (ARF4) initiates Golgi dispersal in response to pathogens and chemical insults (Reiling et al., 2013), and Cdk5 kinase phosphorylation of Golgi matrix protein 130 (GM130) may mediate Golgi dispersal in neurons challenged with beta-amyloid (Sun et al., 2008). It would be exciting to test whether blockade of Golgi-dispersing signals can relieve a-syn toxicity.

THE ENDOLYSOSOMAL SYSTEM AND ALPHA-SYNUCLEIN CLEARANCE

The importance of the endolysosomal system in a-syn toxicity cannot be overstated but will only be briefly introduced. The clearance of a-syn largely depends upon the endosome-lysosome pathway (Flower et al., 2007; Lee et al., 2008; Liu et al., 2009;

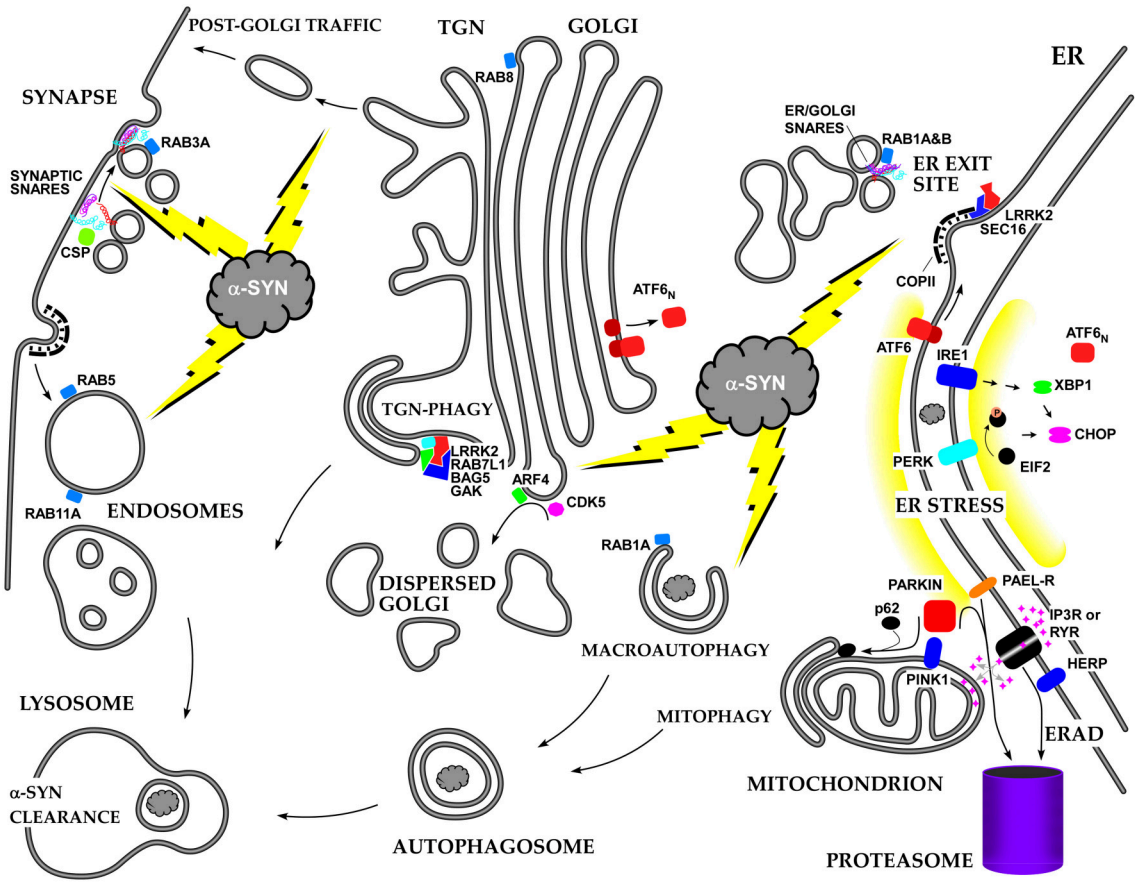

FIGURE 2 | Summary of the roles of alpha-synuclein and other PD-associated proteins in the secretory pathway and the resulting stress responses.

Alvarez-Erviti et al., 2011). This process appears to be Rab11a-dependent (Liu et al., 2009), which likely explains Rab11a's involvement in synucleinopathy (Breda et al., 2015). Alpha-syn over-expression appears to interfere with endosomal trafficking (Soper et al., 2008, 2011; Volpicelli-Daley et al., 2014), and this likely exacerbates a-syn toxicity by potentiating a-syn accumulation. In addition, impaired lysosomal activity could contribute to PD (van Dijk et al., 2013).

Alpha-syn accumulation triggers macroautophagy, the process by which cytosolic proteins and organelles are imported into lysosomes. ERS triggered by a-syn accumulation probably contributes to autophagy activation since the PERK and ATF6 UPR signaling arms induce autophagy genes (He and Klionsky, 2009; Kroemer et al., 2010). However, aggregated or over-expressed alpha-syn inhibits autophagy and results in less efficient clearance, more accumulation and greater cell-to-cell spread of a-syn pathology (Winslow et al., 2010; Lee et al., 2013; Tanik et al., 2013).

OXIDATIVE STRESS

Oxidative stress is another outcome of alpha-syn accumulation, and is closely associated with disruption of ER proteostasis (Exner et al., 2012). Similar to ERS, mitochondrial dysfunction is present in many neurodegenerative diseases (Lionaki et al., 2015), and will be important in disease intervention. Still, a key question remaining is whether the pathological oxidative stress is a downstream manifestation of other stresses, for example perturbed ER calcium or reduced autophagic clearance of defective mitochondria (Chigurupati et al., 2009; Su et al., 2010), or does it belie a more fundamental relationship with alpha-synuclein?

CONCLUSION

We have briefly summarized the multifaceted effects of alpha-syn on cellular membrane trafficking, with special emphasis on the ER-Golgi axis (see **Figure 2**). Many more studies will be required to unravel all of the mechanisms of alpha-syn toxicity, and the early secretory pathway will likely remain a major focus. One of the major issues to resolve is how a-syn manages to play positive as well negative roles in membrane trafficking due to its concentration, aggregation state, and SNARE- and lipid-binding properties. Another major issue is how a-syn triggers its unique kind of ERS and how this stress is modulated by ER/Golgi

transport, ERAD, and the ubiquitin-proteasome system. In addition, it should be resolved whether there is a common pattern of dysfunction in the early secretory pathway lying at the heart of multiple neurodegenerative diseases.

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Golgi fragmentation in Alzheimer's disease

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The Golgi apparatus is an essential cellular organelle for post-translational modifications, sorting, and trafficking of membrane and secretory proteins. Proper functionality of the Golgi requires the formation of its unique cisternal-stacking morphology. The Golgi structure is disrupted in a variety of neurodegenerative diseases, suggesting a common mechanism and contribution of Golgi defects in neurodegenerative disorders. A recent study on Alzheimer's disease (AD) revealed that phosphorylation of the Golgi stacking protein GRASP65 disrupts its function in Golgi structure formation, resulting in Golgi fragmentation. Inhibiting GRASP65 phosphorylation restores the Golgi morphology from Aβ-induced fragmentation and reduces Aβ production. Perturbing Golgi structure and function in neurons may directly impact trafficking, processing, and sorting of a variety of proteins essential for synaptic and dendritic integrity. Therefore, Golgi defects may ultimately promote the development of AD. In the current review, we focus on the cellular impact of impaired Golgi morphology and its potential relationship to AD disease development.

Keywords: Alzheimer's disease, amyloid precursor protein, amyloid beta, Golgi, GRASP65, phosphorylation

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Introduction

The Golgi apparatus is a membranous cellular organelle that mediates proper trafficking, post-translational processing, and sorting of membrane and secretory proteins. The Golgi is often localized to the perinuclear region of the cell, which depends on an intact microtubule network and a motor protein dynein. Golgi membranes form multilayer stacks that are laterally linked into a ribbon. Formation of stable multilayer stacks and ribbons appears to be essential for proper functioning of the Golgi (Rambourg et al., 1987; Ladinsky et al., 1999; Klumperman, 2011; Klute et al., 2011). The Golgi structure is controlled by Golgi matrix proteins, a group of peripheral and membrane proteins localized on the cytoplasmic surface of Golgi membranes, including the coiled-coil domain-containing golgins and the stacking proteins GRASP55/65 (Ramirez and Lowe, 2009; Xiang and Wang, 2011; Wong and Munro, 2014). Depletion of Golgi matrix proteins such as GRASP65 (Xiang et al., 2013; Veenendaal et al., 2014), GRASP55 (Feinstein and Linstedt, 2008; Xiang and Wang, 2010), GM130 (Puthenveedu et al., 2006), golgin-84 (Diao et al., 2003), and golgin-160 (Williams et al., 2006), or treatment of cells with pharmacological drugs such as Brefeldin A (BFA) (Klausner et al., 1992), results in an abnormal, fragmented Golgi morphology. Disruption of normal Golgi morphology directly impacts protein trafficking and processing. For example, breaking down the Golgi ribbon of mammalian cells by nocodazole treatment strongly inhibits intra-Golgi transport of large cargoes without altering the rate of transport of smaller cargoes (Lavieu et al., 2014), while inhibition of stack formation by knocking down GRASP55/65

by RNA interference (RNAi) accelerates protein trafficking, impairs proper glycosylation, and leads to missorting and delivery of lysosomal proteins to the extracellular space (Xiang et al., 2013). Thus, the structure of the Golgi is closely linked to vital cellular processes.

Golgi fragmentation has been observed in neurodegenerative diseases, including Alzheimer's (AD) (Stieber et al., 1996; Huse et al., 2002), Parkinson's (PD) (Mizuno et al., 2001), and Huntington's (HD) (Hilditch-Maguire et al., 2000) diseases and amyotrophic lateral sclerosis (ALS) (Mourelatos et al., 1996; Gonatas et al., 1998; Fujita and Okamoto, 2005). However, the molecular basis of Golgi fragmentation and its role in disease progression remain largely unexplored. Golgi defects may impact the trafficking and processing of many proteins essential for neuronal functions. Thus, morphological and subsequent functional impairments of the Golgi may contribute to the neurotoxicity associated with neurodegenerative diseases. Understanding the mechanisms that cause Golgi fragmentation and its downstream effects on neuronal functions are likely important for understanding the molecular basis of the diseases. Furthermore, targeting Golgi structural defects may represent a novel approach to treating or preventing related diseases with Golgi defects. In this review, we summarize the current literature on the cause and effect of Golgi fragmentation in AD, the leading cause of dementia in adults (Alzheimer's, 2015).

Golgi and Amyloid Plaque Formation

One neuropathological hallmark of AD is the formation of extracellular amyloid plaques by secreted amyloid beta (Aβ) peptides (Nelson et al., 2009), which is highly related to Golgi structure and function (**Figure 1**). Aβ is derived from the amyloid precursor protein (APP), a type I membrane protein that travels through the exocytic and endocytic pathways and undergoes sequential proteolysis by the action of β- and γ-secretases (Vassar et al., 1999). In neurons, APP is transported from the Golgi to many sub-cellular compartments (Haass et al., 1992), including the soma, dendrites and axons, through the exocytic and endocytic pathways. Despite the abundant literature demonstrating the critical role of endosomes in APP processing (for review see Suh and Checler, 2002; Small and Gandy, 2006), it has been indicated that the Golgi (in particular the *trans*-Golgi network) may be a site where Aβ is generated in the cell (Greenfield et al., 1999; Burgos et al., 2010; Choy et al., 2012). In addition, proper functioning of the Golgi is required for trafficking and maturation of both APP and its processing enzymes. For instance, the activity of the γ-secretase depends on the trafficking and maturation of nicastrin (Chung and Struhl, 2001) and other components of the γ-secretase complex through the Golgi (Herreman et al., 2003). Nicastrin is not catalytically active, but is important for the maturation and proper trafficking of the γ-secretase complex (Zhang et al., 2005). Nicastrin functions to stabilize presenilins (PSs), the catalytic subunit of the γ-secretase complex, and mediates PS trafficking to the cell surface by an unknown mechanism (Edbauer et al., 2002; Hu et al., 2002). Nicastrin also binds to the N-terminal domain of APP, and facilitates APP trafficking and cleavage (Yu et al., 2000; Kimberly et al., 2002). APP travels from the endoplasmic reticulum (ER) through the Golgi to the plasma membrane. The majority of APP localizes to the Golgi where it undergoes post-translational modifications and only a small fraction resides in the ER and the plasma membrane (Thinakaran and Koo, 2008). Finally, the α-secretatse ADAM10 is transported from dendritic Golgi outposts to synaptic membranes, a reaction modulated by the synapse-associated protein-97 (SAP97) (Saraceno et al., 2014). Thus, APP processing and Aβ production are intrinsically linked to the proper morphology and functionality of the Golgi.

Golgi and Tau Pathology in AD

Another pathological distinction in AD is the formation of neurofibrillary tangles (NFTs) caused by precipitation of hyperphosphorylated tau protein, which is also related to Golgi structure and function (**Figure 1**) (Morishima-Kawashima and Ihara, 2002). Tau is an abundant protein that binds and stabilizes microtubules in neurons (Gong and Alonso Adel, 2013). Neurons have long protrusions such as axons; transport of membrane organelles and proteins into and from these protrusions highly depends on an intact microtubule network (Baas, 2002). Long microtubules emanate out of the centrosomes and function as the track for rapid transport of membranes by kinesin and dynein motors (Hancock, 2014). Membranes and proteins for synaptic vesicle formation and synaptic function are essentially all transported in this way (Gallant, 2000; Goldstein and Yang, 2000).

In AD, hyperphosphorylation of tau results in NFT formation, which impairs microtubule integrity and blocks membrane transport (Patrick et al., 1999; Avila, 2006; Baloyannis, 2014). Interestingly, the Golgi, whose formation relies on an intact microtubule network (Rios and Bornens, 2003), may also function as an important microtubule organization center (Lewis and Polleux, 2012; Ori-McKenney et al., 2012; Zhu and Kaverina, 2013). Multiple microtubule-binding proteins, such as γ-tubulin (Radulescu et al., 2011), CLASP (Liu et al., 2007; Miller et al., 2009), GMAP-210 (Infante et al., 1999; Roboti et al., 2015), and GM130 (Rivero et al., 2009), are localized on the Golgi membranes and modulate microtubule network formation and membrane trafficking in neurons (Rios, 2014; Tang et al., 2015) and astrocytes (Yoshiyama et al., 2003). It has been reported that tau interacts with Golgi membranes and mediates their association with microtubules (Farah et al., 2006). Overexpression of wild-type and mutant human tau proteins causes Golgi fragmentation in primary hippocampal neurons (Liazoghli et al., 2005), implying that overabundance of tau, and potentially NFT formation, precedes Golgi morphological defects. Additionally, Golgi fragmentation may affect tau phosphorylation (Jiang et al., 2014) and promote NFTs formation in AD (Grundke-Iqbal et al., 1986). Therefore, there is a close connection between Golgi morphology and function, microtubule organization, and tau pathology in AD.

Golgi Morphological Defects in AD

Golgi morphological alterations have been observed in neurons of AD patients (Dal Canto, 1996; Stieber et al., 1996; Gonatas

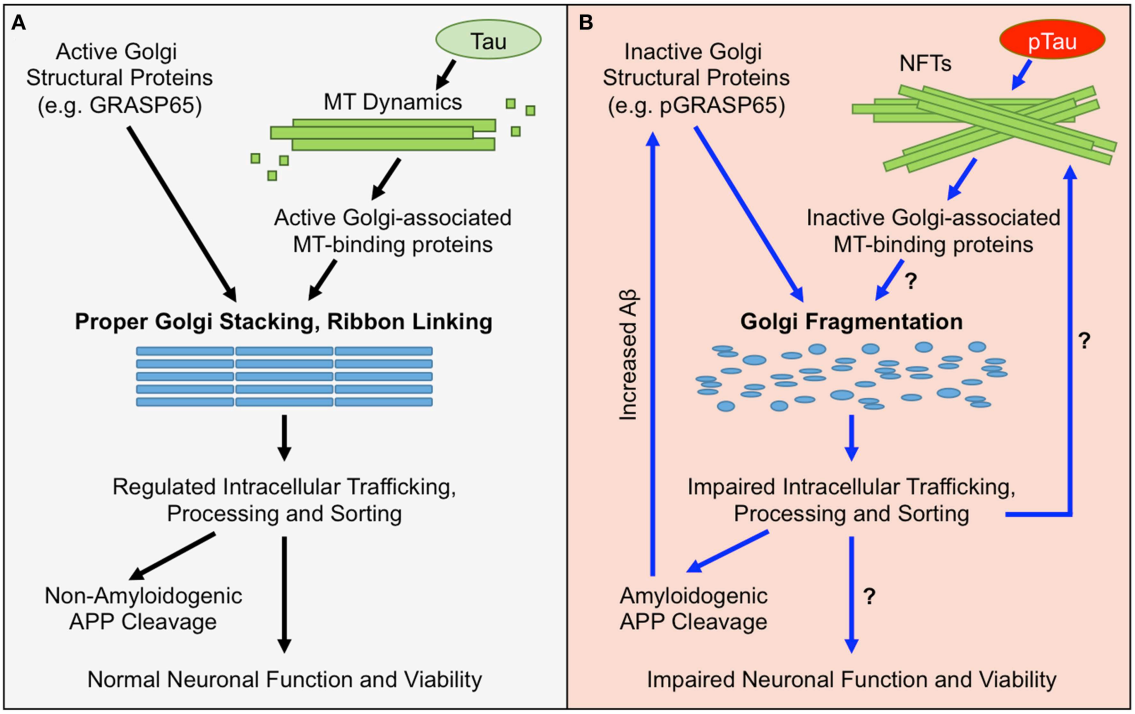

FIGURE 1 | Golgi Morphological and Functional Defects in AD. (A) Under normal conditions, the structure of the Golgi is maintained by active Golgi structural proteins such as GRASP65 (non-phosphorylated) and an intact microtubule (MT) network. Maintaining the Golgi structure is essential for proper trafficking and processing of APP and its processing enzymes. The majority of APP undergoes non-amyloidogenic processing, and cell-surface proteins, lipids, and polysaccharides, which are essential for neuronal function, are properly sorted and transported. Together, these factors maintain neuronal functionality and viability. **(B)** In AD, the Golgi is fragmented due to inactivation of Golgi structural proteins, such as degradation or phosphorylation of GRASP65 (pGRASP65), or tau hyper phosphorylation (pTau) and NFT formation that disrupt MT dynamics and protein trafficking. Golgi fragmentation impairs trafficking, processing, and sorting of APP and APP-processing enzymes, which stimulates amyloidogenic APP cleavage and further inactivates GRASP65. Additionally, Golgi fragmentation is predicted to alter trafficking, processing, and sorting of proteins, lipids, and polysaccharides that are essential for neuronal function; which could ultimately promote neuronal dysfunction and/or cell death.

et al., 1998; Huse et al., 2002), which occur even before the formation of NFTs and neuritic plaques (Baloyannis, 2014). Golgi fragmentation has been recently confirmed in AD tissue culture and mouse models (Joshi et al., 2014). Expression of the Swedish mutant APP (APPswe) and the exon 9-deletion mutant PS1 (PS1ΔE9) in cells and mouse brain, or treatment of neurons with synthetic Aβ peptides, resulted in Golgi fragmentation. Under fluorescence microscopy, Golgi elements are unlinked and dispersed into the cytoplasm. Under electron microscopy (EM), the disconnected Golgi stacks exhibit reduced number of cisternae per stack, shorter cisternae, more vesicles surrounding each stack and a dilated Golgi structure compared with wild type cells and untreated neurons (Joshi et al., 2014). In this study, the direct cause of Golgi fragmentation is Aβ accumulation, as Aβ treatment causes Golgi fragmentation in cultured neurons and other cell types; this effect is reversible upon removal of Aβ from the tissue culture medium (Joshi et al., 2014). Golgi fragmentation has also been observed under physiological conditions, such as during migration, upon growth factor treatment, and in neurons with increased neuronal activity (Bisel et al., 2008; Thayer et al., 2013), suggesting that Golgi fragmentation may not be an immediate pathological response, but rather a compensatory reaction to allow fast transport of proteins to their final destinations when the cells are under stress. Golgi fragmentation, however, impacts protein glycosylation and sorting (Wang et al., 2008; Xiang et al., 2013), as we discuss below.

Mechanisms of Golgi Morphological Defects in AD

The formation and maintenance of the perinuclear localization of the Golgi ribbon rely on an intact microtubule cytoskeleton emanating from the perinuclear centrosomes (Glick and Nakano, 2009; Yadav and Linstedt, 2011; Rios, 2014). The minus end-directed motor dynein associates with the Golgi and moves the membranes along the microtubules toward the centrosomes, leading to the concentration of the Golgi stacks in the pericentriolar region and formation of the Golgi ribbon (Rios and Bornens, 2003; Miller et al., 2009). The basic structural and functional unit of the Golgi is a stack of flattened cisternae. The exact mechanism for Golgi stack formation is not fully understood, but is believed to rely on Golgi structural proteins (Wang and Seemann, 2011; Xiang and Wang, 2011). Among the well characterized Golgi structural proteins, GRASP65 (Wang et al., 2003) and GRASP55 (Xiang and Wang, 2010) play

essential roles in Golgi structure formation, whereas others, including GM130 (Lowe et al., 1998), golgin-160 (Hicks and Machamer, 2002), and p115 (Chiu et al., 2002), are more important for trafficking across the Golgi stack (Xiang and Wang, 2011). For example, GRASP65 forms oligomers that tether the cisternae into stacks (Wang et al., 2003; Tang et al., 2010; Lavieu et al., 2013) and ribbons (Puthenveedu et al., 2006). Recently a GRASP65 knockout mouse has been reported, with defects in *cis*-Golgi ribbon-linking and no apparent neurological phenotype (Veenendaal et al., 2014). One potential concern regarding this mouse strain is that some mRNA encoding exon 1–3 is still present (∼20% of the mRNA level in wild-type mice). This truncated mRNA encodes a 115 aa N-terminal fragment of GRASP65. If translated (although not detected by available antibodies raised against the full length protein), this fragment would be sufficient for Golgi stacking, according to biochemical and structural studies (Tang et al., 2010; Truschel et al., 2011). However, a more recent structure (Feng et al., 2013) indicates that a longer fragment is needed for GRASP65 function in Golgi stack formation (Wang et al., 2003, 2005). The lack of a more prominent Golgi stacking and neurological phenotype may also be due to the complementation by GRASP55, the homolog of GRASP65 that shares some redundancy in stacking (Shorter et al., 1999; Lee et al., 2014).

The mechanism of Golgi fragmentation in AD has not been well studied, but likely involves multiple mechanisms (**Figure 1**). One possibility is through the disruption of the microtubule network by tau precipitation and NFT formation (Liazoghli et al., 2005). Microtubule defects may affect both the central localization of the Golgi in the cell and ER-Golgi-plasma membrane trafficking that indirectly impacts the size and morphology of the Golgi (Fokin et al., 2014). Tau could also affect vesicle trafficking by inhibiting the binding of motor proteins such as kinesins to microtubules (Seitz et al., 2002). Another possibility is through modulation of Golgi structural proteins (**Figure 2**). Both mitotic phosphorylation and apoptotic cleavage of Golgi proteins results in Golgi fragmentation (Wang and Seemann, 2011). For instance, GRASP65 is phosphorylated by mitotic kinases Cdk1 and Polo-like kinase (Plk1) during mitosis (Wang et al., 2003) and cleaved by caspase-3 in apoptosis (Lane et al., 2002), both of which cause Golgi fragmentation (Tang et al., 2008; Wang and Seemann, 2011). In tissue culture and mouse models of AD, GRASP65 phosphorylation was implicated as a major cause of Golgi fragmentation (Joshi et al., 2014; Joshi and Wang, 2015). At the molecular level, Aβ accumulation triggers Ca^{2+} influx (Zempel et al., 2010), which activates Calpain, a protease known to increase the cleavage of p35 to p25 (Lee et al., 2000), p25 then activates Cdk5. It has been previously reported that p35 and Cdk5 are associated with Golgi membranes and regulate membrane traffic (Paglini et al., 2001). Subsequently, Cdk5 (also known to phosphorylate tau in AD) phosphorylates GRASP65, which negatively regulates GRASP65, leading to Golgi fragmentation. Consequently, Golgi fragmentation enhances APP trafficking and increases Aβ production (Joshi et al., 2014). Fragmentation of the Golgi was rapidly reversible by the use of Cdk5-specific inhibitors, or by expression of non-phosphorylatable GRASP proteins, both of which significantly reduced APP trafficking and Aβ production (**Figure 2**). In the same study, degradation of Golgi structural proteins was not detected (Joshi et al., 2014). These results suggest that Golgi fragmentation in AD, at least in the early stage, is caused by phosphorylation of Golgi structural proteins, an event that occurs in parallel with tau hyper-phosphorylation during the development of the disease. Overall, the causes of Golgi structural defects in AD are expected to be manifold and require further investigation to determine the precise mechanisms.

Functional Consequences of Golgi Fragmentation in AD

Proper Golgi structure formation controls the sequence and speed of protein transport through the Golgi membranes for proper trafficking, maturation, sorting, and processing of not only APP, but also many neuronal proteins (Dries and Yu, 2008; Joshi and Wang, 2015). When BACE1 activity is increased or Aβ clearance is decreased, Aβ accumulation induces Golgi fragmentation through modification of GRASP65, and other Golgi structural proteins (Joshi et al., 2014; Joshi and Wang, 2015). Fragmentation of the Golgi, as one possible outcome, enhances vesicle budding from the Golgi membranes, accelerates protein trafficking, and impairs accurate glycosylation (Wang et al., 2008; Xiang et al., 2013), and thus increases Aβ production by enhancing amyloidogenic cleavage (**Figure 2**). APP and the β-secretase BACE1 are sorted by the Golgi into different compartments and the loss of the sorting function of the Golgi by fragmentation in AD results in defective trafficking of APP and BACE1, which promotes Aβ production (Tan and Evin, 2012; Das et al., 2013; Joshi et al., 2014). This deleterious feedback loop may impair the integrity of the secretory pathway for sorting, trafficking and modifications of many essential neuronal proteins. For example, many AD-related proteins that are essential for neuronal function, such as NMDA and AMPA receptors (Pérez-Otaño and Ehlers, 2005; Greger and Esteban, 2007; Shepherd and Huganir, 2007), and synaptic integrity proteins such as neurexins (Fairless et al., 2008; Reissner et al., 2013), are processed and trafficked through the Golgi. Thus, Golgi fragmentation might impair trafficking of membrane receptors and ion channels that regulate neuronal function and/or viability (**Figure 1**).

Long term Golgi defects might also result in a significant change in the composition of proteins, lipids, and polysaccharides at the cell surface (**Figure 1**). A change in the composition of cell-surface molecules could not only directly impair neuronal activity and synaptic integrity, but also potentially trigger an immune response against neurons with Golgi defects that leads to cell death. In addition, Golgi defects may impact microtubule organization, tau function, and cell polarity. The Golgi also functions as a storage reservoir for Ca^{2+} (Pinton et al., 1998; Griesbeck et al., 2001), a small molecule essential for neurotransmission. Ca^{2+} is a key regulator of membrane fusion (Pang and Sudhof, 2010), synaptic plasticity (Neher and Sakaba, 2008), and neurite growth (Tojima et al., 2011). Defects in calcium signaling has been observed

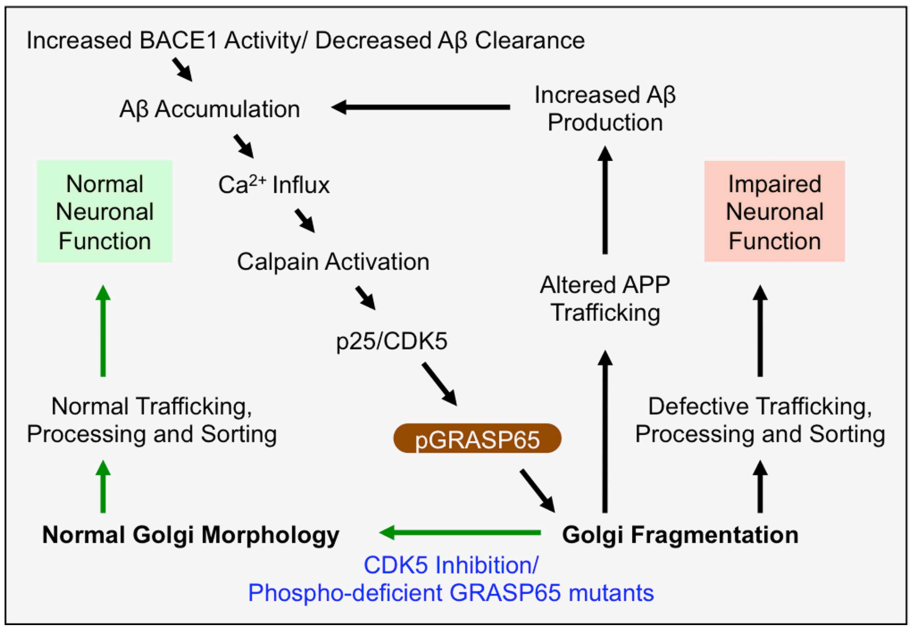

FIGURE 2 | Mechanism of Golgi defects in AD. Increased BACE1 activity and/or decreased Aβ clearance from the extracellular space leads to the accumulation of Aβ. In turn, Aβ induces Ca^{2+} influx, which activates Calpain and induces cleavage of p35 to p25. Consequently, p25 activates CDK5, which in turn phosphorylates and inactivates the Golgi structural protein GRASP65 (i.e., pGRASP65). Inactivation of GRASP65 causes Golgi fragmentation, which alters trafficking of APP, and potentially the secretases, leading to increased Aβ production. This deleterious feedback loop (indicated by black arrows) would impair the integrity of the secretory pathway for sorting, trafficking and modifications of many essential proteins, which may compromise neuronal function, activate inflammatory responses, or cause neuronal cell death. Inhibition of CDK5 or expression of non-phosphorylatable GRASP65 mutants restores the normal Golgi morphology and reduces APP trafficking and Aβ production (indicated by green arrows). Therefore, rescue of the defective Golgi may delay AD development.

in neurodegeneration (Wojda et al., 2008), and prolonged neuronal hyperexcitability and neuronal activity lead to Golgi fragmentation (Thayer et al., 2013). Taken together, Golgi defects may compromise neuron activity and survival through multiple pathways and serve as an important mechanism of pathogenesis in AD and other neurodegenerative diseases.

Conclusion

The Golgi apparatus plays an essential role in trafficking and sorting of proteins that are vital for neuronal functions. The Golgi is fragmented in many neurodegenerative diseases, suggesting that Golgi defects may contribute to neurodegeneration. Golgi fragmentation likely involves multiple mechanisms. In a recent study on AD, Golgi fragmentation is caused by phosphorylation of the Golgi structural protein GRASP65 and Golgi fragmentation results in enhanced APP trafficking and Aβ production. Golgi fragmentation is predicted to impair intracellular trafficking of many proteins that are essential for neuronal function. Restoring Golgi morphological defects might be an attractive approach to treating, or even preventing, AD. Understanding the impact of Golgi pathology and determining the molecular mechanisms that cause Golgi fragmentation is expected to shed insight into novel therapeutic approaches for treating AD and potentially other neurodegenerative disorders.

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Golgi fragmentation in amyotrophic lateral sclerosis, an overview of possible triggers and consequences

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Amyotrophic Lateral Sclerosis (ALS) is an invariably fatal neurodegenerative disorder, which specifically targets motor neurons in the brain, brain stem and spinal cord. Whilst the etiology of ALS remains unknown, fragmentation of the Golgi apparatus is detected in ALS patient motor neurons and in animal/cellular disease models. The Golgi is a highly dynamic organelle that acts as a dispatching station for the vesicular transport of secretory/transmembrane proteins. It also mediates autophagy and maintains endoplasmic reticulum (ER) and axonal homeostasis. Both the trigger for Golgi fragmentation and the functional consequences of a fragmented Golgi apparatus in ALS remain unclear. However, recent evidence has highlighted defects in vesicular trafficking as a pathogenic mechanism in ALS. This review summarizes the evidence describing Golgi fragmentation in ALS, with possible links to other disease processes including cellular trafficking, ER stress, defective autophagy, and axonal degeneration.

Keywords: amyotrophic lateral sclerosis, Golgi fragmentation, ER stress, axonal degeneration, secretory trafficking inhibition, autophagy dysfunction

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INTRODUCTION

The Golgi apparatus (referred to as "Golgi" hereafter) acts as a dispatching station whereby proteins and lipids newly synthesized in the ER are transported to the endosomal system, secretory granules, or plasma membrane. In spite of being a highly dynamic organelle (Griffiths et al., 1989), the Golgi normally maintains a characteristic morphology, consisting of flattened membrane stacks known as cisternae, and associated vesicles. The stacks of Golgi cisternae are interconnected laterally by tubules, forming a ribbon-like network (Rambourg and Clermont, 1990; Polishchuk and Mironov, 2004), usually in the perinuclear region of the cell, adjacent to the centrosome (Linstedt, 2004). The Golgi comprises of three functional compartments: the cis-Golgi, which being the nearest compartment to the ER, forms the entry face to the Golgi, the medial-Golgi, which is responsible for the modification, sorting and packaging of proteins for transportation, and finally the trans Golgi network, which forms the exit face of the Golgi (Rothman and Wieland, 1996; Glick and Nakano, 2009). Specific types of intracellular vesicles are associated with the Golgi. Secretory protein cargo buds from the ER via coat protein complex II (COPII) coated vesicles, to form tubulovesicular structures known as the ER-Golgi intermediate compartment (ERGIC), which eventually fuse with the cis-Golgi (Appenzeller-Herzog and Hauri, 2006). In contrast, the reticular trans-Golgi network (TGN) produces clathrin-coated vesicles which are targeted to endosomes and secretory vesicles in specialized cell types such as neurons (De Matteis and Luini, 2008).

In addition to secretory trafficking, the Golgi is also responsible for the post-translational modification of proteins and lipids, including glycosylation (Stanley, 2011), sulfation (Baeuerle and Huttner, 1987), and proteolytic cleavage (Xu and Shields, 1993).

THE GOLGI IN NEURONS

Neurons are highly specialized cells with unique functional and morphological characteristics. Interestingly, in neurons the Golgi forms specialized "Golgi outposts" localized in axons and dendrites, which are discrete structures that are discontinuous from the somatic Golgi (**Figure 1**). These Golgi outposts are not fully characterized, but are thought to facilitate local secretory trafficking within neurites (Horton and Ehlers, 2003; Merianda et al., 2009). Axonal transport is an important property in neurons which involves trafficking of cellular proteins and vesicles within the axon, towards or away from the cell body. The relationship between axonal transport and transport within the soma is not fully understood, but these processes are clearly linked and involve the Golgi (Hirokawa and Takemura, 2005; Schwarz, 2013).

FRAGMENTATION OF THE GOLGI APPARATUS

The Golgi is capable of undergoing profound morphological changes during normal cellular processes such as mitosis, as well as in pathological conditions. These morphological changes result in disruption of its characteristic ribbon-like network, forming a fragmented Golgi. Golgi fragmentation during mitosis facilitates equal distribution of the Golgi into the resulting daughter cells (Sütterlin et al., 2002). However, irreversible Golgi fragmentation occurs in pathological situations, when apoptosis is activated. Under these conditions, structural proteins within the Golgi are cleaved by the action of caspases (Lane et al., 2002). The Golgi also fragments when vesicular secretory trafficking is perturbed (Dascher and Balch, 1994; Wilson et al., 1994), which may also occur in pathological conditions. The morphological changes evident during fragmentation of the Golgi are attributed to two possibilities, either the Golgi membranes break into smaller dispersed vesicular structures (**Figure 1**), or the Golgi fuses with the ER upon fragmentation, which is then recycled, and it remerges at ER exit sites, dispersed throughout the cytoplasm (Cole et al., 1996; Storrie et al., 1998; Pelletier et al., 2000; Glick, 2002).

Golgi pathology is a feature of neurodegenerative diseases including Alzheimer's disease (Sun et al., 2008), Parkinson's disease (Fujita et al., 2006), Creutzfeldt-Jakob disease (Sakurai et al., 2000), multiple system atrophy (Sakurai et al., 2002), and ALS. Interestingly, Golgi fragmentation is often detected as an early event in these conditions, prior to apoptosis (Gosavi et al., 2002; Liazoghli et al., 2005; Atkin et al., 2014; van Dis et al., 2014), suggesting that Golgi fragmentation could be a trigger for neurodegeneration rather than a simple consequence of neuronal death. We review here the evidence describing Golgi fragmentation in ALS, and discuss recent studies implicating impairment of ER-Golgi mediated vesicular trafficking as a possible trigger. We also predict possible downstream consequences of Golgi fragmentation in ALS, and we examine links to other pathologies, including ER stress, autophagy dysfunction, and axonal degeneration.

AMYOTROPHIC LATERAL SCLEROSIS

Whilst 90% of ALS cases are sporadic, 10% of cases are familial, caused by mutations in genes encoding ubiquitously expressed proteins, including transactive response DNA binding protein (TDP-43), fused in sarcoma (FUS), optineurin, superoxide dismutase 1 (SOD1), and Chromosome 9 open reading frame 72 (C9orf72) (Renton et al., 2014) (**Table 1**). Although, the etiology of ALS remains unknown, RNA dysfunction and disruption to proteostasis are widely implicated as pathogenic mechanisms (Ling et al., 2013). Dysfunction to proteostasis includes protein misfolding and aggregation, ER stress, Golgi fragmentation, autophagy dysfunction, inhibition of cellular trafficking, and axonal degeneration. Like other neurodegenerative disorders, a pathological hallmark of ALS is the accumulation of intracellular inclusions containing misfolded protein aggregates (Wood et al., 2003; Blokhuis et al., 2013). Interestingly, wildtype (WT) forms of TDP-43, FUS, optineurin, and SOD1 may be recruited into ubiquitinated protein inclusions in sporadic ALS patients (Neumann et al., 2006; Deng et al., 2010; Blokhuis et al., 2013). Cytoplasmic accumulation, hyperphosphorylation and/or aggregation of TDP-43 is present in almost all cases of ALS (approximately 97%) (Ling et al., 2013). Transgenic mice overexpressing mutant $SOD1^{G93A}$ are the most widely used animal models of disease, which recapitulate many clinical and pathological features of ALS (Gurney et al., 1994). Increasing evidence now links ALS to frontotemporal dementia (FTD), with recent studies suggesting that ALS and FTD represent opposite ends of the disease spectrum (Ling et al., 2013).

Defects in intracellular trafficking, particularly within the axon, are implicated in ALS (Bilsland et al., 2010; Ikenaka et al., 2012; Alami et al., 2014). The "dying back" or slow degeneration of distal to proximal axons is associated with loss of motor neurons in ALS (Fischer et al., 2004; Dadon-Nachum et al., 2011; Moloney et al., 2014). In SOD1 mice, distal axonopathy and denervation of neuromuscular junctions are observed prior to the onset of clinical manifestations (Fischer et al., 2004). Fast-fatigable motor neurons with the longest axons and highest metabolic demands, are the most susceptible to axonal degeneration (Frey et al., 2000; Fischer et al., 2004). It has been suggested that lack of supply of essential proteins and lipids to distal axons is associated with axonal degeneration (Perlson et al., 2010).

Autophagy is an important proteostatic mechanism to degrade misfolded proteins in post-mitotic neurons (Thomas et al., 2013). It is therefore not surprising that defects in autophagy are present in ALS, however the nature of autophagy defects in ALS remains unclear. Autophagosomes accumulate

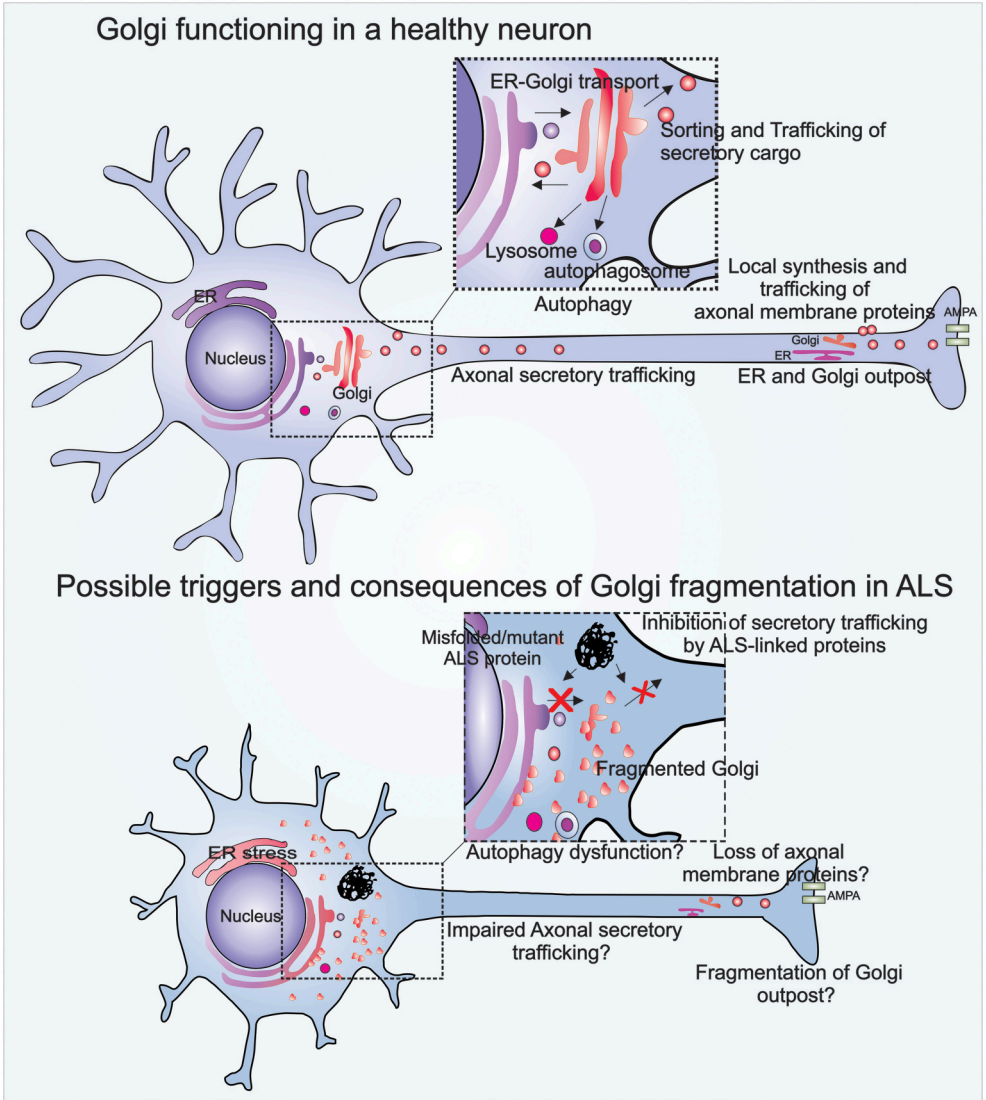

FIGURE 1 | Illustration of Golgi functions in a healthy neuron, and Golgi fragmentation in an ALS-affected neuron. The Golgi in a healthy neuron regulates vesicular trafficking from the ER to the plasma membrane. The Golgi is also involved in the biogenesis of autophagosomes and lysosomes. Golgi outposts in healthy axons are involved in local synthesis and trafficking of axonal membrane proteins. Golgi fragmentation in ALS may be triggered by pathogenic mutant proteins that inhibit vesicular trafficking between the ER-Golgi, and Golgi to plasma membrane. Possible consequences of Golgi fragmentation in ALS include autophagy dysfunction, impaired axonal secretory trafficking, and loss of axonal homeostasis.

in ALS patient brain tissues (Sasaki, 2011), implying that both induction of autophagy and inhibition of clearance of autophagsomes exist in ALS. However, more recent studies have demonstrated that formation of the autophagosome is impaired in cells expressing ALS mutant FUS (Soo et al., 2015b) and in cells with reduced C9orf72 expression (Farg et al., 2014). Furthermore, mutations in proteins involved in endosomal sorting and trafficking which are required for the formation of autophagosomes (VCP, p62, dynactin, and RAB7) are also associated with ALS (Otomo et al., 2012). Activation of the unfolded protein response (UPR) and ER stress are well-documented pathogenic features in human ALS patients (Ilieva et al., 2007; Atkin et al., 2008; Oyanagi et al., 2008; Walker et al., 2010) and in animal/cellular disease models associated with mutant FUS, TDP-43, C9orf72, optineurin, and SOD1 (Atkin et al., 2006; Oh et al., 2008; Walker and Atkin, 2011; Farg et al., 2012; Walker et al., 2013; Zhang et al., 2014; Sundaramoorthy et al., 2015). Interestingly ER stress develops first in the most vulnerable motor neurons in SOD1^{G93A} mice, 60 days before disease onset (Saxena et al., 2009), thus implicating ER stress as an active mechanism inducing cell death in ALS. Similarly, Golgi fragmentation is a prominent pathological feature in human ALS, and appears at a similar time point in SOD1 mice models (Gonatas et al., 1992; Mourelatos et al., 1994; van Dis et al., 2014).

TABLE 1 | List of major ALS genes and ALS-linked proteins with established intracellular and axonal trafficking functions.

Major ALS genes	Chromosome location	Disease feature	Percentage of ALS		References
			Familial	Sporadic	
C9ORF72	9p21	ALS, FTD, ALS with FTD	40	7	DeJesus-Hernandez et al., 2011; Renton et al., 2011
SOD1	21q22	ALS	12	1–2	Rosen et al., 1993
TARDBP	1p36	ALS, FTD, ALS with FTD	4	1	Sreedharan et al., 2008
FUS	16p11	ALS, FTD, ALS with FTD	4	1	Kwiatkowski et al., 2009; Vance et al., 2009
ALS-linked proteins	**Cellular trafficking function**				
C9ORF72	• Possible involvement in Rab-mediated membrane trafficking processes. • Predicted to function as a Rab guanine nucleotide exchange factor (GEF).				Zhang et al., 2012; Levine et al., 2013; Farg et al., 2014
Optineurin	• Vesicular trafficking of secretory proteins, autophagosomes and lysosomes.				Sahlender et al., 2005; Tumbarello et al., 2012; Sundaramoorthy et al., 2015
VCP	• Secretory protein trafficking. • Translocation of misfolded proteins from the ER to cytoplasm for proteosome degradation.				Ballar et al., 2011; Arita et al., 2012; Yi et al., 2012
Profilin1	• Polymerization of the actin cytoskeleton.				Pantaloni and Carlier, 1993
VAPB	• Vesicular ER-Golgi trafficking, dendritic membrane protein trafficking. • Also involved in microtubule organization.				Skehel et al., 2000; Kuijpers et al., 2013
SQSTM1/p62	• Mediates AMPA receptor trafficking at the synapse and and also dynein-linked cellular trafficking. • Mediates Cargo recognition and trafficking in autophagy.				Jiang et al., 2009; Calderilla-Barbosa et al., 2014
Alsin	• Functions as a GEF for Rab5 and Rac1. • Involved in in Rab5-endocytic trafficking and Rac1 regulation of the actin cytoskeleton.				Otomo et al., 2003; Topp et al., 2004
CHMP2B	• Component of endosomal sorting complex required for transport-III (ESCRT-III), essential for endocytic trafficking.				Urwin et al., 2010
Dynactin	• Mediates dynein and kinesin 2 driven intracellular and axonal trafficking on microtubules.				Schroer, 2004; Ross et al., 2008
Neurofilament heavy chain	• Essential for maintaining axon structure and function.				Liu et al., 2004
TUBA4A	• Component of microtubule cytoskeleton.				Oakley, 2000
Peripherin	• Type III neuronal intermediate filament involved in peripheral axon outgrowth and regeneration.				Oblinger et al., 1989
Spatacsin	• Involved in axonal anterograde vesicular trafficking.				Pérez-Brangulí et al., 2014
Phosphoinositide 5 phosphatase	• Associated with late endosome/lysosomal membrane trafficking pathways.				Chow et al., 2007; Ruscher and Wieloch, 2015
Sigma-1 receptor	• Involved in lipid rafts associated transport of proteins and lipids to plasma membrane.				Pabba et al., 2014; Ruscher and Wieloch, 2015

GOLGI FRAGMENTATION IN ALS

Fragmentation of the Golgi was first identified in ALS patient motor neurons over 20 years ago (Gonatas et al., 1992). In contrast to control patients, the Golgi in ALS patients was reduced and fragmented, appearing as disconnected punctate structures, similar to its morphology in cells treated with microtubule depolymerisation agents (Mourelatos et al., 1990; Gonatas et al., 1992). Since then, other studies have confirmed Golgi fragmentation in 10–50% sporadic patients (Gonatas et al., 2006; van Dis et al., 2014) and up to 70% of familial ALS patient motor neurons, bearing SOD1, FUS or optineurin mutations (Fujita et al., 2008; Ito et al., 2011). Interestingly, Golgi fragmentation is more prominent in larger human motor neurons, such as those in the cerebral cortex (Fujita et al., 1999) and anterior horn (Fujita et al., 2000), suggesting they are specifically vulnerable to disturbances in Golgi function.

Golgi fragmentation is also present in spinal anterior horn cells in sporadic ALS patients with cytoplasmic mislocalization of WT TDP-43, implying that a link exists between TDP-43 and Golgi pathologies (Fujita et al., 2008). Similarly, Golgi fragmentation is present in transgenic rats expressing mutant TDP-43^{M337V} (Tong et al., 2012), in mutant SOD1^{G93A} transgenic mice and in neuronal cells expressing SOD1^{G93A,G85R} mutants (Mourelatos et al., 1996; Stieber et al., 2004). Interestingly, Golgi fragmentation precedes SOD1 inclusion formation, neuromuscular denervation, and mitochondrial-mediated apoptosis in low-copy number SOD1^{G93A} transgenic mice, implying it is upstream in pathogenesis (van Dis et al., 2014). Similarly, ALS patients with optineurin mutations (<1% familial cases) demonstrate Golgi fragmentation in ∼70% of

anterior horn cells (Ito et al., 2011). Furthermore, Golgi fragmentation is present in cells expressing ALS-linked mutant FUS, optineurin and vesicle-associated membrane protein B (VAPB) (Teuling et al., 2007; Farg et al., 2013; Sundaramoorthy et al., 2015). However, despite being widely associated with ALS, the cellular events triggering Golgi fragmentation and the resulting consequences are not established. Increasing evidence implicates inhibition of vesicular trafficking between the ER-Golgi in ALS, which may explain the previous observations of Golgi fragmentation.

IMPAIRMENT OF CELLULAR TRAFFICKING IS A TRIGGER FOR GOLGI FRAGMENTATION IN ALS

The ER-Golgi compartments form the first part of the cellular secretory pathway, hence they are sensitive to alterations in the rate of trafficking (Pelletier et al., 2000; Lee et al., 2004), and trafficking inhibition leads to dysfunction in both compartments. In the ER, accumulation of unfolded nascent secretory proteins in the lumen triggers the UPR. The UPR initially aims to reduce protein synthesis and increase protein folding (Graves et al., 2001; Preston et al., 2009). However, when impairment of trafficking persists, prolonged UPR results in activation of apoptosis (Szegezdi et al., 2006; Hetz, 2012). Similarly the organization of the Golgi depends on efficient bidirectional vesicular transport with the ER (Nassif et al., 2010). The formation of Golgi stacks requires continuous recycling of Golgi proteins to/from the ER (Lippincott-Schwartz et al., 2000). Inhibition of protein export from the ER disrupts Golgi organization (Storrie et al., 1998), resulting in the formation of tubulovesicular Golgi clusters, some of which fuse with the ER (Puri and Linstedt, 2003), which can further increase ER stress. Similarly, inhibition of vesicular trafficking from the Golgi to plasma membrane leads to protein accumulation within the Golgi. If prolonged, this can fragment the Golgi (Persson et al., 1992; Zolov and Lupashin, 2005; Zhou et al., 2013). Approximately one-third of the human proteome transverses through the Golgi destined for transmembrane, synaptic, axonal, or extracellular locations (Braakman and Bulleid, 2011). Hence disruption to intracellular trafficking involving the Golgi could severely compromise neuronal function and viability.

We recently demonstrated that Golgi-associated vesicular trafficking is inhibited in cells expressing ALS-mutant proteins: SOD1, FUS, TDP-43, and optineurin, providing an intriguing mechanism explaining Golgi fragmentation in patient tissues (Sundaramoorthy et al., 2013, 2015; Atkin et al., 2014; Soo et al., 2015a). Furthermore, inhibition of ER-Golgi transport by mutant SOD1 preceded all other cellular pathologies examined in neuronal cells, including ER stress, Golgi fragmentation, protein aggregation, inclusion formation, and apoptosis (Atkin et al., 2014). This implies that ER-Golgi trafficking defects may trigger ER-Golgi pathology in SOD1-ALS cases. More recently we demonstrated that mutant forms of both FUS and TDP-43 impair the incorporation of secretory cargo into COPII vesicles budding off from the ER, impeding protein export from the ER, while mutant SOD1 was shown to inhibit ERGIC-Golgi trafficking by destabilizing microtubules (Soo et al., 2015a). Furthermore, we have also demonstrated that misfolded WT SOD1 also impairs ER-Golgi trafficking similar to mutant SOD1, resulting in ER stress and Golgi fragmentation (Sundaramoorthy et al., 2015), although it remains controversial whether misfolded WT SOD1 is present in sporadic ALS tissues (Liu et al., 2009; Forsberg et al., 2010; Grad et al., 2014). However, it is tempting to speculate that impairment of ER-Golgi trafficking is a common trigger for Golgi fragmentation in sporadic and familial ALS. Similarly, we have also shown that expression of ALS-optineurin mutants impair myosin VI-mediated protein trafficking from the Golgi to plasma membrane, also inducing Golgi fragmentation (Sundaramoorthy et al., 2015). Hence these results imply that impairment of distinct protein trafficking pathways by different ALS-linked proteins are specific triggers for Golgi fragmentation in ALS (**Figure 1**).

Consistent with this notion, mutations in genes encoding proteins directly involved in intracellular trafficking are present in familial ALS (**Table 1**). Firstly, mutations in the gene encoding the $p150^{Glued}$ subunit of the dynein/dynactin complex were reported in sporadic and familial ALS (Münch et al., 2004). The ALS causing mutation impedes binding of $p150^{Glued}$ to microtubules, resulting in dysfunctional dynein/dynactin-mediated transport (Levy et al., 2006). Similarly, mutations in proteins directly involved in the ER-Golgi secretory pathway, including VAPB and VCP, are present in ALS (Nishimura et al., 2004; Johnson et al., 2010; Yi et al., 2012; Kuijpers et al., 2013). Recent findings of mutations in ALS-associated genes that encode cytoskeletal associated proteins provide additional evidence for trafficking disruption in ALS. Mutations in profilin 1, which mediates the conversion of soluble G-actin to functional F-actin, (Wu et al., 2012), and in tubulin alpha 4A (TUBA4A), a component of microtubules, were recently reported in familial ALS (Smith et al., 2014).

The identification of hexanucleotide (GGGGCC) repeat expansion mutations in *C9ORF72* as the major cause of familial ALS and FTD (40%), further links cellular trafficking to ALS. Whilst the normal cellular function of C9orf72 was initially unknown, bioinformatics studies first predicted that C9orf72 functions in Rab-mediated trafficking (Zhang et al., 2012; Levine et al., 2013). Rab proteins form a large family of small guanosine triphosphate (GTP)ases that regulate vesicular trafficking at distinct cellular membranes (Stenmark and Olkkonen, 2001). Rab proteins are activated by conversion from an inactive guanosine diphosphate (GDP)-bound state to an active GTP-bound form, which is catalyzed by guanine nucleotide exchange factors (GEFs) (Stenmark and Olkkonen, 2001; Cherfils and Zeghouf, 2013). Bioinformatics predicted that C9orf72 functions as a RabGEF, because of the strong sequence and structural similarity to other evolutionary conserved differentially expressed in normal and neoplastic cells (DENN) domain-containing RabGEFs (Zhang et al., 2012; Levine et al., 2013). Consistent with these predictions, we demonstrated that C9orf72 associates with multiple Rabs including Rab1, which mediates ER-Golgi transport (Farg et al., 2014). Furthermore, we also found that depletion of C9orf72 using siRNA impaired

autophagy and endocytic trafficking from the plasma membrane to Golgi (Farg et al., 2014). Whilst the hexanucleotide repeat expansion is present within an intronic region of *C9ORF72*, expression of C9orf72 protein is reduced in ALS patients causing haploinsufficiency (DeJesus-Hernandez et al., 2011; Haeusler et al., 2014). Hence this would disrupt the normal trafficking function of C9orf72 in ALS. However, recent studies have argued against this mechanism of pathogenesis (Koppers et al., 2015). Nevertheless, we also demonstrated increased association of C9orf72 with Rab7 and Rab11 in C9orf72-ALS patients, implying that intracellular trafficking is dysregulated in C9orf72-ALS, although the mechanism remains unclear (Farg et al., 2014). However, further studies are required to examine the relationship between C9orf72 and trafficking defects, including whether the Golgi is fragmented in C9orf72-ALS patients.

GOLGI FRAGMENTATION AND AUTOPHAGY DYSFUNCTION

The initial step in autophagy is the formation of a double-membraned phagophore, which then expands in size, engulfing defective proteins, damaged cellular organelles, or pathogens, forming the autophagosome (Reggiori and Klionsky, 2005). Although the membranes forming the autophagosome originate from multiple cellular organelles, the ER is implicated as the primary source of membrane because the omegasome, the autophagosome precursor, originates from ER cisternae (Hayashi-Nishino et al., 2009). However, the Golgi is necessary for subsequent autophagosome elongation, and Golgi-mediated trafficking provides membrane components for autophagosome biogenesis. Beclin1, which is located in the trans-Golgi network, recruits other autophagy-related (Atg) proteins for assembly into the autophagosome, (Kihara et al., 2001) and Atg9-positive vesicles cycle from the Golgi to deliver membranes to the developing autophagosome (Young et al., 2006; Webber et al., 2007). Blocking ER to cis-Golgi transport, or transport from the trans-Golgi to plasma membrane/endosomes, reduces autophagosome formation in mammalian cells (Zoppino et al., 2010; Guo et al., 2012). Furthermore, the Golgi recognizes and sorts lysosomal enzymes, which are then packaged into vesicles that bud from the trans-Golgi, forming lysosomes (Griffiths et al., 1988; Kornfeld and Mellman, 1989; Riederer et al., 1994). Hence these observations imply that disruption of Golgi-associated trafficking may impair autophagosome formation.

In contrast, fragmentation of the Golgi has also been shown to increase autophagosome biogenesis by feeding Atg9-positive fragmented Golgi membranes during starvation-induced autophagy (Takahashi et al., 2011). Pharmacological induction of Golgi fragmentation with Brefeldin A or Golgicide increases autophagosome biogenesis and induces accumulation of autophagosomes (Naydenov et al., 2012), but it can also block autophagosome formation in some cases (Nishida et al., 2009). Observations of Golgi fragmentation and autophagy dysfunction imply a possible link between these two pathologies in ALS. Hence examination of the pathological relationship between Golgi fragmentation and autophagy in ALS is warranted.

GOLGI FRAGMENTATION AND AXONAL HOMEOSTASIS

Motor neurons differ from other neurons in that they are exceptionally large, with long axons, up to 1 m in length in an adult human. These distal axons require membrane and cytoskeletal proteins, neurotransmitter receptors, and lipids to maintain synaptic plasticity, synaptogenesis, excitability, dendritic, and neurite outgrowth (Horton and Ehlers, 2004; Tuck and Cavalli, 2010; Ori-McKenney et al., 2012). These components must be transported from the ER/Golgi in the cell body over long distances along the axon. In addition to this traditional route, proteins are also synthesized via axonal ribosomes (Koenig et al., 2000; Kun et al., 2007) and mRNA (Taylor et al., 2009; Jung et al., 2012), thus facilitating local protein synthesis. Proteins synthesized in neurites are processed and secreted via Golgi outposts (Horton and Ehlers, 2003; Merianda et al., 2009) (**Figure 1**). These Golgi outposts share similar molecular markers to the somatic Golgi (Gardiol et al., 1999; Horton and Ehlers, 2003) and they handle secretion of essential axonal/dendritic membrane proteins (Lu et al., 2001; Passafaro et al., 2001). Similarly, cytoskeletal proteins processed via Golgi outposts are essential for axonal regeneration and plasticity of dendritic spines (Matus, 2000; Gu et al., 2008; Tuck and Cavalli, 2010; Shirao and González−Billault, 2013). Fragmentation of the neuronal Golgi would therefore be expected to impair normal axonal functions. In support of this notion, induction of Golgi fragmentation with Brefeldin A reduces synaptic potentiation and AMPA receptor expression on the postsynaptic membrane (Broutman and Baudry, 2001), and reduces axonal outgrowth (Jareb and Banker, 1997). Interestingly, fragmentation of somatic and dendritic Golgi in motor neurons accompanied by trafficking defects, preceded axonal retraction and muscle denervation in mice models of ALS (van Dis et al., 2014). Therefore, Golgi fragmentation may be an important trigger for loss of axonal homeostasis and degeneration of motor neurons in ALS.

CONCLUSION

An emerging concept in ALS is that the diverse mechanisms implicated in pathology are inter-linked, and that disturbances in one pathway induce other pathogenic mechanisms, resulting in neurodegeneration. Increasing evidence links Golgi fragmentation to recent pathological mechanisms implicated in ALS, including disruption of intracellular trafficking and ER stress. This warrants future studies examining the relationship between Golgi fragmentation to other somatic Golgi functions, including autophagy, and specific neuronal functions of the Golgi, such as axonal homeostasis (**Figure 1**). The unique characteristics of neurons and the existence of Golgi outposts may confer additional, more specialized functions of the Golgi in these cells which may render these cells more vulnerable to neurodegeneration in ALS.

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MINI REVIEW
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Defects in the COG complex and COG-related trafficking regulators affect neuronal Golgi function

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The Conserved Oligomeric Golgi (COG) complex is an evolutionarily conserved hetero-octameric protein complex that has been proposed to organize vesicle tethering at the Golgi apparatus. Defects in seven of the eight COG subunits are linked to Congenital Disorders of Glycosylation (CDG)-type II, a family of rare diseases involving misregulation of protein glycosylation, alterations in Golgi structure, variations in retrograde trafficking through the Golgi and system-wide clinical pathologies. A troublesome aspect of these diseases are the neurological pathologies such as low IQ, microcephaly, and cerebellar atrophy. The essential function of the COG complex is dependent upon interactions with other components of trafficking machinery, such as Rab-GTPases and SNAREs. COG-interacting Rabs and SNAREs have been implicated in neurodegenerative diseases like Alzheimer's disease and Parkinson's disease. Defects in Golgi maintenance disrupts trafficking and processing of essential proteins, frequently associated with and contributing to compromised neuron function and human disease. Despite the recent advances in molecular neuroscience, the subcellular bases for most neurodegenerative diseases are poorly understood. This article gives an overview of the potential contributions of the COG complex and its Rab and SNARE partners in the pathogenesis of different neurodegenerative disorders.

Keywords: conserved oligomeric Golgi complex, COG, congenital disorders of glycosylation, neurodegeneration, glycosylation, vesicular trafficking, Rab, SNARE

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INTRODUCTION

The Conserved Oligomeric Golgi (COG) complex is an evolutionarily conserved hetero-octameric protein complex that is a proposed membrane tether during vesicular trafficking at the Golgi apparatus (Lupashin and Ungar, 2008; Reynders et al., 2011; Miller and Ungar, 2012; Willett et al., 2013b). COG is composed of two functionally distinct sub-complexes lobe A (COG1-4) and lobe B (COG5-8) (Fotso et al., 2005; Ungar et al., 2005). Secretory and transmembrane proteins make up 30–50% of all cellular proteins, and are trafficked through the endoplasmic reticulum (ER) to the Golgi for folding and modifications before delivery to their final destination. Secretory cargo molecules are thought to travel through the Golgi complex mostly inside flat cisternae that are constantly maturing in a *cis*-to-*trans* (anterograde) fashion via the so called cisternal maturation mechanism (Glick and Malhotra, 1998). However, resident Golgi proteins and Soluble NSF Attachment protein Receptors (SNAREs) are constantly recycled back in vesicular

carriers to replenish the content of newly formed cis-cisternae. COG regulates the recycling of vesicles containing glycosylation enzymes and other resident Golgi proteins in a *trans*-to-*cis* (retrograde) direction. An intricate assortment of trafficking machineries including small Rab-GTPases, SNAREs, Sec1/Munc18 (SM) proteins, vesicular coat proteins, and tethering proteins are required for vesicular transport (Bonifacino and Glick, 2004). Intracellular pathways rely on these protein families at each step of vesicular transport. Though the functional interaction between the tethers and other trafficking regulators is not completely understood, a multi-subunit tethering complex (MTC), like the COG complex, may coordinate the interactions between all other components of the trafficking machinery at the site of vesicle docking on the target membrane for efficient fusion of the two membranes (Cottam and Ungar, 2012; Willett et al., 2013b).

SNAREs are an essential COG partner. SNAREs catalyze the fusion of the vesicle membrane with the target membrane by the assembly of a quaternary SNARE complex that functions as a zipper to coalesce the opposing lipid bilayers While SNAREs alone have an innate ability to fuse membranes, the SNARE regulatory proteins and tethering complexes are thought to be necessary for physiologically relevant fusion events (Rizo and Südhof, 2012). The COG complex interacts with at least two Golgi SNARE complexes: the *cis*-Golgi STX5/GOSR1(GS28)/YKT6/BET1L(GS15) complex; and the *trans*-Golgi STX16/STX6/VTI1a/VAMP4 complex (Shestakova et al., 2007; Laufman et al., 2009, 2013b). Additionally, SNARE complexes that contain Sec22b, GOSR2(GS27), or SNAP29 are evidenced to interact with COG (Kudlyk et al., 2013; Willett et al., 2013a). SNARE partner transitions connect all compartments of the endocytic and secretory pathways. Thus, it is likely that a defect in one trafficking step may have a cascading effect on all other cargo trafficking steps. In this review we will focus on those SNAREs where deficiency was shown to be associated with neuronal abnormalities, i.e., Ykt6, Sec22b, STX5, SNAP29, GS27, GS28, and Vti1a/b (**Table 1**).

The essential function of the COG complex has been proposed to depend not only upon SNAREs interactions, but also interactions with Rabs (Miller et al., 2013; Willett et al., 2013a; **Figure 1**). Within this context, Rabs are believed to act as molecular switches that cycle between GDP-bound (inactive) and GTP-bound (active) states and regulate cargo trafficking by acting in an intracellular compartment-specific manner. Active (GTP-bound) Rabs regulate trafficking by binding effector molecules like tethering factors and motor proteins (D'Adamo et al., 2014). Binding to some effectors leads to the activation of other Rabs, in a sequence known as the Rab Cascade (Pfeffer, 2013). Nine distinct, Golgi-localized Rabs are known to interact with COG (Miller et al., 2013; Willett et al., 2013b, 2014). The compartment-specific nature of these different Rabs makes them a potential landmark for COG membrane localization and interaction. As with the SNAREs, this review will limit the discussion to COG partners Rab1a, Rab1b, Rab2, Rab4a, and Rab6a that are implicated in neuronal abnormalities (**Table 1**).

NEUROPHYSIOLOGICAL ABNORMALITIES AND COG DEFECTS

Since 2004, defects in seven out of the eight COG subunits have been associated with human Congenital Disorders of Glycosylation (CDG)-type II, a growing family of diseases involving malfunctions in the processing of N- and O-linked glycans and resulting from mutations in proteins involved with glycosylation (Wu et al., 2004; Spaapen et al., 2005; Foulquier et al., 2006, 2007; Kranz et al., 2007; Ng et al., 2007, 2011; Paesold-Burda et al., 2009; Reynders et al., 2009; Richardson et al., 2009; Lübbehusen et al., 2010; Fung et al., 2012; Huybrechts et al., 2012; Rymen et al., 2012, 2015; Kodera et al., 2015; **Table 1**). Glycosylation is a highly dynamic process that occurs in the ER and Golgi which requires an estimated two percent of the human genome to encode the enzymes and trafficking components for the proper maturation of newly formed glycan chains (Freeze et al., 2014). COG deficiency can cause a redistribution of COG-dependent Golgi resident proteins, including glycosylation enzymes. Most COG-CDG patients have defects in sialylation and galactosylation, as indicated by fluorescent lectin staining of plasma membrane glycoconjugates from patient fibroblasts and MALDI-TOF mass spectrometry of serum glycoproteins (Foulquier et al., 2007; Kranz et al., 2007; Paesold-Burda et al., 2009; Reynders et al., 2009; Zeevaert et al., 2009a,b). Along with other multi-system pathologies, COG-CDG patients display mild to severe neurological defects including hypotonia, intellectual disability, developmental delays, epilepsy, and ataxia (**Table 1**). Specific symptoms and the severity of condition appears to relate to the COG subunit that is deficient with COG6 and COG7 patients demonstrating the most severe phenotypes (Rymen et al., 2015). Additionaly, COG defects have not yet been attributed to any other subtype of CDG.

Several CDGs result from mutated COG subunits that are either severely truncated or rapidly degraded. Loss of one COG subunit can destabilize the remaining subunits and reduce their expression and association with the Golgi. Early studies of the COG3 subunit invoked participation of the COG complex in the proper distribution of Golgi enzymes. COG3 depletion by siRNA in HeLa cells causes extensive Golgi fragmentation and destabilization of the COG complex (Zolov and Lupashin, 2005). COG3 and COG7 knockdown generates an accumulation of COG complex-dependent (CCD) vesicles carrying the SNAREs GS15 and GS28, and Golgi enzymes MAN2A1 and GALNT2 (Zolov and Lupashin, 2005; Shestakova et al., 2006). The accumulation of CCD vesicles suggests that in COG deficient cells a significant fraction of Golgi glycosylation enzymes are separated from the proteins they need to modify. COG8-CDG patient fibroblasts have decreased levels of the other lobe B subunits (COG5, COG6, and COG7) all of which have lost their association with the Golgi (Foulquier et al., 2007; Kranz et al., 2007). COG lobe B destabilization was also seen in COG7-CDG patient fibroblasts, resulting in the loss of COG6 association with the Golgi (Kudlyk et al., 2013). The loss of COG also challenges the function of interacting SNAREs. The endosome-to-*trans*-Golgi Network (TGN) SNARE protein STX16 was mislocalized in COG8-CDG patient fibroblasts (Willett et al., 2013a), and the

TABLE 1 | Neurological phenotypes associated with COG and COG-interacting Rabs and SNAREs.

Protein	Disorder	Associated neurological manifestation	References
COG PROTEINS			
COG1	CDG-IIg (COG1-CDG)	Cerebral atrophy, developmental delay, hypotonia	Foulquier et al., 2006
COG2	CDG-II (COG2-CDG)	Developmental delay, epilepsy	Kodera et al., 2015
COG4	CDG-IIj (COG4-CDG)	Developmental delay, epilepsy, hypotonia, lack of speech, nystagmus	Reynders et al., 2009; Ng et al., 2011
COG5	CDG-IIi (COG5-CDG)	Ataxia, cerebral atrophy, developmental delay, epilepsy, hypotonia	Paesold-Burda et al., 2009; Fung et al., 2012; Rymen et al., 2012
COG6	CDG-III (COG6-CDG) Shaheen syndrome (SHNS)	Ataxia, cerebral atrophy, developmental delay, epilepsy, hypotonia, optic nerve atrophy, sensoneural hearing loss Intellectual disability	Lübbehusen et al., 2010; Huybrechts et al., 2012; Shaheen et al., 2013; Rymen et al., 2015
COG7	CDG-IIe (COG7-CDG)	Cerebral atrophy, developmental delay, epilepsy, hypotonia	Wu et al., 2004; Morava et al., 2007; Ng et al., 2007; Zeevaert et al., 2009a
COG8	CDG-IIh (COG8-CDG)	Cerebral atrophy, developmental delay, hypotonia	Foulquier et al., 2007; Kranz et al., 2007
SNARE PROTEINS			
Ykt6	Parkinson's Disease	Trafficking defects and cytotoxicity *in vitro* in NRK and PC12 cell lines	Hasegawa et al., 2003, 2004
Sec22b	Parkinson's Disease	Trafficking defects and cytotoxicity *in vitro* in NRK and PC12 cell lines	Hasegawa et al., 2003, 2004
STX5	Parkinson's Disease Alzheimer's Disease	Trafficking defects and cytotoxicity *in vitro* in NRK and PC12 cell lines Regulates processing of APP in PC12, HeLa, COS-7, and NG108-15 cell lines and primary cultures of rat hippocampal neurons	Suga et al., 2005b; Thayanidhi et al., 2010; Rendón et al., 2013; Suga et al., 2015
SNAP29	CEDNIK-Neuro-cutaneous syndrome	Cerebral Dysgenesis, Neuropathy, Ichthyosis, and Keratoderma	Sprecher et al., 2005; Fuchs-Telem et al., 2011
GS27	Myoclonus epilepsy/early ataxia Parkinson's Disease	Action myoclonus, mild cerebral atrophy, and early ataxia Trafficking defects and cytotoxicity *in vitro* in NRK and PC12 cell lines	Thayanidhi et al., 2010; Corbett et al., 2011
GS28	Neurodegeneration	Retinal degeneration in *in vivo Drosophila* photoreceptors	Rosenbaum et al., 2014
Vti1a/b	Neurodegeneration	Perinatal lethality in double knockouts in an *in vivo* mouse model. Neuronal axon tracks missing, reduced in size or misrouted	Kunwar et al., 2011; Walter et al., 2014
RAB PROTEINS			
Rab1a	Parkinson's Disease	Neuroprotective in *C. elegans*, *D. melanogaster* and primary rat neuron cultures. Rescue from the neurotoxic effects of α-synuclein	Cooper et al., 2006; Gitler et al., 2008
Rab1b	Alzheimer's Disease	Dominant negative mutant of Rab1b blocks trafficking of APP and decreases the secretion of Aβ	Dugan et al., 1995
Rab2	Parkinson's Disease	Reduced expression of Rab2 can rescue Golgi fragmentation in PD models	Rendón et al., 2013
Rab4a	Neumann-Pick disease Alzheimer's Disease Down's syndrome	Reduced Rab4-dependent recycling *in vitro* in Neumann-Pick type A and type C fibroblasts. Postmortem samples: increased Rab4 in patients with AD and mild cognitive disorder Aβ partially co-localizes with Rab4 in a mouse model of Down Syndrome	Cataldo et al., 2000; Choudhury et al., 2004; Arriagada et al., 2010; Ginsberg et al., 2010
Rab6a	Alzheimer's Disease	Dominant negative mutant of Rab6 increases the secretion of soluble APP and decreased Aβ secretion	McConlogue et al., 1996

STX5/GS28/Ykt6/GS15 and STX6/STX16/Vti1a/VAMP4 SNARE complexes were destabilized in both COG7- and COG8-CDG patient fibroblasts (Laufman et al., 2013a). In a non-CDG patient presenting intellectual disability, Shaheen et al. identified a mutation in COG6 which resulted in reduced COG6 and STX6 protein expression (Shaheen et al., 2013). Anterograde trafficking does not appear to be affected in cells with COG mutations, but retrograde trafficking is affected as indicated by a partial resistance to treatment with the transport inhibitor brefeldin A (Steet and Kornfeld, 2006; Foulquier et al., 2007; Kranz et al., 2007; Ng et al., 2007; Paesold-Burda et al., 2009; Reynders et al., 2009) and by endosome-to-TGN trafficking defects elucidated by application of Shiga toxin and SubAB toxin (Zolov and Lupashin, 2005; Smith et al., 2009). Therefore, retrograde intra-Golgi and endosome-to-TGN sorting are particularly impaired by COG deficiency.

NEUROPATHOLOGY AND DEFECTS IN COG-ASSOCIATED PROTEINS

Extensive *in vitro* analyses in control and disease models demonstrate that genetic deficiency in SNARE and Rab COG partners may also result in disintegration of the Golgi apparatus, thereby potentially influencing neurological impairment (**Table 1**). Interestingly, the therapeutic implications of studying neurodegeneration associated with defective COG function is broader than the COG-CDG patient population (D'Adamo et al., 2014; Rymen et al., 2015). Golgi fragmentation is a common feature of neurodegenerative diseases (Gonatas et al., 2006). Current theories argue that the Golgi fragmentation seen in Alzheimer's Disease (AD) and Parkinson's Disease (PD) is either a result of misfolded or aggregated proteins, or that fragmented Golgi causes etiologically important proteins

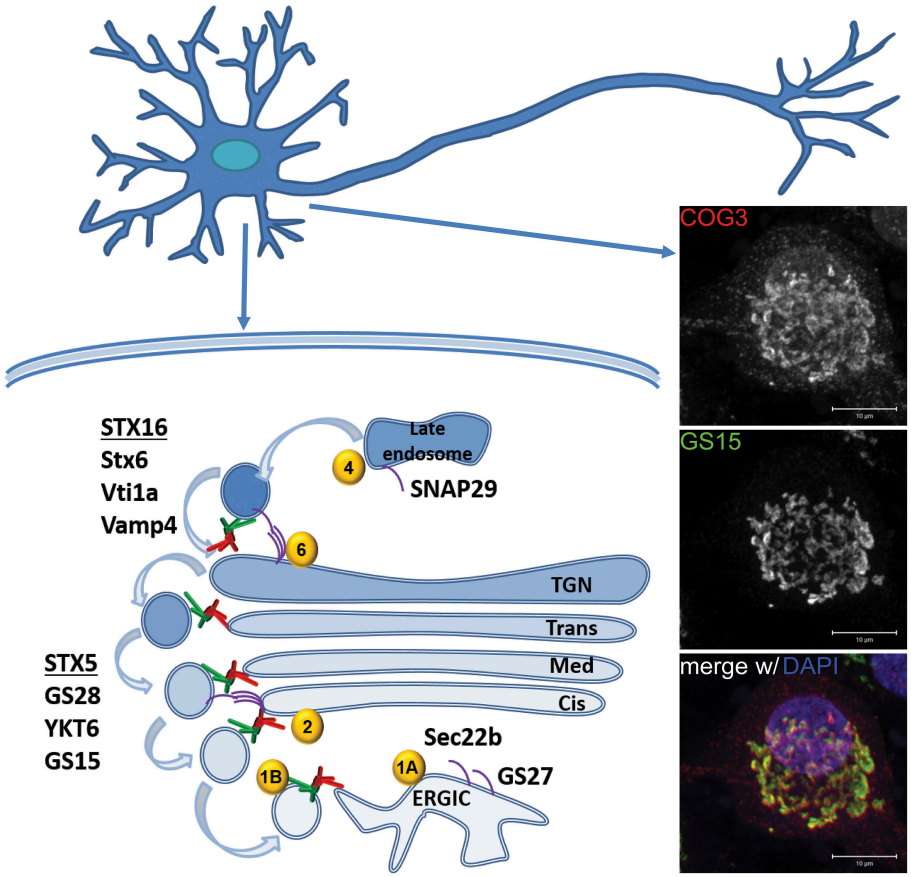

FIGURE 1 | Model of COG trafficking within neurons. A cartoon depicting the proposed roles for the COG complex (depicted as red shapes for lobe A and green shapes for lobe B subcomplexes) and its interacting protein partners in Golgi trafficking: Rabs (yellow circles), and SNAREs (purple lines). Right panel: Immunofluorescence images of COG complex subunit COG3 (top-red) and Golgi SNARE GS15 (middle-green) in rat dorsal root ganglion. Perinuclear (DAPI) co-localization is indicated by yellow in the merged image (bottom). Scale Bar = 10 μm.

to aggregate and misfold leading to further progression of these diseases (Gonatas et al., 2006; Nakagomi et al., 2008; Bellouze et al., 2014; Joshi and Wang, 2015). However, Golgi fragmentation has also been linked to SNARE and Rab proteins making it difficult to pinpoint a single disease progression. Thus, the COG-Rab-SNARE dynamic is important for understanding neurodegenerative phenotypes.

SNARES

Genetic deficiency in SNAREs was shown to be associated with the progression of neurodegenerative diseases like AD and PD. PD is marked by the presence of Lewy bodies which are principally composed of aggregated α-synuclein. Under physiological conditions, α-synuclein may regulate vesicle trafficking and promote synaptic transmission by binding directly to SNAREs and stimulating SNARE complex formation (Burré et al., 2010; Thayanidhi et al., 2010). Overexpression of wild type or the PD-associated mutant of α-synuclein (A53T) leads to cytotoxicity and inhibition of ER-to-Golgi trafficking in *in vitro* models which can be partially suppressed by co-overexpression of SNAREs Ykt6 or Sec22. Ykt6—a protein enriched in neurons—was more protective than Sec22 and suggests a specialized role in mammals (Hasegawa et al., 2003, 2004). *In vitro* binding experiments also point toward the direct interaction of α-synuclein with STX5 and GS27 along with mutant α-synuclein which destabilizes the STX5-GS27-rbet1-sec22b SNARE complex (Thayanidhi et al., 2010). This line of evidence further implicates trafficking defects in bringing about neurodegenerative cytotoxicity.

Although the exact molecular mechanisms connecting Golgi fragmentation and disease mutations is still much under investigation, *in vitro* models can be used to recapitulate Golgi fragmentation seen in neurodegenerative disorders (Suga et al., 2005a). Fragmentation has been recreated *in vitro* and can be rescued by regulating levels of SNAREs. A STX5 knockdown is known to induce Golgi fragmentation in HeLa cells and cultured neurons (Suga et al., 2005a; Amessou et al., 2007). On the other hand, in PC12 cells treated with 6-hydroxydopamine or methamphetamine (an established *in vitro* PD model), a decrease in the level of STX5 rescues Golgi fragmentation (Rendón et al., 2013). This same study also demonstrated

that Golgi fragmentation could intensify disease progression by inducing α-synuclein aggregation and the formation of Lewy bodies.

STX5 defects have also been shown to affect processing of AD-related proteins. In AD, amyloid precursor protein (APP) undergoes a series of proteolytic events by β- and γ-secretases to create the amyloidogenic variants of β-amyloid (Aβ) that are longer and more likely to form aggregates (Peric and Annaert, 2015). Presenilins form a complex with γ-secretase, and mutations in presenilin 1 (PS1) are the most frequently associated mutations found in AD which result in increased production of Aβ, or altered ratios of amyloid peptide species (Hardy, 2006; Saito et al., 2011; De Strooper et al., 2012). Overexpressed STX5 was shown to co-localize with and directly bind to PS1. Further, STX5 overexpression increased the accumulation of APP in the ER and cis-Golgi and inhibited Aβ secretion in a neuroblastoma cell line (NG108-15)(Suga et al., 2005b). In NG108-15 cells expressing the familial AD mutation PS1ΔE9, STX5 was shown to have a decreased association with presenilin. A study of ER stress in an AD model demonstrated that ER stressors can increase synthesis of STX5 and its accumulation in the ER-Golgi intermediate compartment (ERGIC) and transport vesicles. Thus, upregulation of trafficking machinery induced by ER stress could be a cellular mechanism for correcting the accumulation of the amyloidogenic cleavage products of APP (Suga et al., 2015).

As stated above, the *cis*-Golgi SNARE GS27 (GOSR2) binds to α-synuclein and is part of a SNARE complex that is destabilized by mutant α-synuclein. GS27 has also been shown to be associated with a neurological disorder in humans. Six patient were identified baring a mutation that results in improper subcellular localization and loss of function of GS27 leading to symptoms common in COG-CDG patients such as cerebral atrophy, epilepsy, and early ataxia (Corbett et al., 2011; **Table 1**).

GS28 (GOSR1) is a Golgi SNARE involved in both ER-to-Golgi and intra-Golgi transport (Nagahama et al., 1996; Subramaniam et al., 1996), and accordingly has been shown to be associated with three SNARE complexes (Zhang and Hong, 2001; Parlati et al., 2002; Xu et al., 2002; Siddiqi et al., 2010). GS28 mutants have been used to study retinal degeneration in *Drosophila* photoreceptors (Rosenbaum et al., 2014). Lack of expression of GS28 in mutant flies alters trafficking and glycosylation of rhodopsin (Rh1). The photoreceptors in these mutants also exhibit enlarged ER and Golgi membranes and retinal degeneration over time.

Vti1a is a TGN-localized SNARE that functions in vesicle generation and Ca2+ channel trafficking (von Mollard et al., 1997; Lupashin et al., 1997; Walter et al., 2014). A double knockout of Vti1a and Vti1b genes results in progressive neurodegeneration and perinatal lethality in a mouse model (Kunwar et al., 2011). Single knockout of Vti1a or Vti1b does not result in a lethal phenotype indicating overlapping functions of these proteins. The death-after-birth clearly demonstrates that these SNAREs are not required during organismal development, indicating a specialized requirement for Vti1a and Vti1b in neurons leading to neurodegeneration in the double-knockout animals (Walter et al., 2014). Vti1a SNARE partners, STX6 and STX16, are also required for neurite outgrowth (Chua and Tang, 2008; Kabayama et al., 2008).

In humans, a disease known as CEDNIK (Cerebral Dysgenesis, Neuropathy, Ichthyosis, and Keratoderma) syndrome has been linked with loss of function mutations in SNAP29 (Sprecher et al., 2005; Fuchs-Telem et al., 2011). SNAP29 is a member of the SNAP25 family that localizes to the Golgi, endosomal, and lysosomal compartments (Steegmaier et al., 1998). CEDNIK patients exhibit severe neuropathy likely due to the loss of SNAP29 functional involvement in neurotransmission (Pan et al., 2005) and trafficking within neuroglia during active myelination (Schardt et al., 2009).

RABS

As for SNAREs, regulating levels of Rabs has been shown to rescue Golgi fragmentation in multiple *in vitro* models of AD and PD. For example, overexpression of Rab1 can rescue Golgi fragmentation while reduced expression of Rab2 has the same affect in PD models which demonstrates the delicate balance in the regulatory functions of Golgi-associated Rabs (Rendón et al., 2013). Overexpression of Rab1 was shown to be neuroprotective in *Caenorhabditis elegans*, *Drosophila melanogaster* and primary rat neuron cultures (Cooper et al., 2006; Gitler et al., 2008). Rab1 is a key protein in maintaining Golgi architecture and function (Haas et al., 2007). It can also promote the restoration of ER-to-Golgi trafficking and thus afford rescue from the neurotoxic effects of α-synuclein (Cooper et al., 2006; Gitler et al., 2008).

Multiple Rabs are associated with processing of APP. For example, ERGIC and *cis*-Golgi Rab1b-dependent trafficking could modulate the processing of APP as demonstrated in an *in vitro* system in which a dominant-negative mutant of Rab1b blocked trafficking of APP and decreased the secretion of Aβ (Dugan et al., 1995). *trans*-Golgi Rab6A is also implicated in APP trafficking. The dominant negative mutant of Rab6 increased the secretion of soluble APP and decreased Aβ secretion (McConlogue et al., 1996). Rab6 membrane association is dependent upon PS1 based on studies using PS1 knockout fibroblasts, thus implicating PS1 in vesicular trafficking (Scheper et al., 2004).

Golgi-associated and endosomal Rab4 is important for a specific pool of constitutively recycling endosomes which are apparently critical for dendritic spine size. Rab4-dependent recycling is greatly reduced in fibroblasts of patients with type A/B Niemann-Pick disease, a sphingolipid storage disorder which commonly manifests with neurological symptoms such as developmental delay and dementia (Choudhury et al., 2004). Postmortem samples revealed that Rab4 is upregulated in patients with AD and mild cognitive disorder (Cataldo et al., 2000; Ginsberg et al., 2010), and Aβ is known to partially co-localize within Rab4 positive compartments in a mouse model of Down Syndrome (Arriagada et al., 2010) indicating that defects in endosomal sorting may underpin these disorders (Peric and Annaert, 2015).

CONCLUSIONS AND PERSPECTIVES

The COG complex and its Rab and SNARE partners are evolutionarily conserved and ubiquitously expressed across multiple tissues and organ systems in humans. Yet, neurological symptoms are the most debilitating and troublesome clinical manifestations of COG-associated disorders. Why is this the case? We propose that brain-specific manifestations of COG defects result from either COG-dependent (a) glycosylation defects and/or, (b) trafficking defects and/or, (c) a yet unknown neuron/neuroglia-specific function of the COG complex and its partners.

Neuronal function critically depends on coordinated delivery of properly modified ion channels, transporters and components of the synaptic apparatus at the appropriate rates and over long distances, to specific subcellular compartments. Remarkably, the localization of the synaptobrevin homolog Snc1 is altered in COG deficient yeast cells (Whyte and Munro, 2002). In addition, underglycosylated low density lipoprotein receptor is severely destabilized in CHO cells deficient for COG1 or COG2 proteins (Kingsley et al., 1986). Since glycosylation of channels, transporters and transport regulators is essential for their correct delivery, stability and/or activity (Gong et al., 2002; Watanabe et al., 2004; Scott and Panin, 2014), it is reasonable to predict that a majority of underglycosylated ion channels and transporters may similarly be destabilized, thus altering the functionality of COG-CDG patient neurons.

Smooth transport of cargo by the trafficking machinery is very important during development and synaptic transmission. Defective glycosylation of proteins and lipids disrupts development pathways and alters brain function (Freeze et al., 2012). COG deficient cells also display altered glycosphingolipid biosynthesis. Complex gangliosides are sialic acid containing glycosphingolipids synthesized sequentially, beginning with GM3 and then extended by glycosyltransferases to the more elaborate GM1 gangliosides. Biochemical studies revealed decreased levels of sphingomyelin and GM3 gangliosides in COG2 deficient CHO cells (Spessott et al., 2010a,b). Gangliosides are ubiquitously expressed, but in the brain the expression of gangliosides and their glycosyltransferases change dramatically during development, from an abundance in of the precursor GM3 to a greater abundance of GM1 (Yu et al., 1988; Kracun et al., 1991, 1992; Ngamukote et al., 2007). Therefore, altered glycosphingolipid biosynthesis could be another reason for the specific neurological manifestation in COG-related congenital disorders.

An additional factor that may add to the apparently higher neuronal vulnerability to COG deficiency is that unlike many other cells, neurons are non-dividing cells and hence cannot easily dilute toxic proteins, peptides, and organelles. Another possibility is that COG is playing a specific and yet uncovered role in neuronal cells. Although the COG complex has been largely detected at the established perinuclear Golgi location in neurons (**Figure 1**) this does not exclude some sort of moonlighting function for COG subunits at other neuron-specific locations where it could be involved in tethering of specialized vesicles.

Considering the systemic and complex character of COG-related diseases, multiple questions regarding the associated neurological symptoms remain to be addressed. Thus, more *in vivo* studies are needed to dissect the role of COG and its Rab and SNARE partners in these symptoms. Input from COG deficiencies within neuroglia and subtypes of neuroglia presents yet another poorly studied field and avenue requiring further studies. Further research is also needed to establish in which diseases COG-deficiency-associated neurological syndromes are secondary manifestations of some other primary disease state.

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VPS54 and the wobbler mouse

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The wobbler mouse is an animal model for human motor neuron disease, such as amyotrophic lateral sclerosis (ALS). The spontaneous, recessive wobbler mutation causes degeneration of upper and lower motor neurons leading to progressive muscle weakness with striking similarities to the ALS pathology. The wobbler mutation is a point mutation affecting Vps54, a component of the Golgi-associated retrograde protein (GARP) complex. The GARP complex is a ubiquitously expressed Golgi-localized vesicle tethering complex, tethering endosome-derived vesicles to the trans Golgi network. The wobbler point mutation leads to a destabilization of the Vps54 protein and thereby the whole GARP complex. This effectuates impairments of the retrograde vesicle transport, mis-sorting of Golgi- and endosome localized proteins and on the long run defects in Golgi morphology and function. It is currently largely unknown how the destabilization of the GARP complex interferes with the pathological hallmarks, reported for the wobbler motor neuron degeneration, like neurofilament aggregation, axonal transport defects, hyperexcitability, mitochondrial dysfunction, and how these finally lead to motor neuron death. However, the impairments of the retrograde vesicle transport and the Golgi-function appear to be critical phenomena in the molecular pathology of the wobbler motor neuron disease.

Keywords: wobbler, GARP, Vps54, Golgi, vesicle transport, ALS, neurodegeneration

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INTRODUCTION

Vps54 was connected with the wobbler motor neuron degeneration by positional cloning (Schmitt-John et al., 2005; **Figures 1A,B**). Vps54 (vacuolar protein sorting 54) was first identified in yeast in the course of a screening for mutants with mis-sorting of vacuolar proteins (Conboy and Cyert, 2000; Conibear and Stevens, 2000). Vps54 was found to be a component of the Golgi-associated retrograde protein (GARP) complex (Conibear and Stevens, 2000; **Figure 1C**) and a mammalian homolog was reported (Liewen et al., 2005). The GARP complex belongs to the CATCHR (complexes associated with tethering containing helical rods) group of multi subunit tethering complexes (MTCs) like Dsl1-, COG- and Exocyst complexes (Bonifacino and Hierro, 2011) and specifically tethers endosome derived vesicles to the trans Golgi network. The GARP complex interferes with Rab6 protein and supports the vSNARE–tSNARE dependent fusion of the incoming transport vesicles with the trans Golgi membrane (**Figure 1D**) and thus has a function in the retrograde vesicle transport. The identification of the mutation causing the motor neuron degeneration of the wobbler mouse in Vps54 suggested a critical role for Vps54, the GARP complex and the retrograde vesicle transport for motor neuron survival (Schmitt-John et al., 2005; Moser et al., 2013).

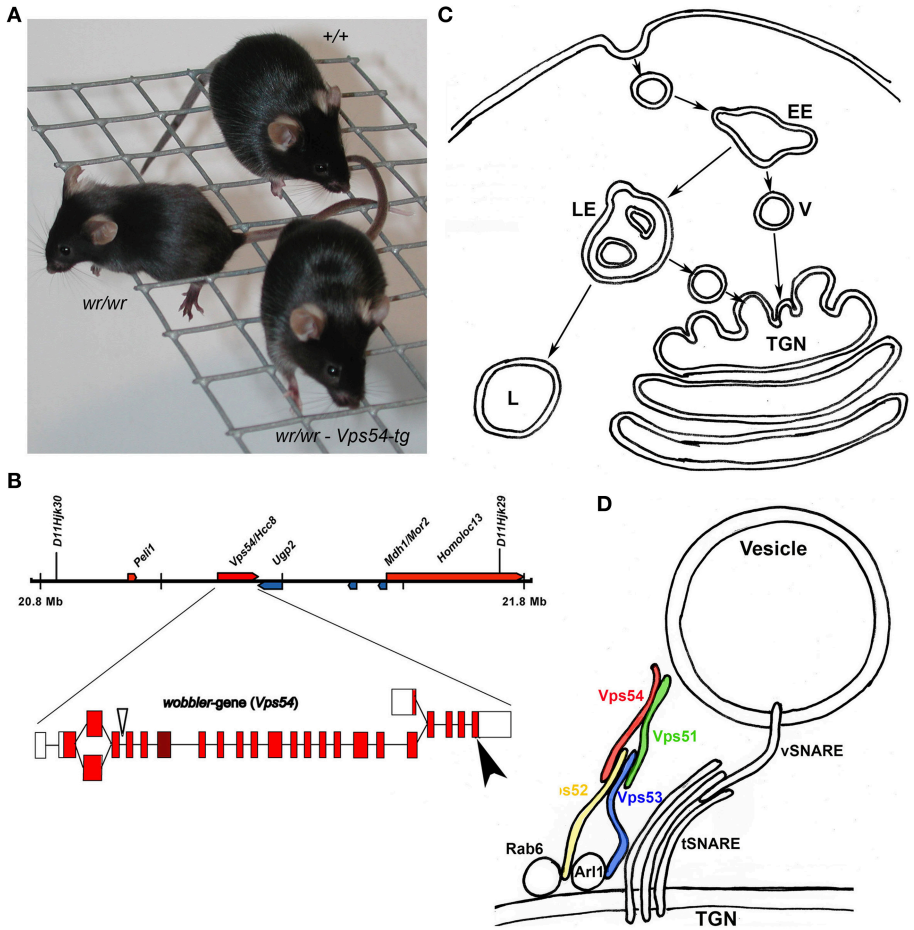

FIGURE 1 | The wobbler loss-of-function mutation of Vps54. The figure summarizes the primary defects of the wobbler mutation. **(A)** A Wildtype mouse (+/+), a homozygous wobbler (wr/wr) mouse with defects in grid walking due to muscle weakness, predominantly affecting the fore limbs and a Vps54 transgenic wobbler mouse (*wr/wr*-Vps54-tg) with wild type appearance (Schmitt-John et al., 2005). **(B)** Vps54 genomic locus on mouse Chr 11, Exon intron structure, the arrow indicates the wobbler point mutation in exon 24. **(C)** The retrograde vesicle transport route from early (EE), and late endosomes (LE); transport vesicles (V) are tethered to the trans Golgi network (TGN); lysosomes (L). **(D)** The GARP complex consists of Vps51, −52, −53, and −54 and tethers endosome-derived vesicles (V) and mediates vSNARE–tSNARE dependent fusion of the vesicle- and target membrane. The GARP complex interferes thereby with GTP effector proteins Rab6 and Arl1. However, the exact docking site to the vesicle membrane is still unknown. The wobbler point mutation destabilizes the GARP complex and thus, the wobbler mutation causes impairments of the retrograde vesicle transport, but it is still unclear how these impairments induce motor neuron death.

Vps54 AND THE WOBBLER PHENOTYPE

The wobbler mouse was first described by Falconer (1956) and has recently been reviewed (Moser et al., 2013; Ott et al., 2015) as animal model for amyotrophic lateral sclerosis (ALS; **Figure 1A**). The spontaneous, recessive wobbler mutation (*wr*) was mapped to the proximal mouse chromosome 11 (Kaupmann et al., 1992) and the critical region was refined to a region homologous to human chromosome 2p13 (Korthaus et al., 1997; Resch et al., 1998; Fuchs et al., 2002). By positional cloning the wobbler mutation was identified as a point mutation in the last exon of Vps54 leading to a single amino acid exchange (Schmitt-John et al., 2005; **Figure 1B**). A successful transgenic rescue approach proved that Vps54 is the wobbler gene (Schmitt-John et al., 2005). A Vps54 null-mutation caused embryonic lethality around day 10.5 of the embryonic development, indicating that the wobbler point mutation is a partial loss-of function allele of Vps54 (Schmitt-John et al., 2005). However, it was demonstrated that the wobbler point mutation causes a destabilization of Vps54 protein and thereby the whole GARP complex (Pérez-Victoria et al., 2010). This leads to a decreased abundance of GARP tethering complexes in wobbler mutant cells, resulting in impairments of the retrograde vesicle transport (Pérez-Victoria et al., 2010; Karlsson et al., 2013). Since the wobbler phenotype closely resembles the human motor neuron disease ALS, the retrograde vesicle traffic is to be considered critical for motor neuron degeneration.

Even though no ALS cases with mutations in VPS54 have been identified yet (Meisler et al., 2008), there is growing evidence that the vesicle transport plays a critical role in human ALS. A subset of familial ALS forms have been attributed to proteins involved in vesicle trafficking, such as Alsin (ALS2; Yang et al., 2001),

VABP (ALS8; Nishimura et al., 2004), and CHMP2B (ALS17; Parkinson et al., 2006). Furthermore, Palmisano et al. (2011) could demonstrate accumulation of APP (amyloid precursor protein) in enlarged endosomes in degenerating motor neurons of both wobbler mice and ALS patients, suggesting similar impairments of the vesicle transport.

In case of wobbler mice the primary cause of motor neuron degeneration is the point mutation of Vps54 (Schmitt-John et al., 2005) leading to a destabilization of the GARP complex (Pérez-Victoria et al., 2010) and impairment of the retrograde vesicle transport (Karlsson et al., 2013), which results in protein mis-sorting and accumulation in enlarged endosomal structures (Palmisano et al., 2011). Despite these facts, it is not that simple to explain how the destabilization of a ubiquitously expressed vesicle tethering factor finally leads to the death of motor neurons.

The wobbler motor neuron degeneration shares a number of pathologic features with human ALS like muscle atrophy, astrogliosis, microgliosis (Duchen and Strich, 1968), hyperexcitability (Nieto-Gonzalez et al., 2011), mitochondrial dysfunction (Santoro et al., 2004), axonal transport defects (Mitsumoto et al., 1990), neurofilament aggregations (Pernas-Alonso et al., 2001), and ubiquitin-positive protein aggregations (Dennis and Citron, 2009), as summarized by Moser et al. (2013). All these cellular effects are directly or indirectly connected to the primary cause, the GARP dysfunction, and contribute more or less to the progression of motor neuron death. It is always difficult to distinguish cause from effect or contributing—from concomitant phenomena. However, all mentioned cellular effects are surely interconnected in a complex manner and the GARP complex and Golgi apparatus appear to play a central role in the wobbler motor neuron pathology.

Vps54 AND THE GARP COMPLEX

Vps54 interacts with Vps51, −52, and −53 in a 1:1:1:1 ratio and forms the GARP complex (Conboy and Cyert, 2000; Conibear and Stevens, 2000) and appears to be predominantly Golgi-localized, tethering retrograde vesicles derived from early and late endosomes (Quenneville et al., 2006). Vps52, −53, and −54 appear to form a core complex, while Vps51 is loosely associated and links the complex to the SNARE Tlg1p (Siniossoglou and Pelham, 2002). Loss-of-function mutations of either of the core components lead to the same phenotype in yeast, decreased growth rate and mis-sorting of vacuolar proteins (Conibear and Stevens, 2000). Double and triple mutants show the same phenotype, while Vps51 mutants display a milder phenotype (Conibear and Stevens, 2000), indicating that the GARP function depends on the function of each of the core components and to a lesser extent on Vps51 (Conibear et al., 2003). In mammals the GARP complex has a similar structure and function (Pérez-Victoria and Bonifacino, 2009; Bonifacino and Hierro, 2011).

Knock down experiments of GARP components in mammalian cell culture (Pérez-Victoria et al., 2008; Pérez-Victoria and Bonifacino, 2009) and the analysis of wobbler mutant cells (Karlsson et al., 2013) clearly indicate that the mammalian GARP complex has an important role for the retrograde vesicle transport and the sorting of proteins, using this transport route as well as sorting receptors like mannose-6-phosphate receptors (Pérez-Victoria et al., 2008). Recent loss-of-function analysis of the GARP complex in HEK293 cells indicated not only retrograde transport defects but also impairments of the anterograde post-Golgi transport of GPI-anchored and transmembrane proteins (Hirata et al., 2015). GARP dysfunction appears to have an impact on both the retrograde and the anterograde vesicle transport and might affect the integrity and function of the whole Golgi apparatus. Complete loss-of-function mutations of murine GARP components cause embryonic lethality. Homozygous deletion of either Vps54 or Vps53 leads to embryonic lethality around day 10.5 of the embryonic development (Schmitt-John et al., 2005; Karlsson et al., 2013), while the null-mutation of Vps52 however, causes even earlier lethality during gastrulation (Sugimoto et al., 2012). This might suggest an additional function of Vps52 in mammals, independent from the GARP complex. Recently, it was reported that Vps51, −52, and −53, but not Vps54, assemble with syndetin to form the EARP complex, which appears to be localized on Rab4-positive recycling endosomes (Schindler et al., 2015). Syndetin appears to fulfil a Vps54-like function in the endosome-associated protein (EARP) complex and binds preferentially to Vps53 (Schindler et al., 2015). Syndetin and Vps54 are probably responsible for the interaction with a specific Rab protein, Rab4 or Rab6 and thereby directing the complex to a specific localization. Heterozygous mutations in Vps53 have been associated with pontocerebellar hypoplasia type 2E (PCH2E) and thus with mental retardation, microcephaly, spastic quadriplegia, and early onset seizures (Feinstein et al., 2014). This finding suggests impairments of both GARP and EARP function due to Vps53 haploinsufficiency. For both PCH2E and the wobbler motor neuron disease swollen vesicles are reported, but no evidence for lysosomal abnormality or lysosomal storage problems was seen (Palmisano et al., 2011; Feinstein et al., 2014).

GOLGI PATHOLOGY AND MOTOR NEURON DISEASE

Golgi function and integrity might have a fundamental significance for neuron function and survival. However, GARP mutant mammalian cells in cell culture show no obvious morphological indications for Golgi-dysfunction or disintegration (Karlsson et al., 2013). In degenerating wobbler motor neurons we observed on electron micrographs enlarged vesicles derived from the Golgi apparatus (**Figure 2**) in early stages of degeneration and massive vacuolization and fragmentation of the Golgi in late stages (Palmisano et al., 2011). Andrews et al. observed electron dense granular material in close proximity to the Golgi in degenerating wobbler motor neurons (Andrews et al., 1974). In final stages the wobbler characteristic vacuolization is very prominent and the vesicular structures appear to originate not only from the Golgi, but also from the ER (Palmisano et al., 2011). The increased blebbing-out of enlarged vesicles from the Golgi or alternatively the

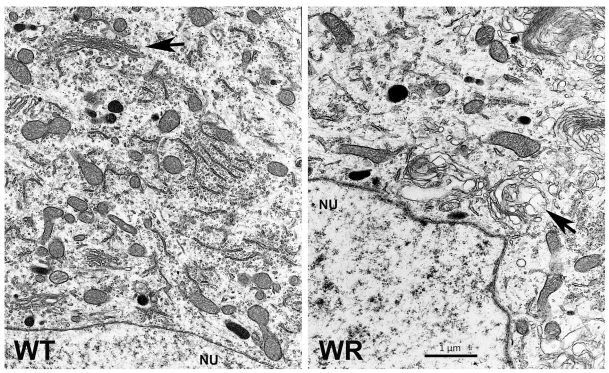

FIGURE 2 | Electron micrograph of spinal motor neurons. Regions near the nucleus (NU) of motor neurons from the cervical spinal cords of wild type (WT) and a symptomatic wobbler mouse (WR) is shown. The normal WT- and morphologically affected WR Golgi are indicated by arrows. The image is a gift from Dr. Peter Heimann and shows swollen areas of the WR Golgi, similar to those published by Palmisano et al. (2011).

abnormal fusion of incoming vesicles in close proximity to the Golgi argue for a Golgi-dysfunction and in later stages a Golgi fragmentation, which might be a hallmark of the wobbler motor neuron degeneration. Whether the enlarged Rab7-positive, APP containing structures, described by Palmisano et al. (2011), are identical with the vacuoles with low electron density (Andrews et al., 1974; Palmisano et al., 2011) is not yet proven but likely, since these structures are found in the same subcellular location and size range.

Golgi fragmentation has been shown for ALS patients (Stieber et al., 1998) and SOD1 G93A transgenic fALS animal models (Mourelatos et al., 1996) by immunostaining for MG160, a Golgi-resident marker protein. It could also been shown that ALS neurons with TDP-43 aggregates also display Golgi-fragmentation (Fujita et al., 2008). For wobbler mice Golgi-fragmentation has not yet been investigated by anti MG160 immunostaining, but the evidence from electron microscopy (Andrews et al., 1974; Palmisano et al., 2011) and the observation of cytoplasmic TDP-43 aggregations in degenerating wobbler motor neurons (Dennis and Citron, 2009), makes it likely that the Golgi-fragmentation in wobbler mice resembles that found in ALS patients and SOD1 transgenic mice.

Taken together, wobbler mice develop motor neuron degeneration, which closely resembles human ALS and the primary cause is a partial loss-of-function mutation of Vps54. The wobbler point mutation destabilizes Vps54 protein and thereby the GARP vesicle tethering complex leading to impairments of the retrograde vesicle transport. While most cell types can cope with these impairments, motor neurons develop a number of pathological phenomena. In motor neurons the retrograde vesicle transport defects lead to enlarged endosomal structures (Palmisano et al., 2011), Golgi-dysfunction, impairments of the anterograde and retrograde axonal transport (Mitsumoto et al., 1990), TDP-43- and ubiquitin positive protein aggregations (Dennis and Citron, 2009), neurofilament aggregations (Pernas-Alonso et al., 1996), mitochondrial dysfunction (Santoro et al., 2004), and hyperexcitability (Nieto-Gonzalez et al., 2011). In the final stage motor neurons display a pronounced vacuolation (Duchen and Strich, 1968; Palmisano et al., 2011). The wobbler motor neuron degeneration is even in early stages associated with pronounced astrogliosis and microgliosis (Laage et al., 1988; Rathke-Hartlieb et al., 1999), which might exacerbate the neurodegeneration. However, it is still unclear how the various phenomena cohere or interdepend and which role the Golgi-dysfunction plays in wobbler motor neuron disease.

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frontiers in Neuroscience

REVIEW
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Golgi Fragmentation in ALS Motor Neurons. New Mechanisms Targeting Microtubules, Tethers, and Transport Vesicles

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Pathological alterations of the Golgi apparatus, such as its fragmentation represent an early pre-clinical feature of many neurodegenerative diseases and have been widely studied in the motor neuron disease amyotrophic lateral sclerosis (ALS). Yet, the underlying molecular mechanisms have remained cryptic. In principle, Golgi fragmentation may result from defects in three major classes of proteins: structural Golgi proteins, cytoskeletal proteins and molecular motors, as well as proteins mediating transport to and through the Golgi. Here, we present the different mechanisms that may underlie Golgi fragmentation in animal and cellular models of ALS linked to mutations in SOD1, TARDBP (TDP-43), VAPB, and C9Orf72 and we propose a novel one based on findings in progressive motor neuronopathy *(pmn)* mice. These mice are mutated in the TBCE gene encoding the cis-Golgi localized tubulin-binding cofactor E, one of five chaperones that assist in tubulin folding and microtubule polymerization. Loss of TBCE leads to alterations in Golgi microtubules, which in turn impedes on the maintenance of the Golgi architecture. This is due to down-regulation of COPI coat components, dispersion of Golgi tethers and strong accumulation of ER-Golgi SNAREs. These effects are partially rescued by the GTPase ARF1 through recruitment of TBCE to the Golgi. We hypothesize that defects in COPI vesicles, microtubules and their interaction may also underlie Golgi fragmentation in human ALS linked to other mutations, spinal muscular atrophy (SMA), and related motor neuron diseases. We also discuss the functional relevance of pathological Golgi alterations, in particular their potential causative, contributory, or compensatory role in the degeneration of motor neuron cell bodies, axons and synapses.

Keywords: Golgi fragmentation, neurodegeneration, ALS, microtubules, SOD1, TDP-43, TBCE, C9orf72

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INTRODUCTION

Amyotrophic Lateral Sclerosis (ALS) is a severe neurodegenerative disease characterized by progressive degeneration of motor neurons in spinal cord, brainstem and cerebral cortex and of their corresponding axons in the corticospinal tract and in peripheral nerves. Degeneration of motor axons and loss of neuromuscular synapses leads to denervation of skeletal muscle fibers

which causes progressive muscle weakness and paralysis and becomes fatal when it reaches critical muscle groups, usually within 2–5 years after disease onset (Robberecht and Philips, 2013).

ALS can be caused by mutations in more than 20 genes, including the major ones SOD1, TARDBP (TDP-43), FUS and C9ORF72, or manifest as apparently sporadic form. In ALS motor neurons, many cellular functions are altered as illustrated by defects in nucleocytoplasmic transport (Freibaum et al., 2015; Jovicic et al., 2015; Zhang et al., 2015), in processing of mRNAs (Lourenco et al., 2015) and miRNAs (Emde et al., 2015), in formation of stress granules (Li et al., 2013), ER stress (Matus et al., 2013), mitochondrial dysfunction (Pasinelli et al., 2004), and alterations in almost all steps of membrane traffic. For instance, autophagy (Ferrucci et al., 2011; Song et al., 2012; Majcher et al., 2015), endocytosis (Rusten and Simonsen, 2008), and secretory function (Gonatas et al., 1998; Nassif et al., 2010) have been shown to be affected. In this review, we will focus on structural and functional alterations of the Golgi apparatus.

The Golgi apparatus is a unique organelle comprising stacks of flattened discrete membrane-bound compartments called cisternae forming the so-called Golgi stacks. In mammalian cells, these stacks are laterally connected by tubules to form a large Golgi ribbon capping the nucleus (**Figure 1A**) and (Képès et al., 2005; Glick and Nakano, 2009). Furthermore, the Golgi apparatus is polarized with a cis-entry site facing the ER and the ERGIC (ER-Golgi intermediate compartment) and a trans-exit face facing the endosomal system (Polishchuk and Mironov, 2004). In motor neurons, the Golgi apparatus forms a very large network that extends into axons and dendrites (Bellouze et al., 2014; Valenzuela and Perez, 2015).

In motor neurons of ALS patients, the Golgi apparatus often appears either fragmented, i.e., transformed into multiple disconnected elements or tubular-vesicular clusters, or atrophied, i.e., reduced in its membrane content (Mourelatos et al., 1990; Gonatas et al., 1992). These pathological changes are detectable in all types of motor neurons located in spinal cord, brainstem and cerebral cortex (Mourelatos et al., 1996; Fujita et al., 1999, 2000). Furthermore, they are common to both sporadic (Gonatas et al., 1992) and familial (Mourelatos et al., 1996; Fujita et al., 1999, 2000) forms of the disease, including ALS with Bunina bodies (Stieber et al., 1998), juvenile ALS (Fujita et al., 2002), and ALS with posterior column involvement (Fujita et al., 2000).

Golgi fragmentation has been found to be closely associated with other neuropathological hallmarks of ALS, such as cytoplasmic basophilic inclusions (Fujita et al., 2002), ubiquitin aggregates (Vlug et al., 2005), SOD1 aggregates (Fujita et al., 2000) and TDP-43 pathology (Fujita et al., 2008). Importantly, Golgi alterations occur at an early preclinical stage in human ALS patients (Maruyama et al., 2010) and in rodent ALS models (Mourelatos et al., 1996; Vlug et al., 2005; Tong et al., 2012; van Dis et al., 2014), suggesting that Golgi fragmentation precedes the degenerative loss of motor neuron cell bodies and axons. By contrast, conspicuous Golgi alterations in motor neurons are not observed after chemical poisoning (Mourelatos et al., 1994) or axonal injury (Bellouze et al., 2014), ruling out that they are a mere consequence of neuronal damage.

The main function of the Golgi apparatus in healthy cells is to ensure the processing (enzymatic modification and proteolytic cleavage) and sorting of proteins from their site of synthesis in the endoplasmic reticulum to their final destination, plasma membrane, extracellular space and endosomal/lysosomal compartments (Bonifacino and Glick, 2004). The Golgi apparatus also ensures the polarity of protein and lipid transport to axons and dendrites (Tang, 2001; Ramírez and Couve, 2011; Bonifacino, 2014). In particular, the Golgi apparatus is required for the assembly and axonal transport of synaptic vesicle precursors (Maas et al., 2012). Alterations of the Golgi apparatus could therefore be accompanied by loss or gain of function in protein sorting, processing and transport to the axons and synapses, leading to their degeneration. Furthermore, Golgi fragmentation leads to activation of apoptotic pathways (Hicks and Machamer, 2005; Machamer, 2015) that can contribute to axonal degeneration and ultimately to cell death.

Despite their early occurrence and high frequency, the precise relevance of these pathological Golgi alterations remains unclear. Future studies have to clarify whether they have a causative, contributory or homeostatic role in motor neuron degeneration, in particular with respect to the loss of cell bodies, axons and synapses. To be able to address this in a meaningful manner, it is crucial to better understand the precise mechanisms of Golgi pathology in ALS.

THREE CLASSES OF PROTEINS ARE IMPORTANT FOR GOLGI MAINTENANCE

In theory, Golgi morphology depends on three classes of proteins: The microtubules (MTs) and MT-associated motor proteins; the structural Golgi proteins; and the proteins of the Golgi transport machinery. Of note, most of the knowledge on these alterations comes from studies in mitotic non-neuronal cells and only few data are available from motor neurons.

The Microtubule Cytoskeleton

It has been known for 30 years that microtubule depolymerization (for instance with nocodazole or colchicine) alters the Golgi morphology of mammalian cells. Instead of the single copy organelle capping the nucleus in non-treated cells, the Golgi ribbon appears fragmented leading to single stacks or group of stacks (Ellinger and Pavelka, 1984; Rogalski and Singer, 1984; Turner and Tartakoff, 1989; Cole et al., 1996; Scales et al., 1997).

It is also increasingly recognized that in addition to the centrosome, the Golgi is a major site of microtubule formation in various cell types (Chabin-Brion et al., 2001; Efimov et al., 2007; Miller et al., 2009), including hippocampal neurons (Stiess et al., 2010; Yau et al., 2014) and motor neurons (Bellouze et al., 2014), see for review (Zhu and Kaverina, 2013; Rios, 2014; Kapitein and Hoogenraad, 2015; Sanders and Kaverina, 2015).

During neuronal differentiation, the centrosome is dismantled and becomes dispensable for the formation of microtubules and for the establishment of proper axonal

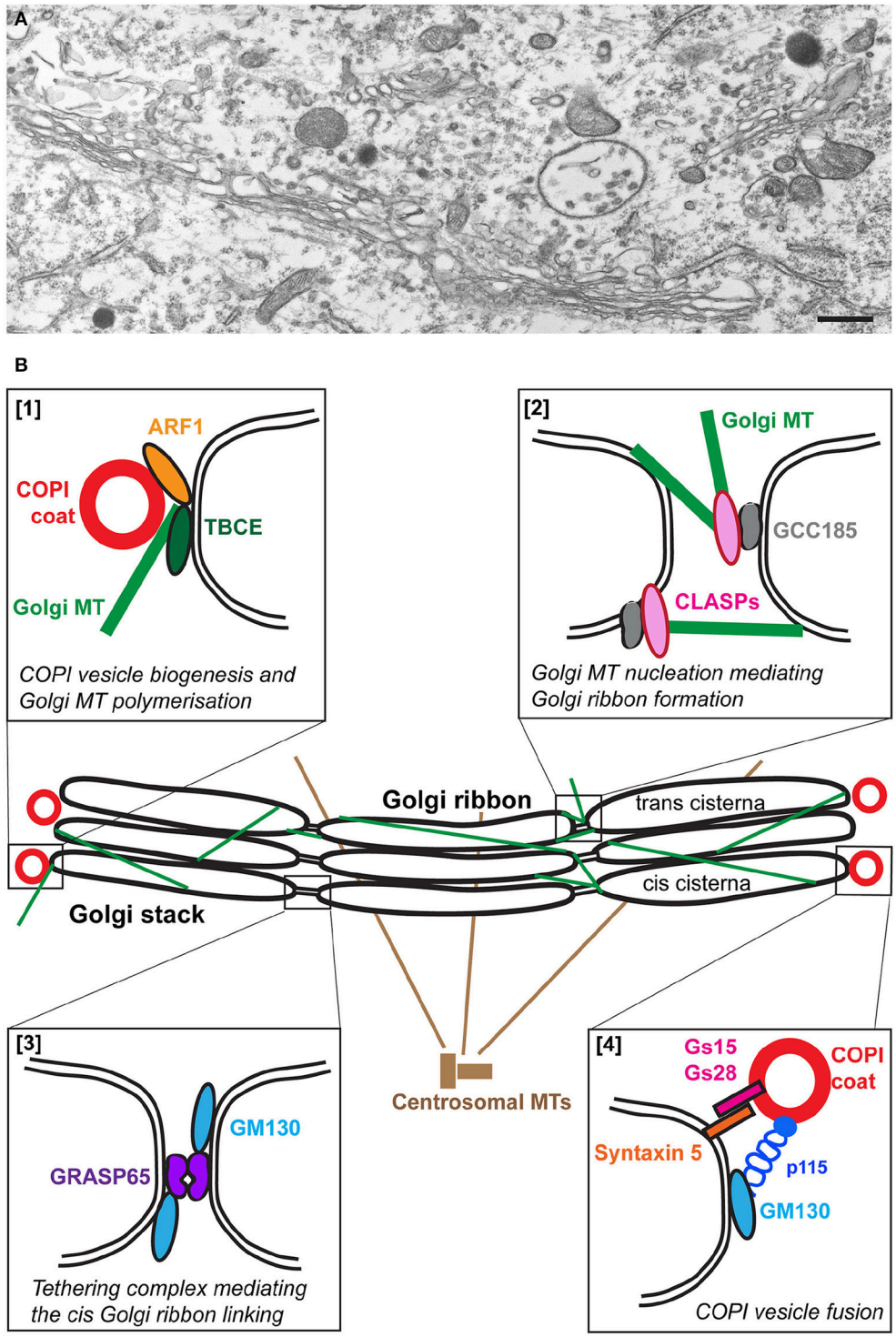

FIGURE 1 | The Golgi apparatus in wild type motor neurons. (A) An electron micrograph showing part of the typical Golgi ribbon in a mouse lumbar spinal cord motor neuron. **(B)** Schematic representation of some of the molecular players involved in the organization of the wild type Golgi. Polymerization of microtubules (MT) at the cis-Golgi depends on TBCE which mediates cross talk with ARF1-mediated COPI vesicle biogenesis [box 1]. Golgi microtubules nucleated by GCC185/CLASPs at the trans-Golgi play a role in Golgi ribbon linking [box 2]. The formation of the Golgi ribbon at the cis side is mediated by the tethering complex GRASP65/GM130 [box 3]. COPI vesicle fusion is mediated by the tethering complex p115/GM130 and SNARE complexes containing GS15, GS28, and Syntaxin 5 [box4].

and neuronal morphology (Basto et al., 2006; Stiess et al., 2010; Nguyen et al., 2011). The Golgi however expands and rearranges (Horton et al., 2005) and may thus become increasingly for the correct formation, nucleation and dynamics of microtubules.

The formation of microtubules involves the folding of alpha- and beta-tubulins, their dimerization and polymerization. The last steps of this complex process are assisted by five tubulin-specific chaperones termed tubulin-binding cofactors TBCA-TBCE (Tian et al., 1996, 1997). Among these, TBCE is expressed at high levels in motor neurons (Schaefer et al., 2007) where it is associated to the cis-Golgi (**Figure 1B**) and is critical for the polymerization of Golgi-derived microtubules (Bellouze et al., 2014). The nucleation of Golgi-microtubules then involves the Golgi proteins GM130 (Kodani and Sütterlin, 2008), AKAP-450 (Rivero et al., 2009), and CLASP1/2 (Efimov et al., 2007) (**Figure 1B**).

The Golgi Structural Proteins

The second class of proteins that could be affected are the so-called Golgi structural proteins. These are the Golgins, extended coiled-coil proteins that form a proteinaceous matrix around the Golgi (Munro, 2011; Gillingham et al., 2014). Their knock down leads to a structural alteration of the Golgi. This is the case for p115, GM130, but also Golgin 84 (Diao et al., 2003) and Giantin (Koreishi et al., 2013). In addition, two non-Golgin proteins have been shown to be critical for the Golgi architecture, GRASP65 and 55 with a role in the formation of tubular connections between Golgi stacks to form the Golgi ribbon (Vinke et al., 2011; Jarvela and Linstedt, 2012, 2014; Veenendaal et al., 2014; **Figure 1B**) as well as in cisternal stacking (Xiang and Wang, 2010). Of note, it is sometimes difficult to tease apart the strict role of these proteins in Golgi structure from their role in trafficking thus precisely pin-pointing the primary cause for Golgi fragmentation.

Interestingly, some of these proteins are the targets of specific modifications that occur at the onset of mitosis when the Golgi becomes physiologically fragmented in a controlled and reversible manner. For instance, GM130 is phosphorylated, leading to dissociation of the GM130/p115 complex. As a result, COPI vesicles are no longer tethered and cannot fuse, leading to Golgi fragmentation and generation of mitotic clusters (Nakamura et al., 1997). Furthermore, GM130 (Walker et al., 2004), p115 (Chiu et al., 2002), and GRASP65 (Lane et al., 2002; Cheng et al., 2010) are targets of apoptotic caspase-mediated proteolysis leading to Golgi fragmentation.

The Golgi Transport Machinery

The third class of proteins that, when altered, could lead to a loss of the typical Golgi architecture are the proteins functioning in the traffic between the ER and the Golgi, especially COPI and COPII coat complexes as well as their tethering/docking/fusion machinery. Trafficking between the ER and the Golgi is mediated by at least two coat complexes.

The COPII coat assembles at specific sites of the ER, called ER exit sites, where COPII coated vesicles form and bud (Miller and Schekman, 2013; Sprangers and Rabouille, 2015). The COPI complex or coatomer assembles at the surface of the Golgi and the ERGIC. The coatomer comprises 6 structural subunits alpha, beta, beta', delta, gamma, and epsilon COP (Beck et al., 2009) and their formation requires the small GTPase Arf1 and its GEF GBF1 (Popoff et al., 2011). There is a strong consensus that COPI coated vesicles mediate the retrograde transport from the Golgi to the ER, but it is also possible that they may also participate in specific anterograde transport steps from the ERGIC to the cis Golgi cisterna and/or within the Golgi cisternae (Aguilera-Gomez and Rabouille, 2015).

After budding from their respective compartments, the COPI and COPII coated vesicles fuse with their target compartments and this requires tethering (p115/GM130), docking (using Rab proteins, Pfeffer, 2013a,b) and fusion proteins, such as the SNAREs. The SNAREs are type II c-tail anchored transmembrane proteins with almost all their mass in the cytoplasm. They belong to two classes, v-SNAREs (present on vesicles) and t-SNAREs (present on target compartments), roughly corresponding to R- and Q-SNAREs. Usually, three Q-SNAREs and one R-SNARE form a tight parallel helix that mediates vesicle fusion (Malsam and Söllner, 2011).

Impairment of the balance between vesicle budding and fusion either at the Golgi or the ER leads to a loss of Golgi architecture and its complete vesiculation. For instance, when COPII vesicle budding is inhibited, the Golgi vesiculates (Prescott et al., 2001). The Golgi appears similarly vesiculated when COPI formation is impaired by BFA treatment or depletion of COPI subunits (Guo et al., 2008; Razi et al., 2009). Last, impaired Golgi SNARE function like depletion of Syntaxin 5 (Suga et al., 2005) also leads to strong Golgi fragmentation, as observed in cellular models of Parkinson disease (Rendón et al., 2013).

Furthermore, at least one caspase (caspase 2) is localized to the Golgi (O'Reilly et al., 2002), suggesting that the Golgi can sense cell death signals and transduce them via cleavage of structural Golgi proteins (Galluzzi et al., 2014).

PROPOSED TRIGGERS OF GOLGI FRAGMENTATION IN ALS AND RELATED MOTOR NEURON DISEASES

The precise mechanisms of Golgi fragmentation in degenerating motor neurons are not fully understood but first insights arise from studies in animal and cellular models of ALS and related motor neuron diseases linked to mutations in SOD1, Dynein/Dynactin, TARDBP (TDP-43), and VAP-B.

Schematically, four mechanisms of Golgi fragmentation can be distinguished: The first one involves defective microtubules or microtubule-dependent transporters due to mutations in SOD1 (Soo et al., 2015), Dynein/Dynactin (Hafezparast et al., 2003; Puls et al., 2003), and BICD2 (Neveling et al., 2013; Peeters et al., 2013). Dynein and BICD2 will not be discussed further as two excellent reviews have just been published on that topic (Jaarsma and Hoogenraad, 2015; Wirth and Martinez-Carrera,

2015). The second one postulates the cleavage of Golgi structural proteins by activation of caspases (SOD1). The third and fourth ones hypothesize that Golgi fragmentation is due to toxic protein aggregates (TDP-43, SOD1) or dysfunctional proteins (VAP-B), which potentially impair trafficking within the early secretory pathway.

Here, we first summarize these data before describing a novel mechanism that involves both microtubules, Golgi vesicles and structural Golgi proteins, and which is potentially shared by several forms of human ALS, spinal muscular atrophy (SMA), and related motor neuron diseases.

Microtubule Disruption by Mutant SOD1

Mutations in the human SOD1 (Cu/Zn superoxide dismutase 1) gene account for ∼5% of familial forms of ALS (Andersen and Al-Chalabi, 2011) and are at the origin of the most widely used animal and cellular models of ALS. Transgenic mice expressing ALS-linked SOD1 mutations manifest signs of motor neuron degeneration such as early axonal dying back and loss of neuromuscular synapses (Fischer et al., 2004; Schaefer et al., 2005; Pun et al., 2006). In these mice, Golgi fragmentation occurs at an early preclinical stage and precedes most other histopathological alterations (Mourelatos et al., 1996; Vlug et al., 2005; van Dis et al., 2014).

Earlier studies suggested that mutant SOD1-linked Golgi fragmentation may originate from microtubule alterations. Indeed, the mean diameter of Golgi cisternae was found to be shorter in mice overexpressing mutant SOD1 G93A when compared to those expressing wildtype SOD1 (Gonatas et al., 1992), an effect similar to that of the microtubule-disrupting drug colchicine (Mourelatos et al., 1990). A recent study further shows that cellular overexpression of mutant SOD1 is associated with unstable microtubules and decreased levels of acetylated tubulins (Soo et al., 2015).

Furthermore, the microtubule-severing protein Stathmin-1 of the Stathmin family that regulates microtubule transition from growing to shrinking phases, some of which are also associated with the Golgi membrane (Gavet et al., 1998) has been found to be up-regulated both at the RNA (Ferraiuolo et al., 2007) and the protein (Strey et al., 2004) level in mutant SOD1-expressing mouse motor neurons. Finally, the most acidic isoform of Stathmin-1, which probably corresponds to the inactive phosphorylated protein, was found selectively down-regulated (Strey et al., 2004).

However appealing, this hypothesis is not yet proven since no conspicuous microtubule alterations have been detected in mutant SOD1 motor neurons with a fragmented Golgi following standard tissue preparation (Strey et al., 2004). To detect SOD1-triggered microtubule alterations *in vivo*, better techniques for microtubule preservation and visualization such as high pressure freezing and EM-based 3D reconstruction (Marsh et al., 2001) may be required. To firmly establish the role of Stathmin-1 and the related Stathmins 2–4 as potential mediators of mutant SOD1-triggered Golgi pathology, it will be necessary to recapitulate Golgi fragmentation by Stathmin overexpression and to rescue mutant SOD1-linked Golgi fragmentation by Stathmin knock down.

Caspase-mediated Cleavage of Structural Golgi Proteins by Mutant SOD1

Mutant SOD1-linked Golgi fragmentation in motor neurons may involve defects in structural Golgi proteins that have been shown to be targets of caspases as described above. We have previously shown that mutant SOD1 sensitizes motor neurons to Fas-triggered cell death involving caspases 8 and 3 (Raoul et al., 2002, 2006). Additional studies have shown that caspases 1, 3, 9, and 12 are activated in mutant SOD1 spinal cord (Li et al., 2000; Inoue et al., 2003; Kikuchi et al., 2006). Whether mutant SOD1 triggers caspase-cleavage of Golgi proteins and thereby contributes to Golgi fragmentation in motor neurons remains however to be investigated.

Protein Aggregates and Golgi Fragmentation: TDP-43

Typical ALS is characterized by cytoplasmic inclusions made of irreversible protein aggregates comprising the nuclear transactivation response (TAR) DNA-binding protein of 43-kDa (TDP-43; Neumann et al., 2006). Furthermore, TARDBP (TDP-43) mutations are associated with sporadic and familial forms of ALS (Sreedharan et al., 2008; Van Deerlin et al., 2008) and were reported to form cytosolic protein aggregates in human postmortem tissues (Lee et al., 2012) and in iPSc-derived motor neurons (Egawa et al., 2012) of patients. The TDP-43 protein aggregates occur in many, albeit not all, neuronal types in patients (Toyoshima and Takahashi, 2014) and in animal models (Tsao et al., 2012). The formation of these inclusions is thought to impair TDP-43 critical nuclear function in neuronal RNA metabolism, including the splicing and stability of numerous RNAs encoding proteins involved in neuronal development, synaptic function and neurodegeneration (Lagier-Tourenne et al., 2012; Jovicic and Gitler, 2014; Lee et al., 2015; Ludolph and Brettschneider, 2015; Scotter et al., 2015; Smethurst et al., 2015). Thus, a loss of these functions through cytoplasmic irreversible protein aggregation is an attractive hypothesis regarding the role of TDP-43 in neurodegeneration (Dewey et al., 2012).

Interestingly, the presence of TDP-43 positive cytoplasmic inclusions has been associated with Golgi fragmentation in spinal motor neurons of ALS patients (Fujita et al., 2008). Similarly, in transgenic rats overexpressing mutant human TDP-43, Golgi fragmentation is detected in a concomitant manner with the formation of TDP-43 aggregates (also positive for ubiquitin) in the same neuron (Tong et al., 2012). Whether and how cytoplasmic TDP-43 protein aggregates are mechanistically linked to Golgi fragmentation is however not known, and the potential Golgi targets and stress signaling pathways of TDP-43 inclusions remain to be identified.

Golgi Fragmentation Due to Impairment in the Early Secretory Pathway Trafficking

Aggregated SOD1 and Golgi Fragmentation

Overexpressed mutant SOD1 in CHO cells is also shown to aggregate, thus trapping key proteins of the early secretory pathway (see below Atkin et al., 2014). In contrast, mutant

SOD1 is seen diffuse in the cytoplasm in Neuro2a cells but its aggregation is triggered upon overexpression of chromogranins, components of neurosecretory vesicles. Interestingly, this aggregation pattern is consistent with the Golgi/TGN that appears disrupted in large vesicles (Urushitani et al., 2006), a result that can be explained by the interaction between chromogranins and mutant SOD1.

This suggests that mutant SOD1 is recruited to the Golgi through its interactions with chromogranins. This could be consistent with an association of SOD1 on the cytoplasmic leaflet of the Golgi membrane, but the authors show that mutant SOD1 is secreted to the extracellular medium together with the chromogranins. This suggests that mutant SOD1 has somehow reached the lumen of these large vesicles, an event proposed to occur early in the early secretory pathway (Urushitani et al., 2008). In this specific case, however, the observed Golgi disruption is not due to expression of mutant SOD1, but to the co-expression of chromogranins. The mechanism for the chromogranin-mediated mutant SOD1 translocation remains to be elucidated.

Several studies also indicate that misfolded mutant forms of SOD1 can impede ER-to-Golgi transport (Stieber et al., 2004). Indeed, using NSC-34 motor neurons cell in culture, expression of mutant SOD1 was shown to first inhibit ER-Golgi trafficking, an event that preceded ER stress (Turner and Atkin, 2006; Atkin et al., 2014), and Golgi fragmentation. Interestingly, four different mutant forms of SOD1 (but not wildtype SOD1) have been shown to bind the COPII subunit Sec23 that co-localizes with aggregated mutant SOD1. This presumably renders it non-functional (Atkin et al., 2014) and leads to Golgi fragmentation that was rescued upon overexpression of the small GTPase Sar1. The interaction between mutant SOD1 and Sec23 was also reported in SOD1 mouse spinal cord at the early age of 10 days. These findings may thus link Golgi fragmentation to the early disruption of ER exit in mutant SOD1 expressing cells (Atkin et al., 2014).

Loss VAP-B Induces Golgi Fragmentation

Atypical forms of familial ALS (ALS8) and late-onset SMA have been associated with a dominant missense mutation (P56S) in the VAPB gene (Nishimura et al., 2004, 2005; Marques et al., 2006; Funke et al., 2010). Additional VAP-B mutations have reported been in few patients with typical ALS (Kabashi et al., 2013).

VAP-B and its homolog VAP-A are members of the highly conserved and ubiquitously expressed VAP (Vesicle-Associated Membrane Protein (VAMP)-Associated Protein) family of ER C-tail anchored proteins. This class of proteins has been shown to interact with the FFAT motif (two phenylalanines in an acidic tract) characteristic of lipid transfer proteins enriched at membrane contact sites between the ER and the Golgi (Mesmin et al., 2013) and other organelles (Levine and Loewen, 2006). In addition, VAP proteins seem implicated in membrane trafficking, ER/cytoskeleton interactions, the unfolded protein response (reviewed in (Lev et al., 2008), calcium homeostasis (De Vos et al., 2012; Stoica et al., 2014), axonal transport of mitochondria (Mórotz et al., 2012) as well as neurite extension and neurotransmitter release (Saita et al., 2009; Ohnishi et al., 2014).

When overexpressed, the ALS-linked VAP-B mutant P56S favors the formation of clusters of paired ER cisternae (Fasana et al., 2010; Kuijpers et al., 2013b) that are located close to the Golgi (Genevini et al., 2014). This leads to ER stress and disrupts ER to ERGIC to Golgi trafficking (Kuijpers et al., 2013b). It also causes Golgi fragmentation in a small number (15%) of primary rat hippocampal neurons (Teuling et al., 2007), although this was not observed (albeit not quantified) in spinal motor neurons on sections (Kuijpers et al., 2013a) or in cell culture (Genevini et al., 2014).

One reason is that overexpression of P56S VAP-B does not completely recapitulate the ALS situation. In fact, P56S VAP-B leads to the degradation of wildtype VAP-B (and probably VAP-A) and a consequent reduced level of the functional protein (Suzuki et al., 2009; Papiani et al., 2012) but not the formation of aberrant ER structures. Similarly, the endogenous mutant P56S allele causes a reduction of VAP-B levels in patient-derived iPSc motor neurons (Mitne-Neto et al., 2011) and its fly equivalent P58S leads to aggregation of wildtype VAP-B (Ratnaparkhi et al., 2008). This is also observed in Vapb P56S knockin mice (Larroquette et al., 2015) where cleaved ubiquitinated VAP-B accumulates in insoluble complexes.

Loss of VAP-B function may also be involved in other forms of ALS since reduced VAP-B levels have been reported in the spinal cord of mutant SOD1 mice and of patients with sporadic ALS (Teuling et al., 2007; Anagnostou et al., 2010). Furthermore, overexpression of VAP-B P56S in mice leads to pathological TDP-43 aggregates both in the nucleus and the cytoplasm of motor neurons (Tudor et al., 2010), which, as reported above, may contribute to disruption of ER to Golgi transport.

Importantly, VAP-B depletion in HeLa cells leads to strong Golgi fragmentation (Peretti et al., 2008). This is likely due to the pleiotropic effects of VAP-B on Golgi-mediated transport pathways (Peretti et al., 2008) where it interacts with numerous Golgi-associated proteins (Huttlin et al., 2015) and microtubules (Skehel et al., 2000). ER stress and autophagy (Larroquette et al., 2015) may be further involved in pathogenesis.

A NOVEL MECHANISM OF GOLGI FRAGMENTATION: DEFECTIVE CROSS TALK BETWEEN THE MICROTUBULE NETWORK AND COPI VESICLES

Progressive Motor Neuronopathy (pmn)

Recently, studies in *pmn* mice carrying a missense mutation in the tubulin-binding cofactor TBCE have led to the elucidation of a mechanism where microtubules, structural Golgi proteins and the Golgi transport machinery are altered, and illustrated a molecular cross talk between these three classes of proteins (Bellouze et al., 2014). Loss of TBCE function in *pmn* mice causes axonal dying back of motor neurons (Schaefer et al., 2007) and loss of cochlear outer hair cells (Rak et al., 2013), growth and mental retardation in human patients with Sanjad-Sakati/Kenny-Caffey syndrome (Parvari et al., 2002) and impaired development of motor axons and neuromucular synapses in Drosophila (Jin et al., 2009). Both the TBCE *pmn* mutation, which leads to protein destabilization and degradation (Martin et al., 2002;

Schaefer et al., 2007), and cellular TBCE depletion (Bellouze et al., 2014) result in a general loss of microtubules including Golgi-derived ones.

Mutant *pmn* mice display clear signs of Golgi fragmentation in lumbar spinal motor neurons at 10 days of age i.e., 5 days before first clinical, electrical or pathological signs of axon degeneration (Schmalbruch et al., 1991; Kennel et al., 1996; Bellouze et al., 2014), confirming the early preclinical occurrence of structural Golgi alterations. During disease course, Golgi membranes are progressively transformed into vesicles and tubules (**Figure 2A**) and the percentage of motor neurons with signs of Golgi fragmentation increases massively (Bellouze et al., 2014).

Biochemical and microscopic analysis of *pmn* associated Golgi defects shows three critical aspects of Golgi fragmentation: a strong decrease in the protein levels of the beta and epsilon COPI vesicle subunits, a dispersion of the Golgi tethers p115 and GM130 away from Golgi membrane and an about 15-fold increase in the protein levels of Golgi v-SNAREs GS15 and GS28 (Bellouze et al., 2014). This accumulation is due to protein stabilization rather than transcriptional up-regulation, and is nevertheless associated with compromised formation of v-/t-SNARE complexes (Bellouze et al., 2014; **Figure 2B**).

Taken together, loss of TBCE leads to loss of Golgi-associated microtubules, a fragmented Golgi and associated molecular defects reported above. This suggests that defects in TBCE-mediated microtubule polymerization at the Golgi impede the COPI coat assembly that in turn causes mislocalization of associated tethers and accumulation of Golgi SNAREs resulting in Golgi fragmentation. This scenario of Golgi fragmentation is further supported by reports showing that depletion of β-COP causes accumulation of small vesicles containing Golgi enzymes at the cell center (Guo et al., 2008) and redistribution of GM130 (Razi et al., 2009), potentially through dysfunction of the ER/Golgi intermediate compartment (ERGIC; Horstmann et al., 2002; Xu and Hay, 2004).

Intriguingly, COPI dynamics feeds back on microtubule polymerization at the Golgi. Expression of constitutionally active GTP-locked ARF1 following TBCE knock down (not knock out) stimulates recruitment of remaining TBCE to the Golgi, polymerization of microtubules and rescue of the Golgi defects (Bellouze et al., 2014). This may be due to an interaction between Arf1 and TBCE, at least upon overexpression of both proteins, making TBCE an Arf1 effector. We therefore propose that the cross talk between microtubules in the cell soma and COPI vesicle dynamics at the Golgi maintains the Golgi structure and that the interruption of this cross talk causes microtubule disruption and Golgi fragmentation observed in *pmn* mice.

Other Mouse Mutants with Motor Neuron Degeneration

Is the interruption of the cross talk also the mechanism of Golgi fragmentation in other disease models? Microtubule and COPI defects may indeed contribute to Golgi fragmentation in *mdf (muscle deficient)* mice mutated in the Scyl1 gene. Scyl1 encodes a protein that normally interacts with α-COP and β-COP (Burman et al., 2008, 2010), suggesting that Golgi fragmentation in motor neurons of *mdf* mice involves defective COPI vesicle formation which may interrupt the COPI/MT cross talk with ensuing Golgi fragmentation.

Furthermore, SNARE-mediated vesicle tethering may be defective in *wobbler* mice with motor neuron degeneration. Indeed the mutant gene product VPS54 (Schmitt-John et al., 2005) is a subunit of the Golgi-associated retrograde protein (GARP) complex that normally tethers vesicle budding from late endosomes to the TGN through interaction with the v-SNAREs Syntaxin-6, Syntaxin-16, and VAMP4 (Pérez-Victoria et al., 2008; Pérez-Victoria and Bonifacino, 2009).

Human ALS Linked to TUBA4A Mutations

Rare forms of human ALS have recently been associated with mutations in the tubulin gene TUBA4A that encodes a major alpha-tubulin isoform of adult spinal cord and brain (Smith et al., 2014). *In vitro* overexpression of several ALS-linked TUBA4A mutations causes severe alterations in the somatic microtubule network (Smith et al., 2014). Additional forms of human ALS and SMA are due to mutations in the microtubule motors Dynein (Hafezparast et al., 2003), its regulator BICD2 (Neveling et al., 2013; Peeters et al., 2013), or in Optineurin (Maruyama et al., 2010), a protein known to bind microtubules and to mediate vesicle transport in the secretory pathway. So, defective microtubule-dependent transport as well as impairment in trafficking through the Golgi may be the basis for Golgi fragmentation in these diseases.

SMA

Defective cross talk between microtubules and COPI vesicles may also be involved in classical SMA caused by deletion of the SMN (Survival Motor Neuron) gene (Lefebvre et al., 1995). Although Golgi fragmentation has not yet been reported in SMN-linked SMA, increased levels of Stathmin-1 (see above) are associated with microtubule loss, axon degeneration and loss of neuromuscular synapses in mouse and cellular models of SMA (Wen et al., 2010, 2013). Furthermore, SMN has been shown to bind the COPI subunit α-COP and to co-traffic with the latter in motor axons (Peter et al., 2011). Interrupting SMN/α-COP interaction leads to SMN accumulation at the Golgi (Ting et al., 2012), reduced neurite outgrowth and altered growth cones (Custer et al., 2013). We therefore hypothesize that the Golgi is fragmented in SMA mice and patients through SMN/COPI/microtubule dysfunction.

ALS Linked to C9Orf72 Mutations

The most frequent forms of human ALS are caused by novel GGGGCC (G_4C_2) hexanucleotide repeat expansions in the first intron of the C9Orf72 gene (DeJesus-Hernandez et al., 2011; Renton et al., 2011). Patients with this form of ALS typically carry several hundred to more than thousand G_4C_2 repeats (Beck et al., 2013; Dols-Icardo et al., 2014), although symptoms may be caused by as few as 20 G_4C_2 repeats (Gómez-Tortosa et al., 2013).

According to the main current hypotheses, G_4C_2 repeat expansions may cause disease through formation of G-quadruplex RNA structures leading to nucleolar stress (Haeusler et al., 2014), formation of nuclear RNA foci sequestering essential RNA-binding proteins (DeJesus-Hernandez et al., 2011; Polymenidou et al., 2012), repeat-associated non-AUG (RAN)

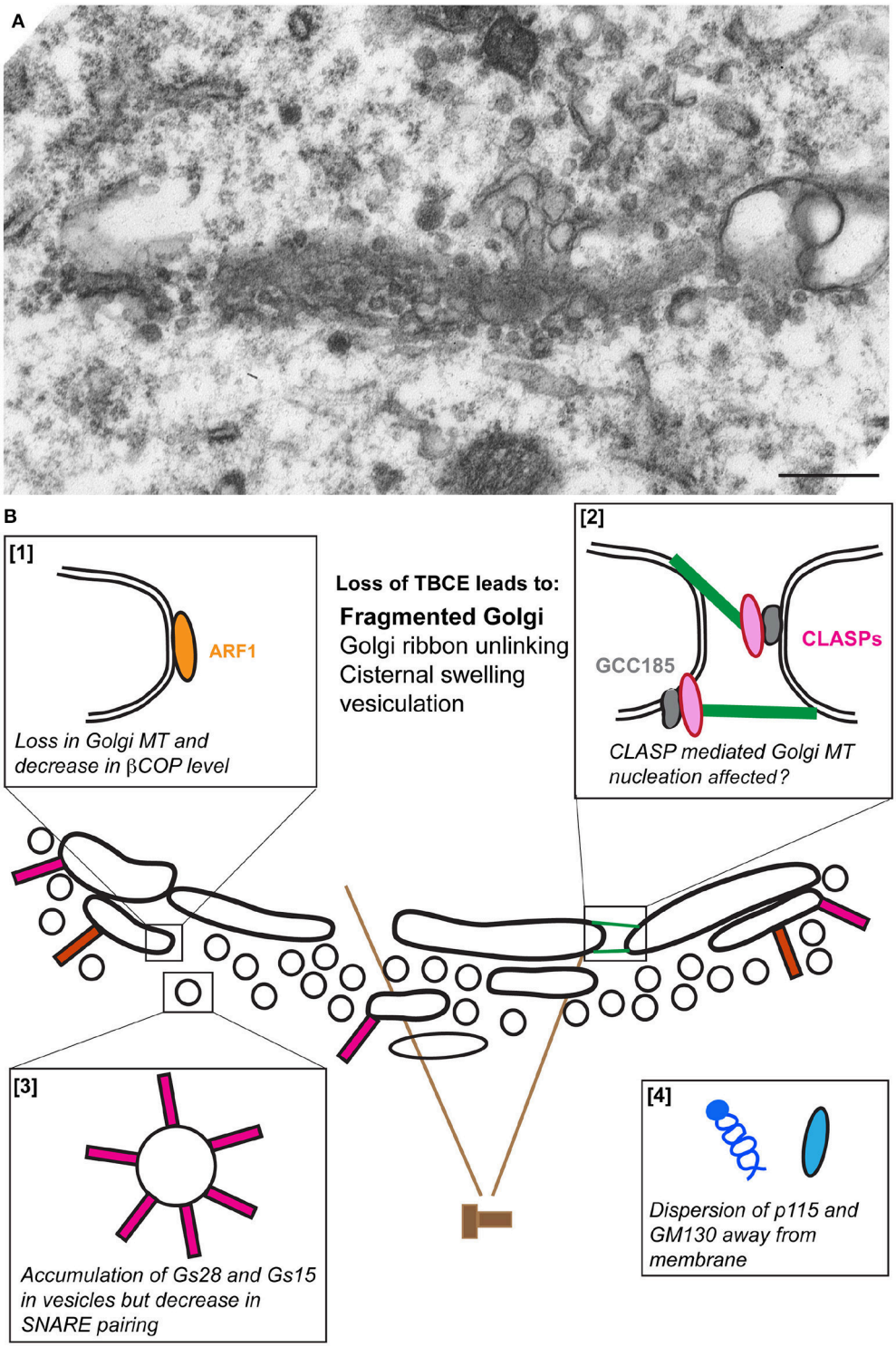

FIGURE 2 | The fragmentation of the Golgi apparatus in *pmn* motor neurons. (A) An electron micrograph showing vesiculation of the Golgi stack in a lumbar spinal cord motor neuron from a *pmn* mouse aged 35 days. **(B)** Schematic representation of the molecular defects leading to Golgi fragmentation. Loss of TBCE function impedes polymerization of microtubules at the cis-Golgi [box 1] and potentially also their GCC185/CLASP-dependent nucleation at the trans-Golgi [box 2]. Levels of β-COP and ε-COP are reduced [box 1], vesicles containing high amounts of Golgi SNAREs GS15 and GS28 accumulate [box3] and tethers p115 and GM130 disperse away from membranes [box 4].

unconventional translation into toxic poly-dipeptides (DPRs; Cleary and Ranum, 2013; Mori et al., 2013; Zu et al., 2013), or by impeding nucleocytoplasmic transport (Freibaum et al., 2015; Jovicic et al., 2015; Zhang et al., 2015).

It should be stressed that Golgi fragmentation has not yet been reported in mutant C9Orf72-linked ALS. However, several findings point to a role of the wildtype C9ORF72 protein in vesicle trafficking. Indeed, bioinformatic analyses predict the presence of DENN (differentially expressed in normal and neoplastic cells) domains in C9Orf72 (Zhang et al., 2012; Levine et al., 2013). DENN domains bear sequence and structural homology with guanine nucleotide exchange factors (GEFs) for Rab GTPases, some of which are involved in regulating vesicle trafficking at the Golgi (Liu and Storrie, 2012). A recent study has reported that the C9Orf72 protein asssociates with Rab1, Rab7 and Rab11 (Farg et al., 2014). In addition to impairment of autophagy and endocytic trafficking upon C9ORF72 knockdown, the GM130 pattern is altered potentially consistent with Golgi alterations (Farg et al., 2014).

Since gain of function rather than loss of function mechanisms appear to drive motor neuron degeneration in C9Orf72-linked ALS (Koppers et al., 2015), future studies need to address the toxic consequences of G_4C_2 repeat expansions and DPRs on vesicle trafficking and maintenance of the Golgi apparatus. The G_4C_2 repeat expansions can be associated with the formation of TDP-43 nuclear and cytoplasmic inclusions (Mori et al., 2013; but see Al-Sarraj et al., 2011), which possibly affects ER to Golgi transport. In addition, the C9Orf72 ALS-associated DPR Gly-Ala has recently been shown to sequester the cargo adaptor Unc119/HRG4 into protein aggregates (May et al., 2014). This may disrupt the ternary complex that is normally formed by Unc119/HRG4, the tubulin-binding co-factor D (TBCD) and the ARF-subfamily member ARL2 (Veltel et al., 2008; Ismail et al., 2012). These data suggest that defects in the MT/COPI cross talk may be implicated in this frequent form of human ALS.

WHAT IS THE RELEVANCE OF GOLGI FRAGMENTATION TO MOTOR NEURON DEGENERATION?

While Golgi fragmentation is now recognized as an early and constant hallmark of degenerating motor neurons in ALS and related motor neuron diseases, its relationship to the neurodegenerative process remains to be clarified. Numerous studies indicate that structural and functional Golgi alterations are not simply a consequence of neurodegeneration. For instance, the drug β,β′-iminodiproprionitrile (IDPN) that causes severe proximal axonopathy does not induce any morphometric changes of the Golgi in rat motor neurons (Mourelatos et al., 1994). Furthermore, surgical transection of the rat facial nerve does not trigger the fine Golgi fragmentation typically observed in human ALS patients (Fujita et al., 2011). Last, transection of the sciatic nerve in mice does not alter the cellular levels or distribution of β-COP, the Golgi tether p115 or the Golgi SNAREs GS28 and GS15 in the corresponding motor neuron cell bodies (Bellouze et al., 2014), suggesting that the microtubule/COPI cross talk is unaffected by axonal injury, in contrast to the situation in *pmn* mice.

Conversely, mounting evidence suggests that Golgi alterations may contribute to, or in some cases even cause, aspects of motor neuron degeneration. In particular, alterations in the microtubule/COPI cross talk may impact on the normal function of the Golgi apparatus in the transport of proteins, lipids and RNAs that are essential for axon and synapse maintenance. The COPI subunits α-, β-, and γ-COP for instance bind numerous mRNAs (Bi et al., 2007; Todd et al., 2013) and facilitate their axonal transport along microtubules (Bi et al., 2007). Furthermore, ARF1 function is critical for axon growth and maintenance (Jareb and Banker, 1997), suggesting a role of COPI in vesicle biogenesis and/or axonal transport.

In conclusion, more work is needed to understand the relevance of Golgi fragmentation for onset and progression of motor neuron degeneration. One way forward is to experimentally inhibit Golgi fragmentation in model systems, whether *in vitro* or *in vivo*, and to subsequently assess the specific consequences on axon degeneration, loss of synaptic vesicles and synaptic dysfunction. To achieve this, we need to gain deeper insights into the mechanisms driving this fragmentation.

Defects in the cross talk between the Golgi microtubule network and the Golgi transport COPI machinery seem an early common mechanism shared by human ALS, SMA, and other motor neuron diseases and thus appear as exciting new molecular targets for the development of biomarkers and pharmacological or gene-based therapies in these severe and currently incurable disorders.

AUTHOR CONTRIBUTIONS

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REVIEW
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Cytoplasmic dynein and its regulatory proteins in Golgi pathology in nervous system disorders

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The Golgi apparatus is a dynamic organelle involved in processing and sorting of lipids and proteins. In neurons, the Golgi apparatus is important for the development of axons and dendrites and maintenance of their highly complex polarized morphology. The motor protein complex cytoplasmic dynein has an important role in Golgi apparatus positioning and function. Together, with dynactin and other regulatory factors it drives microtubule minus-end directed motility of Golgi membranes. Inhibition of dynein results in fragmentation and dispersion of the Golgi ribbon in the neuronal cell body, resembling the Golgi abnormalities observed in some neurodegenerative disorders, in particular motor neuron diseases. Mutations in dynein and its regulatory factors, including the dynactin subunit p150Glued, BICD2 and Lis-1, are associated with several human nervous system disorders, including cortical malformation and motor neuropathy. Here we review the role of dynein and its regulatory factors in Golgi function and positioning, and the potential role of dynein malfunction in causing Golgi apparatus abnormalities in nervous system disorders.

Keywords: Golgi, dynein, dynactin, BICD2, Golgin 160, Lis1, transport, neurodegenerative disorders

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INTRODUCTION

The Golgi apparatus consists of stacks of biochemically and functionally heterogeneous disk-shaped cisternae. In vertebrate cells, the Golgi stacks are laterally connected to form a single continuous membrane system, termed the Golgi ribbon that usually localizes near the centrosome (Klumperman, 2011). Golgi ribbon size and morphology may vary between cell types according to specific requirements of the secretory pathway (Yadav and Linstedt, 2011; Nakamura et al., 2012; Watanabe et al., 2014). In neurons the Golgi ribbon forms an extensive perinuclear network that in multiple neuron types may extend into one or more dendrites (**Figure 1**) (Gardiol et al., 1999; Hanus and Schuman, 2013), and is complemented by Golgi fragments in distal dendrites, designated Golgi outposts (Horton et al., 2005; Ori-McKenney et al., 2012; Quassollo et al., 2015).

The biogenesis and maintenance of the Golgi ribbon structure and position strongly depends on the microtubule cytoskeleton and microtubule motors, in particular cytoplasmic dynein that drives microtubule minus-end transport. Microtubule depolymerization with nocodazole results in the fragmentation of the Golgi ribbon, and the rebuilding of secretion competent ministacks dispersed throughout the cell at endoplasmic reticulum (ER) exit sites. The ministacks are reminiscent of the

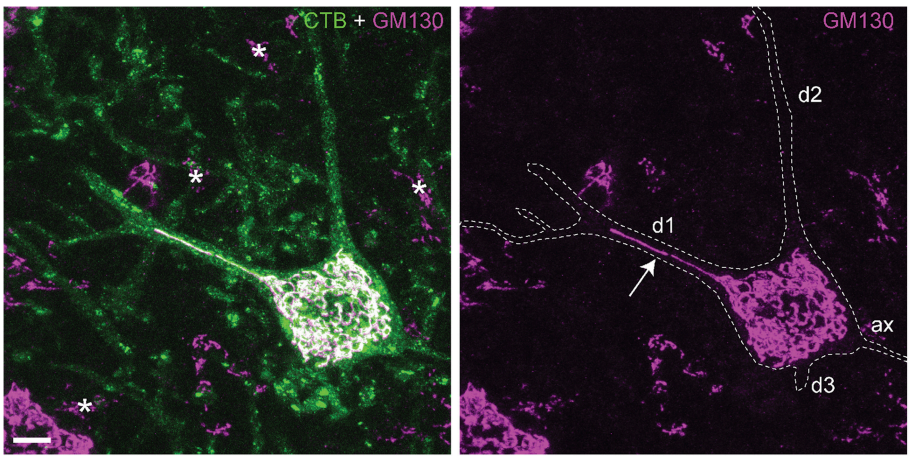

FIGURE 1 | Somatic and dendritic distribution of Golgi apparatus in spinal motor neurons. Projection of confocal sections (optical thickness = 15 μm) of a retrogradely Cholera toxin B (CTB) labeled motor neuron double labeled for cis-Golgi matrix protein GM130 (van Dis et al., 2014). CTB outlines the somato-dendritic compartment of labeled motor neurons, accumulating in the cytoplasm, trans-Golgi and lysosomes. GM130 immunoreactivity is associated with an extensive perinuclear network of thread-like profiles, that in motor neurons may enter into the initial part of one or more dendrites. This example shows one GM130-positive profile extending in the center of the dendrite up to the first branching point (arrow), while other dendrites (d2, d3) do not show Golgi profiles. In other neurons the Golgi profiles may extend up to the second branching point, or multiple profiles running parallel may invade a single dendrite (Gardiol et al., 1999; van Dis et al., 2014). Asterisks, Golgi apparatus of glia cells; Bar = 10 μm.

Golgi apparatus organization found in non-vertebrate organisms, including plants and Drosophila (Cole et al., 1996; Kondylis and Rabouille, 2009). While radial microtubules emanating from the centrosomal microtubule-organizing center mediate pericentrosomal positioning of the Golgi, the vertebrate Golgi apparatus also is a microtubule-organizing center by itself. Golgi-nucleated microtubules, among other functions, are important for assembly of the Golgi ribbon (Zhu and Kaverina, 2013; Rios, 2014).

Also inhibition of cytoplasmic dynein function results in fragmentation of the Golgi ribbon into dispersed, secretion-competent, Golgi ministacks (Palmer et al., 2009; Yadav and Linstedt, 2011). In the Golgi apparatus, cytoplasmic dynein drives the bulk of afferent transport of membrane cargo, i.e., transport from the ER to the cis (entry)-side of the Golgi, and retrograde transport from post-Golgi compartments (endosomes, lysosomes) to the trans (exit)-side of the Golgi (Yadav and Linstedt, 2011; Stephens, 2012; Lord et al., 2013; Lu and Hong, 2014). To accommodate its many functions, a repertoire of factors regulates its subcellular recruitment, cargo interactions, and its mechano-chemical properties (Akhmanova and Hammer, 2010; Fu and Holzbaur, 2014; Jha and Surrey, 2015). The uncovering of Golgin160 as an important dynein adaptor for Golgi membranes has provided basic insight into how dynein may maintain Golgi ribbon structure and position (Yadav et al., 2012). However, knowledge about the role of dynein in the coordinated trafficking of pre-, intra, and post-Golgi membranes, and how mutations in dynein and its regulators affect Golgi function, is still very limited. Here we review the role of dynein and its accessory factors in Golgi membrane trafficking. In addition, since mutations in dynein and its regulators are linked to a variety of nervous system disorders, we will discuss the possible link between disease associated mutations and Golgi abnormalities.

CYTOPLASMIC DYNEIN MOTOR—DYNEIN HEAVY CHAIN

Cytoplasmic dynein 1 (hereafter dynein) is a two-headed microtubule motor that is used for nearly all of the minus-end directed transport, the other dyneins operating in cilia and flagella to power ciliary and flagellar beating (axonemal dyneins) or intraflagellar transport (dynein 2) (Vallee et al., 2012; Carter, 2013; Roberts et al., 2013). Dynein consists of a dimer of the motor-containing heavy chain (DYNHC1H1) and several smaller accessory subunits (**Figure 2**). The motor domain of DYNHC1H1 is in the C-terminus and consists of a ring of six AAA+ ATPase modules, and a 15 nm stalk domain which is responsible for microtubule binding and generating movement along the microtubules (Vallee et al., 2012; Carter, 2013; Roberts et al., 2013). The N-terminal tail domain is responsible for dimerization and cargo interaction.

Dynein heavy chain knockout mice die before implantation and show dispersed Golgi in early embryonic cells (Harada et al., 1998). Heterozygous DYNC1H1 mutations are associated with a spectrum of nervous system abnormalities, with, roughly, tail domain mutations causing a form of spinal muscular atrophy (SMA) or motor-sensory neuronopathies, motor domain mutations causing cortical malformation, and some mutations causing combined phenotypes (Lipka et al., 2013; Peeters et al., 2015; Scoto et al., 2015). These data indicate that mutations may differentially affect the many cellular dynein functions. A tail mutation causing a motor neuronopathy in mice (*Loa* mice) has

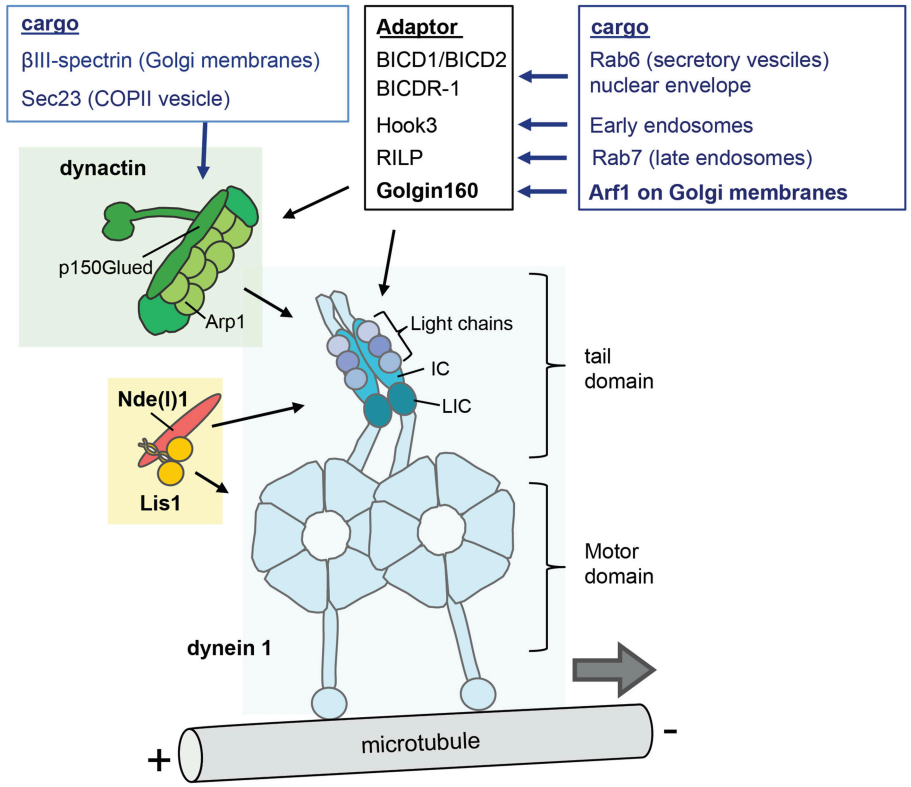

FIGURE 2 | The cytoplasmic dynein complex and its regulators. The dynein 1 molecule is a complex of two heavy chains (HC), two intermediate chains (IC), two light intermediate chains (LIC), and three light chains. The motor domain comprises six AAA+ ATPase modules that form a ring and a stalk that contains the microtubule domain. The N-terminal tail domain interacts with the smaller non-catalytic subunits, and is responsible for dimerization and cargo interaction. The dynactin complex (green) and Lis1/Nde(l)1 (orange/red) are key factors in the regulation of cargo interaction and motor function. Dynactin contains a filament of eight copies of the actin-related protein Arp1, the large subunit p150glued, and 14 additional subunits including one copy of β-actin, Arp11 and p50 dynamitin (Urnavicius et al., 2015). In addition, a few dynein cargo-adaptors are indicated that regulate the interactions with specific cargo's.

been shown to reduce dynein processivity, and to cause impaired retrograde axonal transport, as well as delayed Golgi ribbon reassembly following nocodazole washout (Hafezparast et al., 2003; Vallee et al., 2012). Also mutations in the tail and motor domains associated with combined SMA/cortical malformation cause delayed Golgi ribbon reassembly following nocodazole washout, suggesting impaired Golgi membrane trafficking in many DYNC1H1 patients (Fiorillo et al., 2014). Recently, some dynein tail-mutations (R264L, R598) causing SMA have been found to cause increased binding to the cargo-adaptor BICD2 (see below) (Peeters et al., 2015). Significantly, mutations in BICD2 cause similar motor neuron phenotypes, and are also associated with Golgi abnormalities (Neveling et al., 2013; Peeters et al., 2013).

CYTOPLASMIC DYNEIN MOTOR—DYNEIN ACCESSORY SUBUNITS

The dynein smaller accessory subunits also occur in two copies each, and comprise the intermediate chains (IC), the light-intermediate chains (LIC) and three classes of light chains (**Figure 2**). In primates and rodent two genes encode each of the smaller subunits, while additional diversity is generated by alternative splicing of IC, and phosphorylation of IC and LIC isoforms (Pfister et al., 2006; Allan, 2011). The IC acts as a scaffold between the heavy chains and the light chains and is a major binding platform for dynein interacting proteins including dynactin. Of the two IC isoforms, IC1 and IC2, IC2 is expressed in all cells and is essential for all dynein functions in non-neuronal cells including Golgi apparatus function and positioning (Pfister et al., 2006; Palmer et al., 2009; Raaijmakers et al., 2013). IC1 is predominantly expressed in neurons, with different roles for the various IC1 and IC2 isoforms in axonal transport (Pfister, 2015).

The LIC dimer directly attaches to dynein heavy chain with a Ras-like domain, and can bind cargo adapter proteins such as FIP3, RILP, and BICD2 with its C-terminus (Schroeder et al., 2014). The two LIC subtypes, LIC1 and LIC2, assemble as homodimer into two different populations of dynein. "LIC1-dynein" and "LIC2-dynein" have been implicated in different functions, but the extent to which they are functionally redundant is uncertain (Allan, 2011; Raaijmakers et al., 2013). A knockdown study suggested specific LIC1 functions in ER-Golgi trafficking (Palmer et al., 2009; Brown et al., 2014), but no

Golgi abnormalities were found after LIC1 knockdown by others (Allan, 2011; Tan et al., 2011). Mice, derived from a mutagenesis screen, that were homozygous for a missense mutation (N215Y) in LIC1, and showed mild behavioral changes combined with changes in dendritic morphology also showed abnormalities in Golgi ribbon reassembly following exposure to nocodazole, pointing to a role of LIC1 in Golgi membrane trafficking (Banks et al., 2011). However, LIC1 knockout mice display normal Golgi apparatus, although some evidence suggests a specific role of LIC1 in ER export (Kong et al., 2013). LIC1 knockout mice also show photoreceptor degeneration resulting from impaired dynein dependent transport of Rab11-vesicle trafficking from the Golgi apparatus to the base of the connecting cilium (Kong et al., 2013).

Dynein light chains consists of LL1/2 (also known as LC8-1/2), Roadblock-1/2, and LT1/3 (also known as TCTex1 and TCTex1L or rp3, respectively) that associate with dynein via specific binding sites on the intermediate chains (Pfister et al., 2006; Allan, 2011). The light chains are involved in regulating dynein complex assembly and cargo interactions but in addition may have functions independent of dynein, in particular LL1. Knockdown of LT1 and Roadblock-1 was found to cause Golgi dispersion, while knockdown of LL1 may result in subtle changes in ER to Golgi transport, and LT3 (rp3) knockdown did not alter Golgi markers (Palmer et al., 2009). Another study used rapamycin-inducible ligands that upon dimerization act as molecular traps for LT1 and LL1, respectively (Varma et al., 2008). Both LT1 and LL1 traps induced Golgi dispersion after induction of dimerization. However, despite relatively rapid induction of light-chain inactivation, and, accordingly a dispersion of lysosomes and endosomes within 1 h after light chain trap activation, Golgi dispersion was much slower, suggesting that the effect on Golgi fragmentation is indirect (Varma et al., 2008).

REGULATORS OF DYNEIN ACTIVITY—DYNACTIN

Dynactin is a 1 MDalton multiprotein complex of more than 20 subunits that interacts with the dynein tail domain and mediates dynein-cargo interaction, recruits dynein at microtubule plus-ends, and is required for processive movement (i.e., the ability to make multiple steps before releasing the microtubule) of dynein (Jha and Surrey, 2015; Urnavicius et al., 2015). Major dynactin components include a filament of actin-related protein 1 (Arp1) and a large subunit, p150glued (also designated DCTN1) that mediate dynein interaction (**Figure 2**) (Urnavicius et al., 2015). P150glued can bind microtubules by itself via its N-terminus that contains a conserved CAP-Gly domain. This domain is dispensable for activation of dynein processivity, but is required for other functions such as recruitment of dynein to microtubule plus-ends (Akhmanova and Hammer, 2010; Fu and Holzbaur, 2014; Jha and Surrey, 2015).

Heterozygous point mutations in the p150glued N-terminus cause rare adult onset neurodegenerative disorders (Lipka et al., 2013). Mutations associated with a Parkinsonian disorder (Perry syndrome) afflict microtubule binding and may cause the accumulation of cargo in axon terminals, which may be explained by impaired recruitment of dynactin at microtubule plus-ends in axon terminals (Lloyd et al., 2012; Moughamian et al., 2013; Tacik et al., 2014). Instead a mutation (G59S) associated with a motor neuron disease, is thought to afflict p150Glued stability and to more generally impair dynactin-dynein functions (Lipka et al., 2013). This mutation also may interfere with Golgi apparatus homeostasis, as patient-derived cells show retarded recovery of Golgi after nocodazole treatment (Levy et al., 2006).

Disruption of dynactin or the dynactin/dynein interaction by overexpression of the p50 dynamitin subunit or a dominant p150Glued construct (cc1), respectively is well known to cause Golgi fragmentation and dispersion, and dynactin has been implicated in several steps in ER-to Golgi transport (Yadav and Linstedt, 2011; Lord et al., 2013). Dynactin can directly bind Golgi membranes via its Arp1 subunit that binds βIII spectrin on Golgi membranes (Yadav and Linstedt, 2011). βIII spectrin depletion has been found to cause Golgi fragmentation and impaired Golgi reassembly after nocadozole treatment, consistent with a role in dynein/dynactin function in the Golgi apparatus (Salcedo-Sicilia et al., 2013). Arp1-spectrin interaction also is implicated in dynein dependent transport of Golgi membranes in Drosophila during the cellularization of the larvae (Papoulas et al., 2005). Mutations in βIII-spectrin are associated with a neurodegenerative disease that affects the cerebellar Purkinje cells (SCA5), and βIII-spectrin-deficient mice or mice expressing SCA5-mutant βIII-spectrin develop Purkinje cell degeneration. However, so far there are no neuropathological data indicative of abnormalities in dynein-dependent motility of Golgi membranes in these mice (Armbrust et al., 2014; Clarkson et al., 2014).

Dynactin also can bind specific membrane compartments via its p150glued subunit. P150glued interacts with Sec23 of the COPII vesicle coat complex that sort cargo into budding vesicles at ER exit sites for their delivery at the juxtaposed tubulo-vesicular ER-Golgi intermediate compartment (ERGIC). Although disruption of this interaction slows-down ER exit, the dynactin-Sec23 binding is not essential for ER-ERGIC trafficking (Watson et al., 2005; Yadav and Linstedt, 2011; Lord et al., 2013). A suggested role for dynactin-Sec23 interaction is to aid in the separation of the nascent COPII vesicle (Bacia et al., 2011). Such a role for dynein/dynactin has been suggested in the formation of retromer-coated vesicles at endosomes. Here p150glued binds SNX6 of the SNX-BAR-retromer to first aid with separation of the retromer-coated cargo and then mediate transport from endosomes to the trans Golgi network (TGN) (Wassmer et al., 2009; Stephens, 2012). Interestingly, the interaction between p150glued and SNX6 is negatively regulated by phosphotidylinositol-4-phosphate, a Golgi-enriched phosphoinositide that strongly binds SNX6. Thus, at the TGN phosphotidylinositol-4-phosphate stimulates the dissociation of p150glued and SNX6, providing a mechanism of cargo release by dynein/dynactin (Niu et al., 2013).

REGULATORS OF DYNEIN ACTIVITY—LIS1, NDE1, AND NDEL1

Lis1, Nde1 (nuclear distribution protein E, also known as NudE) and the paralogue Ndel1 are key dynein regulators that can alter its mechanochemical properties, and are involved in most if not all of its functions (**Figure 2**) (Vallee et al., 2012; Roberts et al., 2013). Lis1 is a 45 kD protein that as a dimer can bind the dynein heavy chain motor domain, and can promote a persistent microtubule bound state by uncoupling ATP hydrolysis cycles from microtubule binding affinity changes (Vallee et al., 2012; Toropova et al., 2014). Lis1 is implicated in dynein recruitment and regulation, and is thought to be especially important for high-load dynein functions, including initiation of axonal transport and high load functions in cell migration and mitosis (Yi et al., 2011; Splinter et al., 2012; Vallee et al., 2012; Raaijmakers et al., 2013).

Lis1-dynein interactions are modulated by Nde1 and Ndel1. Nde1 and Ndel1 are important for many dynein functions, either by regulating the recruitment of Lis1, regulating cargo interaction or via other mechanisms (Torisawa et al., 2011; Vallee et al., 2012). In the nervous system, Nde1 and Ndel1 show a largely complementary spatio-temporal expression in neuronal progenitors and post-mitotic neurons, respectively, indicative of different cellular functions, i.e., roles in neurogenesis vs. neuronal migration/morphogenesis, respectively (Sasaki et al., 2005; Pandey and Smith, 2011). The roles of Lis1 and Nde(l)1 are dosage dependent and their concentrations appear to be limiting for several dynein functions. Accordingly, patients and mice with one inactive Lis1 allele display defects in neuronal migration, while additional reduction of Lis1 or coincident reduction of Nde(l)1 results in more severe phenotypes (Sasaki et al., 2005; Youn et al., 2009).

The roles of Lis1 and Nde(l)1 in regulating dynein in the Golgi apparatus are not well understood. Lis1 or combined Nde1-Ndel1 knockdown result in Golgi fragmentation and dispersion in Hela cells, however some conflicting results have been reported in additional studies (Lam et al., 2010). Drosophila Nde1 was found to associate with Golgi membranes and to be required for dendritic targeting of Golgi outposts (Arthur et al., 2015). Notably, Lis1 also has a role as a structural subunit in a phospholipase A enzyme complex, termed PAFAHIb, which deacylates Golgi phospholipids and contributes to morphological remodeling of Golgi membranes. Some evidence indicate that catalytic PAFAHIb subunits compete with Ndel1 for Lis1 binding, and that Lis1 undergoes conformational changes when it switches from a complex with PAFAHIb subunits to one with Ndel1 (Ha et al., 2012).

ACTIVATION OF CYTOPLASMIC DYNEIN MOTILITY

Although dynein subunits, dynactin and Lis1/Nde(l)1 regulate more general aspects of cargo interaction, an additional level of dynein control is provided by cargo-specific adaptor proteins (Akhmanova and Hammer, 2010; Vallee et al., 2012; Jha and Surrey, 2015). A group of these cargo-specific regulatory factors, including BICD1 and BICD2 (nuclear envelope, Rab6 vesicles), BICD-related protein 1 (Rab6 vesicles), Hook3 (early endosomes), Rab11-FIP3 (Rab11 recycling endosomes), RILP (Rab7 late endosomes/lysosome); TRAK1 and 2 (mitochondria), Spindly (kinetochores) and Golgin160 (Arf1 on Golgi membranes) contain coiled coils that bind dynein and/or dynactin (**Figure 2**). Recent studies showed that cargo specific adaptor proteins are also essential cofactors that can activate processive motor motility. Biochemical experiments found that mixing purified mammalian dynein and dynactin with the N-terminal fragment of BICD2 (BICD2-N) results in a stable ternary complex that mediates highly processive unidirectional movement. While dynactin alone is not sufficient for dynein activation, BICD2-N is required to link the two complexes together in order to initiate processive minus-end directed motility (Splinter et al., 2012; McKenney et al., 2014; Schlager et al., 2014a). Structural data show that the BICD2-N coiled coil extends between dynactin and the tail of dynein engaging in multiple reciprocal interactions (Urnavicius et al., 2015). Another study has shown that in inactive non-processive dynein, the two motor heads are stacked together, and that processive motility can be induced by separation of the motor heads (Torisawa et al., 2014). Together the data suggests a model where the binding of the cargo adaptor and dynactin induces conformational changes in the motor domains, promoting dissociation of motor heads and thereby activating directional motility (Urnavicius et al., 2015) (**Figure 3**). Interestingly, recent evidence indicates that different BICD cargo adaptors may differentially regulate speed and processivity of dynein (Schlager et al., 2014b). This can be explained by a mechanism where different adaptors trigger different conformational changes in the dynein motor domains. Thus, different dynein-dynactin cargo adaptors not only differ in cargo binding, but also may differentially modulate motor activity (Schlager et al., 2014b).

DYNEIN REGULATORY FACTORS FOR GOLGI MEMBRANES

Golgin160 has been identified as a dynein cargo adaptor for Golgi membranes (Yadav et al., 2012). Golgin 160 knock-down results in dissociation of dynein from Golgi membranes, fragmentation and dispersion of the Golgi ribbon, and impaired ER to Golgi trafficking of temperature sensitive viral cargo protein (VSVG-GFP). Golgin160 interacts with dynein via its coiled-coil cc7 domain. Like BICD2-N (Hoogenraad et al., 2003), GFP-tagged cc7-domain by itself accumulates at the centrosome, and when attached to mitochondria causes pericentrosomal clustering of mitochondria, indicating that this domain by itself is sufficient to trigger dynein processivity independent of the presence or type of cargo (Yadav et al., 2012). Thus, Golgin160 may coordinate cargo-binding with dynactin-dynein association and initiation of processive motility in a similar way as BICD2. Expression of high levels of BICD2-N or the early endosome cargo-adaptor Hook3 have been shown to cause Golgi dispersion (Hoogenraad et al., 2001; Walenta et al., 2001; Teuling et al., 2008). A possible

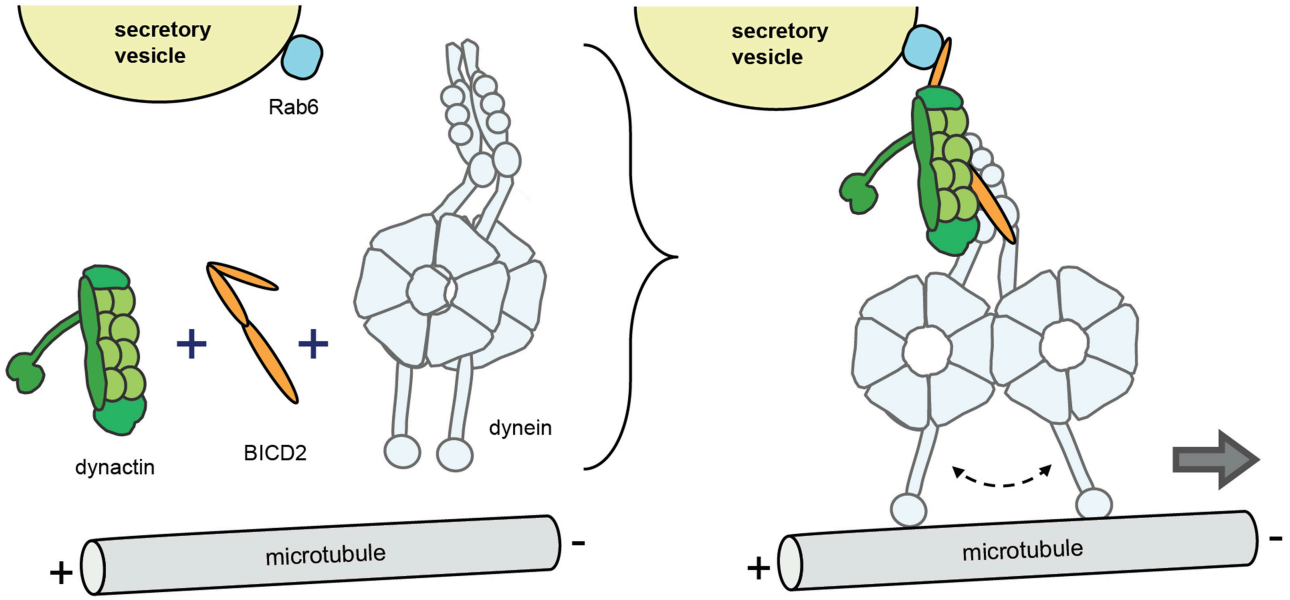

FIGURE 3 | Model of regulation of processive dynein/dynactin motility by cargo adaptors. The model is based on findings with the conserved coiled coil protein BICD2 that operates as a dynein-dynactin cargo adaptor for a variety of cargo including Rab6 vesicles, nuclear envelope, and RNA particles (in Drosophila). Upon cargo binding (in this case Rab6 secretory vesicles), BICD2 co-recruits dynein and dynactin and forms a stable ternary complex to initiate processive minus-end movement. The model implies that dynein in the ternary complex experiences conformational changes in the motor domains, in particular the separation of the motor heads.

explanation is that BICD2-N and hook3 can interfere with Golgin160 binding to dynein-dynactin. This would imply that misregulation of non-Golgi cargo adaptors can indirectly cause abnormalities in Golgi ribbon structure and position, and could explain Golgi abnormalities in cells from patients with BICD2 mutations (Neveling et al., 2013; Peeters et al., 2013). There is no evidence, so far, that wild type BICD2 plays a direct role in Golgi membrane trafficking (Yadav and Linstedt, 2011) and, accordingly, there are no obvious abnormalities in Golgi morphology in BICD2 knockout mice (Jaarsma et al., 2014).

Golgin160 attaches to Golgi membranes via binding of its N-terminus to Arf1, a small GTPase that plays a central role in recruiting factors at ERGIC and Golgi membranes, in particular COPI (Altan-Bonnet et al., 2004; Yadav et al., 2012). Thus, dynein recruitment to Golgi membranes is regulated by activation and deactivation of Arf1, coinciding with the recruitment of other Arf1 effectors and the "Golgification" of ERGIC membranes near the ER exit sites (Yadav et al., 2012). The regulation by GTPases is common to other cargo adaptors which operate as adaptors of Rab6 (BICD1/2, BICDR-1), Rab7 (RILP) and Rab11 (Fip3). Golgin160-Arf1 mediates not only inward motility of peripheral ERGIC membranes, but also clustering of Golgi membranes for assembly of the Golgi ribbon, and maintenance of the Golgi ribbon structure and position near the cell center (Nakamura et al., 2012; Yadav et al., 2012). Interestingly, during mitosis, Golgin160 disassociates from Golgi membranes, which contributes to Golgi disassembly into vesicular structures for proper partitioning between daughter cells (Nakamura et al., 2012; Yadav et al., 2012).

ZW10, an adaptor protein involved in dynactin and dynein recruitment to mitotic kinetochores, has been proposed to act as a linker between dynein/dynactin and Golgi membranes. Inhibition of ZW10 by knock-down, anti ZW10 antibody or dominant-negative overexpression causes Golgi fragmentation and dispersal, and reduced minus-end directed motility of Golgi membranes (Varma et al., 2006). However, other studies suggest that ZW10 has a function in the Golgi independent of dynein (Arasaki et al., 2007; Majeed et al., 2014).

Lava lamp is a large coiled-coil protein, identified in Drosophila, which binds α-Spectrin on Golgi membranes and may also act as a dynein/dynactin adaptor for Golgi membranes (Papoulas et al., 2005). In Drosophila, dynein-dependent motility of Golgi is not needed for maintenance of Golgi apparatus structure and position, as Golgi stacks in Drosophila are dispersed throughout the cytoplasm next to ER exit sites, reminiscent of the dispersed Golgi in mammalian cells after microtubule depolymerization or inhibition of dynein/dynactin (Kondylis and Rabouille, 2009). Instead, dynein is required for specialized processes such as cellularization of synctial embryo's (Papoulas et al., 2005) and the development of complex dendritic arbors in neurons (Ye et al., 2007). In neurons, lava lamp regulates dynein-dependent transport of Golgi outposts to distal dendrites. Inhibition of lava lamp results in a shift of Golgi outposts from distal to proximal dendrites and a concomitant distal to proximal shift in dendrite branching (Ye et al., 2007), and a similar redistribution of Golgi outpost and dendritic branches occur in Drosophila with mutations in IC and LIC dynein subunits and Lis1 (Zheng et al., 2008), as

well as Nde1 knock-down (Arthur et al., 2015). Lava lamp knockdown also causes Golgi outposts to appear in axons and these axons start branching. Analysis of mutants of ER-to-Golgi trafficking, and laser damage of individual Golgi outposts further showed that Golgi outposts are particularly important for dendrite arborization in Drosophila (Ye et al., 2007). A recent study showed that the interaction between lava lamp and dynein at dendritic Golgi outposts is regulated by Leucine-rich repeat kinase (Lrrk), the Drosophila homolog of Parkinson's disease-associated Lrrk2 (Lin et al., 2015). Lrrk loss-of-function Drosophila mutants show increased anterograde movement of Golgi outposts and increased dendritic branching. Lrrk suppresses anterograde (proximo-distal) movement of Golgi outposts via phosphorylation of Lava lamp and inhibiting its interaction with dynein (Lin et al., 2015). The significance of the findings on dynein-dependent motility of Golgi outposts in Drosophila for mammalian neurons remains to be established. A mammalian homolog for lava lamp has not been identified (Munro, 2011), and the extent to which dynein is important for Golgi outpost positioning in hippocampal and cortical neurons is not yet known.

PERSPECTIVES

The uncovering of dynein adaptor proteins and regulatory factors has provided basic insight into how dynein mediates Golgi membrane trafficking and maintains Golgi ribbon structure and position (Yadav and Linstedt, 2011; Yadav et al., 2012). Several important research questions remain to be addressed. Are there other adaptors in addition to Golgin160 implicated in dynein-dependent Golgi membrane motility in mammalian cells? For instance, do specialized adaptors operate in specialized Golgi compartments such as dendritic Golgi outposts? Does Golgin160 co-recruit dynein and dynactin in a similar way as BICD2 (Schlager et al., 2014a; Urnavicius et al., 2015)? How is Golgin160-Arf1 binding coordinated with dynein/dynactin recruitment? Analysis of Drosophila BICD showed that cargo binding triggers conformational changes that promote dynein/dynactin binding, thus coordinating cargo-binding with dynein recruitment (Liu et al., 2013). How do Lis1 and Nde(l)1 operate in Golgi membrane trafficking? Systematic siRNA-based dissection of dynein regulators in mitosis, indicated that Lis1 and dynactin are differentially involved in different mitotic dynein functions. How does dynein-Golgin160 activity cross-talk with other motors, i.e., kinesins and myosin (Barlan et al., 2013)? Knock-down of the kinesin KIF5B results in longer run lengths of ER-to-Golgi membrane carriers, indicating a negative regulation of ER-to Golgi transport by plus-end motors, and the presence of motors of opposite polarity on ER-to-Golgi cargo (Brown et al., 2014). A variety of scaffolds such as huntingtin have been implicated in the coordination of minus- and plus-end motors for specific retrograde and anterograde axonal transport cargo's (Maday et al., 2014). How is dynein-dynactin-Golgin160 motoring integrated with the microtubule organization around the Golgi? This question is particularly relevant in developing and adult neurons with their complex Golgi morphologies and microtubule organization (Horton et al., 2005; Kuijpers and Hoogenraad, 2011; Kapitein and Hoogenraad, 2015).

The importance of proper dynein function for the Golgi apparatus in neurons is illustrated by genetic disorders that combine mutations in dynein subunits and regulatory factors, with nervous system abnormalities and abnormalities in the Golgi apparatus. In addition, some evidence indicates that dynein malfunction in the Golgi apparatus is an early feature in neurodegenerative disorders such as Amyotrophic lateral sclerosis (van Dis et al., 2014) and Alzheimer's disease (Joshi and Wang, 2015). We have recently found that dysregulation of dynein/dynactin function by BICD2-N overexpression increases Golgi fragmentation in SOD1-ALS mice (van Dis et al., 2014). Knowledge about the role of dynein in and around the Golgi will contribute to our understanding of these disorders.

AUTHOR CONTRIBUTIONS

DJ wrote the manuscript and made the figures; CH wrote and edited the manuscript.

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REVIEW
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Dominant spinal muscular atrophy is caused by mutations in BICD2, an important golgin protein

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Spinal muscular atrophies (SMAs) are characterized by degeneration of spinal motor neurons and muscle weakness. Autosomal recessive SMA is the most common form and is caused by homozygous deletions/mutations of the *SMN1* gene. However, families with dominant inherited SMA have been reported, for most of them the causal gene remains unknown. Recently, we and others have identified heterozygous mutations in *BICD2* as causative for autosomal dominant SMA, lower extremity-predominant, 2 (SMALED2) and hereditary spastic paraplegia (HSP). *BICD2* encodes the Bicaudal D2 protein, which is considered to be a golgin, due to its coiled-coil (CC) structure and interaction with the small GTPase RAB6A located at the Golgi apparatus. Golgins are resident proteins in the Golgi apparatus and form a matrix that helps to maintain the structure of this organelle. Golgins are also involved in the regulation of vesicle transport. *In vitro* overexpression experiments and studies of fibroblast cell lines derived from patients, showed fragmentation of the Golgi apparatus. In the current review, we will discuss possible causes for this disruption, and the consequences at cellular level, with a view to better understand the pathomechanism of this disease.

Keywords: BICD2, SMALED2, SMA, Golgi fragmentation, endocytosis, RAB6A, dynein, dynactin

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Spinal muscular atrophy (SMA) is a diverse group of genetic disorders characterized by aberrant development and/or loss of spinal motor neurons, and muscle weakness without sensory neuron involvement (Wee et al., 2010). SMA is usually classified by the pattern of weakness (i.e., proximal or distal) and mode of inheritance (autosomal recessive, autosomal dominant, X-linked).

Autosomal recessive SMA linked to chromosome 5 (5q-SMA) is the most common form accounting for up to 95% of the SMA cases, and is caused by homozygous deletion/mutation of the survival motor neuron 1 (*SMN1*) gene, localized on chromosome 5q12-q13 (Lefebvre et al., 1995; Wirth, 2000). The 5q-SMA is classified into different types based on onset and severity of symptoms. The most severe form of 5q-SMA (type I) has an onset within the first 6 months of life, permanent inability to seat without support and usually death within 2 years (Iannaccone et al., 1993; Rudnik-Schöneborn et al., 1996). The intermediate form of 5q-SMA (type II) has an onset between 6 and 18 months of life, ability to seat but not to walk and death usually occurs after the age of 2 years. A milder form is the 5q-SMA type III with an onset at around 3 years of age, ability to stand and walk but because of progressive muscle weakness, the patients mostly are wheelchair-bound as the disease progresses (Zerres and Rudnik-Schöneborn, 1995; Zerres et al., 1995). The 5q-SMA type IV, also called "adult SMA," is considered the mildest and less severe form with a late onset of around 30 years of age (Zerres et al., 1995). All the mentioned forms of 5q-SMA commonly share proximal muscle weakness with slow progression in case of type II-IV SMA.

In contrast to autosomal recessive 5q-SMA, the autosomal dominant SMA (ADSMA) forms are less frequent, milder, with vague progression and probably, even under-diagnosed. In approx. 70% of individuals with ADSMA the genetic cause is still unknown. However, due to remarkable advances in next generation sequencing and gene discovery, the number of newly described causative genes increases continuously.

Beside autosomal dominant SMA lower extremity-predominant 1 (SMALED1, OMIM #58600) caused by mutations in dynein cytoplasmic 1 heavy chain 1 protein (*DYNC1H1*; OMIM *600112), we and others have recently identified heterozygous variants in *BICD2* (OMIM *609797) as causative for autosomal dominant SMA, lower extremity-predominant, 2, (SMALED2; OMIM #615520) and hereditary spastic paraplegia (HSP) (Neveling et al., 2013; Oates et al., 2013; Peeters et al., 2013).

CLINICAL FEATURES OF INDIVIDUALS CARRYING MUTATIONS IN BICD2

Individuals carrying heterozygous missense variants in *BICD2* exhibit muscle weakness and atrophy predominantly of the proximal lower limbs. However, in some cases the distal lower limbs are also affected, and in few others upper limbs are additionally compromised. The initial reasons for clinical counseling are commonly the difficulties in walking (waddling gait and toe walking), and delayed motor milestones. Contractures are reported frequently, and few patients present congenital hip dysplasia. Notoriously, many patients show evident wasting of the lower limbs and a very broad upper body, which resembles a bodybuilder-like shape. Symptoms are usually present at birth or appear in early childhood, but some cases with adult onset have been also described (**Table 1**; Neveling et al., 2013; Oates et al., 2013; Peeters et al., 2013; Synofzik et al., 2014; Rossor et al., 2015). The course of disease is slowly progressive or non-progressive. Due to the mild phenotype, patients are sometimes diagnosed as SMA type IV, the mildest form of the classical 5q-SMA. However, most cases of SMALED2 present a congenital or early onset with foot deformities and joint contractures, and difficulties when began walking (Frijns et al., 1994; Adams et al., 1998; Oates et al., 2012; Neveling et al., 2013). One infant who died of other causes at 14 months of age showed in the post-mortem examination decreased number of anterior horn cells in the lumber and cervical spine with no peripheral nerve pathology confirming a SMA (Oates et al., 2012).

Two families affected by an HSP phenotype associated to *BICD2* variants, have been described (Oates et al., 2013; Novarino et al., 2014). In the first family, the affected individuals presented features in adulthood, and showed lower-limb spasticity and hyperreflexia as is typical for HSP. It is also reported, a slow progression of contractures, weakness, and wasting (Oates et al., 2013). Regarding the association of this specific variant with the development of HSP but not SMALED2, it would be of particular interest to exclude the involvement of secondary disease-causing genes, resulting then in a multigenic disease, instead of a monogenic form. The second family with HPS linked to *BICD2* is consanguineous, and the affected individuals carry the homozygous variant in *BICD2*, c.G1823A, p.S608L (Novarino et al., 2014). This variant was identified by whole-exome sequencing in combination with network analysis, and is the only homozygous variant reported in *BICD2* so far.

Numerous experimental attempts have been made leading to a better understanding of the physiological role of BICD2 and yielded first insights into possible effects of variants in *BICD2* on a cellular and molecular level (**Table 2**). However, further investigation is needed to fully understand the pathomechanism of how mutations in BICD2 can cause SMALED2 and rarely HSP-like phenotype. Next, we summarize the mutation spectrum and functional knowledge about BICD2, and focus on Golgi fragmentation as a cellular consequence of certain BICD2 mutations, as a possible mechanism to explain failure in neuronal development and maintenance.

GENETIC SPECTRUM OF PATHOGENIC VARIANTS FOUND IN *BICD2* IN PATIENTS WITH SMALED2

BICD2 localizes in chromosomal region 9q22.31 and encodes a canonical isoform of 824 amino acids. All the variants found in patients with SMALED2 are heterozygous and lead to single amino acid substitutions (**Figure 1**). The most common variant reported is the c.320C>T, p.Ser107Leu, found in 29 of 49 cases corresponding to 5 of 13 families described with SMALED2. This variant is located within a CpG dinucleotide (Neveling et al., 2013; Oates et al., 2013; Peeters et al., 2013; Bansagi et al., 2015; Rossor et al., 2015). The cytosine-guanine (CpG) dinucleotide is considered a hotspot for pathological variants. Cytosine is subject for methylation however, spontaneous deamination of 5-methylcytosine may occur, yielding thymine instead (Shen et al., 1994).

BICD2 IS HIGHLY CONSERVED AMONG SPECIES

Bicaudal-D (BicD) was identified first in *Drosophila melanogaster* in a mutant screen for dominant maternal-effect proteins. Loss-of-function mutations in BicD interfere with the determination of the oocyte, while gain-of-function substitutions (e.g., p.Glu224Lys, p.Phe684Ile) disrupt the establishment of anterior and posterior polarity giving rise to bicaudal (two tails) embryos (Mohler and Wieschaus, 1986; Steward, 1987; Schüpbach and Wieschaus, 1991). *BicD* null mutations are recessively lethal (Ran et al., 1994). Further studies show that BicD is a component of dynein-based transport and is implicated in the transport of mRNA (e.g., *clathrin heavy chain* and *osk*) to specific cellular regions of the fly during oogenesis and embryogenesis (**Figure 2**; Bullock and Ish-Horowicz, 2001). Experimental evidence suggested that BicD, together with clathrin heavy chain, mediates endocytosis during oocyte development (Vazquez-Pianzola et al., 2014). BicD is also involved in the regulation of bi-directional transport of lipid droplets (Larsen et al., 2008).

TABLE 1 | Clinical summary of SMALED2 patients carrying BICD2 substitutions.

Variant/ Mutation	Onset	x/y	Motor ability	x/y	Muscle weakness	x/y	Muscle atrophy	x/y	Contractures	x/y	Congenital hip dysplasia. x/y	References
c.320C>T p.Ser107Leu	Congenital <3 years 3–12 years	9/29 10/29 10/29	Delayed motor milestones Waddling gait Toe walking	10/29 25/29 7/29	LL prox and distal LL prox Additional UL prox Additional UL distal	18/29 11/29 1/29 2/29	LL prox and distal LL prox Shoulder girdle	18/29 5/29 10/29	Foot Hips Ankles Achiles tendon	15/29 3/29 7/29 4/29	2/29	Neveling et al., 2013 Peeters et al., 2013 Oates et al., 2013
c.563A>C p.Asn188Thr	<3 years	4/5	Delayed motor milestones Waddling gait Toe walking	1/5 2/5 1/5	LL prox	5/5	LL prox	5/5	no		4/5	Neveling et al., 2013
c.565A>T p.Ile189Phe	Congenital	1/1	Delayed motor milestones Wheelchair dependent	1/1 1/1	LL prox and distal Additional UL	1/1 1/1	LL prox and distal	1/1	Ankles, knees and hips	1/1	1/1	Oates et al., 2013
c.1502G>C p.Arg501Pro	Congenital 3–12 years	2/5 3/5	Delayed motor milestones Waddling gait Toe walking	2/5 3/5 3/5	LL prox and distal LL distal	2/5 2/5	LL prox and distal	4/5	Foot Achiles tendon	3/5 3/5	1/5	Oates et al., 2013
c.1523A>C p.Lys508Thr	Adulthood	1/1	Abnormal gait, difficulty walking	1/1	LL prox and distal	1/1	LL distal	1/1	Hips and knees	1/1	No	Oates et al., 2013
c.1604C>T p.Ala535Val	Congenital	2/2	Delayed motor milestones Waddling gait	2/2 2/2	LL prox and distal	2/2	LL prox and distal	2/2	Foot	2/2	1/2	Rossor et al., 2015
c.2108C>T p.Thr703Leu	Congenital	2/2	Difficulty walking Crutch-assisted	2/2 1/2	LL prox and distal Additional UL distal	2/2 2/2	LL prox	2/2	Foot	2/2	No	Neveling et al., 2013
c.2239C>T p.Arg747Cys	3–12 years Adulthood	1/3 2/3	Toe walking Difficulty walking and climbing	3/3 3/3	LL prox	3/3	LL prox	3/3	no		No	Synofzik et al., 2014
c.2321A>G p.Glu774Gly	3–12 years	1/1	Delayed motor milestones Waddling gait	1/1 1/1	LL prox and distal	1/1	LL prox and distal	1/1	no		No	Peeters et al., 2013

x/y, x of a total of y cases present feature; LL, lower limbs; UL, upper limbs.

TABLE 2 | Summary of possible molecular mechanism involved in SMALED2 due to BICD2 substitutions.

Variant/Mutation	Mechanism proposed	Mechanism proposed by
c.320C>T/ p.Ser107Leu	Higher interaction with DIC and p150Glued subunit of dynactin	Oates et al., 2013; Peeters et al., 2013
c.563A>C/p.Asn188Thr	Slight Golgi fragmentation	Neveling et al., 2013
c.1502G>C/p.Arg501Pro	Higher interaction with DIC and p150Glued subunit of dynactin	Oates et al., 2013
c.2108C>T/p.Thr703Leu	Prominent Golgi fragmentation	Neveling et al., 2013
c.2321A>G/p.Glu774Gly	Less interaction with Rab6a	Peeters et al., 2013

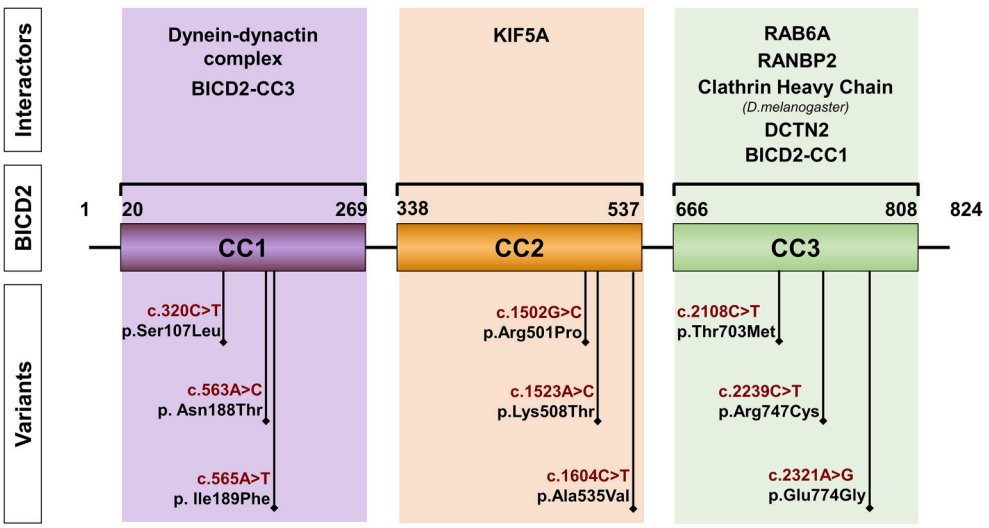

FIGURE 1 | Schematic representation of BICD2. The diagram depicts the coiled-coil domains (CC) of BICD2 indicating the corresponding interacting partners. The position of variants, found in patients with SMALED2 is shown (Neveling et al., 2013; Oates et al., 2013; Peeters et al., 2013; Synofzik et al., 2014; Rossor et al., 2015).

There is only one *BicD* gene in invertebrates, while mammals have two homologs *BICD1* and *BICD2*. BICD1 and BICD2 interact with the dynein-dynactin motor complex (Hoogenraad et al., 2001), and both have been associated with Golgi-ER transport (Matanis et al., 2002). Studies in *Bicd2* knockout mice revealed, that BICD2 has an important role in neuronal cell migration (**Figure 2**) (Jaarsma et al., 2014). The *Bicd2* knockout mice present severe developmental defects of the cerebellar cortex, however other brain regions and spinal cord appear normal (Jaarsma et al., 2014).

BICD2 STRUCTURE AND ORGANIZATION

Studies in *Drosophila* revealed that more than half of the BicD protein consists of heptad repeats. A heptad repeat is a repeating pattern of seven amino acids where hydrophobic residues are preferentially located at positions 1 and 4 (McLachlan and Karn, 1983). These heptad repeats mediate the packaging of one helix against another, forming coiled-coil (CC) structures made up of two or three protein molecules and resulting in homo- or heterodimers or in multimers (Bruccoleri et al., 1986). The BICD protein is predicted to consist of CC domains: the N-terminal domain contains the coiled-coil segment 1 (CC1) and the coiled-coil segment 2 (CC2), and the C-terminal contains the coiled-coil segment 3 (CC3). Studies in *Drosophila* and mammals suggested, that BICD interacts with itself forming homodimers (Oh et al., 2000; Hoogenraad et al., 2001). In the case of BICD2, the segment 1 of the N-terminal domain (CC1, amino acids 10–321) binds to the C-terminal segment (CC3, amino acids 706–810) (Hoogenraad et al., 2001). A hypothetical model proposes that as a result of this interaction between segments, the soluble free BICD2 folds up, and only when C-terminal segment engages in an interaction with other proteins, the N-terminal segment of the BICD2 becomes available for interaction with other proteins (Hoogenraad et al., 2001). These protein interactions seem to be determining for the cellular distribution of BICD2 and the fulfillment of its specific function (**Figures 1**, **2**).

THE ROLE OF BICD2 IN THE GOLGI APPARATUS AND DYNEIN-DYNACTIN COMPLEX

One of the most studied binding partners of BICD2 is the dynein-dynactin complex. Cytoplasmic dynein is the major responsible motor protein for transporting a large variety of cargos toward the minus ends of microtubules (Kardon and Vale, 2009). Dynactin is a protein complex that stimulates dynein processivity and participates in cargo binding (Holleran et al.,

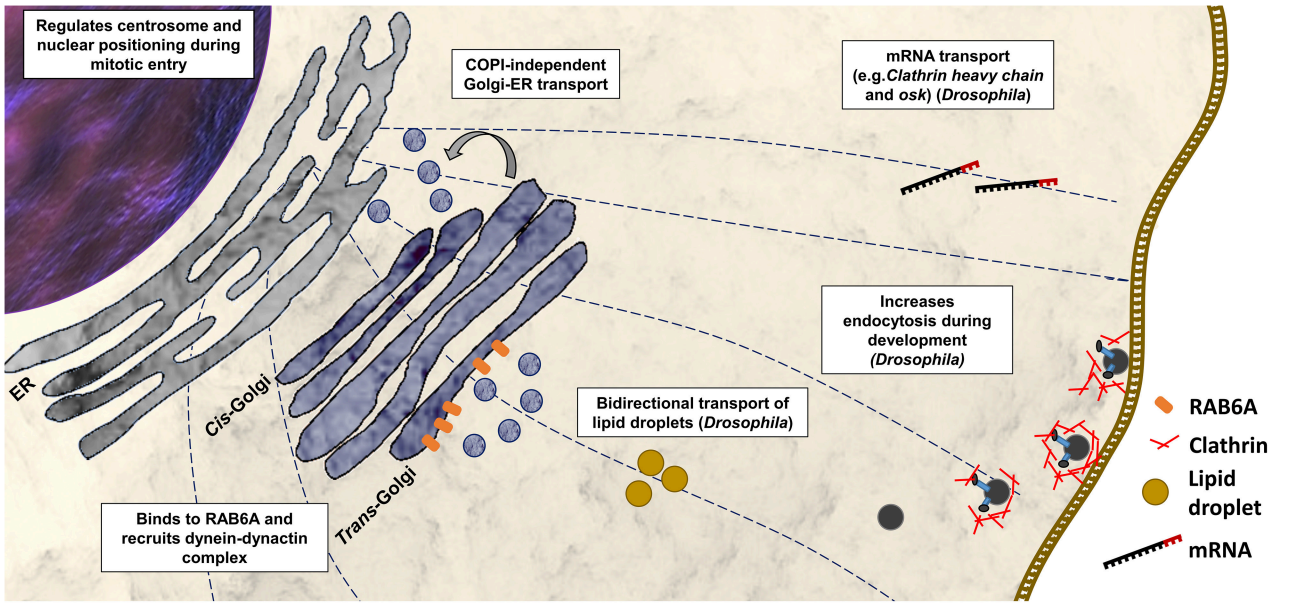

FIGURE 2 | Functions of BICD2 in the cell. Cellular process with BICD2 involvement as described from studies in mammals or *Drosophila* are shown. BICD2 binds to RAB6A and recruits the dynein-dynactin complex to the trans-Golgi to facilitate COPI-independent Golgi-ER transport (Hoogenraad et al., 2001, 2003; Matanis et al., 2002). Prior mitosis (during G2 phase), BICD2 binds to a component of the nuclear pore complex, the RANBP2, and recruits dynein-dynactin to keep centrosomes tethered to the nucleus (Splinter et al., 2012). Studies in *D. melanogaster* have shown that BicD participates in mRNA transport (Bullock and Ish-Horowicz, 2001), endocytosis together with clathrin heavy chain (Vazquez-Pianzola et al., 2014), and in the regulation of bi-directional transport of lipid droplets (Larsen et al., 2008).

1998; Schroer, 2004). Dynein and dynactin directly bind to each other through the interaction of dynein intermediate chain and dynactin subunit p150 (Vaughan et al., 1995; Waterman-Storer et al., 1995; King et al., 2003). The N-terminal domain of BICD2 binds to the dynein-dynactin complex via interaction with the p50 subunit of the dynactin complex (Hoogenraad et al., 2001). Studies *in vivo* and *in vitro* suggested, that the N-terminus of BICD2 promotes a stable interaction between dynein and dynactin (Splinter et al., 2012). In this context it is particularly interesting to mention that mutations in cytoplasmic dynein DYNC1H1 causes SMALED1 (Harms et al., 2012; Tsurusaki et al., 2012; Peeters et al., 2015). See the Review Jaarsma and Hoogenraad (2015).

The C-terminal segment of BICD2 shows the highest degree of evolutionary conservation and is described as the cargo-binding domain (Hoogenraad et al., 2001; Terenzio and Schiavo, 2010). BICD2 is considered a linker protein that acts between a cargo bound to its C-terminal segment and the dynein-dynactin complex associated to its N-terminal segment. The C-terminal segment of BICD2 binds strongly to the active form of the RAB6A GTPase and is responsible for Golgi targeting (Hoogenraad et al., 2001, 2003).

RAB6A is part of a big RAB family of more than 60 members (Zerial and McBride, 2001). RAB proteins shift dynamically between an active membrane-associated GTP-bound form and an inactive cytosolic GDP-bound form (Pfeffer, 2001; Zerial and McBride, 2001). RAB proteins are associated with membranes via geranylgeranyl groups that are attached to cysteine residues at the C-terminus (Stenmark, 2009). Guanine nucleotide exchange factors (GEFs) are responsible for the activation of RAB proteins by catalyzing the exchange of GDP for GTP (Delprato et al., 2004; Stenmark, 2009). Experimental evidence suggested that GEFs are responsible for targeting the RAB proteins to specific membranes (Gerondopoulos et al., 2012; Blümer et al., 2013). The active, membrane-bound RAB proteins are involved in membrane traffic, by binding with specific proteins (Grosshans et al., 2006).

The small GTPase RAB6A is involved in intra-Golgi transport. Several studies support that RAB6A coordinates the retrograde COPI-independent Golgi-ER pathway, which is considered a recycling route for Golgi-resident glycosylation enzymes (Martinez et al., 1997; Girod et al., 1999; White et al., 1999; Storrie et al., 2000). The active form of RAB6A (GTP-bound) recruits BICD2 to the *trans*-Golgi membrane via direct interaction with the C-terminal domain of BICD2 (Hoogenraad et al., 2001; Matanis et al., 2002; Short et al., 2002; Bergbrede et al., 2009; Matsuto et al., 2015). Recent reports suggest that BICD2 may stabilize the active RAB6A, by inhibiting its GTPase activity and thus increasing the GTP-bound membrane-associated RAB6A (Matsuto et al., 2015). Once the C-terminal segment of BICD2 binds to RAB6A, the N-terminal segment of BICD2 becomes available and recruits the dynein-dynactin complex. This recruitment is considered a critical step for the microtubule retrograde traffic. In this manner, a coordinated action between RAB6A, BICD2 and the dynein-dynactin complex controls COPI-independent Golgi-ER transport (Matanis et al., 2002).

Hence BICD2 has an abundant CC structure, localizes at the Golgi complex, and interacts with a member of the RAB family of GTPases (RAB6A), it is considered to be a golgin (Barr and Short, 2003; Short et al., 2005; Goud and Gleeson, 2010).

Golgins are proteins associated with the Golgi apparatus and help to maintain the organized architecture of this dynamic organelle. One type of golgins consists of resident transmembrane-Golgi proteins which are necessary for tethering membranes together (Barr and Short, 2003). Another type of golgins corresponds to proteins that are recruited to the Golgi apparatus, and are associated with components of the trafficking machinery. BICD2 is included in this last class (Barr and Short, 2003).

MUTATIONS IN BICD2 CAUSE GOLGI FRAGMENTATION

The effects of the BICD2 domains on the integrity of the Golgi apparatus have been studied in detail (Hoogenraad et al., 2001, 2003; Splinter et al., 2012). Although the C-terminal domain targets the BICD2 to the Golgi apparatus, the overexpression of the N-terminal segment perturbs the Golgi organization (Hoogenraad et al., 2001). Fragmentation of the Golgi apparatus has been associated with inhibition of dynein function (Burkhardt et al., 1997; Harada et al., 1998; Quintyne et al., 1999).

Based on the association of BICD2 with RAB6A and Golgi targeting, we performed immunofluorescent staining of the Golgi apparatus of fibroblast cells derived from the patients carrying heterozygous mutations in *BICD2*.

We observed a prominent fragmentation of the Golgi apparatus in primary fibroblast cells harboring the p.Thr703Leu substitution (C-terminal) and a milder fragmentation in cells with the p.Asn188Thr substitution (N-terminal) (Neveling et al., 2013) (**Figure 3**). The individuals carrying the mutation p.Thr703Leu show a more severe phenotype including congenital contractures, while the individuals carrying the mutation p.Asn188Thr do not present contractures. Thus, in the case of these two cell lines showing Golgi fragmentation, the grade of fragmentation seems to correlate with the severity of the disease (Neveling et al., 2013). Although, Golgi fragmentation is not a common feature in all the cell lines carrying mutations in BICD2 (Oates et al., 2013; Peeters et al., 2013).

The BICD2 mutations p.Thr703Leu and p.Asn188Thr may cause fragmentation of the Golgi apparatus due to conformational changes of BICD2 that alter protein function and affect the structure of the Golgi. How amino acid substitution at the positions Thr703 and Asn188 impact intramolecular interactions and probably protein folding remains elusive, as there is no crystalloid structure of BICD2 or the region harboring these mutations available. The alteration in the Golgi structure could also be a secondary effect of alteration in the microtubule organization. The integrity and positioning of the

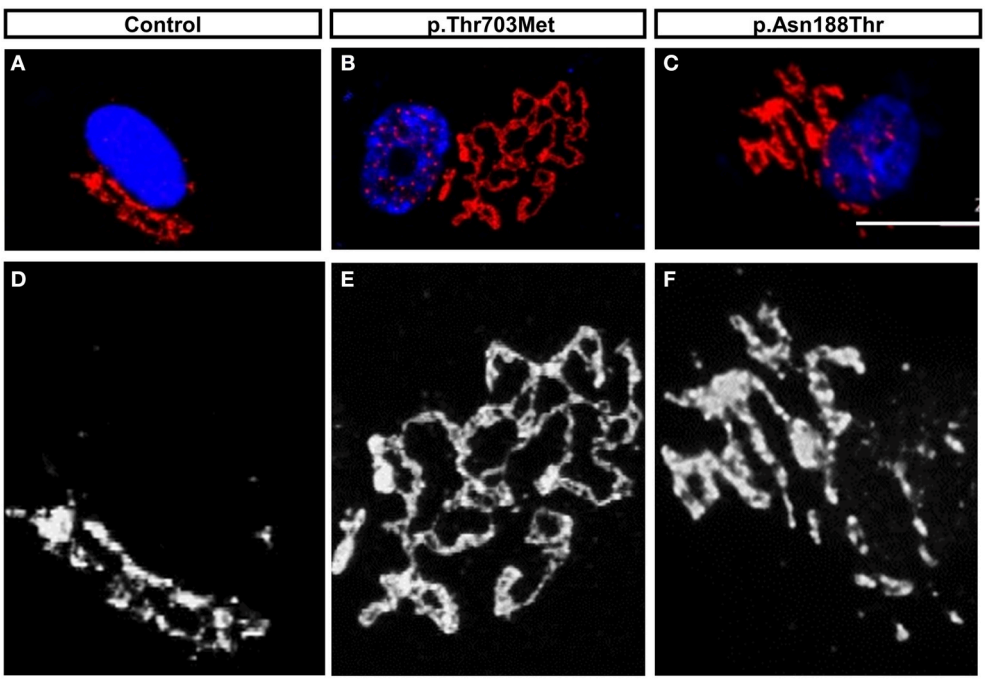

FIGURE 3 | The mutations in BICD2, p.Thr703Leu and p.Asn188Thr, triggered Golgi-fragmentation in Primary Fibroblast Cells. (A–C) Modified data as published previously (Neveling et al., 2013), showing the Golgi apparatus of control fibroblasts and patients fibroblasts. The Golgi apparatus was immunostained with antibody against the Golgi marker ML160. **(D–F)** Magnification in monochrome of **(A–C)** for better visualization and comparison. Please note the prominent unfolded and disperse structure of the Golgi apparatus in cells carrying the mutation p.Thr703Leu and the intermedium or mild dispersion in the cells carrying the mutations p.Asn188Thr. For detail information and quantification, please read (Neveling et al., 2013).

Golgi apparatus seems to have an important role in neuronal polarization and development (de Anda et al., 2005).

POTENTIAL CONSEQUENCES OF GOLGI FRAGMENTATION ON NEURONAL DEVELOPMENT AND MAINTENANCE

Proper development of neurons is vital to fulfill and maintain the precise connectivity of the nervous system. Neurons are among the most polarized cell types and consist of a cell body from which specialized structures emerge, the neurites. In a very coordinated process, only one of the neurites grows long becoming an axon, while the other neurites acquire dendritic identity (Goslin et al., 1989; Craig and Banker, 1994; Polleux and Snider, 2010).

Previous studies have shown that in completely undifferentiated neurons, the centrosomes, Golgi apparatus and late/recycling endosomes cluster together to the area where the first neurite will form, which is in turn opposite from the plane of the last mitotic division (de Anda et al., 2005). The coordinated activity of these organelles is necessary for the polarization of the neuron.

Besides axon development, it has been suggested that the Golgi apparatus has an important role in dendrite maintenance and shape (Horton and Ehlers, 2003; Lewis and Polleux, 2012; Ori-McKenney et al., 2012). Non-neuronal cells contain one Golgi apparatus while many neurons contain several Golgi, the somatic Golgi and the dendritic Golgi centers called "Golgi outposts." These dendritic Golgi outposts function similarly to the somatic Golgi, providing dendrites with substantial secretory capacity. The Golgi outposts appear during early neuronal differentiation, and may form as fragments derived from somatic Golgi that expand into dendrites, as the dendrites grow (Horton and Ehlers, 2003). The Golgi structure, dynamics and localization seem to have an important effect on neuronal development and maintenance, and an alteration in any of them could trigger degeneration or failure in correct maturation.

Additionally to SMALED2, other motor neurodegenerative diseases like ALS, have been associated with Golgi fragmentation (Gonatas et al., 1992; Mourelatos et al., 1994). However, it is still not clear why motor neurons but not other neuronal cells are affected. This effect might be attributable, to specific requirements of motor neurons during development that are different from other neuronal populations.

In conclusion, pathogenic substitutions in BICD2 causes SMALED2 with an autosomal dominant inheritance. Patients with SMALED2 show slowly to non-progressive muscle weakness of proximal and distal mainly of lower extremities, with a quite broad clinical spectrum. The majority of patients show congenital or early onset with foot deformities and joint contractures. However, some patients present a late onset and have very mild and non-progressive appearance. Commonly seems to be a wide aperture of the upper body (body-building shape). BICD2 is a golgin adaptor protein. The various BICD2 substitutions found in patients with SMALED2 impair interaction to other binding partners such as dynein-dynactin, RAB6A etc. and thus impair most likely cellular processes such as axonal transport, vesicular transport, Golgi integrity etc, which however needs further intensive functional studies. It is not understood why these mutations cause specifically loss of spinal motor neurons, since BICD2 is expressed in all neurons, is essential in neuronal migration and in positioning of the nucleus in the cell. There is also a quite broad phenotypic variability even in patients with the same mutation, suggesting the influence of modifying factors, which also need to be investigated.

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Alteration of Golgi Structure by Stress: A Link to Neurodegeneration?

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The Golgi apparatus is well-known for its role as a sorting station in the secretory pathway as well as for its role in regulating post-translational protein modification. Another role for the Golgi is the regulation of cellular signaling by spatially regulating kinases, phosphatases, and GTPases. All these roles make it clear that the Golgi is a central regulator of cellular homeostasis. The response to stress and the initiation of adaptive responses to cope with it are fundamental abilities of all living cells. It was shown previously that the Golgi undergoes structural rearrangements under various stress conditions such as oxidative or osmotic stress. Neurodegenerative diseases are also frequently associated with alterations of Golgi morphology and many stress factors have been described to play an etiopathological role in neurodegeneration. It is however unclear whether the stress-Golgi connection plays a role in neurodegenerative diseases. Using a combination of bioinformatics modeling and literature mining, we will investigate evidence for such a tripartite link and we ask whether stress-induced Golgi arrangements are cause or consequence in neurodegeneration.

Keywords: Golgi, Alzheimers disease, neurodegenration, computational modeling, protein–protein interactions, cellular stress response

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INTRODUCTION

The Golgi apparatus is an organelle that is best known for its roles in post-translational protein modification and in secretory trafficking. In addition, the Golgi is increasingly being viewed as a signaling hub, which is not only able to respond to environmental factors, but it also able to modulate the outcome of signaling cascades by housing signaling molecules (Farhan and Rabouille, 2011; Cancino and Luini, 2013). As will be discussed later in greater detail, one of the signals that the Golgi is responsive to is cellular stress that was shown to induce prominent alterations of Golgi morphology.

Cells across all organisms have evolved adaptive mechanisms to survive adverse environmental conditions such as limited availability of nutrients, too high or low temperatures, non-physiologic pH, changes in tonicity, exposure to oxidative radicals, or the accumulation of toxic protein species. How cells deal with these stressors determines the fate of the cell, which might be survival, death, or malignant transformation. Therefore, it is not surprising that cellular stress responses were suggested to be involved in a plethora of diseases among them neurodegenerative disorders (Bhat et al., 2015; Coppedè and Migliore, 2015), which are the focus of the current work. Most diseases from that spectrum feature structural and functional alterations of the Golgi apparatus. Because stress signaling also affects the Golgi, we are tempted to speculate that the stress-Golgi connection is relevant for neurodegeneration. However, whether this is the case remains unclear.

What has also not been investigated so far is the extent of the connections between stress and Golgi regulators. Another important question in this context is whether the Golgi simply acts as a receiver of stress signals, or whether we can infer evidence for the Golgi sending signals that modulate, stress, neurodegeneration, or both.

The goal of the current work is to use a computational biology approach to build a network of protein–protein interactions (PPI) to identify the extent of connections between stress signaling pathways and putative Golgi regulators. To do so, we will make use of the results of different RNAi screens that uncovered numerous potential regulators of Golgi morphology. The inferred network can be used to search for signatures of relevance for neurodegeneration. We will first review and introduce the relevant literature on the Golgi apparatus in neurodegenerative diseases and then the evidence for stress signaling and its connection to this organelle. We will also briefly introduce the different RNAi screens that were carried out in the past to investigate the secretory pathway and discuss the rationale behind inclusion and exclusion of their hits.

THE GOLGI IN NEURODEGENERATIVE DISEASES

A cellular event that has been observed in several neurodegenerative diseases is the fragmentation of the Golgi apparatus (Fan et al., 2008). In a mouse model of amyotrophic lateral sclerosis (ALS), severe Golgi fragmentation in motoneurons was observed, which was found to be due to loss of the Golgi-localized tubulin-binding cofactor E (TBCE; Bellouze et al., 2014). Depletion of TBCE not only resulted in a defect of microtubules biogenesis at the Golgi, but also affected vesicular transport from the Golgi to the endoplasmic reticulum (ER). This paper provides a potential mechanistic link between functional and structural alteration of the Golgi and a neurodegenerative disorder. The link between Golgi fragmentation and ALS was also reported in other studies. A transgenic mouse ALS model expressing mutated superoxide dismutase 1 (SOD1) displayed an up-regulation of the microtubule-depolymerizing protein stathmin (Strey et al., 2004) and a similar dysregulation of vesicular transport. In addition, Golgi fragmentation was also described as a result of inhibition of ER-to-Golgi trafficking (Cutrona et al., 2013). In Alzheimer's Disease (AD), Golgi fragmentation is linked to the serine/threonine kinase CDK5 shown to phosphorylate Golgi matrix proteins such as GM130 (Sun et al., 2008) and GRASP65 (Joshi et al., 2014). Tau, a microtubule binding protein that forms aggregates in AD was also shown to be phosphorylated by CDK5, an event thought to contribute to formation of neurofibrillary tangles (Castro-Alvarez et al., 2014) as well as leading to Golgi fragmentation (Liazoghli et al., 2005). Phosphorylation of tau subsequent to Golgi fragmentation has also been reported (Jiang et al., 2014). However, while the case of CDK5 in AD is reasonably well understood, we lack a broad and systematic understanding of the signaling pathways that regulate Golgi architecture under conditions relevant to neurodegeneration.

THE GOLGI UNDER STRESS

All organelles of the secretory pathway receive signaling inputs initiated by external stimuli. Thereby, these organelles are capable of sensing the presence or absence of growth factors, nutrients, and amino acids (Farhan et al., 2010; Farhan and Rabouille, 2011; Zacharogianni et al., 2011). Among the signals that the Golgi responds to is cellular stress. Several stresses have been already reported to affect this organelle and alterations induced by osmotic stress were among the first to be reported. Because the plasma membrane is freely permeable to water, cells have evolved evolutionary highly conserved mechanisms to cope with changes in the osmolarity of their surrounding medium. In mammalian cells, hypertonic treatment was reported more than two decades ago to inhibit ER-to-Golgi transport (Docherty and Snider, 1991). Hypotonic stress induces tubulation of Golgi membranes (Lee and Linstedt, 1999), whereas hyperosmotic conditions induce its fragmentation and redistribution of the Golgi back to the ER (Lee and Linstedt, 1999). It remains incompletely understood how these phenomena are generated from a molecular or mechanistic point of view. However, there is experimental evidence that the osmotic shock is perturbing the function of vesicle trafficking machineries, which thereby indirectly results in Golgi fragmentation. For instance, hypo-osmotic shock results in a reduction in the number of export sites on the ER (Lee and Linstedt, 1999). Thus, there is less trafficking from the ER to the Golgi, a condition described by others to result in alterations of Golgi structure (Cutrona et al., 2013). The identity of the signaling pathways that lead to these phenomena remains elusive. In addition, hypo-osmotic conditions seemed to also potentiate retrograde transport from the Golgi to the ER (Lee and Linstedt, 1999). Again, how this effect is mediated is totally unclear. Contrary to this, hyper-osmotic conditions seemed to only affect anterograde transport from the ER to the Golgi, but did not seem to have any major effect on retrograde transport (Lee and Linstedt, 1999). How different osmotic conditions differentially affect membrane trafficking and what the (patho) physiologic significance of his might be remains to be determined.

Recently, DNA damage induced by camptothecin, doxorubicin, or by ionizing radiation was also shown to induce dispersal of the Golgi (Farber-Katz et al., 2014). Of note, this effect was not related to the induction of apoptosis, a condition that also disrupts the Golgi. In support of the notion that this effect is a pure stress response was the elucidation of the signaling pathway that is behind this phenomenon. DNA damage was shown to activate a kinase called DNA-PK, which in turn phosphorylates the Golgi protein GOLPH3 (Farber-Katz et al., 2014). GOLPH3 is a protein that localizes to the trans-Golgi by binding to phosphatidylinositol-4-phosphate. At the Golgi, GOLPH3 mediates an interaction with the actin cytoskeleton by binding to MYO18A (Dippold et al., 2009). Phosphorylation of GOLPH3 by DNA-PK results in increased binding to MYO18A, thereby increasing the tension on Golgi membranes exerted by the actin cytoskeleton, which ultimately leads to Golgi fragmentation. At the same time trafficking from the Golgi to the cell surface is disrupted. This response of the Golgi seems

to mediate a resistance of the cell to the DNA damaging agent (Farber-Katz et al., 2014). An important finding of this work was that the Golgi fragmentation phenotype was persistent and was observed to some extent for a month after the damaging agent was removed (Farber-Katz et al., 2014). This observation could be interpreted in two ways: either Golgi fragmentation is a protective (adaptive) response that persists in order to maintain cell fitness for prolonged periods of time. Alternatively, Golgi fragmentation is a pathologic response and a sign that cell fitness is reduced or that the cell is now more prone to other damaging agents.

Altogether, it appears that the Golgi apparatus is subject to extensive regulation by different classes of stressors. We are aware that it might be relevant to discriminate between different types of stress. However, for the sake of simplicity, we will only generally talk about stress and its connection to the Golgi, without going into detail of the different types of stress.

SYSTEMATIC APPROACHES INVESTIGATING REGULATORS OF THE GOLGI

The advent of systems biology opened up new avenues to study various biological processes in a holistic manner. In the current paper we focus on RNAi screens, as these will form the basis for our computational inference strategy. To date two RNAi screens exist that aimed at identifying genes that when depleted result in a structural alteration of the Golgi (Chia et al., 2012; Millarte et al., 2015). We are aware of other screens of the secretory pathway that were recently discussed (Farhan et al., 2010; Zacharogianni et al., 2011; Simpson et al., 2012; Farhan, 2015) and we will discuss here reasons for their (partial) exclusion. Firstly, the Golgi modulating hits from the first kinome and phosphatome screen of the human secretory pathway (Farhan et al., 2010) are included in a more recent work (Millarte et al., 2015). All other hits in that screen did not affect the Golgi and therefore will not be considered for our analysis. The first screen in *Drosophila* for regulators of the secretory pathway (Zacharogianni et al., 2011) cannot be considered because the structure of the Golgi in fly cells is slightly different from mammalian cells. Indeed, the Golgi in mammalian cells is a single copy organelle that fragments in response to external or internal signals. In *Drosophila*, there are multiple pairs of Golgi stacks per cell (Kondylis et al., 2007; Kondylis and Rabouille, 2009) and therefore it is very difficult to assess the impact of the *Drosophila* screen on the mammalian Golgi apparatus. We therefore, decided not include these hits for further analysis.

We first considered the candidates identified by Chia et al. who screened the human kinome and phosphatome searching for regulators of the Golgi. This work revealed 159 Golgi-regulating genes, with several enriched sub-networks such as phospholipid metabolism or the acto-myosin cytoskeleton (Chia et al., 2012; **Table 1**). While the enrichment of these modules is not surprising in light of previous research, the work also revealed that several components of mitogen activated protein kinase (MAPK) pathways are potentially involved in Golgi regulation. This includes the so-called stress-activated MAPK family members, supporting the notion that the Golgi is a stress-sensing organelle. Another potential Golgi-regulating MAPK family member is ERK8 (also known as MAPK15). ERK8 was found to partially localize to the Golgi and to negatively regulate O-glycosylation (Chia et al., 2014). Notably, ERK8 was found earlier to mediate the stress response of nutrient deprivation to the ER exit sites (Zacharogianni et al., 2011). Therefore, ERK8 might be considered as a potential link between stress signaling and the Golgi.

Second, we have recently screened a focused library of 103 genes for effects on Golgi structure and identified at least 70 potential regulators of Golgi structure (Millarte et al., 2015; **Table 1**). The goal of that screen was to identify new regulators of cell migration, but it nevertheless serves (together with the Chia screen) as a repository for proteins that affect the Golgi, either directly or indirectly.

A third screen from which we partially derive hits for our computational analysis is from the first full-genome screen in human cells for regulators of secretion (Simpson et al., 2012). While this screen identified over 600 hits that regulate the secretion of a transmembrane cargo protein, we largely lack information on the impact of these hits on Golgi morphology. However, we found 78 hits that were tested for their impact on Golgi structure (**Table 1**) and we will include these into our collection of hits that affect Golgi morphology. All these Golgi modulating hits from the three RNA screens will be used to assemble a network of Golgi regulators, which we will link computationally to genes known to be involved in stress signaling.

HYPOTHESIS

As outlined in the introductory section, it is not clear whether the stress-Golgi connection is relevant for the pathogenesis of neurodegenerative disorders. In **Figure 1**, we schematically illustrate this relationship and the known (black arrows) and unknown connections (red arrows). As can be seen, it is unclear whether and how Golgi alteration contributes to neurodegeneration. What is also unknown is whether Golgi alteration mends or deteriorates stress signaling. Based on this illustration the following hypothetical possibilities exist:

Scenario 1: Golgi fragmentation is only a byproduct of neuronal cell death and has no role in the pathogenesis of ND.
Scenario 2: The Golgi is altered consequently to stress signaling, but this is only an epiphenomenon that has no further role in the disease.
Scenario 3: The Golgi fragments in response to stress as a response of the cell to fight the stress. Here Golgi fragmentation is a protective event and interfering with this response is not a desired therapeutic option.
Scenario 4: Stress signals results in Golgi fragmentation, which acts as a positive feedback module that enhances stress signaling and enhances neurodegeneration. Here, blocking Golgi fragmentation is a desired therapeutic strategy.

TABLE 1 | List of Golgi regulating proteins identified from RNAi screens.

A

Hits from Millarte et al. (2015)

ABL1	MGC45428	RYK
AKAP28	NCOA3	SH3RF1
BCR	NEDD9	SNARK
BLK	NYD-SP25	SRC
BMP2K	P38IP	SSH1
CASP10	PAK4	STK22B
CBL	PARD6A	STK22C
CCM2	PDCD10	STYX
CD44	PIK3C2B	TLR4
CDC42	PLCE1	TMED7
CDK4	PLCG1	TRRAP
CLTC	PLCG2	UNC119
DUSP23	PLK3	ZAP70
FYN	PRKACA	
GOLGA2	PRKCA	
HRAS	PRKCM	
KIT	PRKCZ	
LYN	PSPH	
MAPK1	PTPRC	
MAPK10	PTPRN	
MAPK14	RAC1	
MAPK8IP3	RAF1	
MAPK9	RGS2	
MAPRE1	RIPK1	
MBL2	RP6-213H19.1	

B

Hits from Chia et al. (2012)

ACPT	CHKA	EPM2A	LCP2	NPR2	PPP2R2B	ROS1	TXK
ACYP1	CKM	ERBB3	MALT1	NRBP1	PPP2R5E	RP6-213H19.1	ULK4
ADCK1	CLK1	EVI1	MAP2K7	NRG3	PPP3R1	RPRD1A	VRK3
ADCK5	COL4A3BP	EXOSC10	MAP3K13	PAG1	PRAGMIN	RPS6KB1	WNK3
AK3L1	CSNK1A1L	FBP2	MAP3K2	PAK1	PRKAG1	RPS6KB2	YSK4
AK7	CSNK1E	FGFR1	MAP3K8	PAK3	PRKAG3	SCYL3	YWHAH
ALPK2	CSNK1G1	FGFR2	MAP4K2	PANK3	PRKCE	SHPK	
ALPP	CSNK1G2	FLT3	MAP4K3	PAPSS1	PRKCSH	SPHK1	
ANGPT4	CSNK2B	GALK1	MAPK11	PAPSS2	PRKX	SQSTM1	
ANP32E	CXCL10	GAP43	MAPK15	PDGFRA	PRPS1L1	SRMS	
AURKB	DCLK2	GTF2H1	MAPKAPK5	PDK4	PTEN	SRPK2	
AXL	DGKD	HCK	MAPKSP1	PFKP	PTK2	STK32A	
BCKDK	DGKQ	HIPK1	MARK4	PHKG1	PTK7	STK36	
BMPR1B	DGKZ	HIPK3	MAST1	PHKG2	PTP4A1	STK4	
BMX	DLG3	HIPK4	MGC16169	PI4KA	PTP4A3	STK40	
BUB1	DMPK	HK1	MGC42105	PI4KB	PTPN14	TAOK2	
CAMK1	DUSP2	IGBP1	MKNK1	PINK1	PTPRA	TESK1	
CCRK	DUSP22	IGF1R	MKNK2	PIP5K1A	PTPRD	TGFBR1	
CDC2	DUSP6	IKBKE	MPP2	PKLR	PTPRF	TNIK	
CDC25A	DUSP8	INPP1	MTMR1	PPM1D	PTPRN2	TNS3	

(Continued)

TABLE 1 | Continued

B

Hits from Chia et al. (2012)

CDC2L2	EIF2AK2	IPMK	MYO3B	PPM1F	PTPRT	TRIB1
CDC42BPA	ENPP7	ITK	NEK11	PPM1L	PTPRU	TRPM7
CDC42BPG	EPHA1	ITPKA	NEK2	PPP1R11	PXK	TSKS
CDKL2	EPHA8	ITPKB	NLK	PPP1R2P9	RIPK2	TTK
CDKN1B	EPHB1	KHK	NME2	PPP2CA	ROCK1	TWF2

C

Hits from Simpson et al. (2012)

ABHD1	GPT	RADIL
ABHD5	GRIN2C	RHOU
AC068775.1	GSG2	RIOK3
ACTR3	IL18R1	RXRA
AKAP5	INADL	SIRT2
AR	INPP5J	SOS2
ARHGAP12	KIF1C	SPRYD4
ARHGAP32	KIF26A	SRSF1
ARHGAP44	KIFAP3	SST
B3GAT2	KIRREL	STC1
C1orf201	KRT6B	SYT1
C1S	LRP4	SYT7
C22orf45	LUC7L3	TALDO1
CCDC124	LVRN	TANC2
CCR4	MAGIX	TM4SF19
CKAP2	MAPK15	TMTC1
CLPB	MAST3	TRIM41
CLSTN3	MXD4	TSPAN1
COPB2	NT5C	TTC37
COPG	NUDT4	TYRO3
CTGF	PLEKHM2	UBE2E1
DENND4C	PML	WDR75
EPDR1	PPFIA1	YY2
FAM177B	PROC	ZNF503
GLRB	PRR4	ZNF512
GPBP1	PTBP1	ZNF830

In scenarios where Golgi alteration is not linked to the disease (scenarios 1, 2), we expect that while Golgi and stress regulators might form a network, this network will be largely devoid of processes of any relevance to neurodegeneration. In the other scenarios the Golgi is on one hand a receiver of signals (in this case stress signals) and then plays either a protective (scenarios 3) or damaging (scenario 4) role. Our approach is compatible with the Golgi being a receiver of signals in the first line, because the computational inference will be based on hits that were identified as potential regulators of Golgi organization. Thus, the Golgi can be considered a received of signals transduced by these regulators. As for the possibilities that the Golgi sends signals, we would like to emphasize that our approach cannot easily distinguish this possibility from others. Nevertheless, as will been discussed below, we inferred few hypothetical cases where the Golgi might respond to stress signaling, to in turn emit signals that contributes to neurodegeneration.

COMPUTATIONAL INFERENCE OF A NETWORK LINKING STRESS PATHWAYS TO REGULATORS OF GOLGI MORPHOLOGY

PPI orchestrate a wide range of biological process and studying PPI networks has become increasingly important and valuable

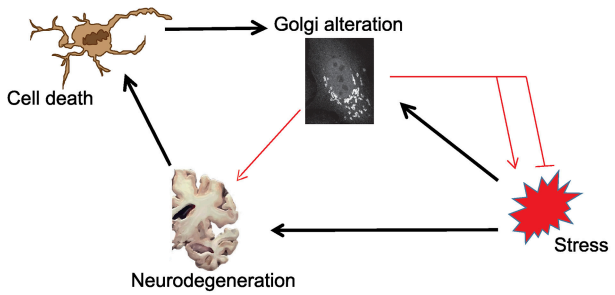

FIGURE 1 | Schematic illustration of the possible connections in the tripartite link between stress, Golgi alterations and neurodegeneration. Black arrows indicate links that are sufficiently supported by evidence from the literature. Red connections are those that are currently of unknown relevance.

in solving biological questions. Based on the three RNAi screens discussed above (Section The Golgi in Neurodegenerative Diseases), we have a collection of 320 hits (**Table 1**) that affect the morphology of the Golgi. In addition, we performed a search of the literature for proteins that were known to be involved in the response to the following types of stress: Osmotic stress, oxidative stress, ER stress (and unfolded protein response), radiation stress, inflammation stress, and DNA damage response producing a list of 76 proteins (**Table 2**). This list includes canonical components of stress signaling pathways. We are aware of studies that analyzed changes of the proteome in neurons that respond to stress (Herrmann et al., 2013). However, the list of up- and down-regulated proteins represents the end-product of stress signaling, rather than components of stress pathways. Our aim is to investigate stress signaling at the Golgi and therefore, we decided to focus on components of stress signaling pathways.

After removing overlapping proteins between the list of stress and Golgi hits, we obtained an input list containing a total of 389 proteins that form the basis of the network inference. In this paper, the background interactome was built upon the BIOGRID database, which only relies on interactions that are supported by experimental evidence, and it is comprised of 6516 proteins and 174411 interactions. The source file can be obtained freely without the need for a license. In order to infer the network connecting the 389 proteins we looked for the connected subnetworks with the minimum number of proteins required to link them. This search was performed by solving, via mathematical programming techniques, the so-called Steiner Tree problem.

The Steiner Tree problem is a classical problem in the field of Combinatorial Optimization (Magnanti and Wolsey, 1995). A network (in our case the entire human interactome) can be defined as N = N(V,E). V are the nodes (all the human proteins) including a subset of so-called terminal nodes T (that is, the input nodes, i.e., Golgi hits and stress genes). E are the interactions between the nodes (the edges linking the nodes). The Steiner Tree problem finds a sub-network connecting all the elements in T (input nodes) using the minimum possible number of edges. Achieving such connection might require the use of additional nodes that were not originally among the input nodes. These additional nodes are called "Steiner nodes" and in this context

TABLE 2 | Stress-related proteins.

DAXX	FADD	MAPK8
ALKBH8	Fas	MAPK9
ALS2	FXYD2	MAPKAPK2
APOE	GADD45A	MAPKAPK3
ATF4	GPX1	MAPT
ATF6	GPX3	MEF2C
ATM	GPX6	NEFH
BAD	GSK3B	NEFL
BCL2	HSPB1	NEFM
BID	HSPB2	NFATC1
CACNA1A	JUN	NFATC3
CAPN1	JUND	NFE2L2
CAPN2	KEAP1	PAK1
CAPNS1	LPL	PPM1B
CASP12	MAP2K3	PPP2CA
CDC25B	MAP2K4	PRKDC
CDK5	MAP2K6	PRPH
CDK5R1	MAP2K7	RELA
CSNK1D	MAP3K1	SOD1
CSNK2A1	MAP3K11	TRAF2
DDIT3	MAP3K5	TXN
DUSP1	MAP3K7	TXNRD1
DUSP4	MAPK10	ZAK
EEF2K	MAPK11	
ERN1	MAPK12	
ERN2	MAPK13	
	MAPK14	

they can be regarded as proteins that enable a functional linkage among the proteins comprised in T.

To solve this optimization problem, we use mathematical programming techniques; in particular, a specially tailored algorithm able to solve the Steiner Tree problem on the considered interactome in few seconds (Fischetti et al., 2014). Note that if it is not possible to connect all the elements in T, then the algorithms seeks for a sub-network spanning as many elements in T as possible, using as few edges as possible. The network resulting from this inference is depicted in **Figure 2A** as a typical "hairball" diagram to illustrate the strong connectivity in the network. Of the 389 input nodes, only 28 nodes do not appear in the network due to poor connectivity (proteins that have no known interaction partners). In addition, 57 additional nodes (Steiner nodes) were included to allow formation of a fully connected network, which indicates that stress pathway components and putative Golgi regulators are reasonably well connected.

We next asked which cellular processes are enriched in this network. This can be performed using a functional annotation clustering of gene ontology (GO) terms. However, we decided not to perform this analysis on the large network, because we will not be able to easily determine to which subnetworks a given enriched cellular process belongs. Therefore, we first aimed at de-bulking the large network and search whether it contains sub-networks. We used the ClustNsee plugin in

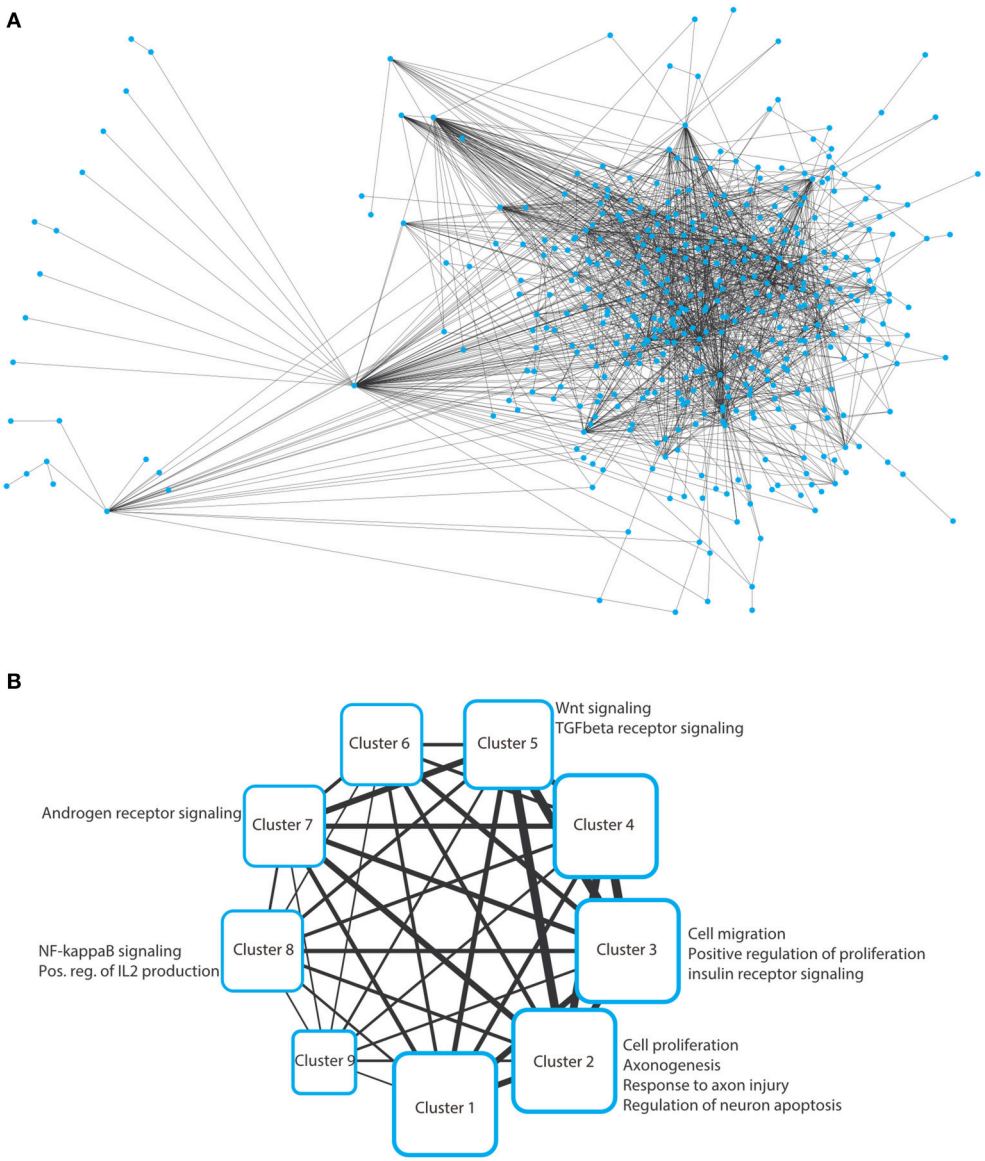

FIGURE 2 | (A) Hairball representation of the network of protein–protein interactions between the Golgi and the stress-related proteins. The network was visualized and arranged using Cytoscape (organic style). The list of the interactions in this network are provided in Supplementary Table S1. **(B)** Nine clusters were obtained by analyzing the network in panel a using ClustNsee. Next to each cluster, we provide the most notable cellular processes that were obtained using a Gene Ontology analysis using the BinGO plugin of Cytoscape. These cellular processes are discussed in detail in the main text.

Cytoscape to search for clusters (Spinelli et al., 2013). The accuracy of the clustering algorithm was verified using a test-network containing very closely related proteins regulating COPI vesicle formation, COPII vesicles formation, and nuclear pore components. ClustNsee could faithfully distinguish subnetworks containing the three aforementioned cellular processes (data not shown). Using the clustering algorithm on our network shown in **Figure 2A** revealed the presence of nine clusters, which revealed several processes and signaling pathways of relevance to neurodegeneration (**Figure 2B**). Next we will discuss these nine clusters.

RELEVANCE OF THE INFERRED SUB-NETWORKS FOR NEURODEGENERATION

We analyzed the different clusters using the BinGO plugin into Cytoscape. BinGO determines the statistical enrichment of GO term annotations in biologic networks. We set the threshold to a statistical significance of ≤ 0.01 after Bonferroni correction. Clusters 1, 4, 6, and 9 did not reveal any notable enrichments of cellular processes and will therefore not be discussed further.

Cluster-2

CDK5

The most notable processes is the enrichment of the GO term "regulation of neuron apoptosis" (**Figure 2**) which included the proteins CDK5, CDK5R1, GPX1, and BCL2. As has been outlined above (Section The Golgi in Neurodegenerative Diseases), CDK5 is a serine/threonine kinase that was implicated by several groups to play a role in neurodegeneration (Liu et al., 2015). With respect to the Golgi, CDK5 was shown to phosphorylate the Golgi matrix protein GM130 and to thereby cause fragmentation of this organelle in a model of AD (Sun et al., 2008). CDK5 is also activated in response to neuronal stress and was shown to result in a defect in axonal transport, which is essential for neuronal viability (Klinman and Holzbaur, 2015).

Therefore, the cumulative evidence suggests that stress-activated CDK5 is involved in inducing neurodegeneration as well as Golgi fragmentation. We therefore looked into the local network of CDK5 within this cluster to search for more potential connections. The local interactome of CDK5 includes several other cyclin-dependent kinases, which are well-known to regulate the cell cycle. This explains why cell proliferation was one of the processes that were enriched in Cluster-2. However, neurons are post-mitotic and the presence of CDK5 in the nucleus was shown to play a role in suppressing the cell cycle in neurons (Zhang et al., 2010). The nuclear import of CDK5 is dependent on p27 (labeled as CDKN2B in the network in **Figure 3**). Inhibition of nuclear localization of CDK5 and its concomitant accumulation in the cytosol is considered an event that triggers neuronal cell death. Treatment of neurons with beta-amyloid, a major pathogenic protein in AD, was shown to disrupt the CDK5-p27 interaction and to result in cytoplasmic accumulation of CDK5 (Zhang et al., 2010). Thus, our approach captured this connection between stress, neurodegeneration and Golgi fragmentation and is therefore an indication that our approach is valid to uncover further evidence for this tripartite connection.

The next question that we asked above is whether the stress-induced Golgi fragmentation is part of the pathologic process. In other words, is the Golgi that receives stress signaling, in turn sending signals that contribute to neurodegeneration? We therefore searched the literature for kinases with known Golgi localization that would be activated by CDK5. Intriguingly, we identified LMTK2 (also known as CPRK) as such a candidate. LMTK2 is kinase with two transmembrane domains that localizes to various intracellular membranes, including the Golgi apparatus and is known to be a target for CDK5 (Kesavapany et al., 2003). LMTK2 is known to bind to and inhibit the protein phosphatase PP1 (Wang and Brautigan, 2002; Manser et al., 2012). Inhibition of PP1 is long considered a contributing factor in the pathogenesis of AD (da Cruz e Silva et al., 2004; Liu et al., 2005). We hypothesize that this is a case where stress signals to the Golgi. This leads to structural and functional changes of this organelle that contribute the neurodegeneration (**Figure 3**). In the current case, stress activates CDK5, which phosphorylates the neurofilament protein tau, an event of relevance to AD (Stoothoff and Johnson, 2005). Concomitantly, stress signaling also targets the Golgi, leading to its fragmentation (Sun et al., 2008) and to activation of a protein at the Golgi (LMTK2) that itself contributes to neurodegeneration. Whether this hypothesis is true requires experimental testing. In addition, whether the fragmentation of the Golgi is itself a contributing factor also remains unclear.

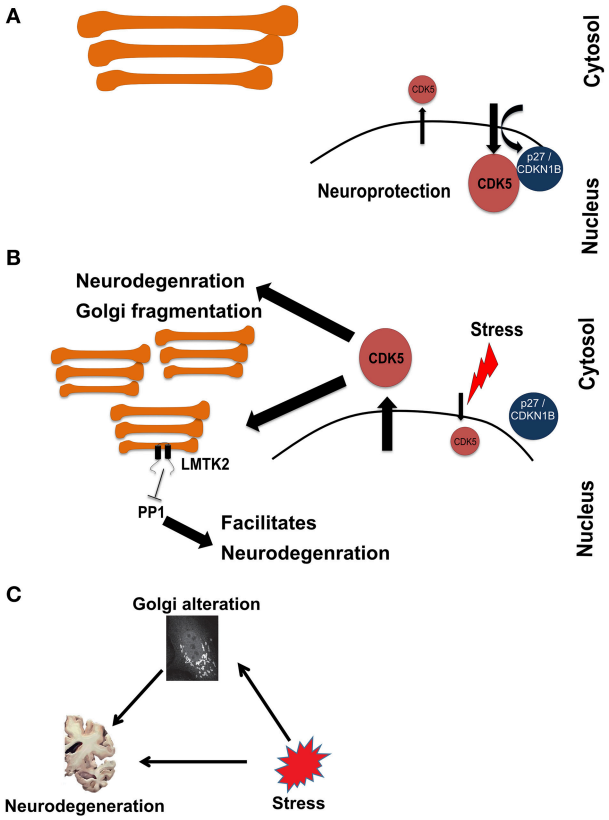

FIGURE 3 | Schematic representation of a hypothetical pathway in neurodegeneration involving stress signaling to and from the Golgi. (A) Under homeostatic conditions, CDK5 is mainly kept in the nucleus in a manner dependent on p27. **(B)** Upon induction of stress, CDK5 accumulates in the cytosol, which is an event that promotes neurodegeneration. At the same time CDK5 signals to fragment the Golgi and activate LMTK2 at this organelle. LMTK2 then in turn signals from the Golgi to inhibit PP1, an event that also promotes neurodegeneration. **(C)** Schematic of the hypothetical connections between stress, Golgi alteration, and neurodegeneration, drawn in analogy to the schematic in **Figure 1**.

APOE

Other processes of interest to neurodegeneration were axonogenesis and the response to axon injury. One of the proteins that plays a role in these processes is APOE, a stress-relevant protein that plays a role in axonogenesis. The human genome encodes for three alleles of ApoE: ApoE2, ApoE3, and ApoE4. The latter allele was linked to AD (Hyman et al., 1996). ApoE4 overexpression in mice results in axonal degeneration (Tesseur et al., 2000). Particles containing Apolipoprotein E (LpE) are produced by astrocytes in the brain and play a crucial role in lipid metabolism in this organ. Apoptosis in response to starvation-stress was prevented by LpE in a manner

dependent on a PLCG1-PKC signaling axis (Hayashi et al., 2009). Of note, we have recently identified PLCG1 as a regulator of secretory trafficking and of Golgi structure (Millarte et al., 2015). Therefore, it might be that ApoE-to-PLCG1 signaling is involved in the modulation of neuronal fitness and of stress response. Whether this is the case remains to be tested in the future.

Among the Golgi hits, APOE is connected to TYRO3 and to MAST1. TYRO3 is a transmembrane tyrosine kinase, with unknown relevance for stress signaling. However, TYRO3 overexpression was found to counteract the production of beta-amyloid production and its deletion in mice increases the number of amyloid plaques (Zheng et al., 2012). MAST1 is a microtubule-associated kinase, that is poorly investigated and so far no clear connection between MAST1 and neurodegeneration or stress exists.

Cluster-3: Small GTPases and PAK

In this cluster, we again obtained processes relevant to proliferation as well as mitogenic signaling (insulin signaling). The main proteins within these processes were components of classical growth factor cascades (HRas, receptor tyrosine kinases, etc…). The enrichment of these processes is probably best explained by the fact that these pathways are survival pathways and therefore act to counteract neuronal death. Another interesting biologic process "cell projection organization," which goes in line with the enrichment of axonogenesis in Cluster-2. However, the enrichment of this process here was mainly due to the presence of the cytoskeletal regulator Rac1, which belongs to the Rho family of small GTPases. We noted the presence of other members, regulators and effectors of Rho GTPase signaling were also found here (yellow nodes in **Figure 4**).

We searched for evidence for a role of Rho GTPase signaling in neurodegenerative diseases. The PAK kinases are activated downstream of the two Rho family GTPases Cdc42 and Rac1 (both present in Cluster-3). The two major neuronal isoforms are PAK1 and PAK3, which are both found in the subnetwork depicted in **Figure 3B**. Mutations of PAK3 were shown to causally underlie nonsyndromic X-linked mental retardation (Allen et al., 1998). PAKs also mediate the pro-survival effect of the Rho family GTPase Rac1 (Johnson and D'Mello, 2005; Loucks et al., 2006). Although the cognitive dysfunction in AD patients correlates well with the formation of amyloid plaques and neurofibrillary tangles, there is a subset of patients who have only little (or no) signs of such amyloid formation. In general the correlation between the histopathological signs of the disease and the clinical symptoms is far from being linear (Nelson et al., 2009). Therefore, it is likely that another factor contributes to the disease in addition to neuronal cell death. One such factor might be the alteration of neuronal function such as the formation of synapses. Based on our network analysis we suggest that stress signaling affects signaling by Rho GTPases and by PAKs leading to alterations of synaptogenesis, which might manifest itself as cognitive defects. In support of this is the observation that DOCK3, an activator for Rac1 exerts a cyto-protective effect on neuronal cells exposed to oxidative stress (Namekata et al., 2013, 2014). Furthermore, Huntingtin-induced neurotoxicity was modulated by Rac1 and PAK2, thus supporting our suggestion that the role of Rho GTPases and PAKs in neurodegeneration should be reexamined in the context of stress signaling.

ALS is a neurodegenerative disease thought to involve inflammation and oxidative stress. The ALS-causing mutant protein Cu(+)/Zn(+) superoxide dismutase SOD1-G93A directly enhances the activity of the main ROS-producing enzyme in microglia, NADPH oxidase 2 (NOX2; Bedard and Krause, 2007). A major activator of NOX2 is the Rho-GTPase family member Rac1 (Hordijk, 2006), implying that Rac1 might be involved in neurodegeneration. Rac1 was also shown to be involved in mediating the neurotoxic effect of 1–42 β-amyloid peptides (Manterola et al., 2013). A similar effect of these amyloidogenic peptides was observed toward Cdc42, an effect that was further shown to affect the actin cytoskeleton (Mendoza-Naranjo et al., 2012). Notably, the GTPase Cdc42 was not only shown to localize to the Golgi, but also to be regulated at this compartment (Baschieri et al., 2014). It is unclear whether stress signaling affects Cdc42 at the Golgi and if so, whether this is of any relevance to neurodegeneration. At the moment, our network inference can only hypothesize about the existence of such a connection and certainly future research on endomembrane signaling of Rho GTPases will reveal more insights.

Cluster-5: Wnt and CK?

From the BinGO analysis of this cluster, we noted the enrichment of Wnt signaling and transforming growth receptor beta (TGF-beta) signaling (**Figure 2B**). It was proposed previously that Wnt signaling might play a protective role in neurodegeneration (for a review see Arrázola et al., 2015). However, our aim was to search for evidence for the relevance of the connection between Golgi, stress, and neurodegeneration. The enriched proteins within Wnt signaling where two members of the casein kinase 1 (CK1) family (CSNK1G1 and CSNK1G2), which were hits that were found to regulate Golgi structure (Chia et al., 2012). CK1 signaling is in general considered to inhibit Wnt signaling. Together with the notion that Wnt signaling is neuroprotective, it might be that activation of CK1 by stress could facilitate neurodegeneration via inhibition of Wnt signaling. With respect to the Golgi, members of the CK1 family were shown to be important for maintaining Golgi integrity and for trafficking to the Golgi (Greer et al., 2014). Cells stressed by misfolded proteins were shown to exhibit CK1-dependent phosphorylation of parkin (Yamamoto et al., 2005), a protein mutated in Parkinson's disease. Whether alzerations of Golgi structure modulate CK1 activity, or whether the modulation of CK1 activity itself has an impact on the Golgi in neurodegeneration cannot be addressed at the moment. Nevertheless, our network inference should motivate toward research on the role of CK1 signaling and its modulation of endomembrane traffic in neurodegeneration.

TGF-beta1 is known to exert a neuroprotective effect and to counteract neurodegenerative disease such as AD (Caraci et al., 2012). However, what is the connection to the Golgi biology and stress signaling? It was shown that a brief ischemic stress of the brain results in a stronger colocalization of TGFbeta1 with Golgi membranes, indicating a defect in its secretory trafficking

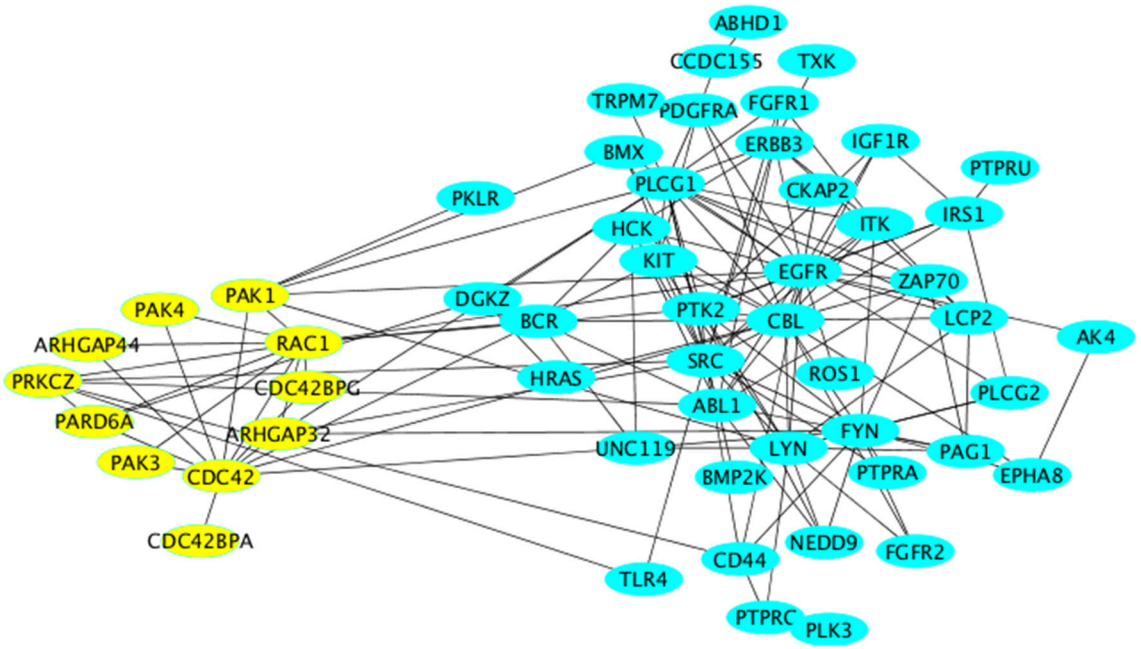

FIGURE 4 | Network representation of Cluster-3 using the "organic" style in Cytoscape. All nodes relevant to Rho GTPase signaling are labeled in yellow and are arranged using the "circular" style in Cytoscape.

out of the Golgi (Hu et al., 2007). Concomitantly, the study also reported aberrant Golgi morphologies. Therefore, hypoxic stress of in neurons might result in alterations in trafficking of TGF-beta and are therefore expected to result in a reduction in TGF-beta signaling and a loss of its neuroprotective effects. In this scenario, alterations of the Golgi are an active part of the disease process. Whether this mechanism is true, or whether it is relevant for other stresses remains to be elucidated in the future.

Cluster-7: The Androgen Receptor Signaling Pathway

In this cluster, we noted the enrichment of "androgen receptor signaling pathway," prompting us to ask whether androgens play a role in the connection between stress, neurodegeneration, and the Golgi. Androgens are known to play a role in the pathogenesis of different neurodegenerative disorders. Disruption of androgen signaling was suggested to play a role in the pathogenesis of ALS and spinal bulbar muscular atrophy (SBMA), because the expression of the androgen receptor was found to be downregulated in motoneurons of diseased mice (Sheean et al., 2015). Because women are at twice as high risk to develop AD (Viña and Lloret, 2010), it has been speculated whether androgens might not represent a protective factor against this disorder. The exact reason for this sex-difference is unclear. However, the androgen receptor is located on the X chromosome, and an interesting hypothesis would be to test whether the risk for Alzheimer's correlates with the X-inactivation patterns (Ferrari et al., 2013). The androgen receptor was a hit in the full-genome screen for regulators of secretion (Simpson et al., 2012).

Nevertheless, a link to the Golgi is not self-evident. We therefore inspected the network around the androgen receptor in **Figure 2A** and noted the tyrosine kinase Src among the interaction partners. Members of the Src family were not only shown to reside at the Golgi, but their activation was also shown to result in Golgi fragmentation (Weller et al., 2010). Therefore, stress-induced Src signaling might result in Golgi fragmentation in neurodegenerative disorders. However, what is the biologic consequence of this Src-induced Golgi fragmentation? With respect to trafficking, Src signaling was recently suggested to activate the small GTPase Cdc42 leading to a facilitation of anterograde transport within the Golgi (Park et al., 2015). This is in line with a study that showed that Golgi fragmentation in neurons exposed to beta-amyloid facilitates secretion of amyloid precursors and thereby increases the deposition of toxic protein species outside cells (Joshi et al., 2014). Altogether, we postulate the following hypothesis: the lower levels of androgens in women and the decline of androgen levels in elder men results in reduced signaling by androgen receptor. Whether and how this modulates Src signaling at the Golgi, in particular in the presence of cellular stress requires experimental testing. We suggest that Src signaling is increased, resulting in Golgi fragmentation, which in turn facilitates the progression of the disease.

Cluster-8: NF-kB

Tis cluster revealed the enrichment of NF-kB signaling as well as the positive regulation of interleukin-2 (IL2) production. Although interesting, the enrichment of these pathways is not surprising. These are two well-established pathways downstream of inflammatory stimuli and the notion that neuroinflammation

contributes to neurodegenerative disease is well documented (see for instance the very recent review by Steardo et al., 2015). Therefore, we will not discuss this in any further detail.

CONCLUDING REMARK

Our computational inference of a network between stress regulators and RNAi screening hits identified as putative Golgi regulators has revealed a clear enrichment of processes relevant for neurodegeneration. We emphasize, that our work is theoretical in nature and cannot replace the validation using "wet lab" experiments. Further, we stress that we are well aware of the limitation that are imposed by using this list of Golgi regulators, because they were identified in cells that were cultured under optimal growth conditions and therefore we might be missing some potential stress-specific regulators. In addition, the screens were performed in HeLa cells and not in neurons. The Golgi in neurons is different from epithelial cells as it was shown to form so called Golgi outposts far away from the somatic Golgi (Ehlers, 2007). Nevertheless, the fundamental organization of the Golgi is similar as every Golgi is organized as a stack of flattened cisternae and so far the Golgi is always involved in protein sorting and post-translational modification. Therefore, insights obtained from screens in HeLa cells are of potential relevance for the functional organization in neurons. In addition, the stress pathways operating in neurons and other cell types are essentially the same. Therefore, despite these limitations we think that our work will provide a starting point for future investigation on the role tripartite connection between the Golgi, stress signaling and neurodegeneration.

Our analysis is supportive of a tripartite link between stress, Golgi alteration, and neurodegeneration. In some instances we found hypothetical evidence for a Golgi not only being a receiver of stress signals, but also that stress signaling to the Golgi triggers another signaling event that might deteriorate neurodegeneration (see scenario in **Figure 3**). Another is the de-regulated signaling by Rho family GTPases at the Golgi and the potential consequences for the pathogenesis of neurodegeneration (see Cluster-7 and Cluster-3). These, and other predictions we made should require experimental testing by the research community.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fnins.2015.00435

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The GOLPH3 pathway regulates Golgi shape and function and is activated by DNA damage

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The Golgi protein GOLPH3 binds to PtdIns(4)P and MYO18A, linking the Golgi to the actin cytoskeleton. The GOLPH3 pathway is essential for vesicular trafficking from the Golgi to the plasma membrane. A side effect of GOLPH3-dependent trafficking is to generate the extended ribbon shape of the Golgi. Perturbation of the pathway results in changes to both Golgi morphology and secretion, with functional consequences for the cell. The cellular response to DNA damage provides an example of GOLPH3-mediated regulation of the Golgi. Upon DNA damage, DNA-PK phosphorylation of GOLPH3 increases binding to MYO18A, activating the GOLPH3 pathway, which consequently results in Golgi fragmentation, reduced trafficking, and enhanced cell survival. The PtdIns(4)P/GOLPH3/MYO18A/F-actin pathway provides new insight into the relationship between Golgi morphology and function, and their regulation.

Keywords: Golgi, phosphatidylinositol-4-phosphate, GOLPH3, MYO18A, secretory trafficking, DNA damage, cancer, neurodegenerative disease

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Introduction

In many mammalian cell types the Golgi complex appears by light microscopy as an extended ribbon that wraps partially around the nucleus. The ribbon is composed of many subresolution stacks of membrane (as observed by electron microscopy) that are linked via Golgi-associated proteins. From first principles we can conclude that the steady-state appearance of the Golgi reflects the balance of forces applied to it. Changes in the shape of the Golgi presumably reflect alterations in the balance of forces applied to the Golgi. Since at least some of the forces that are applied to the Golgi are likely to be important for its function in vesicle trafficking, changes in trafficking machinery might be expected to lead to changes in Golgi morphology. However, it is important to bear in mind that the morphology of the Golgi varies significantly across species (reviewed in Mowbrey and Dacks, 2009; Wei and Seemann, 2010), suggesting that diverse morphologies can still be fully competent for trafficking.

The GOLPH3 pathway provides a link from the trans-Golgi membrane to the actin cytoskeleton that plays a critical role in anterograde trafficking to the plasma membrane (**Figure 1**). The trans-Golgi is highly enriched in phosphatidylinositol-4-phosphate (PtdIns(4)P) (Godi et al., 1999, 2004). In mammalian cells Golgi PtdIns(4)P is produced by the Golgi localized PI-4-kinases, PI-4-kinase-IIIβ (PI4KIIIβ) and PI-4-kinase-IIα (PI4KIIα) (Wong et al., 1997; Wang et al., 2003; De Matteis et al., 2005). PtdIns(4)P levels are reduced by the action of the Golgi/ER localized PtdIns(4)P-4-phosphatase, SAC1 (Foti et al., 2001; Schorr et al., 2001). GOLPH3 localizes to the trans-Golgi via its direct interaction with PtdIns(4)P (Dippold et al., 2009). This interaction is mediated by a binding pocket on the hydrophobic face of GOLPH3. Furthermore, GOLPH3's interaction with PtdIns(4)P

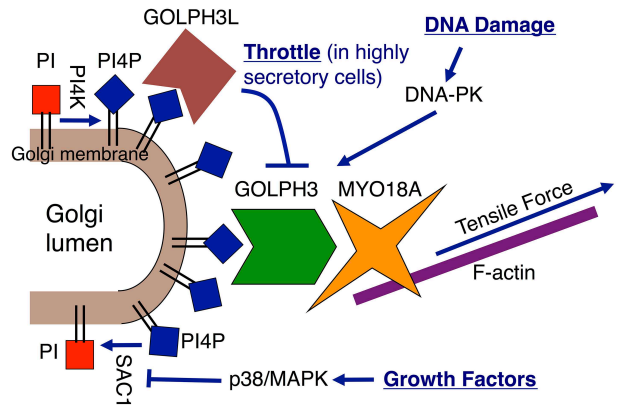

FIGURE 1 | Regulation of the Golgi via the GOLPH3 pathway. The GOLPH3 pathway links the Golgi to the actin cytoskeleton, which applies a tensile force to the Golgi that is essential for anterograde trafficking. The GOLPH3 pathway is subject to regulation by different mechanisms. Growth factor signaling increases Golgi PtdIns(4)P levels through translocation of SAC1 away from the Golgi to the ER (Blagoveshchenskaya et al., 2008). GOLPH3L is a GOLPH3 paralog that acts as a dominant negative inhibitor of the GOLPH3 pathway due to its ability to bind to PtdIns(4)P, while being unable to bind to MYO18A (Ng et al., 2013). GOLPH3L acts as a throttle to Golgi-to-plasma membrane trafficking in highly secretory cells. Upon DNA damage, DNA-PK activates the pathway by phosphorylation of GOLPH3 to enhance its interaction with MYO18A (Farber-Katz et al., 2014).

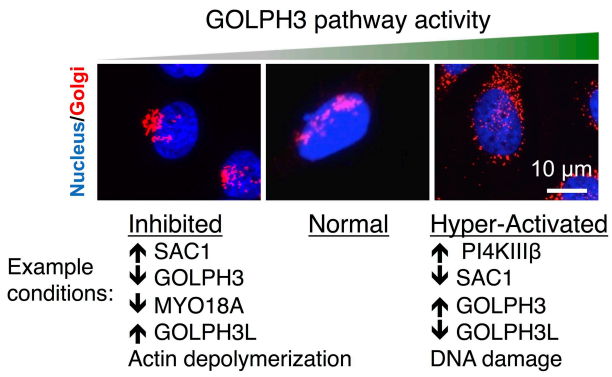

FIGURE 2 | Perturbations of the GOLPH3 pathway alter Golgi morphology. Examples of the Golgi (in red, co-stained with the nucleus in blue) are shown under normal conditions and in response to perturbations to interfere with or to increase the activity of the GOLPH3 pathway. Normally the Golgi appears as an extended ribbon partially wrapped around the nucleus (middle). Inhibition of the GOLPH3 pathway, by overexpression of SAC1 (Dippold et al., 2009), siRNA knockdown of GOLPH3 or MYO18A (Dippold et al., 2009; Ng et al., 2013; Farber-Katz et al., 2014), overexpression of GOLPH3L (Ng et al., 2013), or depolymerization of actin (Dippold et al., 2009), results in compaction of the Golgi (left). Activation of the GOLPH3 pathway, by overexpression of PI4KIIIβ (Hausser et al., 2005) or GOLPH3 (Ng et al., 2013), siRNA knockdown of SAC1 (Liu et al., 2008) or GOLPH3L (Ng et al., 2013), or in response to DNA damage (Farber-Katz et al., 2014), results in extension and dispersal of the Golgi (right).

and its Golgi localization are conserved from yeast to humans (Dippold et al., 2009; Wood et al., 2009). GOLPH3 also binds tightly and specifically to the unconventional myosin, Myosin 18A (MYO18A) (Dippold et al., 2009; Ng et al., 2013; Taft et al., 2013; Farber-Katz et al., 2014), and MYO18A has been shown to bind to F-actin (Isogawa et al., 2005; Guzik-Lendrum et al., 2013; Taft et al., 2013). Thus, GOLPH3/MYO18A serves to link the PtdIns(4)P-rich trans-Golgi membrane to the actin cytoskeleton.

Perturbations of the GOLPH3 Pathway Alter Trafficking and Golgi Morphology

All of the components of the pathway, PtdIns(4)P, GOLPH3, MYO18A, and F-actin, are required for efficient Golgi-to-plasma membrane trafficking. PtdIns(4)P has been shown to be required for Golgi secretory function across species from yeast to humans (Hama et al., 1999; Walch-Solimena and Novick, 1999; Audhya et al., 2000; Wang et al., 2003). Likewise, GOLPH3 and MYO18A are required for Golgi-to-plasma membrane trafficking as measured by VSVG delivery to the plasma membrane (Dippold et al., 2009), total secretory flux by pulse-chase analysis (Ng et al., 2013), secretion of hepatitis C viral particles (Bishé et al., 2012), and for exit of anterograde trafficking vesicles from the Golgi (Dippold et al., 2009). Finally, F-actin has been demonstrated to be required for Golgi secretory function (Hirschberg et al., 1998; Lázaro-Diéguez et al., 2007). Taken together, the data indicate that the majority of trafficking from the Golgi to the plasma membrane depends on the PtdIns(4)P/GOLPH3/MYO18A/F-actin pathway.

Depletion of PtdIns(4)P, GOLPH3, MYO18A, or F-actin results in both a defect in Golgi-to-plasma membrane trafficking and also a striking change in the morphology of the Golgi (**Figure 2**). The normal Golgi ribbon that extends partially around the nucleus condenses into a compact ball at one end of the nucleus upon interference with any component of the GOLPH3 pathway. For example, depletion of PtdIns(4)P by overexpression of SAC1, siRNA knockdown of GOLPH3 or MYO18A, or depolymerization of F-actin by latrunculin B, all cause Golgi compaction (Dippold et al., 2009). From this and other data (see Dippold et al., 2009) we infer that normally the GOLPH3 pathway exerts a tensile force upon the Golgi that contributes to stretching the Golgi around the nucleus. However, the fact that the GOLPH3 pathway is conserved across species, even those with dramatically different Golgi morphologies, suggests that imparting this shape is not the primary role of the pathway, but rather is a side-effect of its role in GOLPH3-dependent Golgi-to-plasma membrane trafficking.

While interfering with the GOLPH3 pathway results in Golgi compaction, increasing the activity of the GOLPH3 pathway causes expansion of the Golgi (**Figure 2**). For example, mild overexpression of GOLPH3 leads to an increasingly extended Golgi ribbon. However, very high levels of overexpression of GOLPH3 result in fragmentation and dispersal of the Golgi throughout the cytoplasm (Ng et al., 2013). Likewise, an increase in PtdIns(4)P at the Golgi by knockdown of SAC1 (Liu et al., 2008) or overexpression of PI4KIIIβ (Hausser et al., 2005) also result in increasing Golgi extension and even dispersal of the Golgi. Moderate increases in GOLPH3 pathway activity lead to Golgi extension and mild increases in anterograde trafficking.

However, large increases in GOLPH3 pathway activity result in dramatic Golgi fragmentation and impaired trafficking (Ng et al., 2013; Farber-Katz et al., 2014).

Regulation of Golgi Morphology and Function via the GOLPH3 Pathway

A growing body of evidence demonstrates regulation of Golgi morphology and function via the GOLPH3 pathway (**Figure 1**). For example, growth factor signaling regulates PtdIns(4)P levels at the Golgi by regulating SAC1 localization. In response to growth factor signaling, activated p38/MAPK phosphorylates SAC1, causing it to translocate from the Golgi to the ER (Blagoveshchenskaya et al., 2008). The resulting reduction of PtdIns(4)P-4-phosphatase activity at the Golgi leads to a rise in Golgi PtdIns(4)P levels, with a consequent increase in Golgi-to-plasma membrane trafficking and extension and dispersal of the Golgi.

The Golgi is also regulated by GOLPH3L, a paralog of GOLPH3 that is expressed in highly secretory cells in vertebrates (Ng et al., 2013). GOLPH3L binds to PtdIns(4)P and localizes to the Golgi similarly to GOLPH3. However, unlike GOLPH3, GOLPH3L does not bind to MYO18A, and thus acts as an endogenous dominant-negative inhibitor of the GOLPH3 pathway. Our evidence suggests that GOLPH3L acts as a throttle in highly secretory cells to prevent overly aggressive anterograde trafficking. Correspondingly, overexpression of GOLPH3L impairs GOLPH3 pathway function, resulting in Golgi compaction and impaired Golgi-to-plasma membrane trafficking. Knockdown of GOLPH3L results in dramatic Golgi dispersal and impaired Golgi-to-plasma membrane trafficking, similar to the effect of dramatic overexpression of GOLPH3.

Regulation of the Golgi by DNA Damage via the GOLPH3 Pathway

A recent study by our lab identified novel signaling that regulates the Golgi in response to DNA damage (Farber-Katz et al., 2014). We found that DNA damage triggers dramatic fragmentation and dispersal of the Golgi. The mechanism involves activation of the GOLPH3 pathway. In response to DNA damage, the DNA-damage response kinase, DNA-PK, phosphorylates GOLPH3 on a conserved TQ motif. The phosphorylation of GOLPH3, in turn, enhances its physical interaction with MYO18A, increasing GOLPH3 pathway-dependent vesiculation of the Golgi. As a consequence of this increased GOLPH3/MYO18A interaction, the Golgi undergoes dramatic fragmentation and dispersal, resulting in impaired Golgi-to-plasma membrane trafficking. Thus, DNA damage regulates Golgi shape and function by activating the GOLPH3 pathway.

DNA damage has been posited to contribute to the pathophysiology of many human diseases, including cancer (reviewed in Lord and Ashworth, 2012) and neurodegenerative diseases (reviewed in Madabhushi et al., 2014). Whether DNA damage-induced regulation of the Golgi contributes to the pathophysiology of these diseases remains unknown.

Relationship between Golgi Morphology and Secretory Function

While perturbations of the GOLPH3 pathway result in predictable and consistent changes in both Golgi shape and secretory function, it does not follow that there is a simple relationship between morphology and function. In fact, perturbations of pathways other than the GOLPH3 pathway have been demonstrated to alter Golgi morphology without affecting secretory trafficking. For example, depolymerization of microtubules by treatment with nocodazole leads to Golgi fragmentation and dispersal (Thyberg and Moskalewski, 1999), but does not alter secretory trafficking (Hirschberg et al., 1998). Similarly, depletion of Rab29, or inhibition of Rab29 function by overexpression of the inactive mutant Rab29-T21N results in fragmentation of the Golgi (Wang et al., 2014). However, despite the dramatic change in Golgi morphology, no change is observed in trafficking of VSVG to the plasma membrane.

Thus, we conclude that there is not a simple relationship between Golgi morphology and secretory function that holds under all circumstances. Instead, the effect of a perturbation on the Golgi is dictated by the specific mechanism of the perturbation. However, for perturbations of the GOLPH3 pathway the consequences to Golgi morphology and secretory function are predictable.

Functional Consequences of Altered Golgi

Since changes in Golgi morphology do not directly relate to its trafficking function, it remains unclear whether changes in Golgi morphology always have cellular consequences. However, it seems safe to predict that perturbations of the Golgi that affect Golgi-to-plasma membrane trafficking will have consequences to the cell. The phenotypes associated with perturbation of the GOLPH3 pathway provide strong examples of the importance of Golgi-to-plasma membrane trafficking to overall cell and organismal biology. Notably, GOLPH3 is an oncogene that is frequently amplified and overexpressed in human cancers, and its increased expression predicts poor patient prognosis (Scott et al., 2009 and reviewed in Buschman et al., 2015). Furthermore, other components of the GOLPH3 pathway have been implicated in oncogenesis as well. Increased expression of PI4KIIα protein and PI4KIIIβ mRNA have been detected in cancer cells (Li et al., 2010; Curtis et al., 2012). A recent unbiased computational analysis of data from The Cancer Genome Atlas for breast cancer has identified MYO18A as a cancer driver (Sanchez-Garcia et al., 2014).

PtdIns(4)P and GOLPH3 have been demonstrated to play important roles in regulating cell motility. Knockdown of SAC1, resulting in elevated Golgi PtdIns(4)P levels, leads to increased cell migration (Tokuda et al., 2014). Conversely, knockdown of PI4KIIIβ, resulting in a reduction of Golgi PtdIns(4)P levels, impairs cell migration. These consequences of

modulation of PtdIns(4)P levels are mediated by its downstream effector GOLPH3. Likewise, perturbation of GOLPH3 itself results in similar effects on cell migration. Increased GOLPH3 promotes cell migration and invasion, while depletion of GOLPH3 impairs cell migration (Isaji et al., 2014; Tokuda et al., 2014).

Our recent study on GOLPH3-mediated Golgi dispersal/fragmentation upon DNA damage has revealed an interesting role of the pathway in promoting cell survival (Farber-Katz et al., 2014). We have demonstrated that the GOLPH3-dependent Golgi response to DNA damage is functionally important for cell survival after DNA damage. Depletion of GOLPH3, MYO18A, or DNA-PKcs not only prevents dispersal of the Golgi, but also significantly increases apoptosis and reduces cell survival after treatment with DNA-damaging agents. Conversely, overexpression of GOLPH3 confers a survival advantage to cells after DNA damage. Importantly, this survival advantage caused by GOLPH3 overexpression requires both GOLPH3 localization to the Golgi and its phosphorylation by DNA-PK. Altered Golgi-to-plasma membrane trafficking that occurs upon Golgi fragmentation after DNA damage is believed to be important for the enhanced survival benefit provided by this pathway, although the critical cargoes have not been identified as of yet. The DNA damage response is a well-known driver of cancer progression (reviewed in Lord and Ashworth, 2012). In addition, the resilience against DNA damage conferred by overexpression of GOLPH3 has particular relevance to cancer, as most standard cancer therapeutic regimens include DNA-damaging agents. Therefore, our findings raise hope of using GOLPH3 expression levels as a clinical marker to predict responsiveness to DNA-damaging cancer therapies. Furthermore, novel agents to interfere with the GOLPH3 pathway may have therapeutic benefit when used in combination with standard DNA-damaging therapeutic agents.

Potential Role of the GOLPH3 Pathway in Neurodegenerative Disease

Whether the GOLPH3 pathway plays a role in the pathophysiology of neurodegenerative disease has not been studied. However, some of the known pathophysiology of neurodegenerative disease makes it reasonable to speculate that the GOLPH3 pathway may be involved. First, dysregulation of the Golgi is a common feature of a variety of human neurodegenerative diseases, including amyotrophic lateral sclerosis, Parkinson's disease, and Alzheimer's disease. The accompanying articles in this issue of Frontiers in Neuroscience provide detailed reviews of the literature implicating Golgi dysregulation in neurodegenerative disease. We note that the changes in Golgi morphology that are associated with these diseases mimic those seen with perturbations of the GOLPH3 pathway. Moreover, knockout of a Golgi-specific PI-4-kinase, PI4KIIα, expected to interfere with GOLPH3 pathway function, leads to late onset neurodegenerative disease in mice (Simons et al., 2009). These knockout mice develop cerebellar Purkinje cell loss and spinal cord axonal degeneration, which closely resembles hereditary spastic paraplegia. Thus, there is at least reason to speculate a role for the GOLPH3 pathway in neurodegenerative disease.

A second link to the GOLPH3 pathway arises from a substantial body of literature implicating the DNA damage response in neurodegenerative disease. Loss-of-function mutations in various DNA damage repair genes cause a range of neurodegenerative diseases (Madabhushi et al., 2014). For example, the DNA damage activated protein kinases ATM and ATR (paralogs of DNA-PK) are mutated in ataxia-telangectasia and ATR-Seckel syndrome, respectively (McKinnon, 2012). Recent data indicate that DNA breaks occur frequently in neurons (Madabhushi et al., 2015), bolstering the concern that dysregulated DNA damage repair may contribute to disease in neurons.

Our discovery that the Golgi is regulated in response to DNA damage via DNA-PK activation of the GOLPH3 pathway provides a plausible link between the evidence implicating DNA damage and altered Golgi function in neurodegenerative disease.

Concluding Remarks

The appearance of the Golgi, at least in part, is a consequence of the forces that act on it for vesicle trafficking. The GOLPH3 pathway, in particular, demonstrates the relationship between Golgi secretory function and Golgi morphology. As might be expected for a pathway with an important role in Golgi function, the GOLPH3 pathway is a target for regulation by multiple signals. For example, the GOLPH3 pathway is activated in response to DNA damage, with consequences for Golgi shape and trafficking function, as well as cell survival. Since many proteins depend on trafficking from the Golgi for their function, alterations in GOLPH3-dependent Golgi-to-plasma membrane trafficking have pleiotropic effects on cellular function, significantly impacting biology and human pathophysiology.

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The Golgi complex in stress and death

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The Golgi complex is a central organelle of the secretory pathway where sorting and processing of cargo occurs. While Golgi structure is important for the efficient processing of secretory cargo, the unusual organization suggests additional potential functions. The Golgi is disassembled after various cellular stresses, and we hypothesize that Golgi disassembly activates a stress signaling pathway. This pathway would function to correct the stress if possible, with irreparable stress resulting in apoptosis. Neurons appear to be particularly sensitive to Golgi stress; early disassembly of the organelle correlates with many neurodegenerative diseases. Here, Golgi stress and potential signaling pathways to the nucleus are reviewed.

Keywords: Golgi complex, disassembly, stress, apoptosis, signaling

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GOLGI STRUCTURE AND FUNCTION

The Golgi complex plays a central role in processing and sorting of biosynthetic cargo in all eukaryotic cells. In mammals, the Golgi complex consists of sets of flattened cisternal membranes arranged in stacks with associated tubules and vesicles, which are usually collected at the microtubule organizing center (MTOC) in a ribbon structure (Klumperman, 2011). This structure is not essential for the known functions of the Golgi, and may suggest additional functions. Golgi structure is also quite dynamic; the organelle is disassembled at mitosis and then reassembled (Wang and Seemann, 2011). The organelle can also accommodate cargo of different shapes and sizes (Machamer, 2013). We previously hypothesized that mammalian Golgi organization may have evolved in part to sense and transduce specific stress signals to the nucleus (Hicks and Machamer, 2005).

Golgi structure in mammalian cells is maintained by the cytoskeleton, and GRASPs and golgins (**Figure 1A**). GRASP65 and GRASP55 form homo- or hetero-oligomers and mediate stacking and can contribute to the Golgi ribbon structure (Ramirez and Lowe, 2009; Xiang and Wang, 2010). The golgin family comprises a group of peripheral Golgi membrane proteins with long coiled coil domains. Some golgins are vesicle tethers, some function in Golgi stack structure, and others may be involved in trafficking of specific cargo molecules (Munro, 2011). Disassembly of the Golgi in mitosis or apoptosis results from reversible phosphorylation of GRASPs and golgins or irreversible cleavage, respectively.

GOLGI DISASSEMBLY AND STRESS

Golgi fragmentation is commonly observed in cells subjected to "stress," including pharmacological and oxidative stress. Fragmentation can be the result of perturbation of microtubules, or phosphorylation or cleavage of Golgi structural proteins. Golgi stacks can be dispersed (mini-stacks) or completely disassembled, depending on the perturbation

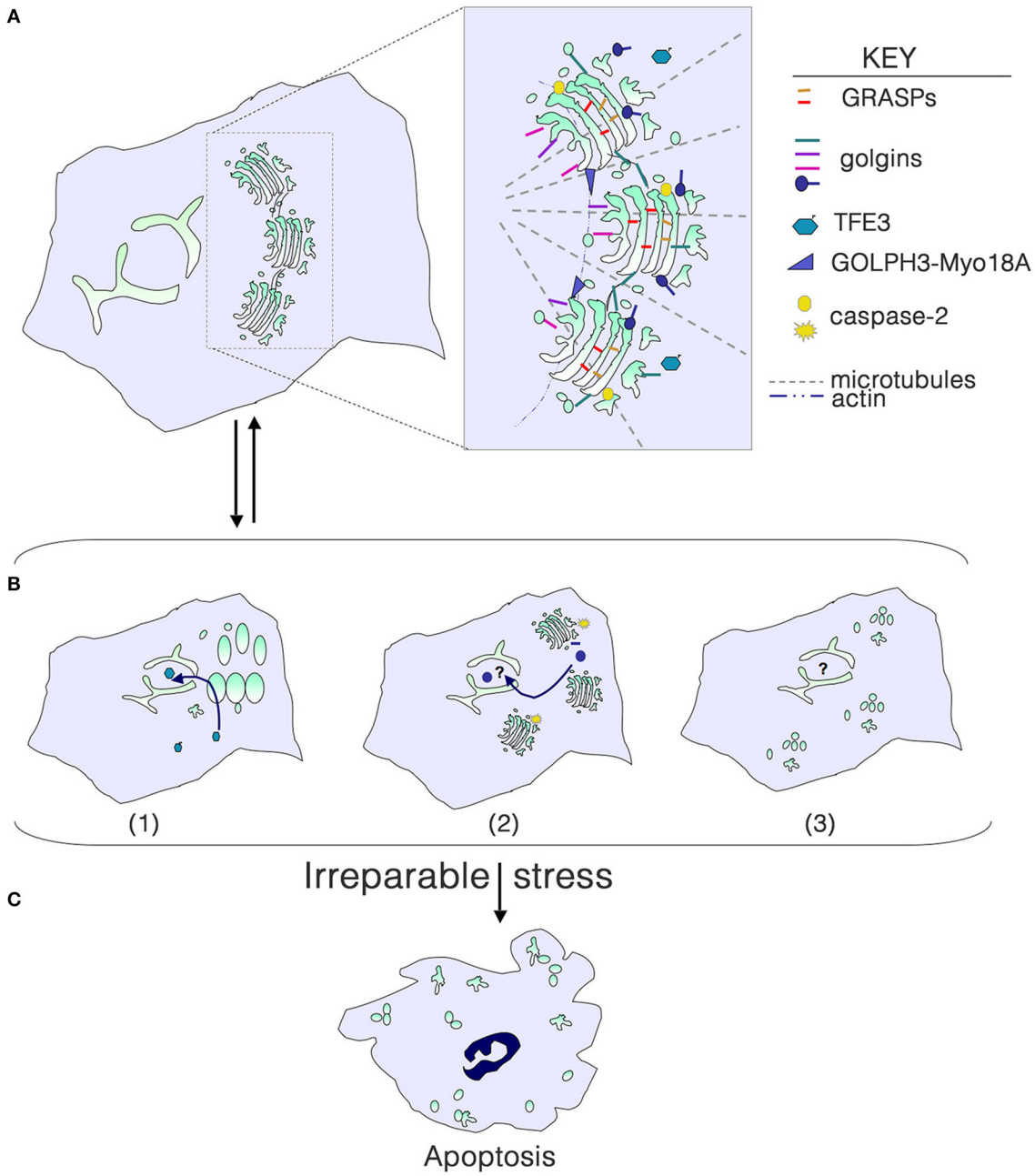

FIGURE 1 | Golgi structure in life, stress and death. (A) Golgi morphology in a typical mammalian cell, with the key structural players shown in the inset. For simplicity, individual golgins and GRASPs are not indicated. **(B)** Golgi stress due to cargo load or size, ionic imbalance, infection with intracellular pathogens, or perturbation of glycosylation or the cytoskeleton results in structural alterations that can signal to the nucleus to help repair the stress. (1) Dephosphorylation of TFE3 and tranlocation to the nucleus results in transcription of genes with a GASE, including some glycosyltransferases and trafficking components. (2) Activation of local caspase-2 cleaves select golgins, and fragments enter the nucleus to perform an unknown function. (3) Phosphorylation of GRASPs and golgins or their cleavage can result in a more complete disassembly of the Golgi, although the consequences for signaling to the nucleus are unknown. **(C)** With irreparable stress, the Golgi is completely disassembled as the cell undergoes apoptosis.

(**Figure 1B**). Although the term "Golgi stress" has been frequently used in the literature (e.g., Jiang et al., 2011; Oku et al., 2011; Reiling et al., 2013), there is no clear understanding of what Golgi stress entails. Can Golgi stress be activated in the absence of endoplasmic reticulum (ER) stress? Similar to the well-documented unfolded protein response in the ER (Walter and Ron, 2011), a Golgi stress response pathway should serve to help alleviate the stress, and only result in cell death if the stress is irreparable (**Figure 1C**). Pharmacological inhibitors of glycosyltransferases, glycosidases, proton and calcium pumps,

and perturbation of luminal pH have all been shown to alter the structure of the Golgi complex. High levels of cargo or large cargo passing through the Golgi may be the most physiological type of stress. But do any of these insults result in outcomes that would help eliminate the stress?

One of the most extensively studied types of cellular stress is pro-apoptotic stress. In apoptosis, extrinsic, or intrinsic pathways lead to programmed disassembly of the cell. Cysteine proteases called caspases are activated and cleave a select set of cellular proteins during programmed cell death. Different types of stress activate specific initiator caspases, which then activate effector caspases (Boatright and Salvesen, 2003). Not all caspases are involved in cell death however.

We previously reported that procaspase-2 is partially localized at the cytoplasmic face of the Golgi complex (Mancini et al., 2000), and golgin-160 and several other golgins are caspase-2 substrates (Mancini et al., 2000; Lowe et al., 2004). Caspase cleavage of golgin-160 is predicted to inhibit its function in promoting efficient trafficking of specific cargo molecules (Bundis et al., 2006; Hicks et al., 2006; Williams et al., 2006). Caspase-2 is an unusual caspase in that it possesses a long prodomain like inititator caspases, but does not activate effector caspases (Fava et al., 2012). Recent evidence suggests non-apoptotic roles for caspase-2 in maintaining genome stability, checkpoint regulation in the cell cycle, response to oxidative stress, tumor suppression and in aging (Olsson et al., 2015).

Another stress that acts at the Golgi complex is inhibition of O-glycosylation. It was shown that treatment of fibroblasts with benzyl 2-acetamido-2-deoxy-a-d-galactopyranoside (GalNAc-bn) to inhibit this post-translational modification induced upregulation of HSP47, an ER chaperone (Miyata et al., 2013). HSP47 apparently protects cells from Golgi fragmentation and death when O-glycosylation is blocked because knock-down of HSP47 in GalNAc-bn treated cells led to Golgi vacuolization and eventual apoptosis. Interestingly, caspase-2 appears to be activated here as well. The mechanism by which HSP47 leads to protection is unknown, but it has been reported to be a collagen-specific chaperone (Mala and Rose, 2010). HSP47 may regulate the level of this abundant secretory protein that enters the Golgi, whereas in its absence collagen might accumulate in the Golgi when it cannot be O-glycosylated, resulting in Golgi structural perturbations and eventual apoptosis.

GOLGI-NUCLEUS SIGNALING PATHWAYS

Signals from the nucleus can lead to phosphorylation of Golgi proteins and subsequent Golgi disassembly. For example, GOLPH3 is an oncogene that is upregulated in a number of cancers. It is a peripheral Golgi protein that binds phosphoinositol-4-phosphate at the *trans*-Golgi as well as the myosin Myo18A (Dippold et al., 2009). Normal Golgi ribbon structure is maintained in part by interaction of the Myo18A-GOLPH3 complex with actin, which keeps the ribbon extended. DNA damage results in Golgi fragmentation, which was shown to occur through phosphorylation of GOLPH3 by DNA-dependent protein kinase (DNA-PK). Phosphorylated GOLPH3 interacts more strongly with Myo18A and thus leads to Golgi dispersal (Farber-Katz et al., 2014). Although this dispersal leads to a reduction in cargo traffic through the Golgi, it is not yet clear how this connects to DNA damage or cell survival. Since DNA-PK can activate caspase-2 (Shi et al., 2009), it will be important to determine if caspase-2 cleavage of Golgi proteins contributes to this response.

But can signals from the Golgi lead to changes in gene expression in the nucleus? Membrane-anchored transcription factors can be released from Golgi membranes by site 1 and 2 proteases (Fox and Andrew, 2015), allowing nuclear translocation and transcription of specific genes. However, these transcription factors are trafficked to the Golgi by specific events signaled in the ER. Several golgins contain cryptic nuclear localization signals, and fragments of these proteins (generated by caspases or other proteases) can be targeted to the nucleus. But to date, none of these fragments has been shown to induce gene expression that might alter Golgi function. A caspase cleavage fragment of p115, a vesicle tethering protein localized at the *cis*-Golgi, is targeted to the nucleus and promotes cell death in a p53-dependent pathway (Chiu et al., 2002; How and Shields, 2011). However, cleavage of p115 is not observed in all pro-apoptotic settings (Lowe et al., 2004).

A cryptic nuclear localization signal is present in a fragment of golgin-160 generated by caspase-2 cleavage, and this fragment accumulates in the nucleus when expressed exogenously (Hicks and Machamer, 2002). We hypothesized that Golgi stress leads to low levels of caspase-2 activation, and that the nuclear fragments of golgin-160 participate in a stress repair pathway (Hicks and Machamer, 2005). The nuclear fragments do not resemble transcription factors *per se*, so may serve as transcriptional enhancers or repressors. Interestingly, cells expressing caspase-resistant golgin-160 were less sensitive to apoptosis induced by ER stress and death receptor ligation compared to cells expressing wild-type golgin-160. However, these cells responded normally to other pro-apoptotic stresses (Maag et al., 2005). Our recent data support the idea that the stable lines expressing caspase-resistant golgin-160 adapted during selection because a block in golgin-160 cleavage prevented normal response to stress. Although it is interesting that only stress within the secretory pathway depends on golgin-160 cleavage, we still lack direct evidence that golgin-160 is cleaved during Golgi stress and mediates subsequent changes in gene expression.

Extended treatment with the ionophore monensin was shown to increase the levels of certain Golgi glycosylation enzymes and vesicle transport components (Oku et al., 2011). Monensin collapses sodium and proton gradients, and thus raises the pH and induces swelling of low pH compartments, including the Golgi complex. The authors identified a "Golgi apparatus stress element" (GASE) in the promoters of several Golgi resident proteins that was required for their upregulation. Further work identified TFE3, a basic helix-loop-helix transcription factor, which binds to the GASE (Taniguchi et al., 2015). They showed that under normal growth conditions, TFE3 was phosphorylated and remained cytoplasmic, but after monensin treatment, TFE3 was dephosphorylated and it was transported to the nucleus. The signaling pathway resulting in dephosphoylation of TFE3 remains to be determined. Nuclear

translocation of TFE3 and transcription of GASE-containing genes was also activated when proteoglycan synthesis was inhibited or the CMP-sialic acid transporter was depleted (Taniguchi et al., 2015), suggesting that TFE3 regulation of Golgi homeostasis involves glycosylation. It will be interesting to see if this pathway is activated by increased flux of cargo during differentiation of secretory cells or trafficking of large cargo, or if different regions of the Golgi activate different stress response pathways, as recently proposed (Sasaki and Yoshida, 2015).

Another signaling pathway recently identified that may impact Golgi stress due to pathogen infection involves ADP-ribosylation factor (ARF)-4 and the transcription factor CREB3 (Reiling et al., 2013). Originally identified through a screen for brefeldin A-resistance, ARF4 levels increase when cells are exposed to Golgi disrupting treatments, and this requires the CREB3 transcription factor. Cells depleted of ARF4 or CREB3 contain increased levels of ARF1, 3, and 5, and are more resistant to infection with Chlamydia and Shigella. These results suggest that ARF4 may be induced by infection with these pathogenic bacteria to promote Golgi structural rearrangements required for efficient growth. How Golgi perturbation activates CREB3 is unknown. An earlier finding of caspase-2 involvement in infection with pathogenic bacteria is also intriguing in this light (Jesenberger et al., 2000).

GOLGI DISRUPTION IN NEURODEGENERATIVE DISEASES

Golgi fragmentation has been observed for decades in neurons from patients and in animal models of neurodegenerative diseases, including amyotrophic lateral sclerosis, Alzheimer's disease, Creutzfeld-Jacob disease and spinocerebellar ataxia type 2 (Gonatas et al., 2006). But does the fragmentation induce neuron dysfunction or is it simply a downsteam effect? Most evidence suggests that Golgi disruption occurs prior to cell death or disease phenotypes. In cortical neurons undergoing excitotoxicity or oxidative or nitrosyl stress, Golgi fragmentation precedes cell death, and both fragmentation and death could be blocked when Golgi structure was rescued with expression of a C-terminal GRASP65 fragment (Nakagomi et al., 2008). The C-terminal GRASP65 fragment allows formation of GRASP oligomers but cannot be phosphorylated, which is required for disassembly. Golgi fragmentation usually results in decreased or blocked trafficking to the cell surface. This would be expected to impair cellular function and could contribute to apoptosis.

In cell and animal models of Alzheimer's disease, increased Aβ processing from the amyloid precursor protein (APP) leads to Golgi fragmentation before cell death (Gonatas et al., 2006). A recent study shows that phosphorylation of GRASP65 by Cdk5 activated by Aβ (possibly through calcium signaling) results in reversible disassembly of the Golgi complex (Joshi et al., 2014). By contrast to other situations where Golgi fragmentation results in decreased cargo trafficking, the Wang group has shown that Aβ-induced fragmentation actually increases cargo trafficking. This results either directly or indirectly in positive feedback, where production of Aβ is increased and thus increases Golgi fragmentation. However, increased Aβ processing may block further accumulation of Aβ. It was recently shown that the intracellular domain of APP (released after Aβ processing and translocated to the nucleus) leads to reduced levels of machinery required for APP trafficking out of the Golgi, and thus reduced production of Aβ (Ceglia et al., 2015). Clearly, there is still much to learn about the role of the Golgi complex in Alzheimer's disease.

ACBD3 (also known as GCP60) is upregulated in cell culture models expressing the huntingtin (Htt) protein with expanded polyglutamine repeats, as well as in the brains of mice that model Huntington disease and in the striatum of Huntington's patients (Sbodio et al., 2013). ACBD3 is a ubiquitous peripheral Golgi protein that interacts with golgins and may regulate trafficking of fragments of golgin-160 generated by caspase cleavage (Sbodio et al., 2006). Interestingly, as mentioned above, ACBD3 is one of the genes shown to be upregulated by Golgi stress induced by monensin (Oku et al., 2011), which requires TFE3 (Taniguchi et al., 2015). In striatal neurons, increased levels of ACBD3 may lead to increased neurotoxicity due to its interaction with both Rhes and Htt. Rhes is a small G protein that is associated with Htt pathogenicity. Rhes is specifically expressed in the striatum (unlike Htt, which is widely expressed) and is thus thought to limit the site of degeneration in Huntington's disease to this region of the brain. Caspase-2 cleavage of Htt has also been implicated in pathology (Hermel et al., 2004). It will be interesting to see if any other binding partners of ACBD3 and caspase-2 cleavage at the Golgi are involved in Huntington's pathology.

OUTLOOK

Elucidation of the types of stresses that originate at the Golgi complex as well as the resulting signaling pathways will be required for a complete understanding of the role of this important organelle in neurodegeneration as well as other diseases. The identification of transcription factors that respond to Golgi stress, such as TFE3 and CREB3, will greatly aid the dissection of the signaling pathways. Determining how perturbation of Golgi structure and cleavage fragments of Golgi structural proteins influence gene expression is crucial. Finally, the consequences of Golgi fragmentation on cell homeostasis, including membrane traffic, need to be assessed for each type of Golgi stress uncovered.

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