Phage λ Red-Mediated Adaptive Mutation

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Replacement of the *recBCD* genes of *Escherichia coli* with the *red* recombination genes of bacteriophage lambda results in a strain in which adaptive mutation occurs at an elevated frequency. Like RecBCD-dependent adaptive mutation, Red-mediated adaptive mutation is dependent upon *recA* and *ruvABC* functions.

Adaptive mutation is a process by which an organism under nonlethal selective pressure produces mutations that relieve the selective pressure (see reference 5 for a review). It is illustrated particularly well by *Escherichia coli* strain FC40, a strain bearing an F′ episome carrying a *lac* allele with a frameshift mutation that makes it phenotypically Lac⁻. When FC40 is plated on lactose-minimal medium, Lac⁺ revertants appear at a high rate over several days, largely in the absence of cell growth, death, or turnover (2, 4). The formation of these adaptive mutations, unlike mutations that occur during growth under nonselective conditions, depends on the RecA-RecBCD recombination pathway (2, 8).

E. coli strains in which the RecBCD function is replaced by the Red recombination system of bacteriophage λ exhibit a hyperrecombination phenotype (13, 14). The possibility that the activity of the cell's recombination system might be a rate-limiting factor in the production of adaptive revertants led us to test whether Red⁺ bacteria might produce such revertants at an elevated rate. We report here that they do.

Bacterial strains. P1 transduction was used to replace the *recC-ptr-recB-recD* gene cluster in FC40 and FC691 with a sequence bearing both P_{tac}-*gam-bet-exo* and the *cat* gene. (The P_{tac}-*gam-bet-exo* sequence is designated *red*.) Recombinants were selected for chloramphenicol resistance, producing strains TP694 and TP730. Strains TP705 and TP732 were made by transforming TP694 and TP730 with linear DNA resulting from the digestion of plasmid pTP822 (16). Recombinants, in which Δ*recBCD*::*red-cat* was replaced by Δ*recBCD*::*red-pae-c*I822, were selected for immunity to phage λ and screened for sensitivity to chloramphenicol and kanamycin. These and other strains employed in this study are described in Table 1.

Media. The lysogeny broth (LB) used contained, per liter, 10 g of tryptone, 5 g of yeast extract, 5 g of NaCl, and 1 ml of 1 M NaOH. It was solidified with 15 g of agar per liter and supplemented as necessary with 25 μg of tetracycline/ml, 20 μg of chloramphenicol/ml, 0.1 mM isopropylthiogalactopyranoside (IPTG), or 80 μg of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal)/ml.

M9 salt solution contained, per liter, 6.3 g of Na_2HPO_4, 3.0 g of KH_2PO_4, 0.5 g of NaCl, and 1.0 g of NH_4Cl. M9 glycerol-minimal medium consisted of M9 salt solution plus 1 mM $MgSO_4$, 5 μg of thiamine/ml, 0.1 mM $CaCl_2$, 10 μg of gelatin/ml, and 1 mg of glycerol/ml. M9 lactose-minimal agar consisted of M9 salt solution plus 1 mM $MgSO_4$, 5 μg of thiamine/ml, 1 mg of lactose/ml, and 15 mg of agar/ml.

Measurement of stationary-phase reversion to Lac⁺. The strains to be tested were scraped from frozen cultures in storage vials and streaked on LB plates supplemented with appropriate antibiotics. A single colony was used to inoculate 5 ml of LB broth, and the culture was grown with aeration at 30 or 37°C to apparent saturation. The LB culture was diluted 10^5-fold with M9 glycerol-minimal medium, with a final 100-fold dilution into three to six tubes containing 1 ml each. These cultures in M9 glycerol-minimal medium were grown with aeration at 30 or 37°C to apparent saturation, and 0.01 to 0.5 ml of each culture was plated on one M9 lactose-minimal plate with approximately 2×10^9 scavenger cells. Scavenger cells (FC29) were grown to saturation in M9 glycerol-minimal medium and concentrated by centrifugation and resuspension in 0.1 volume of M9 salt solution; thereafter, 0.1 ml was plated. The plates were incubated at 37°C; colony counts were recorded on days 2 through 6 or 7. (Lac⁺ cells present in the culture at the time of plating take 2 days to form colonies.) The few plates with large numbers of preexisting revertants were ignored. Titers of the tested cultures were determined by plating on LB agar at 37°C.

Red-mediated reversion of episomal *lac*. A derivative of the F′ Φ(*lacI33-lacZ*)-bearing *E. coli* strain FC40, in which the *recBCD* gene cluster is replaced by the *red* system of phage λ, exhibits an elevated rate of adaptive mutation to Lac⁺ relative to its *recBCD*⁺ parent (Fig. 1a). As in *recBCD*⁺ bacteria (7, 9), the rate is significantly lower in the absence of the *ruv* genes (Fig. 1b) and lower still in the absence of *recA* (Fig. 1c). Deletion of *recG* from the *red*-substituted strain mildly increases the rate of reversion (Fig. 1d). The *red*⁺ *recG* strain and its *recBCD*⁺ *recG* counterpart exhibit approximately equal rates of reversion (data not shown).

Although Red stimulates the rate of adaptive reversion, it apparently has little effect on the rate of mutation occurring during nonselective growth. Revertants arising during the growth of the cultures form visible colonies in 2 days. The average numbers of colonies appearing on day 2 were 3.5 per 10^8 cells plated for the wild type (average of 8 experiments)

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TABLE 1. Bacterial strains used in this study

Strain	Relevant genotype	Reference or construction
FC29	F′ Δ(*lacI lacZ*)	2
FC40	F′ Φ(*lacI33-lacZ*)	2
FC691	F⁻ Φ(*lacI33-lacZ*)	18
JC15329	Δ(*srl-recA*)306::Tn*10*	A. J. Clark
KM32	AB1157 *recBCD*Δ::*red-cat*	16
N4454	Δ*ruvABC*::*cat*	12
TP538	Δ*recG*::*tet6200*	15
TP547	KM32 *lacZ*::*tet805*	KM32 × linear DNA
TP694	FC40 Δ*recBCD*::*red-cat*	FC40 × P1(TP547)
TP705	FC40 Δ*recBCD*::*red-pae-cI822*	TP694 × linear DNA
TP709	TP705 Δ*recG*::*tet6200*	TP705 × P1(TP538)
TP710	TP705 Δ(*srl-recA*)306::Tn*10*	TP705 × P1(JC15329)
TP724	TP705 Δ*ruvABC*::*cat*	TP705 × P1(N4454)
TP725	FC40 Δ*ruvABC*::*cat*	FC40 × P1(N4454)
TP732	FC691 Δ*recBCD*::*red-pae-cI822*	FC691 × linear DNA
TP734	TP732 Δ*recG*::*tet6200*	TP732 × P1(TP538)

TABLE 2. Reversion of chromosomal *lac* mutants

Strain	Relevant genotype	Rate of reversion[a]
FC691	F⁻ Φ(*lacI33-lacZ*)	0.63 ± 0.22
TP732	FC691 Δ*recBCD*::*red-pae-c*I822	2.47 ± 0.34
TP734	TP732 Δ*recG*::*tet6200*	2.39 ± 0.69

[a] Rates are given as mean numbers of revertants per day per 10^9 cells plated ± standard errors of the means for 5 to 12 cultures. Newly arising colonies were counted between 4 and 7 days after plating. Plating conditions were the same as those for the F′ Φ(*lacI33-lacZ*)-bearing strains, except that greater numbers of cells were plated (1×10^9 to 2×10^9 per plate), and no scavenger cells were used.

and 3.9 per 10^8 cells plated for the *red*-substituted strain (average of 10 experiments) (data not shown).

Red-mediated adaptive reversion appears to depend on the same *E. coli* recombination functions as does Red-mediated recombination, and like Red-mediated recombination, it is apparently mildly inhibited by RecG function (15). RecBCD-mediated adaptive mutation, however, differs from RecBCD-mediated recombination in that it is greatly inhibited by RecG function while RecBCD-mediated recombination is mildly stimulated by RecG function (7, 9).

The DNA segment replacing *recBCD* in the strains used in these experiments includes genes encoding the PaeR7 restriction-modification system and the λ repressor, in addition to the λ *red* system. These additional functions apparently do not contribute to the elevated rate of reversion; TP694, another *red*-substituted strain, lacking *pae* and *cI*, reverts at an equally high rate (data not shown).

Red-mediated reversion of chromosomal *lac*. *E. coli* FC691 is F⁻ and carries the Φ(*lacI33-lacZ*) allele on its chromosome (18). A derivative of FC691 in which the *recBCD* gene cluster is replaced by the *red* system of phage λ also exhibits an increased rate of stationary-phase mutation to the Lac⁺ phenotype, as shown in Table 2. The observed increase was approximately fourfold. The deletion of *recG* did not appear to increase the rate of reversion further (Table 2).

Stability of the Red-generated revertants. If Lac⁺ revertants of the episomal *lac* allele are generated by amplification, they form sectored colonies when streaked on nonselective medium containing X-Gal (1, 10). To determine whether amplification underlies the high rate of Red-mediated stationary-phase reversion, 10 late-arising Lac⁺ revertants of strain TP705 and 2 of FC40 were streaked on LB plates supplemented with X-Ga1 and IPTG and incubated at 37°C. The great majority of the colonies in all cases were solid blue. A few pure-white colonies
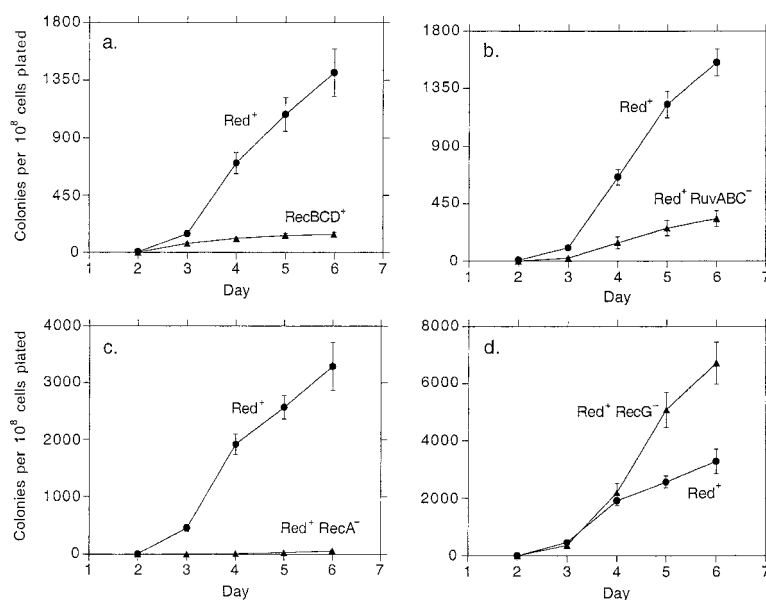

FIG. 1. Rates of reversion to Lac⁺. Points represent the means of the numbers of independent cultures specified below. Error bars (some of which are smaller than the symbols) represent the standard errors of the means. (a) Six cultures each of TP705 (circles) and FC40 (triangles); (b) six cultures of TP705 (circles) and five of TP724 (triangles); (c) three cultures of TP705 (circles) and six of TP710 (triangles); (d) three cultures of TP705 (circles) and six of TP709 (triangles).

were observed as well, possibly representing the scavenger strain. Of approximately 100 well-isolated TP705 revertant colonies, only one appeared to have a mixed phenotype, but its appearance could be accounted for by a pure-white colony and a pure-blue colony formed in close proximity. By the sectoring test, therefore, the great majority of the late-arising Lac⁺ colonies of the *red⁺* F′ F(*lacI33-lacZ*) strain appear to represent true reversion events rather than amplifications of the mutant allele. In contrast, when this test was repeated with Red-mediated revertants of the chromosomal *lac* allele, 7 of 12 late-arising colonies tested gave rise to numerous sectored colonies. Thus, Red increases stable amplification on the chromosome but not on the episome.

Amplification is involved in the reversion of the episomal *lac* allele, although its true role is debated (4, 10, 11, 17). Since adaptive reversion of the chromosomal *lac* allele in FC691 is not dependent on RecA and therefore does not involve amplification (6), it appears that Red may induce an entirely new pathway for adaptive change on the chromosome. Alternatively, the high frequency of sectored colonies among Red-mediated chromosomal Lac revertants may reflect not a greater frequency of formation but a greater stability of amplifications on the chromosome in the Red⁺ background. Although the chromosomal amplifications have not been characterized, based on previous reports, they are expected to be the same type of tandem repeats as are the episomal amplifications (3).

REFERENCES

1. **Anderson, D. I., E. S. Slechta, and J. R. Roth.** 1998. Evidence that gene amplification underlies adaptive mutability of the bacterial *lac* operon. Science **282:**1133–1135.
2. **Cairns, J., and P. L. Foster.** 1991. Adaptive reversion of a frameshift mutation in *Escherichia coli*. Genetics **128:**695–701.
3. **Edlund, T., and S. Normark.** 1981. Recombination between short DNA homologies causes tandem duplication. Nature **292:**269–271.
4. **Foster, P. L.** 1994. Population dynamics of a Lac⁻ strain of *Escherichia coli* during selection for lactose utilization. Genetics **138:**253–261.
5. **Foster, P. L.** 1999. Mechanisms of stationary phase mutation: a decade of adaptive mutation. Annu. Rev. Genet. **33:**57–88.
6. **Foster, P. L., and J. M. Trimarchi.** 1995. Adaptive reversion of an episomal frameshift mutation in Escherichia coli requires conjugal functions but not actual conjugation. Proc. Natl. Acad. Sci. USA **92:**5487–5490.
7. **Foster, P. L., J. M. Trimarchi, and R. A. Maurer.** 1996. Two enzymes, both of which process recombination intermediates, have opposite effects on adaptive mutation in *Escherichia coli*. Genetics **142:**25–37.
8. **Harris, R. S., S. Longerich, and S. M. Rosenberg.** 1994. Recombination in adaptive mutation. Science **264:**258–260.
9. **Harris, R. S., K. J. Ross, and S. M. Rosenberg.** 1996. Opposing roles of the Holliday junction processing systems of *Escherichia coli* in recombination-dependent adaptive mutation. Genetics **142:**681–691.
10. **Hastings, P. J., H. J. Bull, J. R. Klump, and S. M. Rosenberg.** 2000. Adaptive amplification: an inducible chromosomal instability mechanism. Cell **103:** 723–731.
11. **Hendrickson, H., E. S. Slechta, U. Bergthorsson, D. I. Anderson, and J. R. Roth.** 2002. Amplification-mutagenesis: evidence that "directed" adaptive mutation and general hypermutability result from growth with a selected gene amplification. Proc. Natl. Acad. Sci. USA **99:**2164–2169.
12. **McGlynn, P., and R. G. Lloyd.** 2000. Modulation of RNA polymerase by (p)ppGpp reveals a RecG-dependent mechanism for replication fork progression. Cell **101:**35–45.
13. **Murphy, K. C.** 1998. Use of bacteriophage λ recombination functions to promote gene replacement in *Escherichia coli*. J. Bacteriol. **180:**2063–2071.
14. **Poteete, A. R., and A. C. Fenton.** 1993. Efficient double-strand break-stimulated recombination promoted by the general recombination systems of phages λ and P22. Genetics **134:**1013–1021.
15. **Poteete, A. R., and A. C. Fenton.** 2000. Genetic requirements of phage λ Red-mediated gene replacement in *Escherichia coli* K-12. J. Bacteriol. **182:** 2336–2340.
16. **Poteete, A. R., A. C. Fenton, and K. C. Murphy.** 1999. Roles of RuvC and RecG in phage λ Red-mediated recombination. J. Bacteriol. **181:**5402–5408.
17. **Powell, S. C., and R. M. Wartell.** 2001. Different characteristics distinguish early versus late arising adaptive mutations in *Escherichia coli* FC40. Mutat. Res. **473:**219–228.
18. **Rosche, W. A., and P. L. Foster.** 1999. The role of transient hypermutators in adaptive mutation in Escherichia coli. Proc. Natl. Acad. Sci. USA **96:** 6862–6867.