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Inorganic Polyphosphate in Host and Microbe Biology

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Abstract

Inorganic polyphosphate (polyP) is produced by both bacteria and their eukaryotic hosts, and appears to play multiple important roles in the interactions between those organisms. However, the detailed mechanisms of how polyP synthesis is regulated in bacteria and how it influences both bacterial and host biology remain largely unexplored. In this review, we examine recent developments in the understanding of how bacteria regulate the synthesis of polyP, what roles polyP plays in controlling virulence in pathogenic bacteria, and the effects of polyP on the mammalian immune system, as well as progress on developing drugs that may be able to target bacterial polyP synthesis as novel means of treating infectious disease.

Keywords

Polyphosphate; Bacterial Survival; Pathogenicity; Immune Regulation

Why Study Polyphosphate?

Inorganic polyphosphate (polyP) is a universally conserved biomolecule composed of covalently linked units of phosphate monomers, as depicted in Figure 1A, with functions as widely varied as life itself. In bacteria, polyP has been linked to a myriad of functions, including energy storage, metabolic regulation, stress responses, viability, colonization, pathogenicity, virulence, mobility, and antibiotic resistance [1–14]. PolyP is involved in the movement of large magnetotactic bacteria in suboxic zones of the Black Sea, and has recently been investigated as a basis for a photo-microbial fuel cell [15, 16]. In eukaryotes, polyP plays roles in everything from oxidative and divalent cation stress responses in *Saccharomyces cerevisiae* to blood coagulation, macrophage differentiation, leukocyte proliferation, neutrophil recruitment, and platelet functions in mammalian systems [17–22], as well as playing a protective role in neuronal signaling by preventing glutamate excitotoxicity [23]. PolyP is important in archaea, as well. In *Methanosarcina mazei*, for example, polyP has been shown to accumulate under phosphate starvation conditions, acting to regulate the transcription of multiple phosphate metabolism and transport genes including

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those encoding the *pstSCAB-phoU* complex [24], while in several species of *Sulfolobales*, polyP is involved in archaeal motility, adhesion, and biofilm formation in [25].

With such diverse functions, it is of little surprise that new studies are steadily discovering new and reimagined roles for polyP. It is the purpose of this review to discuss advances in the field of polyP biology over the past few years, emphasizing the importance of understanding polyP functions in host-microbe interactions and how polyP represents a promising, but complicated target for clinical applications.

Overturning Established Models of Polyphosphate Regulation

There are two unrelated kinases utilized by many bacteria to synthesize polyP: **PPK1** or **PPK** (see Glossary) and **PPK2** [1]. PPK1 predominately catalyzes the synthesis of polyP from ATP. PPK2 is different in that it can efficiently use GTP or ATP to synthesize polyP and is more efficient at hydrolyzing polyP to synthesize nucleotide triphosphates [26]. Some bacteria possess **homologs** of both PPK1 and PPK2; others possess only one, or in some cases, neither. While this review focuses primarily on polyP synthesis by PPK1, it is important to note that some species (such as *Francisella tularensis)* have both PPK1 and PPK2 homologs, and that most studies we highlight only focus on one of these enzymes. For brevity, and because most recent studies examine PPK1 functions, it is the intent of this review to focus largely on PPK1. For clarity, however, we will take care to indicate when PPK2 is also present in a system.

Many bacteria, including the model Gram-negative bacterium *Escherichia coli* (*E. coli*), which has a single PPK1 homolog (called simply PPK) [27], activate polyP synthesis in response to environmental stress [28], and the many roles of polyP in stress tolerance [4, 6, 8, 9, 11, 12, 29–36] are central to the function of polyP in host-microbe interactions. Nevertheless, the molecular mechanisms of polyP regulation in bacteria have been largely ignored since early studies in *E. coli* by Arthur Kornberg's lab in the late 1990s [37, 38]. Those studies proposed a model in which the repression of polyP-degrading exopolyphosphatase (**PPX**) activity by the stringent response alarmone **(p)ppGpp** [39] was the key regulatory step in polyP synthesis [38, 40], and this idea was generally accepted until very recently [41]. However, we have recently demonstrated that induction of polyP synthesis in *E. coli* is, in fact, independent of (p)ppGpp synthesis [42], although it does depend on the presence of the RNA polymerase-binding stringent transcription factor **DksA** as well as on the alternative sigma factors **RpoN** and **RpoE**, which are best known for their roles in responding to nitrogen starvation and cell envelope disruption stresses, respectively [42, 43]. We do not currently know what gene or genes regulated by these transcription factors directly influence polyP levels, since *ppk* transcription is not activated under stress conditions in *E. coli* [43], although there does appear to be transcriptional control of *ppk* in some other bacteria in response to stress [44, 45]. Our lab has also recently published a study describing point mutations in *E. coli* PPK that strongly activate polyP synthesis *in vivo* without affecting the enzyme's active site or *in vitro* activity [46] suggesting a role for some form of post-translational regulation of PPK activity, either by post-translational modification of PPK itself or by interaction with other proteins, but this remains to be tested. It has been known for some time that the phosphate transport regulators **PhoU** and **PhoB**

regulate polyP accumulation in many bacteria [40, 47–49], but how they do so and whether they interact with other regulatory systems remains to be determined. In summary, as shown in Figure 1B, there is a great deal remaining to be discovered about the genes and proteins involved in controlling polyP accumulation, even in *E. coli*, which has a long history of polyP research.

Bacterial Polyphosphate and Lon Protease

One of the areas ripe for exploration is the relationship between bacterial polyP and **Lon** protease, one of the major protein-degrading enzymes of bacteria [50]. It is well established, at least in *E. coli*, that polyP interacts with Lon. The effects of this interaction, though, remain incompletely understood. Early on, Kornberg reported that polyP activates Lon-mediated degradation of a subset of ribosomal proteins, contributing to recovery from amino acid starvation [51–53]. Recently, the Konieczny lab has shown that polyP regulates DNA replication initiation by acting on Lon during the stringent stress response in *E. coli* [54]. In this report, the researchers showed both *in vitro* and *in vivo* that polyP activates Lon protease to target and degrade the essential replication initiation protein DnaA. DnaA inhibits replication initiation when bound to ATP and stimulates replication when bound to ADP [55, 56]. Gross and Konieczny demonstrated that polyP associates with DnaA-ADP, but not with DnaA-ATP, and that this association is necessary for the Lon protease to degrade DnaA. This results in depletion of DnaA-ADP, while enriching the DnaA-ATP repressor pool. However, polyP inhibits the Lon-dependent degradation of some other proteins, including the model protein α-casein [57] and, notably, the cell division inhibitor SulA [53]. This suggests a general Lon-dependent network by which polyP accumulation inhibits cell division. PolyP, Lon, DnaA, and ribosomal proteins are all very highly conserved among bacteria, and DnaA is a conserved target for Lon degradation [58]. Importantly, Lon is implicated in multiple polyP-dependent functions, some of which will be discussed below. The mechanism by which polyP affects Lon targeting and activity is currently unknown. Further deciphering of the global effect of polyP on Lon targeting and activity, and therefore on the bacterial proteome, is essential for understanding polyP biology and represents an exciting area for future research.

Polyphosphate in Pathogenic Bacteria

Much of the research in the field focuses on pathogenic bacteria and how polyP is involved in disease. PolyP production is required for virulence in many pathogens [2, 3, 8, 12–14, 35], and recent research has now begun to establish more detailed molecular mechanisms for these roles.

For example, one report found that polyP synthesis is essential for the virulence of *Salmonella enterica* serovar Typhimurium and its survival in *Dictyostelium discoideum,* a social amoeba used as a model for macrophage interactions [59]. The developmental cycle of *D. discoideum* progresses through three stages (aggregation, elevation, and culmination), which *S. enterica* sv. Typhimurium delays by inducing a starvation-like transcriptional response while selectively impairing expression of genes required for chemotaxis and aggregation [60–62]. *S. enterica* sv. Typhimurium, like *E. coli*, has only a single PPK1

homolog (called simply “PPK”) and lacks PPK2. The study by Varas *et al* found that *Δppk S. enterica* sv. Typhimurium were deficient in impairing developmental progress of *D. discoideum* [59]. Proteomic profiling of infected amoebae revealed that the WT strain triggered a robust response, including the expression of DNA repair enzymes, but the *Δppk* strain did not induce the expression of DNA repair enzymes. Importantly, while the WT strain was able to replicate and survive in the amoeba, the *Δppk* mutant was not, despite being internalized at significantly higher levels. This suggests that *S. enterica* sv. Typhimurium induces DNA damage through an unknown pathway that depends on continued bacterial survival, and that polyP synthesis is essential for this survival. What mechanisms are specifically regulated by polyP in *S. enterica* sv. Typhimurium and how those mechanisms allow it to evade host defenses remain to be explored; however, *D. discoideum* is clearly a powerful model system for deciphering these kinds of bacterial-eukaryotic interactions.

Another example of a newly-discovered role for polyP in bacterial pathogenesis is the recent report by Tang-Fichaux *et al*. showing that production of the DNA-damaging toxin colibactin by a variety of strains of *E. coli* is dependent on the presence of *ppk* [63]. The loss of polyP, either by deletion of *ppk* or by chemical inhibition of PPK activity with mesalamine (see The Discovery and Testing of Microbial Polyphosphate Kinase Inhibitors section below) decreased expression of the promoter driving the expression of colibactin synthesis genes, reducing the genotoxicity of *E. coli*.

There are many reports of polyP acting as a pro-virulence factor in bacteria. However, Rohlfing *et al*. tell a different story [64]. They investigated the role of PPK1 in *Francisella tularensis,* which possesses both PPK1 and PPK2. In *F. tularensis* major pathogenic elements are expressed from the *Francisella* Pathogenicity Island (**FPI**), which is regulated in a (p)ppGpp-dependent manner. Rohlfing *et al*. found that a *Δppk1* mutant had higher expression of the FPI genes observed, suggesting that *ppk* expression actually antagonized *Francisella* pathogenicity. A Lon deletion strain (*Δlon*) also showed increased transcript levels of multiple virulence genes, though not to the level of the *Δppk1* mutant. FPI expression in a *Δlon Δppk* strain was comparable to the *Δppk1* single mutant. **Western blots** revealed that the level of an FPI protein was higher in the *Δlon*, *Δppk1*, and *Δlon Δppk1* strains than in WT, further arguing that the expression of polyP represses the expression of FPI pathogenicity elements, likely through a Lon-dependent mechanism. These results emphasize the importance of understanding both the polyP-Lon relationship and the individual mechanisms utilized by different pathogenic species.

Regulation of virulence gene expression and protein stability are not the only roles polyP may play in pathogenic bacteria’s survival in a host. Roewe *et al*. have recently reported on the effect of bacterial polyP on innate host defense in an *E. coli* sepsis model in mice [34]. They found that polyP directly affects innate immune response in a chain-length dependent manner. Injecting long-chain polyP (lcPolyP, ~300–1000 phosphate units [31]), similar to that synthesized by bacterial PPK, into a host along with bacteria resulted in markedly increased mortality. High levels of polyP reduced the ability of neutrophils and macrophages to phagocytize bacteria while also reducing the expression of macrophage-attracting chemokines (such as **CCL2** and **CXCL10**) and cytokines like **INFβ**. Moreover,

lcPolyP bound to the surfaces of macrophages and was internalized, resulting in drastic alterations to gene expression and macrophage polarization from an M1 (pro-inflammatory) to an M2 (anti-inflammatory) phenotype. Interestingly, lcPolyP not only drove a M2 phenotypic response, but it also overrode the LPS-induced M1 response by enhancing M2 genes in LPS-activated macrophages while simultaneously antagonizing M1 genes, even to the extent of impairing the expression of **iNOS** genes and the secretion of NO_2^- into the supernatant. LcPolyP also altered the type I interferon response to LPS, resulting in a less responsive macrophage population. As a final blow to the innate response, lcPolyP interfered with antigen presentation by suppressing the expression of the Major Histocompatibility Complex (**MHC**) invariant chain as well as the important costimulatory proteins **CD80** and **CD86**. Importantly, the reduction in the expression of the MHC-invariant chain was also seen *in vivo*, demonstrating interference by lcPolyP in the interplay between the innate and adaptive immune systems.

Supporting the idea that bacterial polyP plays an important role in modulating innate immune responses, Rijal *et al.* have recently reported that pathogenic *Mycobacterium* species, including *M. tuberculosis* and *M. smegmatis* (both of which possess PPK1 and PPK2, although in this study only PPK1 was examined) secrete polyP and that this secreted bacterial polyP increases survival of these bacteria after phagocytosis by either human macrophages or *D. discoideum* amoeba [65]. In these experiments, polyP inhibited both phagosome acidification and lysosome activity. Intriguingly, in *D. discoideum*, the putative polyP receptor GrlD was required for these effects, suggesting that eukaryotic cells may possess signaling pathways directly responsive to bacterial polyP. There is evidence that at least some other pro-inflammatory pathogenic bacteria can secrete or maintain substantial extracellular levels of polyP [66, 67] so, while important questions about physiological polyP concentration and sources during natural infections remain unresolved, the observation that bacterial-type polyP can repress the innate immune response is both important and informative for the study of polyP biology and suggests a molecular mechanism underlying the essentiality of polyP production for virulence in many pathogens.

Polyphosphate War: Clashing Functions in Host-Pathogen Interactions

PolyP is produced by both bacteria and eukaryotes. Not only does polyP play a role in bacterial survival, but it is also involved in host defense and repair mechanisms. For example, Suess *et al.* found that polyP plays multiple roles in wound healing and leukocyte biology [22]. They demonstrated that fibrocyte differentiation depended on platelet-derived polyP and that low concentrations of polyP (1 – 2 pM) promoted fibrocyte differentiation in peripheral blood mononuclear cell (**PBMC)** cultures, while higher (30 – 125 pM) appeared to decrease fibrocyte differentiation while concurrently increasing macrophage differentiation in the absence of serum. Interestingly, they also found that 0 – 10 pM polyP gradients acted as chemoattractants for neutrophils, suggesting a novel role for recruiting neutrophils to sites of tissue damage. Finally, they found that extracellular polyP played a role in proliferation, as concentrations of 100 μM or higher inhibited proliferation of PBMCs *in vitro*. In this study, Suess *et al.* convincingly demonstrated that polyP is highly involved in the innate response, playing roles in maturation of fibroblasts and macrophages while also acting as a chemoattractant for neutrophils into areas of inflammation.

The relationship between polyP and neutrophils takes on a new level of significance when considering neutrophilic responses to bacterial sepsis. In bacterial sepsis, neutrophils release traps composed of neutrophilic proteins (such as neutrophil elastase), DNA, and histones into the microvasculature in multiple tissues [68]. These Neutrophil Extracellular Traps (NETs) capture circulating bacteria while stimulating inflammation and coagulation in the surrounding tissue. While this serves as an effective mechanism to capture bacteria, the subsequent clotting and inflammation often cause significant tissue and organ damage. In 2017, McDonald *et al.* investigated the role of NETs, **histone H4**, and polyP in sepsis-induced intravascular coagulation [69]. To investigate the specific role of polyP in the neutrophilic response and to determine if H4 histone-driven polyP release from platelet granules drove coagulation, they treated septic mice with a monoclonal blocking antibody against polyP. While blocking polyP did not affect the quantity of NETs or platelets in the microvasculature of the liver in septic mice, it significantly reduced the amount of thrombin cleavage activity and subsequent clotting, suggesting that polyP is a crucial factor in the platelet-NET-coagulation response. Taken with Suess *et al.*'s more recent results, a more informative - yet complicated - picture of polyP's role in the innate immune responses begins to emerge, where polyP both draws neutrophils to sites of infection to commence the innate response, while also driving the coagulation and inflammatory response in the area by regulating the activity of thrombin as well as the differentiation of macrophages and fibroblasts.

Given all of this, host polyP has become a target of great interest for potential medical treatments. One target of interest is inositol hexakisphosphate kinase I (**IP6K1**), which is known to regulate the levels of polyP produced by platelets in mice [70]. Recently, Hou *et al.* reported that impairing host IP6K1 substantially reduced the production of polyP from platelets, which resulted in enhanced host bacterial killing while reducing pulmonary neutrophil accumulation, thus minimizing tissue damage induced by highly active neutrophilic responses [71]. This reduction in lung damage was observed when mice were challenged with *E. coli*, *Staphylococcus aureus*, or purified LPS. The decrease in platelet-derived polyP resulted in fewer neutrophil-platelet aggregations (NPAs), which ultimately led to the reduction of neutrophil accumulation in the alveolar tissue. They found that by using an IP6K1-specific inhibitor they could induce the reduction of NPAs in both mice and a culture of human primary neutrophils and platelets, demonstrating a clinical significance to their work. This identification of a polyP-driven immune mechanism that can be altered to enhance bacterial clearance and reduce host damage demonstrates the importance of understanding polyP on a larger scale.

In general, the data we have discussed in this section demonstrates multiple roles for polyP in host immune responses, including recruiting neutrophils, controlling fibroblast and macrophage differentiation, and altering the local microenvironmental chemistry. How this interacts with the immunomodulatory effects of polyP discussed in the previous section remains to be determined. There is clearly a delicate and complicated relationship between host and pathogen polyP usage, which has been summarized in Figure 2 (Key Figure).

Polyphosphate: Too Much of a Good Thing?

PolyP accumulation is not entirely beneficial for the host. As discussed above, it can lead to increased coagulation, heightened inflammation, and greater infiltration of neutrophils into tissue that may be damaged more by the innate response than by the bacteria that triggered it. Independent of bacterial triggers, however, polyP has been implicated in several disorders and diseases. In humans, for example, polyP has been linked to cancer-associated thrombosis (reviewed in [72]). Conversely, polyP has also been associated with beneficial results, including inhibiting metastasis and inducing apoptosis (reviewed in [73]). Indeed, polyP is often assigned counterproductive functions in the same system. For example, polyP accelerates the formation of amyloid fibrils *in vitro*, which are associated with a wide range of human disease such as Alzheimer's disease, Parkinson's disease, and dialysis-related amyloidosis [74], but polyP can also be neuroprotective, by preventing over excitement of neurons by glutamate signaling [23]. While the focus of this article is primarily on the importance of polyP in microbes and in host-microbe interaction, the literature on polyP biology is far more extensive and demonstrates the importance of approaching this ancient, universal molecule with an open mind.

The Discovery and Testing of Microbial Polyphosphate Kinase Inhibitors

The enzymology of polyP production in mammals is not well understood (although one recent paper suggests that the mitochondrial **F_1F_0 ATPase** synthesizes polyP [75]), but the well-characterized prokaryotic enzymes of microbial polyP metabolism [1] offer a unique opportunity to develop therapeutics targeting a multitude of infectious diseases. The idea that bacterial polyP metabolism could be a useful target for antimicrobial therapy is not new [76–79]. Several groups have recently reported substantial progress in this area and have identified a chemically diverse group of PPK1 inhibitors (Table 1), with exciting new data showing that bacterial polyP synthesis is targetable *in vivo* and that doing so may have useful anti-virulence effects. Only a small handful of studies have explored PPK2 as a target for inhibitors [77, 78, 80], and this remains an intriguing area for future work.

Using *in silico* modeling in combination with an *in vivo* screen of *Pseudomonas aeruginosa* virulence with *D. discoideum* as a host, the Chavez lab identified five compounds that potently inhibit PPK1 *in vitro* (**IC50** < 10 μM) and also reduce bacterial virulence, mimicking the effect of a *P. aeruginosa ppk1* knockout mutation [81], although this is complicated somewhat by the fact that *D. discoideum* is one of the few eukaryotes with a PPK1 homolog [82], and that polyP production by *D. discoideum* is involved in phagocytosis [83]. Usefully, however, the same compounds also inhibit *E. coli* PPK *in vitro* [84]. Another *in silico* screen for potential PPK inhibitors by the Bardaweel group identified two compounds that mimicked the effects of a *ppk* null mutation on both *E. coli* metabolism (as determined using the Biolog™ platform) and reduced total biofilm production [85], but they did not report the effect of these compounds on *in vitro* PPK activity. In contrast, an *in vitro* screen of bisphosphonic acid derivatives (a family of compounds which contains many clinically important enzyme inhibitors) by the Berlicki lab identified two compounds with IC50s for PPK1 of 50 – 60 μM, but did not test their effect on living bacteria [80]. Most recently, exciting new results from the Sun lab have identified two more PPK inhibitors, also

from an initial *in silico* screen, that not only increase the stress sensitivity of uropathogenic *E. coli* (UPEC) under lab growth conditions, but also significantly reduce bacterial burden in an *in vivo* mouse model of UPEC infection [86]. Neither of these compounds are especially potent inhibitors of PPK activity *in vitro* (IC50 > 320 μM), but the fact that they are effective anti-virulence treatments *in vivo* is extremely encouraging. Surprisingly, none of the above studies directly measured the effect of inhibitors on bacterial polyP content, which will be an important control in future experiments.

By screening a library of FDA-approved drugs for inhibitory activity against *E. coli* PPK, the Jakob lab identified the front-line inflammatory bowel disease drug mesalamine (5-aminosalicylic acid) as a PPK inhibitor [87]. Although its inhibitory activity *in vitro* was also modest (increasing the $\mathbf{K_m}$ of PPK for ATP by 4-fold at 1 mM mesalamine), at concentrations comparable to those used therapeutically, mesalamine significantly reduced polyP accumulation by cultures of *E. coli*, *P. aeruginosa*, and *Vibrio cholerae*, as well as in the gut microbiota of mesalamine-treated mice and humans. The mechanism(s) by which mesalamine reduces inflammation have been debated for many years [88], but these results may suggest that, in fact, we actually have been using bacterial polyP as a therapeutic target for quite some time. It remains to be seen, however, whether inhibiting polyP production will be a therapeutically useful strategy for dealing with other bacterial infections in humans, especially in light of the multiple roles of polyP in both host and microbe biology.

Concluding Remarks

Along with the renaissance of studies on the biology of polyP have come substantial improvements in the tools and assays for studying polyP in biological systems. These have been thoroughly reviewed recently [89], and include new and streamlined extraction and quantification techniques [90–93] as well as convenient biochemical methods for length determination and end-labeling of polyP [94, 95]. We expect that these and related technologies will be increasingly important as the community of researchers interested in polyP continues to grow.

The first description of polyP in living organisms was over a century ago [96], but our understanding of how it fits into cellular physiology has been slow in coming. There are no easy answers when studying polyP biology, but there is a bounty of questions to be explored (see Outstanding Questions), and a dynamic and growing community of researchers asking those questions. It’s an exciting time for polyP, and we are eager to see what new insights will be revealed about this ancient molecule.

Glossary

CCL2	C-C Motif Chemokine Ligand 2. One of several chemokine receptors utilized by monocytes and basophils to detect and direct migration towards areas with high C-C chemokine receptor 2 (CCR2) concentration.
CD80	An immunoglobin expressed on antigen presenting cells that binds to a T cell’s CD28 receptor to provide essential

	costimulatory signals for activation. Closely related to CD86.
CD86	An immunoglobin expressed on antigen presenting cells that binds to a T cell's CD28 receptor to provide essential costimulatory signals for activation. Closely related to CD80.
CXCL10	A pro-inflammatory cytokine that binds to CXCR3 on monocytes, Natural Killer cells, and T cells to stimulate pleiotropic effects related to antimicrobial activity.
DksA	RNA polymerase-binding transcription factor involved in bacterial stringent response
F_1F_0 ATPase	protein complex responsible for ATP synthesis in mitochondria
FPI	*Francisella* Pathogenicity Island, genetic locus encoding multiple factors necessary for *F. tularensis* virulence
histone H4	one of the five histones involved in DNA packaging. Its presence outside of the host cell triggers immune activity as it should only be inside healthy cells.
homolog	a species-specific version of a gene or protein that is found among multiple species that share a common ancestor.
IC50	concentration of an inhibitor which halves activity of the target
INFβ	antiviral chemokine secreted by many immune cells. It stimulates macrophages and natural killer cells.
iNOS	inducible Nitric Oxide Synthase; produces nitric oxide which acts to regulate the host immune response
IP6K1	Canonically converts inositol hexakisphosphate to diphosphoinositol pentakisphosphate but has recently been shown to be involved in the production or regulation of mammalian polyP.
K_m	Michaelis-Menten constant; the concentration of substrate at which an enzyme acts at half its maximal velocity
Lon	major bacterial protease involved in protein turnover and regulation
LPS	lipopolysaccharide; strongly immuno-stimulatory outer membrane lipid of Gram-negative bacteria

MHC	major histocompatibility complex; utilized by cells to present antigen fragments to T and B cells
PBMC	the portion of blood cells containing the mononuclear lineages, which include the lymphocytes (T cells, B cells, NK cells) and monocytes.
PhoB	bacterial transcription factor that positively regulates phosphate uptake
PhoU	negative regulator of phosphate uptake in bacteria
(p)ppGpp	guanosine penta- and tetraphosphate; second messengers that are global regulators of starvation stress response
PPK1	or PPK, family of polyphosphate kinases, synthesizes polyP from ATP
PPK2	family of polyphosphate kinases, synthesizes polyP from NTPs
PPX	exopolyphosphatase; breaks down polyP to orthophosphate
RelA	(p)ppGpp synthase
RpoE	global regulator of bacterial cell envelope stress responses
RpoN	global regulator of bacterial nitrogen starvation stress response
RpoS	global regulator of bacterial general stress response
SpoT	(p)ppGpp synthase/hydrolase
stringent response	bacterial starvation stress response mediated by (p)ppGpp and DksA
Western blot	technique for detecting and quantifying proteins using specific antibodies

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Outstanding Questions Box

- What are the pathway(s) by which bacteria regulate polyP accumulation in response to different environmental stresses? How well-conserved are the regulatory mechanisms being identified in *E. coli*?
- How does the interaction of polyP and Lon protease change the global proteome of bacteria under different conditions? How does this differ between species?
- Is the anti-virulence effect of polyP unique to *Francisella,* or do other bacteria also use similar mechanisms?
- What are the immunologically-relevant levels of short- and long-chain polyP in an active infection site? What is the balance between host and bacterial polyP during infection?
- What receptors do eukaryotic cells, including neutrophils, use to sense and respond to polyP? Can they distinguish between short- and long-chain polyP, and if so, how?
- How and under what circumstances do bacteria release polyP? Do they actively secrete polyP or is it released by cell lysis? Does this vary from species to species?
- Can PPK inhibitors or other polyP-manipulating drugs be used to treat infections or modulate immune responses in a clinically useful way?

Highlights

- PolyP plays multiple roles in bacterial regulation, including controlling proteolysis by the Lon protease and regulating virulence factors, both positively and negatively
- PolyP plays a major role in host repair by facilitating thrombin-driven coagulation and in defense mechanisms by recruiting neutrophils and driving M1 macrophage differentiation
- Bacteria use polyP to manipulate host immune responses, impairing phagocytic cell functions, modulating inflammatory responses, and impairing antigen presentation
- *Dictyostelium discoideum* is emerging as a powerful model system to investigate polyP biology
- Multiple drugs influencing polyP levels in different organisms have recently been discovered

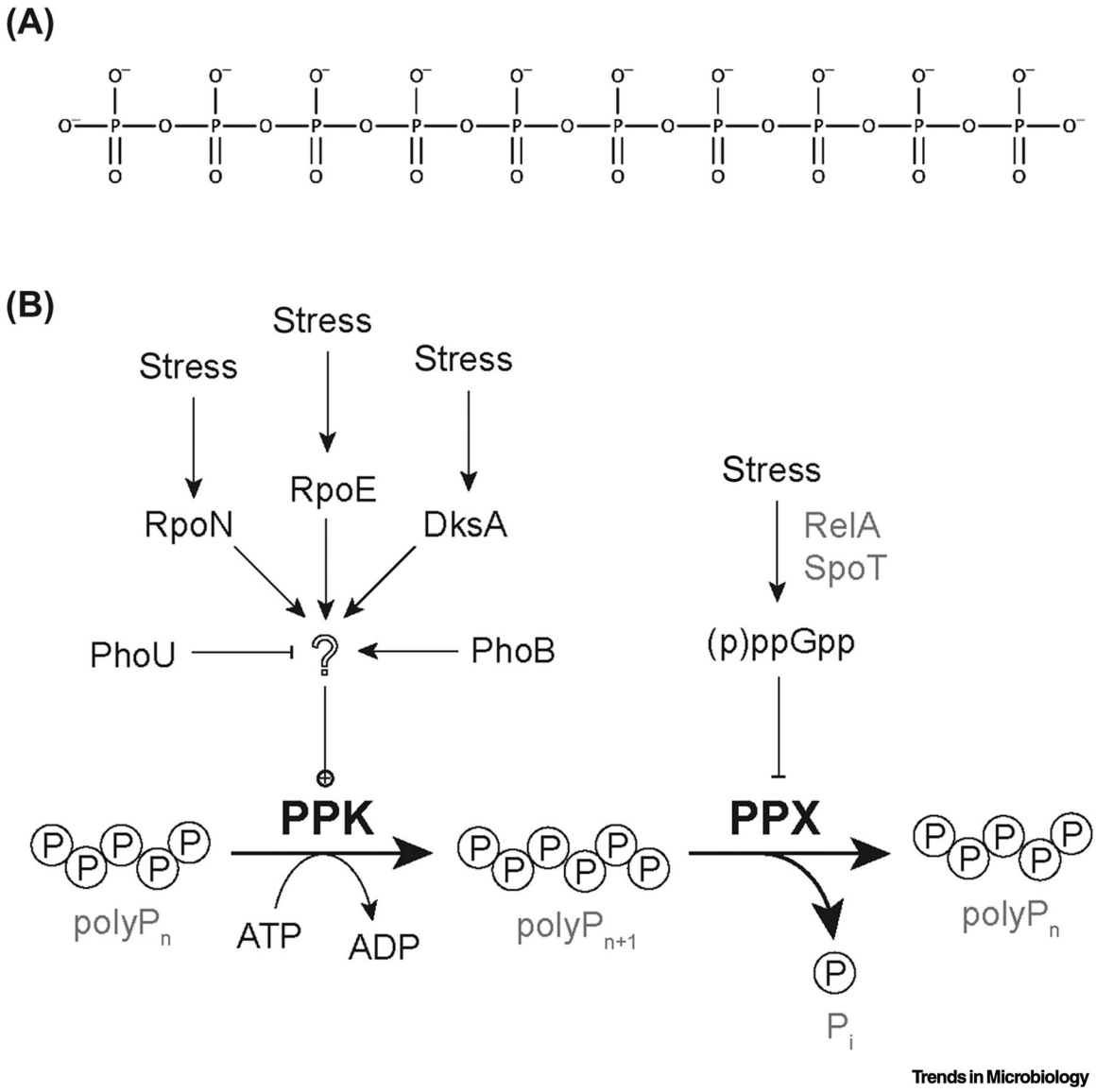


Figure 1. The structure and regulation of polyP in bacteria.

(A) The structure of polyP. A short polyP molecule (polyP$_{10}$). Bacterial polyP can be up to 1000 phosphate units long, while eukaryotic polyP is usually substantially shorter. (B) The current model for polyP regulation in *Escherichia coli*. PolyP is synthesized by polyP kinase (PPK) and degraded by exo-polyPase (PPX). Starvation stress stimulates **RelA** and **SpoT** to synthesize (p)ppGpp, which inhibits PPX, but does not influence PPK activity and is not required for induction of polyP synthesis. Induction of polyP synthesis does depend on the transcription factors RpoN, RpoE, DksA, and PhoB, although none of these activate transcription of the *ppk* gene itself. PhoU is a negative regulator of both phosphate transport and polyP synthesis. None of the mechanism(s) by which these regulators affect PPK activity are currently known.

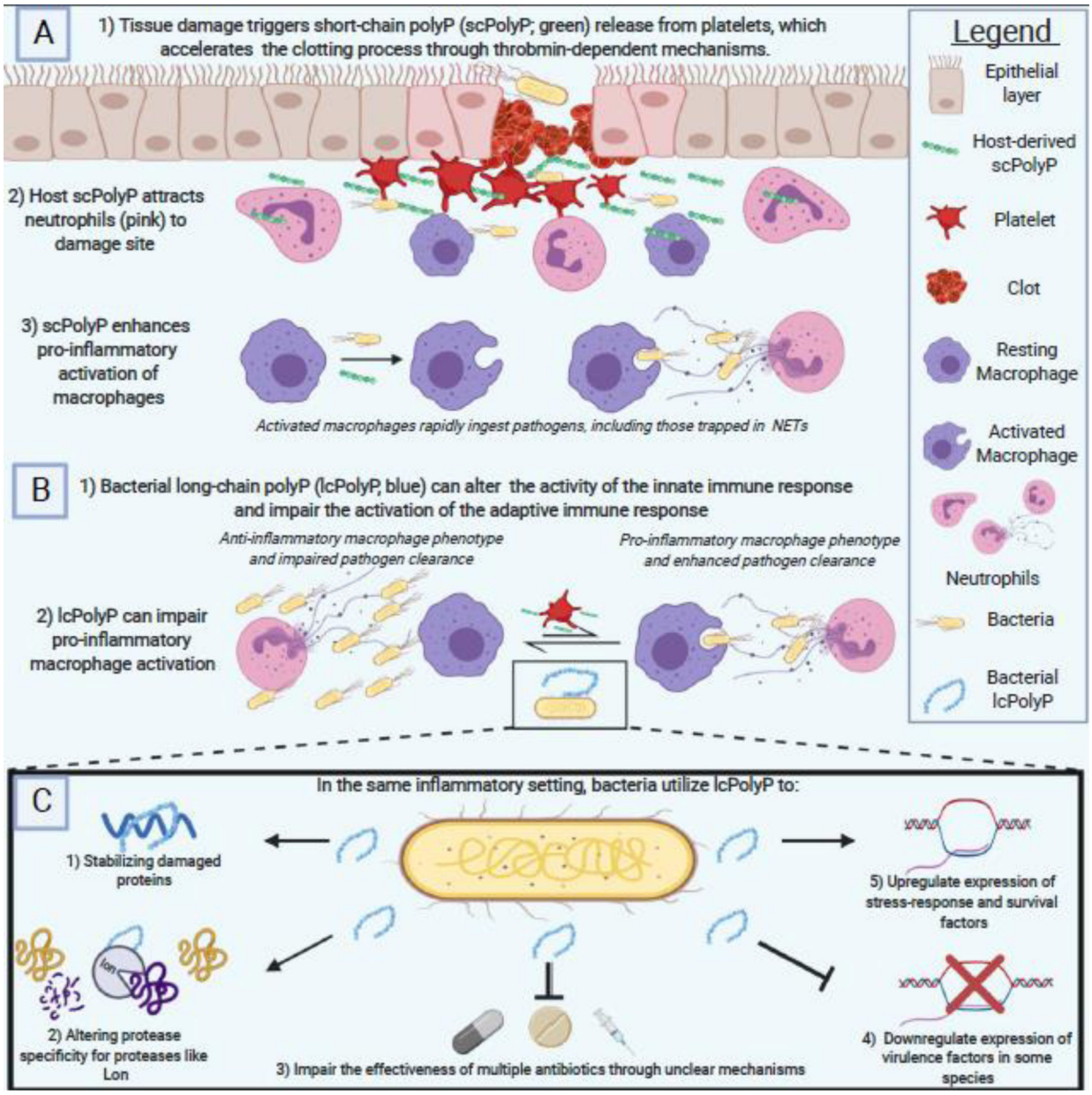


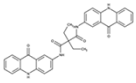
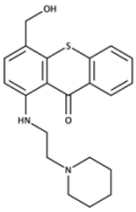
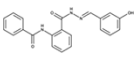
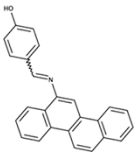
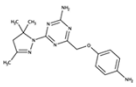
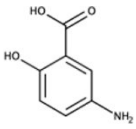
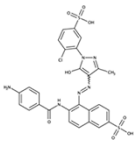
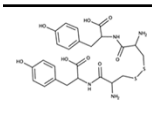
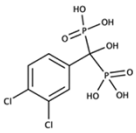
Figure 2. The PolyP Wars: The Struggle Between Host and Pathogen PolyP.

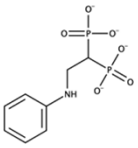
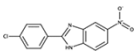
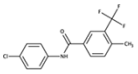
A) Host polyP utilization begins as soon as damage is detected, with platelets releasing short-chain polyP (scPolyP) to accelerate thrombin-dependent clotting and neutrophil recruitment. Host polyP also drives macrophage differentiation into the pro-inflammatory M1 phenotype that facilitates rapid phagocytosis and clearance of pathogens. B) Meanwhile, pathogenic bacteria utilize long-chain polyP (lcPolyP) to impair the host immune response, driving the anti-inflammatory M2 activation of macrophages while also impairing the expression of MHC Class II molecules to hamper the adaptive immune response. C) Internally, bacteria utilize lcPolyP for a wide variety of functions from stabilizing damaged proteins to regulating expression of crucial stress response and virulence factors. The two

sides clash in an age-old contest using an ancient biomolecule as their weapon of choice. Figure created using BioRender.

Table 1.

Structures and activities of PPK1 inhibitors

Structure	Chemical ID	Target Species	*In vitro* PPK1 Inhibition[a]	*In Vivo* Effects
	NSC618160	*P. aeruginosa*	+++	modestly reduced virulence in *D. discoideum* [81]
	NSC166366	*P. aeruginosa*	+++	reduced virulence in *D. discoideum* [81]
	NSC205574	*P. aeruginosa*	+++	strongly reduced virulence in *D. discoideum* [81]
	NSC141672	*P. aeruginosa*	+++	strongly reduced virulence in *D. discoideum* [81]
	NSC696924	*P. aeruginosa*	+++	strongly reduced virulence in *D. discoideum* [81]
	mesalamine (5-amino-salicylic acid)	*E. coli*	+	reduced polyP, mimics *ppk* phenotypes [87]
	NSC75963	*E. coli*	ND	mimics *ppk* phenotypes [85]
	NSC333714	*E. coli*	ND	mimics *ppk* phenotypes [85]
	[(3,4-dichlorophenyl) (hydroxy)phosphonatomethyl]phosphonate	*E. coli*	++	ND [80]

Structure	Chemical ID	Target Species	*In vitro* PPK1 Inhibition[a]	*In Vivo* Effects
	[2-(phenylamino)-1-phosphonatoethyl]phosphonate	*E. coli*	++	ND [80]
	2-(4-chlorophenyl)-5-nitro-1H-benzo[d]imidazole	*E. coli*	+	mimics *ppk* phenotypes, treats UPEC infections [86]
	N-(4-chlorophenyl)-4-methyl-3-(trifluoromethyl)benzamide	*E. coli*	+	mimics *ppk* phenotypes, treats UPEC infections [86]

[a] +++ = IC50 < 10 μM, ++ = IC50 < 70 μM, + = IC50 > 100 μM, ND = not determined.