JOURNAL OF BACTERIOLOGY, Jan. 1993, p. 288–290
0021-9193/93/010288-03$02.00/0

Vol. 175, No. 1

NOTES

Host RecJ Is Required for Growth of P22 *erf* Bacteriophage

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Received 12 June 1992/Accepted 31 October 1992

Growth of bacteriophage P22 *erf* is known to require host RecA recombination function. We show that the RecA function is necessary but not sufficient to restore the plaque-forming ability of phage P22 *erf*; such mutant phage also requires host RecJ function. The residual efficiency of plaquing of P22 *erf* in a *recJ* background (0.03%) is completely abolished in *recJ recB* hosts (<0.001%), suggesting that the RecBCD nuclease can provide an alternative function allowing phage growth. One tentative explanation is that circularization of P22 *erf* DNA mostly proceeds through the RecF pathway of recombination; however, less efficient circularization via the RecBCD pathway may also occur. In a *recJ* background, lysates obtained upon induction of an *erf* prophage show reduced yield (10%), suggesting that growth of P22 *erf* may require host RecJ in a step(s) other than circularization of phage DNA.

The genome of bacteriophage P22 encodes a recombination function (Erf) which is necessary for growth on recombination-deficient mutants of *Salmonella typhimurium* (1, 12, 17, 20). The product of the *erf* gene is a single-strand-specific DNA-binding protein that can promote annealing of homologous single strands in vitro (13). In vivo, circularization of the infecting DNA appears to be the step for which recombination is required (1); in *recA* hosts infected with *erf* phage, DNA synthesis stops after one round of replication, suggesting that circularization is a topological requirement for late replication (1, 19). The RecA requirement for growth of *erf* mutants indicates that host RecA can promote recombination between the redundant ends of phage P22 DNA, thereby substituting for the phage-encoded recombinase (12). The experiments described below show that, in addition to RecA, growth of P22 *erf* phage also requires host RecJ function.

Effect of *recJ* mutations on plaque formation by P22 *erf* bacteriophage. Phage P22 *erf* mutants require host RecA function to form plaques (1). To determine whether other host recombination functions are needed in this process, we plated phage P22 *erf* on a series of recombination-deficient strains of *S. typhimurium* (listed in Table 1). Bacteriophage used were isogenic phages P22 HT105/1 (*erf*$^+$) and P22 NBP182 HT105/1 *erf* (*am*H1173), obtained from Nick Benson (Department of Biology, University of Utah, Salt Lake City). Culture media, growth conditions, and plaquing assays were as described in reference 9; further details are given in Table 2. P22 *erf*$^+$ showed similar efficiencies of plaquing (EOP) in the wild type and in recombination-deficient strains. In contrast, the EOP of phage P22 *erf* greatly decreased when the host strain lacked either RecA or RecJ (or both). The EOP of P22 *erf* on a *recJ* host showed a 30-fold reduction; moreover, the lytic plaques were extremely small. The combinations *recA recJ* and *recB recJ* caused a reduction in EOP of over 1,000-fold; a similar reduction is observed in a *recA* background. These results, summarized in Table 2, indicate that the RecA protein is necessary but not sufficient to restore plaque formation by phage P22 *erf*: host RecJ function is also needed.

Complementation of the plaque-forming defect of P22 Erf$^-$ phage by cloned *erf* and *recJ* genes. To confirm that the phage P22 *erf* plaque-forming defect observed in *recA* and *recJ* host strains was due to the lack of Erf function, we complemented the Erf defect with a recombinant plasmid containing the wild-type *erf* gene. The plasmid used was pTP531, a pMC7 derivative which contains the P22 *erf*$^+$ gene expressed from an inducible *tac* promoter (Table 1). Assays to complement *erf* phage with Erf product provided by pTP531 can be summarized as follows. (i) Efficient complementation always resulted in both increased EOP and increased plaque size. (ii) When the recipient strain contained plasmid pTP531, the EOP of phage P22 *erf* increased to wild-type levels (or above) in the presence of 1 mM IPTG (isopropyl-β-D-thiogalactopyranoside) (Table 2). Since the Erf recombinase has a RecA-like (but not RecJ-like) activity, it is not surprising that *recA* mutations are more efficiently complemented than those of *recJ*; increases in EOP over wild-type levels can be attributed to *erf* expression from the multicopy plasmid. (iii) A small but significant increase in the EOP of P22 *erf* was observed in the absence of IPTG, presumably because leaky transcription from the *tac* promoter occurred (data not shown). As expected, both the plaque-forming ability and the plaque size of phage P22 *erf* were restored when the host strain was a *recJ* mutant complemented with the *recJ*$^+$ plasmid pJC765 (strain TT15302; data not shown).

A model for the role of RecJ in phage P22 *erf* growth. RecJ nuclease, also known as Exo IX, is a single-strand, 5′ exonuclease (8). In *Escherichia coli*, RecJ is known to play a role in the RecF pathway of recombination (2–6). In *S. typhimurium*, RecJ appears to limit the activity of the RecBCD enzyme during P22 replication: in the absence of the phage-encoded anti-RecBCD function (Abc), host RecJ is required for plaque formation (9). An interesting observa-

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TABLE 1. Bacterial strains

Strain	Genotype	Reference or source
LT2	Wild type	Laboratory collection
TT11289	*recA1 srl-202*::Tn*10d*-Cm	J. Roth
TT13229	*recB497*::MudJ *hisD1447*	10
TT15302	*recJ504*::MudJ/pJC765 (*recJ*$^+$)[a]	9
TT16817	*recJ504*::Mud-Cm	L. Miesel
TT16846	*recB497*::MudJ *recJ504*::Mud-Cm *hisD1447*	9
SV1202	LT2/pTP531[b]	This paper
SV1203	*recA1 srl-202*::Tn*10d*-Cm/pTP531[b]	This paper
SV1204	*recB497*::MudJ *hisD1447*/pTP531[b]	This paper
SV1206	*recJ504*::Mud-Cm/pTP531[b]	This paper
SV1207	*recA1 recJ504*::Mud-Cm	This paper
KRS2321	*recF522*::Tn*5*	15

[a] Plasmid pJC765, obtained from S. T. Lovett, is a pBR322 derivative containing the wild-type *recJ* gene of *E. coli* (see reference 7). This plasmid confers ampicillin resistance.

[b] Plasmid pTP531, obtained from A. R. Poteete, is a pMC7 derivative containing the P22 *erf*$^+$ gene expressed from a *tac* promoter. This plasmid contains a *lacI*^{q} allele and confers tetracycline resistance.

tion is that *recBCD* mutations are epistatic to *recJ*, indicating that the inability of phage P22 *abc* to form plaques is directly dependent on the presence of the RecBCD nuclease. Presumably, RecJ function can limit the digestion of RecBCD-sensitive substrates produced during P22 *abc* replication either directly, by interacting with the RecBCD enzyme or protecting RecBCD-sensitive ends, or indirectly, by allowing P22 to replicate in a RecBCD-insensitive fashion (9). Consistent with this suggestion, Poteete and coworkers (12, 14) have shown that P22 Abc functions not only inhibit RecBCD enzyme activity and RecBCD-mediated recombination but also appear to activate the *E. coli recF* pathway of recombination.

In contrast to phage P22 *abc*, lack of both RecJ and RecBCD products shows an additive effect on growth of phage P22 *erf*: plaque formation is completely abolished in a *recJ recB* background, as it is in *recA*. A simple interpretation of these results is that growth of *erf* phage requires both RecJ and RecA; in the absence of RecJ, plaque formation occurs with lower efficiency and requires RecBCD and RecA.

Since RecJ is thought to be involved in presynaptic events which precede recombination via the RecF pathway (reviewed in reference 2), we propose that host-dependent circularization of P22 *erf* DNA mostly proceeds through the RecF pathway of homologous recombination. This is consistent with the ability of the RecF pathway to repair double-strand breaks (reviewed in reference 18). Generation of 3′ single-stranded ends at the redundant termini of the linear P22 DNA might create the substrate for the initial annealing reaction. In a *recJ* background, circularization might proceed through the RecBCD pathway. This pathway must also operate, albeit with low efficiency, in a RecF-proficient (*recA*$^+$ *recF*$^+$ *recJ*$^+$) strain, since the presence of a *recB* mutation causes a fivefold reduction in the EOP of P22 *erf* phage (Table 2). Thus, phage DNA might follow two alternative ways for circularization.

TABLE 2. EOP of phage P22 NBP182 HT105/1 *erf* (*am*H 1173) on *S. typhimurium* recombination mutants

Strain	Relevant genotype	Relative EOP[a]
LT2	*rec*$^+$	1
TT11289	*recA1*	$<10^{-3}$
TT13229	*recB497*::MudJ	0.2
KRS2321	*recF522*::Tn*5*	0.3
TT16817	*recJ504*::Mud-Cm	0.03
SV1207	*recA1 recJ504*::Mud-Cm	$<10^{-3}$
TT16846	*recB497*::MudJ *recJ504*::Mud-Cm	$<10^{-3}$
SV1202	LT2/pTP531 *erf*$^+$	6.0
SV1203	*recA1 srl-202*::Tn*10d*-Cm/pTP531 *erf*$^+$	7.3
SV1204	*recB497*::MudJ/pTP531 *erf*$^+$	5.4
SV1206	*recJ504*::Mud-Cm/pTP531 *erf*$^+$	1

[a] Relative EOP refers to the titers obtained on strain LT2, with a P22 *erf* phage broth containing around 10^7 PFU/ml, prepared as described by Maloy (11). All experiments were carried out in the presence of 1 mM IPTG. Only plates containing 50 to 500 lytic plaques were used for counts. Every relative EOP value is the average of three to seven experiments.

A test for the involvement of the RecF pathway in P22 *erf* circularization provided a seemingly contradictory result: the EOP of phage P22 *erf* in a *recF*::Tn*5* mutant of *S. typhimurium* showed a threefold reduction, in contrast to the 30-fold reduction found on a *recJ* host (Table 2). However, the contradiction may be only apparent, since P22 is known to contain a gene homologous to the *recF*-equivalent gene *orf* of bacteriophage lambda (16). Thus, the crucial test for our hypothesis would require the use of P22 *orf* mutants, which are not available.

Optimal phage growth upon induction of a P22 *erf* prophage requires host RecJ product. To ascertain whether host RecJ was required only for phage DNA circularization or also for other steps of phage growth, we determined the yield of P22 *erf* lysates obtained upon induction of isogenic *recJ*$^+$ and *recJ* lysogens. The rationale for this experiment was that prophage excision and circularization must proceed via a site-specific recombination event, thereby overcoming the need for nucleolytic processing at linear DNA ends. For prophage induction, overnight cultures were diluted 1/10 and incubated for 1 h; mitomycin (Sigma) was then added, at a final concentration of 2 mg/liter. Incubation was continued for 4 to 6 h. Phage was harvested when lysis was observed. The yield of plaque-forming phage was reduced 10-fold after induction of a *recJ* lysogen. Although such a reduction was smaller than that found upon infection, the existence of a 10-fold difference from the wild type suggests that optimal phage growth requires host RecJ product for a step(s) other than DNA circularization. The simplest hypothesis is that RecJ may also play a role in phage replication, as previously suggested for growth of P22 *abc* mutants (9).

Two of us (M.J.M. and J.C.) became interested in recombination while in the laboratory of John Roth, to whom we are deeply indebted for his advice, encouragement, and support. We also thank Nick Benson, Anthony Poteete, Susan Lovett, and Lynn Miesel for providing strains and Andrés Aguilera for critical reading of the manuscript.

A.G. is the recipient of a predoctoral fellowship from the Regional Government of Andalusia, Spain.

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