Inorganic polyphosphate essential for lytic growth of phages P1 and fd

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Transduction frequency with phage P1 had been observed to be very low in *Escherichia coli* K-12 mutants lacking the operon (*ppk1-ppx*) responsible for the synthesis of inorganic polyphosphate (poly P). We now find that these mutants, for lack of poly P, are lysogenic for P1 and when infected with phage P1 produce only ≈1% the number of infective centers compared with the WT host. Both phage adsorption and release were unaffected. The host-encoded P1 late-gene transcriptional activator, SspA, failed to show the transcriptional increase in the mutant, observed in the WT. UV induction of a P1-infected mutant resulted in a 200-fold increase in the production of infectious phage particles. The lysogenized P1 (P1*mut*) and P1 progeny from the mutant host (Δ*ppk1-ppx*) produced plaques of differing morphologies, whereas P1 progeny from the WT yielded only small, clear plaques. Two discernable variants, one producing small and clear plaques (P1*small*) and the other large plaques with turbid rims (P1*large*), had broader host range and produced larger burst sizes in WT compared with P1. Transmission electron microscopy showed P1*mut* had contractile sheath defects. Thus, the lack of poly P/PPK1 in the mutant host resulted in the formation of defective P1 particles during intracellular growth. A filamentous phage, fd, also failed to produce plaques on a mutant lawn. Although fd adsorbed to the F-pilus, its DNA failed to enter the mutant host.

contractile sheath | lysogeny | lytic replication | Stringent Starvation protein A

Inorganic polyphosphate (poly P), a linear polymer of orthophosphate residues linked by high-energy phosphoanhydride bonds, ranges in length from tens to hundreds of residues. Poly P is evolutionarily conserved and has been found in all organisms (1, 2). Poly P accumulation may be very high in many bacteria and fungi, accounting for as much as 10–20% of dry weight (3, 4). Intracellular accumulation of poly P in these organisms is an essential response to stress and for survival (4, 5). The principal poly P-synthesizing enzyme in several prokaryotic species, including *Escherichia coli*, is polyphosphate kinase 1 (PPK1), which catalyzes the transfer of the terminal phosphate group of ATP to a growing chain of poly P (6). A null mutant of PPK1 is defective in transcription, cell motility, quorum sensing, biofilm formation, and survival (7–9). In *E. coli* infected with the temperate bacteriophage λ, the p_R promoter is negatively regulated by guanosine 3′,5′-bisdiphosphate (ppGpp), at the level of transcription (10). Phage P1 is a temperate phage like λ, but the P1 prophage exists as an episome (11). Although its replication in *E. coli* under stringent response has not been specifically examined, mutants in the stringent starvation protein A (*sspA*) are defective in P1 lytic replication (12). Previously, we observed a very low transduction frequency ($<10^{-6}$) when we attempted to transfer the *ppk1-ppx* mutation into *E. coli* and *Shigella* spp. (4, 5). To understand how the cell physiology of the *ppk1-ppx* null mutant affected P1 replication, we investigated the P1 lytic cycle in the mutant. We report here the episomal existence of P1 in our *ppk1-ppx*-null mutant and studies on its intracellular growth, morphology, and failure in lytic replication.

The filamentous phage fd is a member of the Ff single-stranded DNA phages that include M13 and f1. Infection is mediated by gene 3 protein (g3p), a minor coat protein (13). The D2 domain of g3p is required for binding to the F-pilus, whereas the D1 domain is required for interactions with TolA, the fd coreceptor needed for DNA entry (14–16). TolA is a component of the Tol-Pal membrane complex implicated in the import of group A colicins that effects cell death (17). In this study, we provide evidence for defect in fd DNA entry in a *ppk1-ppx*-null host that prevents any phage replication.

Results

Transduction of the Δ*ppk1-ppx* Mutation to a New Host Produces P1-Lysogenized Mutants. That the mutant CF5802 (MG1655 Δ*ppk1-ppx*) harbored a lysogenized P1 emerged from studies of P1 infection of WT and the mutant. Spent media obtained by centrifuging overnight (≈18 h) cultures of MG1655 (WT) and CF5802 in LB were plated on either a WT or CF5802 lawn. The WT spent medium did not produce any plaques on the mutant lawn, but that of the mutant produced large plaques with turbid rims on the WT lawn. This phage variant, designated P1*mut*, was present only in the mutant. Furthermore, as a lysogenized cell population, there was an equilibration of extracellular phage (EP) with the bacterial population. After most (99.6%) of the EP population was removed by washing the mutant cells grown overnight, the concentration of EP was assayed at various time intervals during growth of the washed cells in fresh medium. The EP concentration increased exponentially along with the cell number toward their respective equilibrium values achieved in overnight cultures: 3.1×10^6 pfu/ml for phage and 6.1×10^9 cfu/ml for the host. Thus, the CF5802 is a P1-lysogenized cell population. Kuroda *et al.* (18) initially introduced the Δ*ppk1-ppx* mutation into an *E. coli recBC sbc* strain, and subsequently transduced it into MG1655 by using P1 to construct CF5802; the acquisition of P1 was therefore due to transduction and lysogenization. Generally, the lysogenized P1 is curable by repeated streaking of colonies over several generations. However, the attempts to cure CF5802 were not successful (data not shown). Further, all Δ*ppk1-ppx*::K_m transductants obtained by transducing either MG1655 or LL1000 strains with phage propagated on CF5802 were found to carry lysogenized P1.

P1 Lytic Replication Differs in WT and the Mutant. To investigate P1 lytic replication in WT and in the mutant, spot tests were performed on the respective host lawns. The mutant host required a P1 concentration ≈10^5-fold greater than that of the WT to achieve visible plaque formation. To corroborate this

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Abbreviations: poly P, inorganic polyphosphate; PPK1, polyphosphate kinase 1; moi, multiplicity of infection; ppGpp, guanosine 3′,5′-bisdiphosphate; TEM, transmission electron microscopy.

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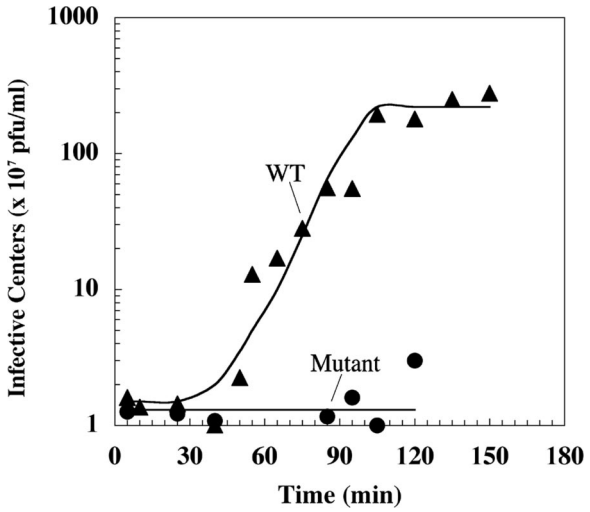

Fig. 1. One-step growth in the WT and the mutant hosts. P1 was used to infect the WT (▲) and the mutant (●) exponential phase cultures at an moi of 1:10. WT was used as indicator. These results are representative of three independent experiments.

observation, a one-step growth of P1 showed that the number of phage particles increased ≈100-fold in WT, but <3-fold in the mutant (Fig. 1).

P1 Adsorption Is Unaffected in the Mutant. P1 adsorption was measured by the increase in the number of infective centers in the cell pellets and by the decrease in the number of unadsorbed phage in the supernatant. Although the rates of decrease in unadsorbed phage in the two hosts were not significantly different (Fig. 2*A*), the mutant produced 40-fold fewer infective centers than the WT host (Fig. 2*B*). Thus, a postadsorption event is responsible for the decrease in lytic replication in the mutant.

Intracellular P1 in the Mutant Is UV-Inducible. The mutant was more sensitive to UV irradiation than the WT [supporting information (SI) Fig. 7]. However, the number of infective centers increased >200-fold in the mutant, whereas that of the WT increased <4-fold (Table 1). The modest rise in the phage titer in the WT was because most cells were P1-lysed before UV irradiation, whereas lysis in the mutant was due to the induced phage.

Transcription of *sspA* Is Altered in the Mutant. sspA is a host-encoded transcriptional activator of P1 late genes (19). To determine whether the level of this protein in the mutant is altered, a promoter fusion between P*ssp* and the β-gal gene was introduced into the WT and mutant by plasmids. The levels of β-gal activity, as an indicator of *sspA* transcription showed that it was significantly higher ($P < 0.01$) in WT than in the mutant during stationary phase (Fig. 3).

P1 Superinfection in the Mutant Produced Different Plaque Morphologies. P1 yields plaques with specific morphologies depending on the phage lysate and the host it infects. P1*mut*, isolated from the mutant host, produced mostly large plaques with a turbid rim on the WT lawn, and the P1 lysate produced small clear plaques on both WT and mutant lawns (data not shown). Although P1 propagated in the mutant host produced small clear plaques on the same host, two distinct plaque morphologies were visible when tested on the WT lawn (data not shown). One of the plaque types was indistinguishable from the small clear plaque (P1*small*) produced by P1 grown in WT; the other plaque type (P1*large*), which varied from 10% to 20% in number, was larger in size and

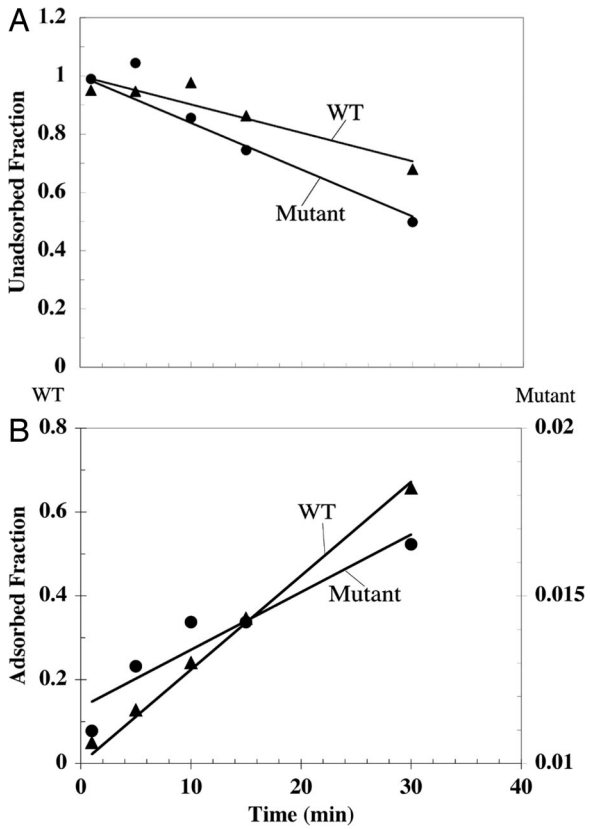

Fig. 2. P1 adsorption is unaffected in mutant hosts. The number of P1 in the supernatant (*A*) and pellet fractions (*B*) of infected WT (▲) and the mutant (●) exponential phase cultures within the first 30 min of infection were determined by plaque assays and normalized with respect to the initial number of phage in the supernatant. The decrease in P1 in the supernatant was not statistically significant ($P = 0.345$) using a paired *t* test at 95% confidence level.

contained a turbid rim. The infections using P1 and P1*mut* on WT and the mutant, and their corresponding plaque morphologies are represented in SI Fig. 8. Although the two P1 variants emerged from the mutant, they are not separate genotypes. Individual plaques picked from the mutant lawn, when plated on WT lawn, gave rise to both plaque morphologies. Thus, the mutant propagates a heterogeneous population of P1 variants.

P1*large* is supervirulent in the WT host, but both P1*large* and P1*small* variants replicate less efficiently in the mutant host. Inasmuch as the host range and burst size of a phage progeny may vary, the P1 variants from the mutant host were examined with spot tests and one-step growth. Spot tests showed that P1*large* required a $\approx 10^7$-fold higher concentration to produce individual plaques on the mutant lawn than on WT lawn. These observations suggest that a variant of P1 may be selected to overcome lytic deficiencies in the mutant, and that this phage

Table 1. UV-induced P1 lysis

	Ratio (infective centers/bacterial cells)	
	WT host	Mutant host
Before (1 h) UV induction	2.1	0.0028
After (1 h) UV induction	7.5	0.65
Factors of change	3.6*	230

The hosts were infected with P1 before UV induction.
*A large fraction of WT cells were already P1-lysed before the UV induction at 1 h.

BIOCHEMISTRY

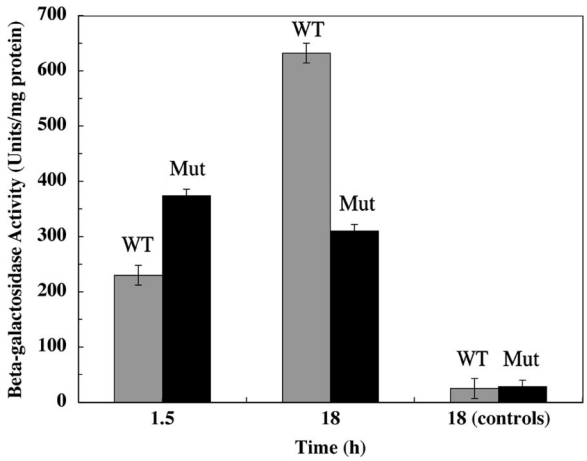

Fig. 3. Transcription pattern of *sspA* is altered in the mutant. β-Gal activity was measured at 1.5 and 18 h in WT (gray) and in the mutant (black) transformed with pLL101. WT and mutant without pLL101 were used as controls.

population shows hypervirulence in WT. One-step growth experiments were performed for the phage variants using either WT or the mutant as hosts. P1 propagated in WT and P1*small* and P1*large* with a similar latent period and the rise period beginning at ≈45 min. However, the burst sizes differed significantly: P1 had a low burst size of ≈10 pfu/cell; the burst size of P1*small* was $\approx 10^2$ pfu/cell, but that of P1*large* was $\approx 10^4$ pfu/cell.

In the mutant host, P1 exhibited a low burst size as in WT, ≈10 particles per cell, but P1*small* and P1*large* both did not release any significant number of phage particles within the time assayed (160 min). For P1*large*, the lack of phage production was consistent with its low lytic ability as seen by the spot test. Although P1*small* showed clear lysis on the spot test, it lacked phage production in a one-step growth experiment. Thus, the replication of this phage variant in the mutant was most likely delayed. In both cases, P1 variants from the mutant were defective in replicating in its host even though both showed enhanced lytic ability in WT.

Both P1*large* and P1*small* Have a Broader Host Range Compared with P1. The host ranges of P1*large* and P1*small* were compared with that of P1 by examining their lytic ability. Dilutions of phage lysates were spotted onto selected bacterial lawns. The P1 lysate prepared from the mutant host, as well as P1*large* and P1*small* lysates prepared from WT, produced single plaques on the lawn of a host lacking SspA (DJ626). Moreover, P1 lysate from the mutant, P1*large*, and P1*small* were able to infect the lysogenic strain BW14333 containing prophage P1*cm.clr100*, whereas the P1*vir* could not.

P1*mut* and P1 Have Different Structural Morphologies. The genomic difference between P1*mut*, the lysogenized P1 in the mutant host, and P1 is accompanied by changes in structural morphology of the phage. The transmission electron microscopy (TEM) images (Fig. 4*A*) show that P1 has the classical features, an icosahedral head attached to one end of a sheathed tail tube and six kinked tail fibers to the other end (20). Two variants with distinguishable head sizes were observed in P1, as reported by Walker and Anderson (21). One variant had an average head size of 201 ± 11 nm, a thick and rigid contractile tube (47 ± 5 nm across), long tail fibers, and an average tail length of 567 ± 32 nm (Fig. 4*A Right*). The other variant had a smaller average head size of 154 ± 20 nm, a thinner contractile tube (33 nm across), short tail fibers, and a similar average tail length of 570 ± 40 nm (Fig. 4*A Left*). The larger structural variant constituted the majority of P1 particles (data not shown). P1*mut* also had a

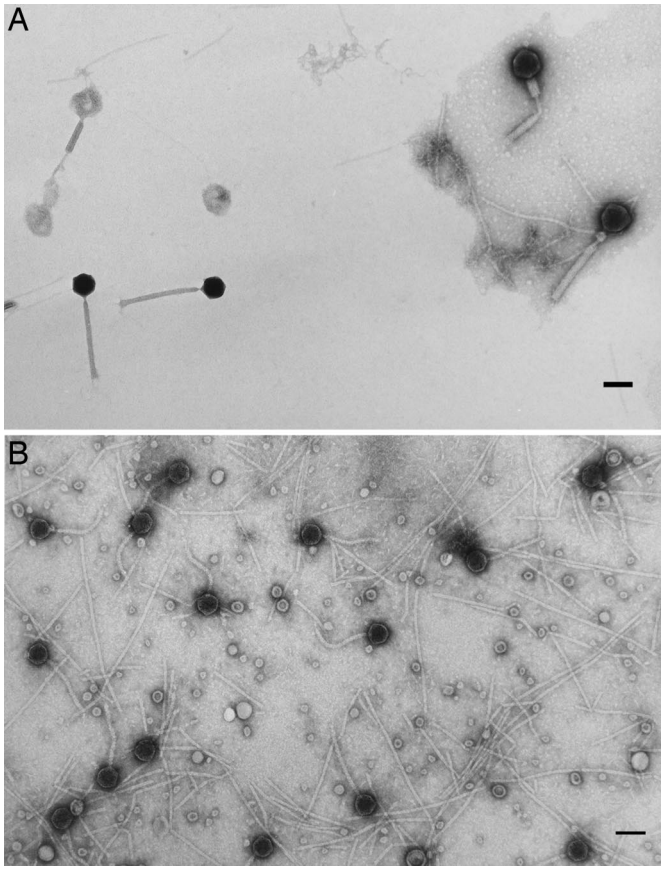

Fig. 4. TEM images of P1 and P1*mut*. P1 (*A*) and P1*mut* (*B*) were purified and viewed. (Scale bars, 200 nm.)

smaller average head size of 144 ± 10 nm, but its tail structure lacked an outer sheath (25 ± 3 nm across) and was slightly shorter (516 ± 66 nm) and more flexible (Fig. 4*B*). Furthermore, one phage particle (Fig. 4*A Right*) showed a broken tail structure, revealing the inner core of P1. The core averaged 22 ± 1 nm in diameter. Although P1*mut* also had the icosahedral head and the tail, its tail fibers were less discernible. Its head size was comparable to that of the smaller variant from the P1 lysate. The most notable difference from P1 was the absence of a thick outer tail sheath, allowing for more flexibility in the tail tube.

Phage fd Cannot Produce Plaques on the Mutant Lawn, and Its DNA Fails to Enter. Spot tests using fd phage did not show any plaque formation on the mutant LL1001 lawn, whereas plaques were visible even at the 10^{-12} dilution on the WT LL1000 lawn (data not shown). To determine whether fd enters the mutant host at all, P-32-labeled fd (see *SI Text*) was used to infect WT LL1000 and mutant LL1001. Although the mutant, as compared with the WT host, had significantly higher number of radioactive counts ($P < 0.05$) in the sheared F-pilus fraction, it had significant lower counts ($P < 0.05$) in the pellet fraction (Fig. 5), indicating that fd DNA was defective in entering the mutant.

TolA Gene Expression and Colicin-Mediated Cell Lysis Are Unaffected. To determine the expression of *tolA* in WT LL1000 and mutant LL1001, a promoter fusion between P*tolA* and the β-gal gene was introduced into hosts by plasmid pTC480. The levels of β-gal activity, as an indicator of *tolA* transcription (SI Fig. 9), were not significantly different between the two hosts. Next, to determine whether TolA is still functional, the cell lysis mediated by colicin A was measured by turbidity in exponential cultures of WT

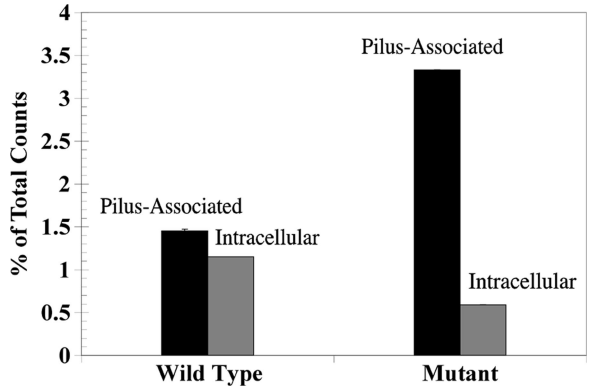

Fig. 5. Fd DNA entry. Fd DNA in the sheared F-pilus fraction (black) and the intracellular fraction (gray) was determined by radioactive counts and normalized with respect to the total counts.

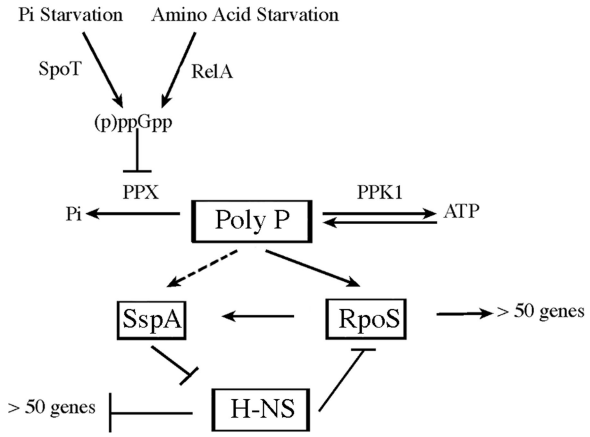

Fig. 6. Scheme for interactions among poly P, SspA, RpoS, and H-NS.

LL1000 and mutant LL1001 (data not shown). In both the early and late exponential phase, WT LL1000 and mutant LL1001 cultures responded similarly to the addition of colicin A, which prevented bacterial population increase.

Discussion

E. coli mutant lacking the *ppk1-ppx* operon fails to accumulate poly P (22), which, in effect, reduces resistance to several stresses including nutritional starvation, heat shock, oxidative stress, and UV irradiation (2). Phage infection may be perceived as a stress, because its replication draws on the resources of the host bacterium. In a rich medium like LB, the growth of the mutant was comparable to that of WT. However, the deficiencies in the mutant become visible only during stationary phase and nutritional stringency. Despite the resources that a rich medium can provide, phage infection diverts the resources from their use in cell maintenance and channels them for phage reproduction instead. Thus, the observation that the mutant host is deficient in supporting normal P1 lytic replication is consistent with the responses of the mutant to other stress conditions.

The presence of lysogenized P1 in the poly P-deficient mutant as a remnant of transduction may be attributed to lack of PPK1, which results in the depletion of intracellular poly P. Because P1 goes through lytic cycle when the multiplicity of infection (moi) is less than one, in P1 transduction, lysogenization in the mutant is favored. Spontaneous phage release into the surrounding media during growth of the mutant in LB suggested that lytic replication might still be functional, but P1 remained lysogenized in the mutant. Even after superinfecting the mutant with P1, phage particles could be recovered only upon UV induction. Additionally, an increased release of P1 in the mutant host could not be fully accounted for by the lysogenized P1, because mechanical lysis showed only a modest increase in P1 infective centers. Thus, lytic functions are still intact, even though they may be more inefficiently induced. The direct lack of poly P/PPK1, or the physiological changes resulting from it, reduces the lytic capacity of P1 in its host. Furthermore, because P1 lysogenizes in the mutant host, poly P/PPK1 may be a contributing factor in the lytic vs. lysogeny decision. A similar system of decision making has already been described in phage λ, which uses ppGpp, a metabolite that is involved in intracellular poly P accumulation (23).

Because infecting the mutant with P1 produced a few infective centers, the reduced lytic replication in this host must be due to more than lysogeny alone. Inasmuch as adsorption rates in WT and mutant hosts were not significantly different (Fig. 2*A*), the defect in replication in the mutant appeared to be in the postadsorption stages. One way in which poly P might affect P1 replication is in the modulation of the level of SspA, a host-encoded late-gene transcriptional activator of P1. Expression of *sspA* is positively regulated by *relA*, which is required for (p)ppGpp synthesis and is induced during nutritional stress and during stationary phase growth (24). Intracellular poly P also accumulates during nutritional stress that increases the (p)ppGpp level (18). SspA modulates NTP levels (25), inhibits the accumulation of histone-like protein H-NS during stationary growth, and confers acid resistance (26). PPK1 can serve as a nucleotide diphosphate kinase using poly P and consequently can influence NTP levels (27). Furthermore, poly P is involved in acid resistance (5) and as polyanion likely regulates the levels of histone-like proteins in chromosome condensation as well. Thus, poly P and SspA are likely to be involved in similar pathways.

Although P1 was defective in lytic replication in the *sspA* mutant, its lysogenic growth, DNA replication, and transduction efficiency were unaffected (12). SspA is a transcriptional activator of P1 late genes by facilitating the σ^S-independent binding of RNAP to the P1 late promoter P*s* (12, 19). Consequently, P1 replication may be sensitive to the intracellular level of SspA. Although the Δ*ppk1-ppx* mutant had higher *sspA* transcription in the early exponential phase compared with the WT, it had significantly lower transcription in the stationary phase. Possibly the transcriptional differences are due to the lysogenized P1, which represses *sspA* transcription to deter the lytic cycle. However, more likely, the *sspA* level differs from the WT that induced P1 lysogeny during transduction. Poly P induces *rpoS* (3), which is required for the expression of genes including *sspA* specific for stationary phase of growth. Combined with results from previous studies on the relationships between SspA and H-NS, poly P and RpoS, and H-NS and RpoS (3, 26, 28), we propose a scheme of interactions among these four components (Fig. 6). In Fig. 6, poly P may directly or indirectly up-regulate the levels of SspA and RpoS. SspA, in turn, down-regulates the H-NS level, which releases the inhibitory effect of H-NS on RpoS. Because of the interactions between poly P and these global regulators, the poly P-deficient mutant is likely to have undergone significant physiological and transcriptional changes.

The phage progeny produced by the mutant, whether from induced P1-lysogen or by P1 superinfection, displayed altered plaque morphology, generally larger in size compared with that produced from P1 progeny of WT. Progeny from the mutant may be more virulent than from P1 in the WT. This modification of the host was reflected on during the multiplication of phage in this host lacking poly P. To overcome lytic defects, modifications may be introduced to improve replication efficiency. The turbid rims of the large plaques indicate the persistency of lysogenesis.

Table 2. Bacterial strains, plasmids, and phages

Strain name	Relevant genotype	Ref.
Bacteria		
MG1655	F⁻ arcA⁻1655 fnr⁻1655	18
CF5802	MG1655 Δppk1-ppx::Km	18
DJ626	MG1655 ΔsspA::Neo	19
LL624	MG1655 Δppk1-ppx::Km	This study
BW14333	DE3(lac) X74Δ (phoA532)	19
P1Cmclr.100		
LL1000	*E. coli* K-12; Hfr C	American Type Culture Collection (ATCC) 15669
LL1001	LL1000 Δppk1-ppx::Km	This study
W3110	F⁻; contains pColA9	43
Plasmids		
pLL101	pQF50 Pssp-lacZ	This study
pTC480	pJEL250 ϕtolA-lac at 2735	44
Phages		
P1	P1kc, P1vir	ATCC 25404-B1
P1small, P1large	Derived from infecting CF5802 with P1	This study
P1mut	Lysogenized in CF5802	This study
Fd	Filamentous phage	ATCC 15669-B2

Examining the lytic cycle of the phage variants, P1*large* and P1*small*, we found that these phages exhibited greater burst sizes than did P1 grown in the WT. Although increased burst size can account for the large plaque sizes, the possibility remains of a delayed or extended replication cycle for P1*small*.

P1 produced from the mutant has an altered tail structure. Inasmuch as transcription of the tail structure genes is late in phage replication, it is likely that SspA regulates these genes; lack of SspA may have affected some of these genes. TEM showed P1*mut* lacking an outer tail sheath present in P1. The gene encoding a sheath protein is gp22 (20), but relatively little is known about the function of this protein in P1. However, the well studied phage T4 may offer insights into the role of the outer sheath, which assembles last, after that of the base, the tail fibers, and the inner core. The assembly of the sheath starts from the base and works its way up toward the phage T4 head (29). The T4 tail protein is also suggested to bind GTP (30). Assuming that the outer sheath composition and assembly of P1 are similar to those of T4, then the assembly of the sheath proteins may require poly P as a component or as a scaffold for sheath polymerization.

In addition, phage and its respective bacterial host coevolve. Comparative studies have shown that genomes of double-strand DNA tailed phages are highly mosaic, mostly due to horizontal gene transfers. These exchanges may introduce new functions such as morons (i.e., autonomously replicating genes), homologous functions, and analogous functions into the phage genome (31, 32). Bacteria also acquire phage genes from lysogenized phages and from chance recombination with the components of the phage genome (33). Moreover, the bacteria develop resistance by altering their receptors or their restriction-modification system (34, 35). Because phage replication depends on the bacterial host physiology, changes in the host population would introduce new selection pressures on the phage. Thus, poly P deficiency, which leads to defects in various stress pathways and cellular processes, is likely to influence phage replication as well.

The filamentous phage fd, as another example, failed to produce plaques on mutant lawn. Although fd adsorbed to the F-pilus, as seen from radiolabeling in the pilus fraction, the DNA failed to enter the host. Because the expression of TolA and the colicin A-mediated cell lysis were unaffected in the mutant, it is likely that the function of TolA as a coreceptor is unaffected. The *ppk1* mutants in *Pseudomonas aeruginosa* (8) and *Myxococcus xanthus* (36) are both defective in motility that requires type IV pili. Considering the similarity of the F-pilus to type IV pili in assembly and disassembly, it may be that the defect lies in the transfer of fd phage from the pilus tip to the coreceptor. The defect may involve pilus retraction in pulling fd toward the host membrane or may be in the fusion between fd and the host cell. Because PPK1 associates with the bacterial membrane, the enzyme, or its product poly P, may interact directly with membrane components required for pilus disassembly or for membrane fusion.

Finally, in view of the ubiquity and importance of poly P in cellular metabolism, its role in phage replication may be widespread. Thus, poly P may be crucial in the replication of other phages as well, with specific roles depending on the specific phage and the host. As phages coevolve to use host components necessary for the survival of the host in its natural environment, phage replication will likely depend on the highly conserved poly P. Moreover, this conservation may extend to its role in the virulence and latency of viruses in higher organisms. Indeed, certain species of poly P has already been shown to inhibit HIV-1 adsorption in mammalian cells (37).

Materials and Methods

Bacterial Strains, Phages, and Plasmids. The *E. coli* strains, phages, and plasmids used are in Table 2. MG1655 (WT) is a K-12 strain, a derivative of W1485 (38). P1 was propagated in MG1655 grown on LB plates and was used for all transduction experiments (39).

Construction of pLL101. The promoter region P*ssp* of the *ssp* operon (40) was amplified from genomic DNA of MG1655 using the primers 5′-GCGAGGATCCGTCAACTATTTCAGACT-3′ (forward) and 5′-GCGGAAGCTTTGGCCAGTCAGAAGTTGTGT-3′ (reverse), containing BamHI and HindIII restriction sites, respectively, at the 5′ end. The PCR fragments cloned into Topovector were transformed into "One Shot" (Invitrogen, Carlsbad, CA) *E. coli* cells. Subsequently, the fragments produced after BamHI and HindIII digestion of the vector containing the insert were ligated into plasmid pQF50. The sequence (40) of P*ssp* in pLL101 was confirmed by DNA sequencing (Cell Imaging Core Facility, Stanford University, Stanford, CA).

Media and Growth Conditions. *E. coli* was grown aerobically overnight (≈18 h) at 37°C in LB broth. The media used for the mutant contained kanamycin (50 μg/ml). Cells of the overnight cultures were inoculated into 125-ml Erlenmeyer flasks contain-

ing 12.5 ml of fresh media with an initial OD_{540} of ≈0.05. The cultures were then grown at 37°C, aerated at 250 rpm in a Gyrotory Water Bath Shaker Model G76 (New Brunswick Scientific, Edison, NJ), and monitored by measuring turbidity at 540 nm.

One-Step Growth. The procedure was based on that of Hadas *et al.* (41). Cultures were grown in LB broth to exponential phase (OD_{540} ≈0.3) as described above. Phage was then added with an moi of 0.5, unless otherwise indicated. After 4 min of incubation at 37°C, sodium citrate was added to a final concentration of 0.01 M. The infected cultures were centrifuged at 13,000 rpm for 3 min in a Beckman Microfuge Lite tabletop centrifuge (Beckman Coulter, Fullerton, CA), and the supernatant was discarded. The pellets were resuspended in 1 ml of LB broth, and after 4 min, each culture was diluted 10^4-fold to a final volume of 10 ml in a 125-ml Erlenmeyer flask. The flasks were incubated at 37°C, and at various time intervals, a 0.5-ml sample was withdrawn, diluted if necessary, mixed with 0.1 ml of the chosen host cells contained in 3 ml of 0.6% LB top agar, and plated on LB agar. The plates were incubated at 37°C for 12 h, and plaques were counted.

UV Induction of Phage P1. Cultures were grown in LB broth to exponential phase (OD_{540} ≈0.3) as described above. Phage P1 at an moi of 1:10 was added to these cultures, which were then incubated at 37°C with agitation. Samples (0.5 ml) of each culture were removed after 10 min, suitably diluted, mixed with 3 ml of 0.6% LB top agar, and spread evenly on LB plates for bacterial colony count. Part of the culture (3 ml) was treated with sodium citrate (10 mM final concentration) to inactivate unadsorbed phage. The supernatants, after appropriate dilution, were plated on a WT lawn to enumerate unadsorbed phage. The pellets were resuspended in 10 mM $MgSO_4$ and exposed to UV radiation. Both phage titers and bacterial counts were determined before and after UV treatment.

***β*-Gal Activity Assay.** The assay was performed as described by Zhang and Bremer (42).

TEM. Purified phage particles (see *SI Text*) stained with uranyl acetate were examined (Cell Imaging Core Facility).

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