The exoribonuclease Polynucleotide Phosphorylase influences the virulence and stress responses of yersiniae and many other pathogens

Jason A. Rosenzweig[1,2]* and Ashok K. Chopra[3,4,5,6]*

[1] Department of Biology, Center for Bionanotechnology and Environmental Research, Texas Southern University, Houston, TX, USA
[2] Department of Environmental and Interdisciplinary Sciences, Texas Southern University, Houston, TX, USA
[3] Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX, USA
[4] Sealy Center for Vaccine Development, University of Texas Medical Branch, Galveston, TX, USA
[5] Institute of Human Infections and Immunity, University of Texas Medical Branch, Galveston, TX, USA
[6] Galveston National Laboratory, University of Texas Medical Branch, Galveston, TX, USA

Microbes are incessantly challenged by both biotic and abiotic stressors threatening their existence. Therefore, bacterial pathogens must possess mechanisms to successfully subvert host immune defenses as well as overcome the stress associated with host-cell encounters. To achieve this, bacterial pathogens typically experience a genetic re-programming whereby anti-host/stress factors become expressed and eventually translated into effector proteins. In that vein, the bacterial host-cell induced stress-response is similar to any other abiotic stress to which bacteria respond by up-regulating specific stress-responsive genes. Following the stress encounter, bacteria must degrade unnecessary stress responsive transcripts through RNA decay mechanisms. The three pathogenic yersiniae (*Yersinia pestis*, *Y. pseudo-tuberculosis*, and *Y. enterocolitica*) are all psychrotropic bacteria capable of growth at 4°C; however, cold growth is dependent on the presence of an exoribonuclease, polynucleotide phosphorylase (PNPase). PNPase has also been implicated as a virulence factor in several notable pathogens including the salmonellae, *Helicobacter pylori*, and the yersiniae [where it typically influences the type three secretion system (TTSS)]. Further, PNPase has been shown to associate with ribonuclease E (endoribonuclease), RhlB (RNA helicase), and enolase (glycolytic enzyme) in several Gram-negative bacteria forming a large, multi-protein complex known as the RNA degradosome. This review will highlight studies demonstrating the influence of PNPase on the virulence potentials and stress responses of various bacterial pathogens as well as focusing on the degradosome-dependent and -independent roles played by PNPase in yersiniae stress responses.

Keywords: RNA decay, yersiniae, type three secretion system, host-cell induced stress response, degradosome, oxidative stress response, cold stress response

INTRODUCTION

Earlier, mRNA decay was considered a non-specific, rapid, and inevitable default pathway for all transcripts, regardless of their size and/or secondary structure (Deutscher and Li, 2001; Kushner, 2002). Presently, profound advances in our understanding of RNA decay/maturation processes and their regulation have been made (Deutscher and Li, 2001; Kushner, 2002; Silva et al., 2011). The majority of RNA decay studies have employed *Escherichia coli*, with limited (but a growing number of) studies performed in Gram-positive organisms (Condon and Bechhofer, 2011; Silva et al., 2011) including *Bacillus subtilis* (Even et al., 2005; Mäder et al., 2008; Bechhofer, 2011; Lehnik-Habrink et al., 2012), *Streptococcus pyogenes* (Barnett et al., 2007), *Streptococcus pneumoniae* (Domingues et al., 2009), and *Staphylococcus aureus* (Huntzinger et al., 2005; Boisset et al., 2007). Found in both Gram-positive and -negative organisms, ribonucleases are enzymes that degrade ribonucleotides.

Endoribonucleases cleave RNA molecules within the transcript while exoribonucleases degrade RNA molecules in a 3′ to 5′ or 5′ to 3′ direction (Silva et al., 2011). Of all the Gram-positive organisms interrogated, *B. subtilis* has been most extensively studied regarding ribonucleases (Even et al., 2005; Mäder et al., 2008; Bechhofer, 2011; Lehnik-Habrink et al., 2012) and PNPase specifically (Oussenko et al., 2005; Lehnik-Habrink et al., 2012).

RNase E AND THE DEGRADOSOME

The 1061 amino-acid residue long ribonuclease E (RNase E) is the predominant endoribonuclease in *E. coli* and is essential for cell viability. It is absent in Gram-positive organisms which instead rely on endoribonuclease/exoribonuclease J (RNase J1/J2) (Condon, 2010; Silva et al., 2011). The first 500 amino-terminal RNase E residues include both the catalytic and S1 RNA-binding domains. The carboxy-terminus (residues 734–1060) serves as a scaffolding region upon which the

various components of the multi-protein degradosome complex bind, including the exoribonuclease polynucleotide phosphorylase (PNPase), the RNA helicase RhlB or RhlE, and enolase (a glycolytic enzyme) (Carpousis et al., 1994; Vanzo et al., 1998; Khemici and Carpousis, 2004). Specialized degradosomes of differing composition (including the association of additional accessory proteins) as well as a eukaryotic degradosome-like machine (the exosome) have also been reported in *Saccharomyces cerevisiae* (Lawal et al., 2011 and references therein). More specifically, a degradosome containing RNase E, the exoribonuclease RNase R, and RhlE (an RNA DEAD box helicase) was identified in the psychrotrophic bacterium *Pseudomonas syringae* Lz4W (Purusharth et al., 2005). Additionally, a "cold-shock degradosome" was identified in *E. coli* consisting of the cold-inducible DEAD box helicase CsdA/DeaD, PNPase, and other degradosome components (Prud'homme-Généreux et al., 2004).

Despite physical evidence of degradosome assembly observed in a number of organisms, the precise physiological functions carried out by these large multiprotein complexes/hyperstructures have yet to be fully elucidated (Norris et al., 2012). Using bacterial two-hybrid analysis as well as co-immunoprecipitation techniques, we recently determined that a *Yersinia pseudotuberculosis* degradosome, comprised of RNase E, PNPase, and RhlB helicase associates (Henry et al., 2012), extending an earlier report of physical interaction between only PNPase and RNase E in the aforementioned organism (Yang et al., 2008).

PNPase

Originally identified in *E. coli*, PNPase, encoded by the *pnp* gene, is an ∼80 kDa homo-trimeric exoribonuclease that poorly degrades structured RNA molecules (Guarneros and Portier, 1991; Symmons et al., 2000). The *Yersinia pestis* bv. Antique's highly conserved PNPase (UniProt Entry: Q1C3L8) is a slightly smaller enzyme (∼78 kDa) but shares 89% sequence similarity with that of its *E. coli* counterpart. Additionally, PNPase acts as a polymerase when the concentration of nucleotide di-phosphates is greater than inorganic phosphate, as shown in *E. coli* (Deutscher and Li, 2001). Historically, PNPase played a large part in determining the genetic code (Grunberg-Manago et al., 1955), winning Dr. Severo Ochoa the 1959 Nobel Prize in Physiology or Medicine. As mentioned above, PNPase is also a degradosome constituent in *E. coli* and *Y. pseudotuberculosis*, but exactly what percentage of PNPase's degradative effort is spent while associated with the degradosome has yet to be determined (Lawal et al., 2011). To date, the synthetic/polymerase activity of PNPase has not been demonstrated in the yersiniae. PNPase consists of two catalytic domains as well as both structurally conserved KH and S1 RNA-binding domains in its extreme carboxy-terminus.

PNPase, NON-CODING sRNAs, AND Hfq (RNA-BINDING PROTEIN)

With the discovery of functional, small non-coding RNA molecules (sRNAs) involved in regulating gene expression, the entire paradigm associated with "what constitutes a gene" was shifted. sRNAs became increasingly studied when they were found to influence bacterial physiology and even virulence (Gottesman et al., 2001; Shimoni et al., 2007; Repoila and Darfeuille, 2009; Silva et al., 2011; Storz et al., 2011). More specifically, in *E. coli*,

PNPase was found to stabilize several regulatory sRNAs including: RyhB, SgrS, and CyaR. Further, RNase E appeared responsible for their increased turnover/decay in the absence of PNPase (De Lay and Gottessman, 2011). These findings strongly suggest that the degradosome assembly (which includes the presence of PNPase) is required for stabilizing some regulatory sRNAs in *E. coli*. Perhaps the degradosome is protecting virulence-regulating sRNAs in bacterial pathogens as well?

In *Y. pseudotuberculosis*, 150 sRNA species (Koo et al., 2011) have been identified, many of which are involved in *Yersinia* virulence (Koo and Lathem, 2012; Lathem, 2012). Upon proteome profiling, the SraG sRNA was found to regulate expression of 16 different genes as evidenced by differential protein expression in *Y. pseudotuberculosis*. Interestingly, SraG appeared to positively regulate levels of PNPase, one of the 16 differentially expressed proteins (Lu et al., 2012). These powerful sRNAs used for modulating gene expression complement other post-transcriptional regulating factors including: ribonucleases (e.g., PNPase), RNA-binding proteins, and thermal riboswitches (e.g., the low calcium response at 37°C) (Schiano and Lathem, 2012).

In many instances, Hfq (an RNA-binding protein) will bind to sRNAs either promoting their stabilization or degradation (Silva et al., 2011). In the case of *Yersinia pestis*, 31 unique sRNAs were discovered, and the majority of those required the presence of Hfq for their expression (Beauregard et al., 2013). A separate global analysis of sRNAs revealed as many as 43 species in *Y. pestis* (Qu et al., 2012). Further, of the two *Y. pestis* iron-responsive sRNA RyhB homologs, only RhyB1 required Hfq for stabilization while RyhB2's stability was Hfq-independent (Deng et al., 2012). Further, PNPase has been shown to play a crucial role in the degradation of sRNAs that do not associate with Hfq (Andrade et al., 2012).

However, in *E. coli*, Hfq was shown to be necessary for PNPase-mediated degradation of the Cold shock Protein A encoding transcript (*cspA*) during cold shock (Hankins et al., 2010). Hfq also influences the virulence of a number of pathogenic bacteria (Chao and Vogel, 2010) including *Y. pestis* and *Y. pseudotuberculosis* (Geng et al., 2009; Schiano et al., 2010), while also influencing the growth of the latter (Bai et al., 2010). Perhaps Hfq is the link between yersiniae PNPase, sRNAs, and bacterial virulence?

PNPase AND COLD SHOCK/COLD GROWTH

Bacterial responses to low temperature stress are generally referred to as "cold shock." PNPase confers cold growth capabilities to a number of bacteria (including several pathogens) including: *E. coli*, the yersiniae, *Salmonella enterica*, *Campylobacter jejuni*, *S. aureus*, and *B. subtilis* (Jones et al., 1987; Wang and Bechhofer, 1996; Goverde et al., 1998; Clements et al., 2002; Rosenzweig et al., 2005, 2007; Anderson and Dunman, 2009; Haddad et al., 2009, 2012). Considering that *C. jejuni* and several pathogenic yersiniae are contracted via the fecal oral route, PNPase could serve to promote bacterial persistence on processed meats that are shipped at refrigerated temperatures (thereby facilitating foodborne illness).

During the cold shock response in *E. coli*, cold inducible genes, including six cold shock protein genes (*csp*'s), become upregulated (Jones et al., 1987; Goldstein et al., 1990). Additionally,

cold-inducible *pnp* and *rnr* genes, encoding PNPase and RNase R respectively, were also found to be cold-inducible in *E. coli* (Jones et al., 1987; Cairrão et al., 2003). Interestingly, RNase R was required for specifically degrading *ompA* transcript (encoding outer membrane protein A—OmpA) during stationary phase growth of *E. coli* (Andrade et al., 2006), strongly suggesting that RNase R is not only cold-inducible but also growth-phase inducible.

During acclimation, immediately following cold-shock, PNPase is required for degradation of unnecessary *csp* (that likely trap ribosomes) and other cold-inducible transcripts in both *E. coli* and *Yersinia enterocolitica* (Goverde et al., 1998; Neuhaus et al., 2000; Yamanaka and Inouye, 2001; Polissi et al., 2003). More specifically, in *Y. enterocolitica* PNPase, together with RNase E, influences cold growth adaptation through its recognizing and cleaving AGUAA motifs (termed cold-shock-cut boxes) in *csp* transcripts (Neuhaus et al., 2003). Furthermore, the ability of PNPase to promote cold growth in the yersiniae absolutely required the catalytic activity of PNPase and to a lesser extent its RNA-binding domains, the S1 RNA-binding domain more than the KH RNA-binding domain (Rosenzweig et al., 2005). Surprisingly, we found that PNPase was not required for chemical stress responses associated with antibiotic exposures that target protein translational machinery, the cellular membrane, and the yersiniae cell wall (Henry et al., 2012), indicating that PNPase might not be a global stress regulator but rather a regulator of specific encountered stresses in yersiniae, like the cold temperature stress.

PNPase AND BACTERIAL VIRULENCE

PNPase influences the virulence of number of human pathogens as well as one animal pathogen. In the Gram-negative *Dichelobacter nodosus* (the etiological agent of foot root in sheep), PNPase was found to negatively regulate virulence/twitching motility, primarily through its S1 RNA-binding domain (Palanisamy et al., 2010). Whether animal or human pathogens, when Gram-negative pathogens, including the yersiniae and salmonellae, are confronted by threatening innate immune cells, a rapid genetic re-programming occurs resulting in anti-host type three secretion system (TTSS) genes becoming upregulated (Cornelis and Van Gijsegem, 2000; Cornelis, 2002; Viboud and Bliska, 2005). It has now been firmly established that a number of ribonucleases (e.g., PNPase, RNase R, RNase E, RNase III) influence bacterial virulence in a wide range of Gram-negative and -positive pathogens (Anderson and Dunman, 2009; Lawal et al., 2011; Jester et al., 2012; Matos et al., 2012). Specifically, PNPase was required for the optimal virulence of several bacterial human pathogens (**Table 1**), in several instances by targeting TTSS targets.

In *S. enterica*, PNPase is required for optimal virulence in both mouse and cell culture infection models (the latter causing acute infections). However, in a seemingly counter-intuitive observation, PNPase works to negatively regulate macrophage invasion and expression of type TTSS genes involved in macrophage invasion (Clements et al., 2002). Additionally, PNPase works to also negatively regulate *Salmonella* plasmid virulence genes (*spv*) transcript levels by acting through the *spv* activator/regulator *spvR*,

Table 1 | List of PNPase influences on various bacterial pathogens.

Organism	PNPase role	References
Salmonella enterica	Promotes cold growth	Clements et al., 2002
	Promotes acute infection and virulence in murine infections	Clements et al., 2002
	Suppresses *Salmonella* plasmid virulence genes	Ygberg et al., 2006
Yersinia enterocolitica	Promotes cold growth	Goverde et al., 1998
Yersinia pseudotuberculosis	Promotes cold growth	Rosenzweig et al., 2005
	Promotes virulence in murine and cell culture infections	Rosenzweig et al., 2007
Yersinia pestis	Promotes virulence in cell culture infection and optimal TSSS function	Rosenzweig et al., 2005
	Promotes virulence in Swiss Webster murine IP infections	Lawal et al., 2011
Dichelobacter nodosus	Suppresses virulence and twitching motility	Palanisamy et al., 2010
Campylobacter jejuni	Promotes cold growth	Haddad et al., 2009, 2012
Staphylococcus aureus	Promotes cold growth	Anderson and Dunman, 2009
Streptococcus pyogenes	Degrades virulence transcripts during late exponential phase	Barnett et al., 2007
Streptococcus mutans	Is upregulated during acid-shock	Len et al., 2004

TTSS, Type three secretion system.

thereby reducing intracellular survival of *S. enterica* (Ygberg et al., 2006). In *Salmonella*, PNPase might be acting as a molecular switch, whereby a persistent infection is caused unless active PNPase promotes acute infections.

As was the case for the cold shock response, following host immune cell stress, the yersiniae must resume pre-stress gene expression profiles through the removal of unwanted and unnecessary TSSS-associated transcripts. Is PNPase, and, by extension, the degradosome involved in this post-TTSS acclimation following the host-cell encounter. This question becomes even more interesting when considering that both the RNA degradosome and the T3SS are large multi-protein hyperstructure complexes that could be interacting (Norris et al., 2012). Of the 11 known Gram-negative *Yersinia* spp., only *Y. enterocolitica*, *Y. pseudotuberculosis*, and *Y. pestis* are human pathogens. While fecal-oral transmission of *Y. pseudotuberculosis* and *Y. enterocolitica* causes self-limiting gastroenteritis (Galindo et al., 2011), *Y. pestis* (transmitted by the bite of an infected flea) has caused

three major human pandemics as well as the great plagues of London in the 1660s (Inglesby et al., 2000). In both *Y. pestis* and *Y. pseudotuberculosis*, PNPase was required for optimal virulence in cell culture infections (as measured by bacterial proliferation vis a vis mouse macrophage-like cells and degree of induced HeLa cell rounding, an indirect measure of TTSS function) (Rosenzweig et al., 2005) as well as in murine models of infection (Rosenzweig et al., 2007; Lawal et al., 2011). Interestingly, there was no evidence of PNPase reducing TTSS promoter activity in *Y. pseudotuberculosis* wild type and Δ*pnp* deletion mutants that harbored a *yopE::gfp* (Rosenzweig et al., 2005). In contrast, PNPase was required for robust Yop D and E secretion during an early kinetic TTSS induction window (between 0 and 20 min), but not for optimal Yop production levels (Rosenzweig et al., 2005). Counter-intuitively, PNPase promotes robust Yop secretion during initial TTSS induction despite negatively regulating T3SS transcript and protein production levels (Rosenzweig et al., 2007).

Interestingly, different PNPase determinants were required for various yersiniae stress responses (**Table 2**). Whereas PNPase's catalytic activity was dispensable for inducing HeLa cell rounding, it was absolutely required during cold stress (Rosenzweig et al., 2005). However, it was PNPase's S1 RNA-binding domain that proved necessary for inducing optimal HeLa cell rounding. In fact, S1 RNA-binding domains (derived from exoribonucleases PNPase, RNase II, and RNase R) alone were sufficient in complementing the yersiniae Δ*pnp* deletion mutants when expressed *in trans* (Rosenzweig et al., 2005). Furthermore, the minimal S1 domain region required for Δ*pnp* complementation in TTSS function and HeLa cell cytotoxicity was later determined to be amino acid residues 65–125 (Rosenzweig et al., 2007).

Within the required 65–125 amino acid S1 domain region, we were able to identify the specific S1 domain residue (F638), which proved necessary for not just TTSS function (as measured by HeLa cell rounding) but also cold growth (Rosenzweig et al.,

2007). The yersiniae PNPase F638 is highly conserved and present not just in the S1 domain of *E. coli* PNPase but also in the S1 domain of *E. coli* RNase E at reside F57 (Diwa et al., 2002). Further, a co-crystal structural determination revealed that within the catalytic core of *E. coli*'s RNase E that includes its S1 domain, the F57 residue (conserved with PNPase's F638) might play a facilitating role in binding RNA while residues F67 and K112 actually contact the RNA molecule (Callaghan et al., 2005). Since our previous studies revealed that both the cold-stress response as well as the HCISR require the S1 RNA-binding domain of PNPase (Rosenzweig et al., 2005, 2007; Henry et al., 2012), perhaps an as of yet unidentified upstream regulator (or novel factor) will converge on the PNPase S1 domain's RNA-binding property?

When considering that PNPase confers cold growth capabilities to a number of bacterial pathogens including *Y. pestis*, *Y. enterocolitica*, *Y. pseudotuberculosis*, *S. enterica*, *C. jejuni*, and *S. aureus* (Goverde et al., 1998; Clements et al., 2002; Rosenzweig et al., 2005, 2007; Anderson and Dunman, 2009; Haddad et al., 2009, 2012), PNPase acts as a "virulence empowering factor" by enhancing the pathogens' survival during adverse/hostile environmental conditions. In another striking example of this, PNPase becomes upregulated ~4-fold in the dental pathogen, *Streptococcus mutans* during acid-shock/encounters with low pH (Len et al., 2004). Perhaps, in the same way PNPase serves as a "virulence empowering factor" during low temperature exposure, it may be enhancing the survival of an oral pathogen when local environments on tooth surfaces become acidic during decay? In another Gram-positive pathogen, PNPase appeared to play a role in *S. pyogenes sag A* (a virulence transcript encoding a streptolysin) and *sda* (encoding an anti-neutrophil DNase) degradation during late-exponential growth phase (Barnett et al., 2007). Taken together, PNPase appears to influence the virulence potential and/or survival of a broad range of pathogens, including the pathogenic yersiniae.

Table 2 | List of PNPase determinants in yersiniae stress responses.

Organism	Degradosome-dependence	PNPase role	References
Yersinia pestis	?	Catalytic activity plays an essential role in cold growth	Rosenzweig et al., 2005
	?	Required for virulence in a catalytic-activity-independent manner	Rosenzweig et al., 2005
	?	S1 domain sufficient for mediating virulence	Rosenzweig et al., 2005
	?	The first 15 aa residues of the S1 domain are dispensable for virulence; however, the first 30 aa residues are required for virulence	Rosenzweig et al., 2007
	?	Amino acid residue F638 within the PNPase S1 domain is required for virulence and cold growth	Rosenzweig et al., 2007
Yersinia pseudotuberculosis	No	S1 and KH domains also contribute to cold growth.	Rosenzweig et al., 2005
	No	Degradosome-independent role in cold growth	Henry et al., 2012
	Yes	Degradosome assembly required for virulence	Yang et al., 2008
	Yes	Degradosome-dependent role in oxidative stress response	Henry et al., 2012
	No	PNPase is dispensable for antibiotic/chemical stress responses	Henry et al., 2012

S1, RNA-binding domain; KH, RNA-binding domain.

DO RIBONUCLEASES OTHER THAN PNPase CONTRIBUTE TO BACTERIAL STRESS RESPONSES?

PNPase is not the sole ribonuclease involved in bacterial stress responses. As mentioned earlier, RNase R was shown to be up-regulated in *E. coli* as well as being necessary for optimal cold growth (Cairrão et al., 2003). However, in *Y. pestis* neither RNase R nor RNase II was necessary for cold growth in the yersiniae (Lawal et al., 2013). That does not, however, necessarily mean that RNase R and/or RNase II are not also up-regulated in the yersiniae during cold stress, something that has yet to be determined. Interestingly, RNase R has been shown to play a role in the HCISR of *S. flexnari* (Tobe et al., 1992), *Aeromonas hydrophila* (Erova et al., 2008), and *H. pylori* (Tsao et al., 2009). In contrast, RNase R did not appear necessary for *Y. pestis* virulence (Rosenzweig et al., 2005). With regards to the yersiniae, it seems that PNPase specifically influences cold (Rosenzweig et al., 2005, 2007; Henry et al., 2012), oxidative (Henry et al., 2012), and host-cell induced stress responses (Rosenzweig et al., 2005, 2007).

DEGRADOSOME DEPENDENCE FOR YERSINIAE VIRULENCE AND DURING OXIDATIVE STRESS BUT NOT DURING COLD STRESS

Using bacterial two-hybrid analysis as well as co-immunoprecipitation techniques, the *Y. pseudotuberculosis* degradosome was determined to contain RNase E, PNPase, and RhlB helicase (Henry et al., 2012), extending an earlier report of physical interaction between only PNPase and RNase E in the aforementioned organism (Yang et al., 2008). To create a degradosome assembly-incompetent *Y. pseudotuberculosis* strain, a dominant-negative, truncated RNase E variant (residues 1–465), containing only its amino terminal catalytic and S1 domains, was ectopically expressed (Briegel et al., 2006; Yang et al., 2008). RNase E autoregulates itself post-transcriptionally; however, when the aforementioned dominant-negative variant was expressed, its autoreagulatory activity was inhibited (Briegel et al., 2006; Yang et al., 2008). Since the carboxy-terminal degradosome scaffolding region was absent when the above mentioned dominant-negative variant was expressed, the yersiniae degradosome could not assemble, as was seen in *Y. pseudotuberculosis* (Yang et al., 2008).

To determine whether degradosome assembly was required for yersiniae virulence, both a proliferation assay in co-culture with macrophage-like cells as well as a TTSS functional evaluation were carried out. Interestingly, *Y. pseudotuberculosis* proliferation was significantly reduced when the degradosome was unable to assemble (Yang et al., 2008). In fact, the degree of reduced proliferation mirrored that of the yersiniae Δ*pnp* deletion mutants observed earlier (Rosenzweig et al., 2005). Furthermore, Yang et al. (2008) were able to demonstrate that degradosome assembly was also necessary for optimum TTSS function, as measured by YopE secretion. Again, this diminished TTSS function resembled that of the yersiniae Δ*pnp* deletion mutants (Rosenzweig et al., 2005, 2007). Taken together, these data collectively demonstrated that the yersiniae requirement for PNPase to achieve optimal virulence is degradosome-dependent.

Was the PNPase contribution to yersiniae cold-growth degradosome-dependent or -independent, and did PNPase contribute to the oxidative stress response? To address this, the dominant-negative, truncated RNase E variant, residues 1–465 (Yang et al., 2008), was again employed. Interestingly, the *Y. pseudotuberculosis* PNPase confers cold-growth capability in a degradosome-independent manner while PNPase responds to oxidative stress, in the form of H_2O_2 exposure, in a degradosome-dependent manner (Henry et al., 2012). The yersiniae degradosome dependence for optimal virulence (Yang et al., 2008) does, however, logically align itself with our finding that the *Yersinia* oxidative stress response also requires PNPase in a degradosome-dependent manner (Henry et al., 2012) since, during the host-cell stress, the yersiniae are likely to encounter host-cell generated reactive oxygen species.

In sharp contrast, the PNPase requirement of *E. coli* during oxidative stress was degradosome-independent since an RNase E truncated variant devoid of the degradosome scaffolding region did not demonstrate compromised growth when exposed to H_2O_2 (Wu et al., 2009). In *E. coli*, PNPase independently prevented the accumulation of oxidatively damaged RNA (Wu et al., 2009). Curiously, however, PNPase, itself, was required for the optimal oxidative stress response in both *E. coli* and *Y. pseudotuberculosis* (Wu et al., 2009; Henry et al., 2012). Taken together, these data clearly demonstrate that PNPase contributes to various stress responses differently, at times by associating with the degradosome hyperstructure (as in the case of the yersiniae oxidative and host-cell stress response).

CONCLUSION

Throughout the literature, many examples of how ribonucleases impact bacterial virulence are apparent (Lawal et al., 2011; Matos et al., 2012; Jester et al., 2012). Whereas RNase R and RNase II have been evaluated for their impacts on bacterial virulence and various stress responses in numerous organisms, detailed studies of the aforementioned ribonucleases in the yersiniae have yet to be carried out. However, PNPase has been well-characterized in the yersiniae and found to be essential for cold growth (Goverde et al., 1998; Rosenzweig et al., 2005), required for optimal T3SS activity (Rosenzweig et al., 2005, 2007), oxidative stress responses (Henry et al., 2012), and yersiniae virulence (Rosenzweig et al., 2005). In fact, the yersiniae are not alone when it comes to dependence on PNPase for optimal virulence. *S. enterica* and *D. nodosus* also require PNPase for optimal virulence, while PNPase works to enhance survival of *C. jejuni*, *S. aureus*, *S. pyogenes*, and *S. mutans* during unfavorable conditions (**Table 1**). With regards to PNPase's contributions to yersiniae virulence, degradosome assembly was required, indicating that PNPase likely mediates its effect through the larger degradosome hyperstructure. Furthermore, this implicated RNase E, the chief endoribonuclease and scaffold upon which the degradosome assembles, as a virulence-associated factor (Yang et al., 2008).

Despite our furthering the aforementioned study, to determine that yersiniae degradosome assembly was also necessary for oxidative stress responses but not cold stress responses (Henry et al., 2012), still much remains unknown, specifically about the role of other ribonucleases during yersiniae stress responses. Furthermore, the precise mechanism by which PNPase (either in a degradosome-dependent or -independent manner) mediates the specific stress responses mentioned earlier also remains

unclear. For example, whether PNPase is degrading a repressor/negative regulator or binding to (through its S1 RNA-binding domain) and protecting an activator/positive regulator remains unknown during the various stress responses. Although progress has been made on understanding the roles the ribonucleases and RNA metabolism during various bacterial stress responses, a broader survey of additional ribonucleases should be carried out in the yersiniae, possibly revealing novel chemotherapeutic targets and/or novel vaccine candidate strains.

ACKNOWLEDGMENTS

REFERENCES

Anderson, K. L., and Dunman, P. M. (2009). Messenger RNA turnover processes in *Escherichia coli*, *Bacillus subtilis*, and emerging studies in *Staphylococcus aureus*. *Int. J. Microbiol.* 2009, 525491. doi: 10.1155/2009/525491

Andrade, J. M., Cairrão, F., and Arraiano, C. M. (2006). RNase R affects gene expression in stationary phase: regulation of *ompA*. *Mol. Microbiol.* 60, 219–228. doi: 10.1111/j.1365-2958.2006.05092.x

Andrade, J. M., Pobre, V., Matos, A. M., and Arraiano, C. M. (2012). The crucial role of PNPase in the degradation of small RNAs that are not associated with Hfq. *RNA* 18, 844–855. doi: 10.1261/rna.029413.111

Bai, G., Golubov, A., Smith, E. A., and McDonough, K. A. (2010). The importance of the small RNA chaperon Hfq for growth of epidemic *Yersinia pestis*, not *Yersinia pseudotuberculosis*, with implications for plague biology. *J. Bacteriol.* 192, 4239–4245. doi: 10.1128/JB.00504-10

Barnett, T. C., Bugrysheva, J. V., and Scott, J. R. (2007). Role of mRNA stability in growth phase regulation of gene expression in the group A *Streptococcus*. *J. Bacteriol.* 189, 1866–1873. doi: 10.1128/JB.01658-06

Beauregard, A., Smith, E. A., Petrone, B. L., Singh, N., Karch, C., McDonough, K. A., et al. (2013). Identification and characterization of small RNAs in *Yersinia pestis*. *RNA Biol.* 10, 397–405. doi: 10.4161/rna.23590

Bechhofer, D. H. (2011). *Bacillus subtilis* mRNA decay: new parts in the toolkit. *Wiley Interdiscip. Rev. RNA* 2, 387–394. doi: 10.1002/wrna.66

Boisset, S., Geissmann, T., Huntzinger, E., Fechter, P., Bendridi, N., Possedko, M., et al. (2007). *Staphylococcus aureus* RNAIII coordinately represses the synthesis of virulence factors and the transcription regulator Rot by an antisense mechanism. *Genes Dev.* 21, 1353–1366. doi: 10.1101/gad.423507

Briegel, K. J., Baker, A., and Jain, C. (2006). Identification and analysis of *Escherichia coli* ribonuclease E dominant-negative mutants. *Genetics* 172, 7–15. doi: 10.1534/genetics.105.048553

Cairrão, F., Cruz, A., Mori, H., and Arraiano, C. M. (2003). Cold shock induction of RNase R and its role in the maturation of the quality control mediator SsrA/tmRNA. *Mol. Microbiol.* 50, 1349–1360. doi: 10.1046/j.1365-2958.2003.03766.x

Callaghan, A. J., Marcaida, M. J., Stead, J. A., McDowall, K. J., Scott, W. G., and Luisi, B. F. (2005). Structure of *Escherichia coli* RNase E catalytic domain and implications for RNA turnover. *Nature* 437, 1187–1191. doi: 10.1038/nature04084

Carpousis, A. J., Van Houwe, G., Ehretsmann, C., and Krisch, H. M. (1994). Copurification of *E. coli* RNAase E and PNPase: evidence for a specific association between two enzymes important in RNA processing and degradation. *Cell* 76, 889–900. doi: 10.1016/0092-8674(94)90363-8

Chao, Y., and Vogel, J. (2010). The role of Hfq in bacterial pathogens. *Curr. Opin. Microbiol.* 13, 24–33. doi: 10.1016/j.mib.2010.01.001

Clements, M. O., Eriksson, S., Thompson, A., Lucchini, S., Hinton, J. C., Normark, S., et al. (2002). Polynucleotide phosphorylase is a global regulator of virulence and persistency in *Salmonella enterica*. *Proc. Natl. Acad. Sci. U.S.A.* 99, 8784–8789. doi: 10.1073/pnas.132047099

Condon, C. (2010). What is the role of RNase J in mRNA turnover? *RNA Biol.* 7, 316–321. doi: 10.4161/rna.7.3.11913

Condon, C., and Bechhofer, D. H. (2011). Regulated RNA stability in the gram positives. *Curr. Opin. Microbiol.* 14, 148–154. doi: 10.1016/j.mib.2011.01.010

Cornelis, G. R. (2002). *Yersinia* type III secretion: send in the effectors. *J. Cell Biol.* 158, 401–408. doi: 10.1083/jcb.200205077

Cornelis, G. R., and Van Gijsegem, F. (2000). Assembly and function of type III secretory systems. *Annu. Rev. Microbiol.* 54, 735–774. doi: 10.1146/annurev.micro.54.1.735

De Lay, N., and Gottessman, S. (2011). Role of polynucleotide phosphorylase in sRNA function in *Escherichia coli*. *RNA* 17, 1172–1189. doi: 10.1261/rna.2531211

Deng, Z., Meng, X., Su, S., Liu, Z., Ji, X., Zhang, Y., et al. (2012). Two sRNA RyhB homologs from *Yersinia pestis* biovar microtus expressed *in vivo* have differential Hfq-dependent stability. *Res. Microbiol.* 163, 413–418. doi: 10.1016/j.resmic.2012.05.006

Deutscher, M. P., and Li, Z. (2001). Exoribonucleases and their multiple roles in RNA metabolism. *Prog. Nucleic Acid Res. Mol. Biol.* 66, 67–105. doi: 10.1016/S0079-6603(00)66027-0

Diwa, A. A., Jiang, X., Schapira, M., and Belasco, J. G. (2002). Two distinct regions on the surface of an RNA-binding domain are crucial for RNase E function. *Mol. Microbiol.* 46, 959–969. doi: 10.1046/j.1365-2958.2002.03231.x

Domingues, S., Matos, R. G., Reis, F. P., Fialho, A. M., Barbas, A., and Arraiano, C. M. (2009). Biochemical characterization of the RNase II family of exoribonucleases from the human pathogens *Salmonella typhimurium* and *Streptococcus pneumoniae*. *Biochemistry* 48, 11848–11857. doi: 10.1021/bi901105n

Erova, T. E., Kosykh, V. G., Fadl, A. A., Sha, J., Horneman, A. J., and Chopra, A. K. (2008). Cold shock exoribonuclease R (VacB) is involved in *Aeromonas hydrophila* pathogenesis. *J. Bacteriol.* 190, 3467–3474. doi: 10.1128/JB.00075-08

Even, S., Pellegrini, O., Zig, L., Labas, V., Vinh, J., Bréchemmier-Baey, D., et al. (2005). Ribonucleases J1 and J2: two novel endoribonucleases in *B. subtilis* with functional homology to *E. coli* RNase E. *Nucleic Acids Res.* 33, 2141–2152. doi: 10.1093/nar/gki505

Galindo, C. L., Rosenzweig, J. A., Kirtley, M. L., and Chopra, A. K. (2011). Pathogenesis of *Y. enterocolitica* and *Y. pseudotuberculosis* in Human Yersiniosis. *J. Pathog.* 2011, 182051. doi: 10.4061/2011/182051

Geng, J., Song, Y., Yang, L., Feng, Y., Qiu, Y., Li, G., et al. (2009). Involvement of the post-transcriptional regulator Hfq in *Yersinia pestis* virulence. *PLoS ONE* 4:e6213. doi: 10.1371/journal.pone.0006213

Goldstein, J., Pollitt, N. S., and Inouye, M. (1990). Major cold shock protein of *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.A.* 87, 283–287. doi: 10.1073/pnas.87.1.283

Gottesman, S., Storz, G., Rosenow, C., Majdalani, N., Repoila, F., and Wassarman, K. M. (2001). Small RNA regulators of translation: mechanisms of action and approaches for identifying new small RNAs. *Cold Spring Harb. Symp. Quant. Biol.* 66, 353–362. doi: 10.1101/sqb.2001.66.353

Goverde, R. L., Huis in't Veld, J. H., Kusters, J. G., and Mooi, F. R. (1998). The psychrotrophic bacterium *Yersinia enterocolitica* requires expression of pnp, the gene for polynucleotide phosphorylase, for growth at low temperature (5 degrees C). *Mol. Microbiol.* 28, 555–569. doi: 10.1046/j.1365-2958.1998.00816.x

Grunberg-Manago, M., Ortiz, P. J., and Ochoa, S. (1955). Enzymatic synthesis of nucleic acid-like polynucleotides. *Science* 122, 907–910. doi: 10.1126/science.122.3176.907

Guarneros, G., and Portier, C. (1991). Different specificities of ribonuclease II and polynucleotide phosphorylase in 3' mRNA decay. *Biochimie* 73, 543–549. doi: 10.1016/0300-9084(91)90021-R

Haddad, N., Burns, C. M., Bolla, J. M., Prévost, H., Fédérighi, M., Drider, D., et al. (2009). Long-term survival of *Campylobacter jejuni* at low temperatures is dependent on polynucleotide phosphorylase activity. *Appl. Environ. Microbiol.* 75, 7310–7318. doi: 10.1128/AEM.01366-09

Haddad, N., Tresse, O., Rivoal, K., Chevret, D., Nonglaton, Q., Burns, C. M., et al. (2012). Polynucleotide phosphorylase has an impact on cell biology of *Campylobacter jejuni*. *Front. Cell. Infect. Microbiol.* 2:30. doi: 10.3389/fcimb.2012.00030

Hankins, J. S., Denroche, H., and Mackie, G. A. (2010). Interactions of the RNA binding protein Hfq with *cspA* mRNA, encoding the major cold shock protein. *J. Bacteriol.* 192, 2482–2490. doi: 10.1128/JB.01619-09

Henry, A., Shanks, J., van Hoof, A., and Rosenzweig, J. A. (2012). The *Yersinia pseudotuberculosis* degradosome is required for oxidative stress, while its PNPase

subunit plays a degradosome-independent role in cold growth. *FEMS Microbiol. Lett.* 336, 139–147. doi: 10.1111/j.1574-6968.12000.x

Huntzinger, E., Boisset, S., Saveanu, C., Benito, Y., Geissmann, T., Namane, A., et al. (2005). *Staphylococcus aureus* RNAIII and the endoribonuclease III coordinately regulate spa gene expression. *EMBO J.* 24, 824–835. doi: 10.1038/sj.emboj.7600572

Inglesby, T. V., Dennis, D. T., Henderson, D. A., Bartlett, J. G., Ascher, M. S., Eitzen, E., et al. (2000). Plague as a biological weapon: medical and public health management. Working Group on Civilian Biodefense. *JAMA* 283, 2281–2290. doi: 10.1001/jama.283.17.2281

Jester, B. C., Romby, P., and Lioliou, E. (2012). When ribonucleases come into play in pathogens: a survey of gram-positive bacteria. *Int. J. Microbiol.* 2012, 592196. doi: 10.1155/2012/592196

Jones, P. G., VanBogelen, R. A., and Neidhardt, F. C. (1987). Induction of proteins in response to low temperature in *Escherichia coli*. *J. Bacteriol.* 169, 2092–2095.

Khemici, V., and Carpousis, A. J. (2004). The RNA degradosome and poly(A) polymerase of *Escherichia coli* are required *in vivo* for the degradation of small mRNA decay intermediates containing REP-stabilizers. *Mol. Microbiol.* 51, 777–790. doi: 10.1046/j.1365-2958.2003.03862.x

Koo, J. T., Alleyne, T. M., Schiano, C. A., Jafari, N., and Lathem, W. W. (2011). Global discovery of small RNAs in *Yersinia pseudotuberculosis* identifies *Yersinia*-specific small, noncoding RNAs required for virulence. *Proc. Natl. Acad. Sci. U.S.A.* 108, E709–E717. doi: 10.1073/pnas.1101655108

Koo, J. T., and Lathem, W. W. (2012). Global discovery of small noncoding RNAs in pathogenic *Yersinia* species. *Adv. Exp. Med. Biol.* 954, 305–314. doi: 10.1007/978-1-4614-3561-7_38

Kushner, S. R. (2002). mRNA decay in *Escherichia coli* comes of age. *J. Bacteriol.* 184, 4658–4665. doi: 10.1128/JB.184.17.4658-4665.2002

Lathem, W. W. (2012). Identification of small, noncoding RNAs in pathogenic *Yersinia* species: implications for evolution and virulence. *Virulence* 3, 154–156. doi: 10.4161/viru.19155

Lawal, A., Jejelowo, O., Chopra, A. K., and Rosenzweig, J. A. (2011). Ribonucleases and bacterial virulence. *Microb. Biotechnol.* 4, 558–571. doi: 10.1111/j.1751-7915.2010.00212.x

Lawal, A., Kirtley, M. L., van Lier, C. J., Erova, T. E., Kozlova, E. V., Sha, J., et al. (2013). The effects of modeled microgravity on growth kinetics, antibiotic susceptibility, cold growth, and the virulence potential of a *Yersinia pestis ymoA*-deficient mutant and its isogenic parental strain. *Astrobiology* 13, 821–832. doi: 10.1089/ast.2013.0968

Lehnik-Habrink, M., Lewis, R. J., Mäder, U., and Stülke, J. (2012). RNA degradation in *Bacillus subtilis*: an interplay of essential endo- and exoribonucleases. *Mol. Microbiol.* 84, 1005–1017. doi: 10.1111/j.1365-2958.2012.08072.x

Len, A. C., Harty, D. W., and Jacques, N. A. (2004). Stress-responsive proteins are upregulated in *Streptococcus mutans* during acid tolerance. *Microbiology* 150, 1339–1351. doi: 10.1099/mic.0.27008-0

Lu, P., Zhang, Y., Li, L., Hu, Y., Huang, L., Li, Y., et al. (2012). Small non-coding RNA SraG regulates the operon YPK_1206-1205 in *Yersinia pseudotuberculosis*. *FEMS Microbiol. Lett.* 331, 37–43. doi: 10.1111/j.1574-6968.2012.02548.x

Mäder, U., Zig, L., Kretschmer, J., Homuth, G., and Putzer, H. (2008). mRNA processing by RNases J1 and J2 affects *Bacillus subtilis* gene expression on a global scale. *Mol. Microbiol.* 70, 183–196. doi: 10.1111/j.1365-2958.2008.06400.x

Matos, R. G., Bárria, C., Pobre, V., Andrade, J. M., and Arraiano, C. M. (2012). Exoribonucleases as modulators of virulence in pathogenic bacteria. *Front. Cell. Infect. Microbiol.* 2:65. doi: 10.3389/fcimb.2012.00065

Neuhaus, K., Anastasov, N., Kaberdin, V., Francis, K. P., Miller, V. L., and Scherer, S. (2003). The AGUAAA motif in *cspA1/A2* mRNA is important for adaptation of *Yersinia enterocolitica* to grow at low temperature. *Mol. Microbiol* 50, 1629–1645. doi: 10.1046/j.1365-2958.2003.03795.x

Neuhaus, K., Rapposch, S., Francis, K. P., and Scherer, S. (2000). Restart of exponential growth of cold-shocked *Yersinia enterocolitica* occurs after down-regulation of *cspA1/A2* mRNA. *J. Bacteriol.* 182, 3285–3288. doi: 10.1128/JB.182.11.3285-3288.2000

Norris, V., Menu-Bouaouiche, L., Becu, J. M., Legendre, R., Norman, R., and Rosenzweig, J. A. (2012). Hyperstructure interactions influence the virulence of the type 3 secretion system in yersiniae and other bacteria. *Appl. Microbiol. Biotechnol.* 96, 23–36. doi: 10.1007/s00253-012-4325-4

Oussenko, I. A., Abe, T., Ujiie, H., Muto, A., and Bechhofer, D. H. (2005). Participation of 3′-5′ exoribonucleases in the turnover of *Bacillus subtilis* mRNA. *J. Bacteriol.* 187, 2758–2767. doi: 10.1128/JB.187.8.2758-2767.2005

Palanisamy, S. K., Fletcher, C., Tanjung, L., Katz, M. E., and Cheetham, B. F. (2010). Deletion of the C-terminus of polynucleotide phosphorylase increases twitching motility, a virulence characteristic of the anaerobic bacterial pathogen *Dichelobacter nodosus*. *FEMS Microbiol. Lett.* 302, 39–45. doi: 10.1111/j.1574-6968.2009.01831.x

Polissi, A., De Laurentis, W., Zangrossi, S., Briani, F., Longhi, V., Pesole, G., et al. (2003). Changes in *Escherichia coli* transcriptome during acclimatization at low temperature. *Res. Microbiol.* 154, 573–580. doi: 10.1016/S0923-2508(03)00167-0

Prud'homme-Généreux, A., Beran, R. K., Iost, I., Ramey, C. S., Mackie, G. A., and Simons, R. W. (2004). Physical and functional interactions among RNase, E, polynucleotide phosphorylase and the cold-shock protein, CsdA: evidence for a 'cold shock degradosome'. *Mol. Microbiol.* 54, 1409–1421. doi: 10.1111/j.1365-2958.2004.04360.x

Purusharth, R. I., Klein, F., Sulthana, S., Jäger, S., Jagannadham, M. V., Evguenieva-Hackenberg, E., et al. (2005). Exoribonuclease R interacts with endoribonuclease E and an RNA helicase in the psychrotrophic bacterium *Pseudomonas syringae* Lz4W. *J. Biol. Chem.* 280, 14572–14578. doi: 10.1074/jbc.M413507200

Qu, Y., Bi, L., Ji, X., Deng, Z., Zhang, H., Yan, Y., et al. (2012). Identification by cDNA cloning of abundant sRNAs in a human-avirulent *Yersinia pestis* strain grown under five different growth conditions. *Future Microbiol.* 7, 535–547 doi: 10.2217/fmb.12.13

Repoila, F., and Darfeuille, F. (2009). Small regulatory non-coding RNAs in bacteria: physiology and mechanistic aspects. *Biol. Cell* 101, 117–131. doi: 10.1042/BC20070137

Rosenzweig, J. A., Chromy, B., Echeverry, A., Yang, J., Adkins, B., Plano, G. V., et al. (2007). Polynucleotide phosphorylase independently controls virulence factor expression levels and export in *Yersinia* spp. *FEMS Microbiol. Lett.* 270, 255–264. doi: 10.1111/j.1574-6968.2007.00689.x

Rosenzweig, J. A., Weltman, G., Plano, G. V., and Schesser, K. (2005). Modulation of *yersinia* type three secretion system by the S1 domain of polynucleotide phosphorylase. *J. Biol. Chem.* 280, 156–163. doi: 10.1074/jbc.M405662200

Schiano, C. A., Bellows, L. E., and Lathem, W. W. (2010). The small RNA chaperone Hfq is required for the virulence of *Yersinia pseudotuberculosis*. *Infect. Immun.* 78, 2034–2044. doi: 10.1128/IAI.01046-09

Schiano, C. A., and Lathem, W. W. (2012). Post-transcriptional regulation of gene expression in *Yersinia* species. *Front. Cell. Infect. Microbiol.* 2:129. doi: 10.3389/fcimb.2012.00129

Shimoni, Y., Friedlander, G., Hetzroni, G., Niv, G., Altuvia, S., Biham, O., et al. (2007). Regulation of gene expression by small non-coding RNAs: a quantitative view. *Mol. Syst. Biol.* 3, 138. doi: 10.1038/msb4100181

Silva, I. J., Saramago, M., Dressaire, C., Domingues, S., Viegas, S. C., and Arraiano, C. M. (2011). Importance and key events of prokaryotic RNA decay: the ultimate fate of an RNA molecule. *Wiley Interdiscip. Rev. RNA* 2, 818–836. doi: 10.1002/wrna.94

Storz, G., Vogel, J., and Wassarman, K. M. (2011). Regulation by small RNAs in bacteria: expanding frontiers. *Mol. Cell* 43, 880–891. doi: 10.1016/j.molcel.2011.08.022

Symmons, M. F., Jones, G. H., and Luisi, B. F. (2000). A duplicated fold is the basis for polynucleotide phosphorylase catalytic activity, processivity, and regulation. *Structure* 8, 1215–1226. doi: 10.1016/S0969-2126(00)00521-9

Tobe, T., Sasakawa, C., Okada, N., Honma, Y., and Yoshikawa, M. (1992). *vacB*, a novel chromosomal gene required for expression of virulence genes on the large plasmid of *Shigella flexneri*. *J. Bacteriol.* 174, 6359–6367.

Tsao, M. Y., Lin, T. L., Hsieh, P. F., and Wang, J. T. (2009). The 3′-to-5′ exoribonuclease (encoded by HP1248) of *Helicobacter pylori* regulates motility and apoptosis-inducing genes. *J. Bacteriol.* 191, 2691–2702. doi: 10.1128/JB.01182-08

Vanzo, N. F., Li, Y. S., Py, B., Blum, E., Higgins, C. F., Raynal, L. C., et al. (1998). Ribonuclease E organizes the protein interactions in the *Escherichia coli* RNA degradosome. *Genes Dev.* 12, 2770–2781. doi: 10.1101/gad.12.17.2770

Viboud, G. I., and Bliska, J. B. (2005). *Yersinia* outer proteins: role in modulation of host cell signaling responses and pathogenesis. *Annu. Rev. Microbiol.* 59, 69–89. doi: 10.1146/annurev.micro.59.030804.121320

Wang, W., and Bechhofer, D. H. (1996). Properties of a *Bacillus subtilis* polynucleotide phosphorylase deletion strain. *J. Bacteriol.* 178, 2375–2382.

Wu, J., Jiang, Z., Liu, M., Gong, X., Wu, S., Burns, C. M., et al. (2009). Polynucleotide phosphorylase protects *Escherichia coli* against oxidative stress. *Biochemistry* 48, 2012–2020. doi: 10.1021/bi801752p

Yamanaka, K., and Inouye, M. (2001). Selective mRNA degradation by polynucleotide phosphorylase in cold shock adaptation in *Escherichia coli*. *J. Bacteriol.* 183, 2808–2816. doi: 10.1128/JB.183.9.2808-2816.2001

Yang, J., Jain, C., and Schesser, K. (2008). RNase E regulates the *Yersinia* type 3 secretion system. *J. Bacteriol.* 190, 3774–3778. doi: 10.1128/JB.00147-08

Ygberg, S. E., Clements, M. O., Rytkönen, A., Thompson, A., Holden, D. W., Hinton, J. C., et al. (2006). Polynucleotide phosphorylase negatively controls *spv* virulence gene expression in *Salmonella enterica*. *Infect. Immun.* 74, 1243–1254. doi: 10.1128/IAI.74.2.1243-1254.2006

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