Phylogenetic and Functional Analysis of the Bacteriophage P1 Single-Stranded DNA-Binding Protein

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Bacteriophage P1 encodes a single-stranded DNA-binding protein (SSB-P1), which shows 66% amino acid sequence identity to the SSB protein of the host bacterium *Escherichia coli*. A phylogenetic analysis indicated that the P1 *ssb* gene coexists with its *E. coli* counterpart as an independent unit and does not represent a recent acquirement of the phage. The P1 and *E. coli* SSB proteins are fully functionally interchangeable. SSB-P1 is nonessential for phage growth in an exponentially growing *E. coli* host, and it is sufficient to promote bacterial growth in the absence of the *E. coli* SSB protein. Expression studies showed that the P1 *ssb* gene is transcribed only, in an *rpoS*-independent fashion, during stationary-phase growth in *E. coli*. Mixed infection experiments demonstrated that a wild-type phage has a selective advantage over an *ssb*-null mutant when exposed to a bacterial host in the stationary phase. These results reconciled the observed evolutionary conservation with the seemingly redundant presence of *ssb* genes in many bacteriophages and conjugative plasmids.

Single-stranded DNA-binding proteins (SSBs) have been found in all investigated organisms from bacteria to humans (7, 36). These proteins have no enzymatic activity, but as their name indicates, they bind nonspecifically and stoichiometrically to single-stranded DNA, hold the single strands in an open conformation, and protect them from degradation by nucleases. In *Escherichia coli*, the assembly and propagation of the DNA replication fork, RecA-mediated homologous recombination, and the DNA base excision repair mechanism depend on the presence of SSB (see Chase [6], Meyer and Laine [29], and Lohmann and Ferrari [26] for reviews on *E. coli* SSB). SSB is an essential protein, and a functional copy of an *ssb* gene is present in every viable bacterial cell. Despite this fact, many *E. coli* bacteriophages and conjugative plasmids encode their own SSBs (9). Most of these episomal SSBs show strong conservation of key residues important for SSB function (24), and among them, several were shown to be able to reverse the temperature-sensitive growth defect of an *ssb-1* mutant *E. coli* strain (9, 10, 12, 24, 29, 34). The exact function of the episomal SSBs, however, was unclear, and the fact that most of them appeared to be dispensable remained puzzling (7, 10, 12).

Under harsh natural conditions, bacteria more often than not encounter limited resources allowing only minimal or no growth (33). *E. coli* drastically adapts the expression of its genetic information in order to endure and survive such periods of stationary-phase growth (17, 48). The response of bacteriophages to such growth conditions varies (1). Some phages are unable to grow or acquire a stationary-phase dormant state, called pseudolysogeny (37), and resume growth once the

host cells obtain fresh nutrients (21, 37). Others seem to be able to grow, albeit with reduced efficiency compared to exponential growth conditions (19, 42). Large bacteriophages like T4 (20) or P1 (47) contain a plethora of genes which are not absolutely required for normal phage development under standard laboratory conditions. Though the function(s) of most of these genes is unknown, many are proposed to ensure the survival of the phage in different habitats or under various growth conditions (16).

In this study we tried to address the question of whether the *ssb* gene of bacteriophage P1 has an essential function or plays an accessory role. Our results indicated that the P1 SSB protein is evolutionarily distinct, but functionally equivalent, to the SSB protein of its host, *E. coli*. Expression studies showed that the production of SSB-P1 correlates with the onset of stationary-phase growth of the bacterial host but is independent of the host stationary-phase sigma factor RpoS (4, 22). Growth experiments demonstrated that wild-type P1 replicates more efficiently than a mutant phage carrying a disrupted *ssb* gene when the bacterial host was in the stationary phase. These results provided the base for a plausible model to account for the evolutionary conservation and preservation of the *ssb* gene in bacteriophage P1.

MATERIALS AND METHODS

Standard procedures. Standard DNA techniques, liquid media, and agar plates were used as described by Sambrook et al. (40). Antibiotics were added as appropriate at concentrations of 100 µg/ml for ampicillin, 50 µg/ml for chloramphenicol, 25 µg/ml for kanamycin, and 10 µg/ml for tetracycline.

Bacterial strains. The *E. coli* K-12 strains used were UT580 (F⁺ Tet^r *tra*Δ36 *lacI*^qΔ(*lacZ*)M15 *proA*⁺*B*⁺/*supD* *thi* Δ(*lac-proAB*) (13), wild-type strains WA3110 (16) and MC4100 (43), the *rpoS* strain RH90 (23), and strain RDP268/pRPZ146 (35). The last strain is a derivative of AB1157 (F[−] *thr-1* *leuB6* *proA2* *his-4* *argE3* *thi-1* *ara-14* *lacY1* *galK2* *xyl-5* *mtl-1* *tsx-33* *supE44* λ[−]) carrying a kanamycin cassette (*aphA*) inserted in the chromosomal *ssb* gene. This strain is only viable in the presence of a helper plasmid carrying an alternative copy of *ssb* (35).

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Sequence alignments. Available alignment methods generally assume the same differentiation in all parts of a protein and use the same gap penalties and weight matrices throughout a sequence. While the DNA-binding part of the SSB proteins is well conserved among most prokaryotes, the carboxy-terminal end is highly differentiated. Consequently, these two parts were aligned separately with CLUSTAL W (44) and adjusted manually. In order to improve the alignment, the data matrix was also divided into subsets of similar taxa, which were first aligned separately and later fused to form the complete data matrix. When nucleotide sequences were used in the phylogenetic analyses, they were first aligned with CLUSTAL W and then adjusted manually, to match the indels of the amino acid alignment.

Phylogenetic analysis. Minimum evolutionary trees and bootstrap consensus trees were constructed with PAUP*, version 4.0b8 [PAUP*, phylogenetic analysis using parsimony (*and other methods), D. L. Swofford, Sinauer Associates, Sunderland, Mass.], with maximum parsimony criteria. Gaps in the alignment were not included, and characters were not weighted before or reweighted during the analyses. The idea behind the phylogenetic analysis is that genes sharing the same variable character at the same site in a sequence are thought to be related, and the actual character is thought to be inherited by all from a common ancestor. A tree is constructed based on many such homologous characters in a way that tries to minimize the number of character state transitions. Characters that contradict the tree and indicate another phylogenetic relationship are said to be homoplasious. In general, homoplasy could be caused by selection (convergent evolution), recurring mutation, or recombination between distant genes in the tree. In our analyses, recombination among the taxa in a tree was detected by visual inspection of the distribution of informative characters and by a Sawyer's runs test (41) with the program geneconv. If one of two identical copies of a gene recombine with a third, more-distantly related gene, the first gene will be identical with the second up to the recombination point and with the third after that point. The first and second genes will be homologous on one side of the point and homoplasious on the other side. Homoplasious characters will consequently appear in clusters. The geneconv program performs pairwise comparisons of all nucleotide sequences, finds the longest identical fragment, and assesses the probability to get such a long fragment by Monte Carlo simulation. Two probabilities can be calculated, either by comparing lengths of fragments between randomly shuffled discordant sites in the actual pair only, called a pairwise *P* value, or with lengths randomly generated by using the entire alignment, called a global *P* value. Given enough time, extensive recombination will break up long identical fragments. We consequently allowed mismatches at the lowest level (*gscale* = 1) within fragments to make it possible to detect recombination events of different ages.

Since a strictly bifurcating tree could be misleading if there has been recombination between the genes of the taxa in a tree, yet another tree constructing method was applied. The method used by the program SplitsTree (2, 14) does not assume a bifurcating tree but tries to detect phylogenetically conflicting signals and constructs a figure that shows these more-complex relationships. If there has been recombination, this method ideally will represent the result in a net-like structure.

In the first round of analyses, all available SSB amino acid sequences were used. In later analyses, both the amino acid and the corresponding nucleotide sequences were analyzed for different subsets of taxa consisting of P1 and a selection of enterobacteria, transmissible plasmids of *E. coli*, and human pathogenic bacteria.

Plasmid replacement experiments. The strain RDP268/pRPZ146 (34) was transformed with either the compatible plasmid pHAL253, carrying the P1 *ssb* gene and conferring ampicillin resistance or the parent of pHAL253, pUC19 (46), lacking an *ssb* gene. Strains carrying pRPZ146 and either of the incoming plasmids were subjected to several rounds of purification in the presence of ampicillin but in the absence of tetracycline, which would select for pRPZ146. A strain, RDP268/pHAL253, which had lost the pRPZ146 plasmid could be isolated, but pRPZ146 could not be replaced by pUC19.

Construction of a P1 *ssb* strain. In order to assay whether the P1 *ssb* gene was essential for phage development, the P1 gene was inactivated by the insertion of a kanamycin-resistance cassette. To this end, a pACYC184 (46) derivative, carrying the P1 *ssb* gene with a kanamycin-resistance cassette inserted into the unique *Bgl*I site within *ssb*-P1, was constructed (see reference 24 for a detailed map of *ssb*-P1). This plasmid, pHAL254, was then transformed into a strain carrying the temperature-sensitive prophage P1c1ts (15). A culture of the resulting strain was grown at 30°C to an optical density at 600 nm of 0.2, and then the prophage was induced to lytic growth by a shift to 42°C. During phage development, general recombination between the plasmid- and phage-carried copies of *ssb*-P1 could occur, allowing the kanamycin-resistance cassette to be transferred onto the P1 chromosome. Following cell lysis, phage particles were isolated and

screened for the ability to transduce the kanamycin-resistance marker. Transducing phages were readily isolated, and the insertion of an 1,100-bp cassette within the *ssb* gene of P1c1ts-*ssb*::Km was confirmed by PCR (see below).

Growth phase-dependent expression of *ssb*-P1. The construction of plasmid pHAL252, carrying an *ssb*-P1::lacZ indicator fusion was described previously (24). The *rpoS* strain RH90 and the isogenic wild-type strain MC4100 were transformed with pHAL252. Fresh overnight cultures of the plasmid-carrying strains were diluted 1/1,000 and allowed to grow exponentially for five to six generations until they reached an optical density at 600 nm of 0.1. Aliquots were then removed at regular intervals and assayed for both optical density and β -galactosidase activity according to the method of Miller (31).

Mixed infection experiments. Exponentially growing cultures, cultures poisoned with chloramphenicol, and stationary-phase cultures, the latter aged for 96 h, of the host UT580, were infected with 1:1 mixtures of P1c1ts and P1c1ts-*ssb*::Km. The chosen multiplicity of infection was 0.01 or lower, in order to reduce the probability of single cells being infected by both phages. Five minutes after infection, the cultures were carefully washed to remove any residual, nonadsorbed phage particles. The cultures were then reincubated and assayed for both P1 DNA replication and the production of progeny particles.

P1-specific DNA replication was monitored with the help of a colony PCR protocol (45). Aliquots harvested at different times after infection served directly as templates in PCRs with the two previously described primers SSB1 and SSB3 (24). With all other parameters kept constant, the resulting amount of PCR product was expected to be directly proportional to the amount of template DNA present in the cultures. The two *ssb* alleles, wild-type *ssb* and *ssb*::Km, could easily be distinguished, as the latter carried an 1,100-bp insertion.

To monitor the production of viable phage particles, aliquots were harvested at different times after infection, extracted with chloroform to liberate phage particles not yet released by cell lysis, and assayed by titration (15). The genotype of plaque-forming phages was analyzed by isolating phage DNA from single plaques, which then served as templates in PCRs as described above. All PCR products were analyzed by standard agarose gel electrophoresis (40).

RESULTS

Phylogenetic analysis of *ssb*-P1. The presence of a functional copy of *ssb* in the genome of bacteriophage P1 could be rationalized if the phage gene were recently acquired from the host bacterium *E. coli*. To address questions about the evolutionary relationship between the phage and host genes, extended alignments of both *ssb* genes and SSB proteins were constructed. A maximum parsimony phylogenetic analysis of all available SSB amino acid sequences showed that the SSB of phage P1 was related to that of enteric bacteria and distinct from the SSBs of transmissible plasmids (Fig. 1). However, bootstrapping of the nucleotide sequences of the DNA-binding part of the gene for this subset of taxa revealed that this position in the tree was weakly supported. Thus, according to the analysis, P1 was not closely related to any particular bacterium but to enteric bacteria and bacteria in general. A closer look at the homoplasies revealed that they were clustered, indicating recombination between almost all taxa, as in many other cases of microbial genes (8, 30).

There was less homoplasy within the *ssb* genes of the transmissible plasmids of *E. coli* and only a few runs of homoplasious characters between some of them. The bootstrap value for the transmissible plasmid node was always close to 100%, which, together with the small amount of homoplasy, spoke for a monophyletic origin. Consequently, these genes were left out of the following analysis.

A Sawyer's runs test of P1 together with enteric bacteria did not result in any significant global fragments and only six significant ($P < 0.05$) pairwise fragments. However, one of these, a 23-bp-long fragment, was found in the pair P1-*Haemophilus influenzae*. This fragment was also identical to a significant fragment in the pair *E. coli*-*H. influenzae*. Apart from showing

FIG. 1. Phylogenetic tree. Nucleotide sequences of *ssb* genes were analyzed with the program PAUP*, version 4.0 (Sinauer Associates). The numbers on the branches indicate bootstrap percentages of 100 replicates. Only groups compatible with a 50% majority rule consensus during the bootstrap procedure were included in the tree.

that recombination had indeed occurred between these genes, the test also indicated that one of these recombination events had happened before the *ssb* genes of P1 and *E. coli* differentiated into separate genes.

The nucleotide sequences of P1 and the bacteria which appeared to be most closely related in the maximum parsimony phylogenetic analysis were analyzed with the program Splitstree in an attempt to visualize recombination between the *ssb* genes. In the resulting figure, the *ssb* gene of P1 was connected at a node in the middle of a net of several bacterial genes (Fig. 2), indicating a complex pattern of recombination between all taxa, including that of P1. The surrounding bacteria were those previously shown to have about the same degree of homoplasy in pairwise comparisons with P1. A simple χ^2 homogeneity test of the nucleotide base composition of *ssb* for the taxa shown in Fig. 2 resulted in a statistically highly significant difference ($P < 0.001$). The AT percentage in *ssb* of P1 was not like that of *E. coli* but more like that of *Vibrio cholerae*, but the test of the pair P1-*E. coli* did not result in a statistically significant difference ($P = 0.12$). The P1 *ssb* gene uses almost all codons, including some that are rare compared to those of *E. coli*, and had the greatest codon diversity of all the *ssb* genes. Thus, both methods of phylogenetic analyses and the codon usage pattern all indicated that *ssb*-P1 is an ancient gene of bacteriophage P1 and was not recently acquired from a host bacterium. The negative result of the Sawyer's runs test on recombination events between the phage and *E. coli* *ssb* genes implied that they apparently coexist without frequent recombination occurring between them.

SSB-P1 can substitute for *E. coli* SSB. Previous experiments showed that SSB-P1 was able to restore growth to a temperature-sensitive mutant of *E. coli* (29) at the nonpermissive temperature of 42°C (18, 24). The defect in the *E. coli* SSB, leading to the temperature-sensitive phenotype, interferes with the essential ability of SSB to form stable tetramers (29). As very small amounts of SSB-P1 were sufficient to restore growth, it was likely that SSB-P1 formed heterotetramers with the mutant *E. coli* SSB and thereby restored thermal stability (24). In

order to address the question of whether SSB-P1 could not only rescue an *E. coli* mutant but fully support *E. coli* growth in the absence of bacterial SSB, we made use of strain RDP268/pRPZ146 constructed by Porter et al. (34, 35). In this strain, the chromosomal copy of the *ssb* gene was inactivated by the insertion of a kanamycin cassette. Such an insertion is only tolerated in the presence of an alternative copy of an *ssb* gene, located on plasmid pRPZ146 for the above strain (34). The plasmid pHAL253 carries the P1 *ssb* gene under the control of its native promoter. By transforming strain RDP268/pRPZ146 with pHAL253, followed by selection for the maintenance of pHAL253 but no selection for pRPZ146, we managed to isolate strain RDP268/pHAL253. This strain was viable and showed a generation time of 32 ± 1 min, which was only insignificantly slower than the 30 ± 1 min observed for RDP268/pRPZ146. Also, in sensitivity to UV-irradiation, these two strains showed no significant difference (data not shown). The fact that *E. coli* can grow exclusively with SSB-P1 demonstrated that the host and phage proteins are functionally interchangeable.

P1 *ssb* gene is dispensable for phage growth in an exponentially growing host. In order to assay whether P1 might show a specific requirement for SSB-P1, we attempted to disrupt the phage gene by insertion of a kanamycin-resistance cassette (see Materials and Methods). A P1c1ts *ssb*::Km phage could readily be isolated. Figure 3 shows that the lysis profile of P1c1ts *ssb*::Km was not significantly different from the profile of the parent strain P1c1ts. The onset of lysis was slightly delayed for P1c1ts *ssb*::Km, but the lysis efficiency was not affected. Also, the burst size in a single-burst experiment, as shown in Fig. 3, did not differ between P1c1ts and P1c1ts *ssb*::Km. The latter phage showed no defect in lysogenizing host cells, and there was also no detectable difference in the growth rates of P1c1ts and P1c1ts *ssb*::Km lysogenic strains. Thus, bacteriophage P1 showed no specific requirement for SSB-P1 but could replicate exclusively by using the SSB protein provided by the host.

SSB-P1 is expressed during stationary-phase growth. Prior expression studies showed that the P1 *ssb* gene was strictly repressed by P1 immunity functions during lysogenic growth, and thus a role for SSB-P1 during lytic replication was postu-

FIG. 2. Splitstree. Distance tree generated by the program Splitstree (14). Branches are drawn proportionate to the Juke-Cantor distances, between taxa, of the nucleotide sequences of the DNA-binding part of the *ssb* genes. Gaps in the alignment were eliminated.

FIG. 3. Lysis curves. Cultures of the strains UT580 (P1c1ts) (triangles) and UT580 (P1c1ts *ssb::Km*) (squares) were grown to the exponential-growth phase at 30°C. At the time point indicated by the arrow, the cultures were shifted to an incubation temperature of 42°C in order to induce the temperature-sensitive prophages to lytic growth. The values shown represent averages of at least four independent measurements.

lated (24). Surprisingly, however, no expression of SSB-P1 could be detected during a single-burst growth experiment (24). The expression of *ssb*-P1 was therefore reexamined, assaying an *ssb*-P1::*lacZ* indicator fusion in the absence of any P1-specific proteins. Figure 4 shows the results of these experiments. SSB-P1 showed a growth phase-dependent expression profile. It was not expressed during exponential growth, but

expression started at the onset of, and persisted during, stationary-phase growth. Such an expression profile was reminiscent of genes regulated by the stationary-phase sigma factor *rpoS* (22). To test whether the expression of *ssb*-P1 was *rpoS* dependent, the indicator fusion was assayed in an *rpoS* mutant host. The observed expression pattern (Fig. 4) did not differ between wild-type and mutant hosts, indicating that *ssb*-P1 expression was *rpoS* independent.

SSB-P1 provides growth advantage during stationary-phase infections. As the above result strongly suggested a role for *ssb*-P1 during stationary-phase growth, we tried to address this question by using liquid infection experiments. The P1 isolates in our hands and in most laboratories around the world (25) showed poor growth when stationary-phase bacteria were infected. Using 10^6 phage/ml to infect 10^8 stationary-phase cells/ml, our optimal harvest was 200 phage/ml 3 h after infection. In comparison, when 10^6 phage/ml were used to infect 10^8 exponentially growing cells/ml, the yield was 10^8 phage/ml 1 h after infection. The low yield during the stationary phase made it theoretically problematic to get statistically relevant data, as already small differences in the culture conditions or in the presence of GASP (growth advantage in stationary phase) mutants (48) could significantly affect the progeny yield. However, with mixed infection experiments, these parameters could be kept constant for both phage strains, avoiding these problems.

When exponentially growing cells were infected with a 1:1 mixture of P1c1ts and P1c1ts *ssb::Km*, injected DNA of both

FIG. 4. Growth phase-dependent expression of *ssb*-P1. The wild-type strain MC4100/pHAL252 (squares) was compared to its *rpoS* derivative RH90/pHAL252 (triangles). The plasmid pHAL252 carries an *ssb*-P1::*lacZ* indicator fusion allowing the detection of *ssb*-P1 expression (24). Both growth curves (filled symbols) and LacZ expression levels (open symbols) are shown. The values represent averages of six independent measurements.

FIG. 5. Detection of injection and phage DNA replication. Host cells growing either exponentially or in the stationary phase were infected with either P1c1ts, P1c1ts-*ssb*::Km, or a 1:1 mixture of those two phage strains. Cell aliquots harvested at different times after infection served directly as templates in PCRs (see reference 45 and Materials and Methods). Amplified PCR products were separated by agarose gel electrophoresis, stained with ethidium bromide, and detected with UV.

phages could be detected by PCR, as shown in Fig. 5. A sample harvested 40 min after infection indicated that both phages were able to replicate about equally well. Progeny phage was harvested and allowed to form plaques on agar plates. A total of 100 plaques (20 each, picked from five independent infection experiments) were analyzed for their *ssb* genotypes. A 1:1 mixture (52:48) of P1c1ts and P1c1ts *ssb*::Km was found.

A different picture was observed for the stationary-phase infections. Again, a 1:1 mixture of both phages was used, and injection could be detected after 5 min (Fig. 5). However, in a sample harvested 180 min after infection, some replication of the wild-type phage DNA was detectable while no obvious replication of the mutant phage DNA was found. Once more, progeny phage was grown on plates, and 100 plaques were analyzed. A 9:1 mixture (87:13) of P1c1ts and P1c1ts *ssb*::Km was found. The number of mutant phage recovered from the stationary-phase cultures did not vary drastically between the five independent experiments (4, 3, 3, 2, and 1 per 20, respectively), arguing against the possible risk that rare events like a double infection might have skewed the result. Also, due to the very low progeny yield in these stationary-phase infection experiments, it was imperative to avoid the carryover of residual unadsorbed phage particles. Infected cells were therefore carefully washed 5 min following infection. That the phage analyzed indeed represented newly synthesized progeny particles was confirmed in a control experiment. Cells poisoned with chloramphenicol were infected with a 1:1 mixture of both phages as described above, and no progeny phage was produced, even after prolonged incubation, indicating that de novo protein synthesis was required in order to produce the

low yield of progenies found (data not shown). These experiments thus corroborated the idea that the presence of a functional *ssb* gene provides bacteriophage P1 with a selective advantage when exposed to a stationary-phase host.

DISCUSSION

The data presented in this report indicated that it is unlikely that the *ssb* gene of P1 has been recently laterally transferred from any bacterium or transmissible plasmid analyzed here. The gene does not cluster with any particular bacterial *ssb* gene in the phylogenetic analyses but is about equally different from all enteric bacterial genes. Inspection of parsimoniously informative characters showed runs of homoplasy indicating recombination between P1 and enteric bacteria as well as between different enteric bacteria. A Sawyer's runs test found several statistically significant fragments, but these could only explain part of the amount of homoplasy. The conclusion was that *ssb*-P1 probably originated in a bacterium ancestral to enteric bacteria after the split from other parasitic groups of bacteria but before the split of *E. coli* and *Salmonella enterica* serovar Typhimurium. However, recombination and recurring mutation have made it impossible to track down the exact evolutionary path. The results implied that P1 has harbored an *ssb* gene for a long time, maybe even since the split between enteric and other parasitic groups of bacteria.

Repeated and random recombination is difficult to detect and quantify (27). Figure 2 may not be an entirely correct picture of *ssb* evolution but it reflects the homology and homoplasy patterns better than a traditional bifurcating tree. This

tree building method is more suitable when recombination is reciprocal, i.e., when a homoplasious region is found in another taxon. In this case, such regions are very short, but the net-like structure is present even if P1 is removed, which indicated that the figure reflects a true relationship. Another feature was that the branch leading to P1 from the bacterial net is fairly long, which illustrated that SSB-P1 is about equally different from, or equally homoplasious to, the SSBs of all enteric bacteria.

Bacteriophages have a limited capacity for storing genetic information as a consequence of the space restrictions encountered in the viral particle. Genes with no selective advantage for the phage are expected to rapidly accumulate mutations and eventually be deleted. That bacteriophage P1 carries an ancient, intact copy of an *ssb* gene implies selective pressure to maintain it. Theoretically, P1 could show a specific requirement for its own SSB, but our results indicated that this is not the case. During a single-step growth experiment, the host-encoded SSB protein was sufficient to support phage growth. The distinct expression pattern observed for *ssb*-P1 pointed towards a role of the phage protein during infections targeting stationary-phase hosts. There is some evidence that bacteria like *E. coli* (32) and *Bacillus subtilis* (J. Bernhardt, personal communication) downregulate the expression of their *ssb* genes upon entry into the stationary phase, and the host SSB pool might thus be limiting for phage-specific DNA replication under such circumstances. The mixed infection experiments showed a clear-cut growth advantage of the wild-type phage over an *ssb* mutant, confirming the hypothesis that *ssb*-P1 provides a selective advantage during stationary-phase infections. P1 phage particles have no means of detecting the physiological state of their target cells prior to infection. Adsorption to and DNA injection into stationary-phase hosts thus readily occur. Increased DNA replication supported by the phage SSB protein appears to be sufficient to increase the number of phage particles produced in such an ill-fated infection cycle, thus increasing the general fitness of the phage.

Since the isolation of P1 half a century ago (3), phage stocks have been prepared almost exclusively in rich media. However, when grown on agar plates on a lawn of host cells (39), the last few infection cycles during plaque formation most likely encounter stationary-phase growth conditions. Thus, continued selection for the maintenance of *ssb*-P1 might be present even under laboratory conditions.

In the plasmid replacement experiment we could show that an *E. coli* strain carrying a chromosomal *ssb* deletion is perfectly viable in the presence of a plasmid-carried copy of *ssb*-P1. In retrospect, this result is surprising, as *ssb*-P1 turned out to be exclusively expressed at the onset of stationary-phase growth. However, the exact conditions that lead to the expression of *ssb*-P1 are not yet known. It is imaginable that the expression of *ssb*-P1 is triggered whenever the intracellular SSB pool drops below a critical threshold, maybe sensed via stalling replication forks. In RDP268/pHAL253, a pulse of SSB-P1 produced under such conditions might be sufficient to support growth for one or more cell division cycles, until the SSB pool is depleted again, triggering the next pulse of gene expression.

It would be interesting to study whether the *ssb* genes present on conjugative plasmids are expressed under condi-

tions similar to those of *ssb*-P1. Facilitated plasmid replication and conjugative transfer under stationary-phase conditions could provide the selective advantage necessary to account for the presence and conservation of these genes. The SSB protein specified by the fertility factor F might even play an as yet unappreciated role in the phenomenon called adaptive mutation (see reference 38 and references therein). SSB is essential for the SOS response (29), and the level of SSB-F produced in starving cells might thus well influence the outcome of SOS-controlled, recombination-dependent, genome-wide hypermutation (5, 11, 28).

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REFERENCES

1. Ackermann, H.-W., and M. S. DuBow. 1987. Viruses of prokaryotes. CRC Press, Boca Raton, Fla.
2. Bandelt, H. J., and A. W. Dress. 1992. Split decomposition: a new and useful approach to phylogenetic analysis of distance data. *Mol. Phylogenet. Evol.* **1**:242–252.
3. Bertani, L. E. 1951. Studies in lysogenesis. I. The mode of phage liberation by lysogenic *Escherichia coli*. *J. Bacteriol.* **62**:293–300.
4. Bohannon, D. E., N. Connell, J. Keener, A. Tormo, M. Espinosa-Urgel, M. M. Zambrano, and R. Kolter. 1991. Stationary-phase-inducible “gearbox” promoters: differential effects of *katF* mutations and role of σ^{70} . *J. Bacteriol.* **173**:4482–4492.
5. Bull, H. J., G. J. McKenzie, P. J. Hastings, and S. M. Rosenberg. 2000. Evidence that stationary-phase hypermutation in the *Escherichia coli* chromosome is promoted by recombination. *Genetics* **154**:1427–1437.
6. Chase, J. W. 1984. The role of *E. coli* single-stranded DNA binding protein in DNA metabolism. *Bioessays* **1**:218–222.
7. de Vries, J., and W. Wackernagel. 1994. Cloning and sequencing of the *Proteus mirabilis* gene for a single-stranded DNA-binding protein (SSB) and complementation of *Escherichia coli* *ssb* point and deletion mutations. *Microbiology* **140**:889–895.
8. Feil, E. J., E. C. Holmes, D. E. Bessen, M. S. Chan, N. P. Day, M. C. Enright, R. Goldstein, D. W. Hood, A. Kalia, C. E. Moore, J. Zhou, and B. G. Spratt. 2001. Recombination within natural populations of pathogenic bacteria: short-term empirical estimates and long-term phylogenetic consequences. *Proc. Natl. Acad. Sci. USA* **98**:182–187.
9. Golub, E. I., and K. B. Low. 1985. Conjugative plasmids of enteric bacteria from many different incompatibility groups have similar genes for single-stranded DNA-binding proteins. *J. Bacteriol.* **162**:235–241.
10. Golub, E. I., and K. B. Low. 1986. Derepression of single-stranded DNA-binding protein genes on plasmid derepressed for conjugation, and complementation of an *E. coli* *ssb*[−] mutation by these genes. *Mol. Gen. Genet.* **204**:410–416.
11. Harris, R. S., G. Feng, K. J. Ross, R. Sidhu, C. Thulin, S. Longrich, S. K. Szigety, P. J. Hastings, M. E. Winkler, and S. M. Rosenberg. 1999. Mismatch repair is diminished during stationary-phase mutation. *Mutat. Res.* **437**:51–60.
12. Howland, C. J., C. E. D. Rees, P. T. Barth, and B. M. Wilkins. 1989. The *ssb* gene of plasmid ColIb-P9. *J. Bacteriol.* **171**:2466–2473.
13. Hübner, P., P. Haffter, S. Iida, and W. Arber. 1989. Bent DNA is needed for recombinational enhancer activity in the site-specific recombination system *Cin* of bacteriophage P1. The role of FIS protein. *J. Mol. Biol.* **205**:493–500.
14. Huson, D. H. 1998. SplitsTree: analyzing and visualizing evolutionary data. *Bioinformatics* **14**:68–73.
15. Iida, S., and W. Arber. 1977. Plaque forming, specialized transducing phage P1: isolation of P1CmSmSu, a precursor of P1Cm. *Mol. Gen. Genet.* **153**:259–269.
16. Iida, S., R. Hiestand-Nauer, H. Sandmeier, H. Lehnher, and W. Arber. 1998. Accessory genes in the *darA* operon of bacteriophage P1 affect antirestriction function, generalized transduction, head morphogenesis, and host cell lysis. *Virology* **251**:49–58.
17. Ishihama, A. 1997. Adaptation of gene expression in stationary phase bacteria. *Curr. Opin. Genet. Dev.* **7**:582–588.

18. Johnson, B. F. 1982. Suppression of the *lexC* (*ssbA*) mutation of *Escherichia coli* by a mutant of bacteriophage P1. *Mol. Gen. Genet.* **186**:122–126.
19. Kokjohn, T. A., G. A. Sayler, and R. V. Miller. 1991. Attachment and replication of *Pseudomonas aeruginosa* bacteriophages under conditions simulating aquatic environments. *J. Gen. Microbiol.* **137**:661–666.
20. Kutter, E., K. Gachechiladze, A. Poglazov, E. Marusich, M. Shneider, P. Aronsson, A. Napuli, D. Porter, and V. Mesyanzhinov. 1995. Evolution of T4-related phages. *Virus Genes* **11**:285–297.
21. Kutter, E., E. Kellenberger, K. Carlson, S. Eddy, J. Neitzel, L. Messinger, J. North, and B. Guttman. 1994. Effects of bacterial growth conditions and physiology on T4 infection, p. 406–418. *In* J. D. Karam (ed.), *Molecular biology of bacteriophage T4*. American Society for Microbiology, Washington, D.C.
22. Lange, R., and R. Hengge-Aronis. 1991. Growth phase-regulated expression of *bolA* and morphology of stationary-phase *Escherichia coli* cells are controlled by the novel sigma factor σ^S . *J. Bacteriol.* **173**:4474–4481.
23. Lange, R., and R. Hengge-Aronis. 1991. Identification of a central regulator of stationary-phase gene expression in *Escherichia coli*. *Mol. Microbiol.* **5**:49–59.
24. Lehnher, H., J. D. Bendtsen, F. Preuss, and T. V. Ilyina. 1999. Identification and characterization of the single-stranded DNA-binding protein of bacteriophage P1. *J. Bacteriol.* **181**:6463–6468.
25. Lennox, E. S. 1955. Transduction of linked genetic characters of the host by bacteriophage P1. *Virology* **1**:190–206.
26. Lohmann, T. M., and M. E. Ferrari. 1994. *Escherichia coli* single-stranded DNA-binding protein: multiple DNA-binding modes and cooperativities. *Annu. Rev. Biochem.* **63**:527–570.
27. Maynard-Smith, J. 1999. The detection and measurement of recombination from sequence data. *Genetics* **153**:1021–1027.
28. McKenzie, G. J., R. S. Harris, P. L. Lee, and S. M. Rosenberg. 2000. The SOS response regulates adaptive mutation. *Proc. Natl. Acad. Sci. USA* **97**:6646–6651.
29. Meyer, R. R., and P. S. Laine. 1990. The single-stranded DNA-binding protein of *Escherichia coli*. *Microbiol. Rev.* **54**:342–380.
30. Milkman, R., E. A. Raleigh, M. McKane, D. Cryderman, P. Bilodeau, and K. McWeeny. 1999. Molecular evolution of the *Escherichia coli* chromosome. V. Recombination patterns among strains of diverse origin. *Genetics* **153**:539–554.
31. Miller, J. H. 1972. *Experiments in molecular genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
32. Moreau, P. L. 1987. Effects of overproduction of single-stranded DNA-binding protein on RecA protein-dependent processes in *Escherichia coli*. *J. Mol. Biol.* **194**:621–634.
33. Morita, R. Y. 1997. *Bacteria in oligotrophic environments. Starvation-survival lifestyle*. Chapman & Hall, New York, N.Y.
34. Porter, R. D., and S. Black. 1991. The single-stranded-DNA-binding protein encoded by the *Escherichia coli* F factor can complement a deletion of the chromosomal *ssb* gene. *J. Bacteriol.* **173**:2720–2723.
35. Porter, R. D., S. Black, S. Pannuri, and S. Carlson. 1990. Use of the *Escherichia coli* *ssb* gene to prevent bioreactor takeover by plasmidless cells. *Bio/Technology* **8**:47–51.
36. Raghunathan, S., C. S. Ricard, T. M. Lohman, and G. Waksman. 1997. Crystal structure of the homo-tetrameric DNA binding domain of *Escherichia coli* single-stranded DNA-binding protein determined by multiwavelength x-ray diffraction on the selenomethionyl protein at 2.9 Å resolution. *Proc. Natl. Acad. Sci. USA* **94**:6652–6657.
37. Ripp, S., and R. V. Miller. 1998. Dynamics of the pseudolysogenic response in slowly growing cells of *Pseudomonas aeruginosa*. *Microbiology* **144**:2225–2232.
38. Rosenberg, S. M. 1997. Mutation for survival. *Curr. Opin. Genet. Dev.* **7**:829–834.
39. Rosner, J. L. 1972. Formation, induction, and curing of bacteriophage P1 lysogens. *Virology* **48**:679–689.
40. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. *Molecular cloning: a laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
41. Sawyer, S. 1989. Statistical tests for detecting gene conversion. *Mol. Biol. Evol.* **6**:526–538.
42. Schrader, H. S., J. O. Schrader, J. J. Walker, T. A. Wolf, K. W. Nickerson, and T. A. Kokjohn. 1997. Bacteriophage infection and multiplication occur in *Pseudomonas aeruginosa* starved for 5 years. *Can. J. Microbiol.* **43**:1157–1163.
43. Silhavy, T. J., M. L. Berman, and L. W. Enquist. 1984. *Experiments with gene fusions*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
44. Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**:4673–4680.
45. Trower, M. K. 1996. A rapid PCR-based colony screening protocol for cloned inserts. *Methods Mol. Biol.* **58**:329–333.
46. Yanisch-Perron, C., J. Vieira, and J. Messing. 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene* **33**:103–119.
47. Yarmolinsky, M. B., and M. B. Lobocka. 1993. Bacteriophage P1, p. 1.50–1.61. *In* S. J. O'Brian (ed.), *Locus maps of complex genomes*. Cold Spring Harbor Laboratory Press, New York.
48. Zinser, E. R., and R. Kolter. 1999. Mutations enhancing amino acid catabolism confer a growth advantage in stationary phase. *J. Bacteriol.* **181**:5800–5807.