EXTENDED PDF FORMAT
SPONSORED BY
usb
www.usbweb.com

Evidence That Gene Amplification Underlies Adaptive Mutability of the Bacterial lac Operon

Dan I. Andersson, *et al.*

Science **282**, 1133 (1998);
DOI: 10.1126/science.282.5391.1133

The following resources related to this article are available online at www.sciencemag.org (this information is current as of April 22, 2009):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:
http://www.sciencemag.org/cgi/content/full/282/5391/1133

This article **cites 26 articles**, 20 of which can be accessed for free:
http://www.sciencemag.org/cgi/content/full/282/5391/1133#otherarticles

This article has been **cited by** 83 article(s) on the ISI Web of Science.

This article has been **cited by** 35 articles hosted by HighWire Press; see:
http://www.sciencemag.org/cgi/content/full/282/5391/1133#otherarticles

This article appears in the following **subject collections**:
Genetics
http://www.sciencemag.org/cgi/collection/genetics

Information about obtaining **reprints** of this article or about obtaining **permission to reproduce this article** in whole or in part can be found at:
http://www.sciencemag.org/about/permissions.dtl

Downloaded from www.sciencemag.org on April 22, 2009

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005. Copyright 1998 by the American Association for the Advancement of Science; all rights reserved. The title *Science* is a registered trademark of AAAS.

Evidence That Gene Amplification Underlies Adaptive Mutability of the Bacterial *lac* Operon

Dan I. Andersson,* E. Susan Slechta, John R. Roth†

Adaptive mutability is the apparent alteration in specificity or rate of mutability seen in bacteria during stress. A model is proposed by which gene amplification during selective growth can give the appearance of adaptive mutability without requiring any change in mutability. The model is based on two assumptions, that a mutant *lac* locus with residual function allows growth if its copy number is increased, and that true reversion events are made more likely by replication of chromosomes with many copies of the locus. Apparent directed mutability, its recombination requirement, and its apparent independence of cell growth are all accounted for by the model. Evidence is provided for the required residual function and gene amplification.

In 1988 J. Cairns and co-workers described an experimental system in which bacteria appeared to show "adaptive mutability" (*1*), defined here as an alteration in the level or target-specificity of mutability as part of a programmed response to stress. Initially the results were interpreted to suggest that bacteria might direct mutability to selectively valuable sites (*2–4*). More recent results have been interpreted as reflecting a generalized hypermutable state induced in some cells in response to stress (*5*), a model originally proposed by Hall (*3*). Cairns's experimental system involved reversion of a *lac* frameshift mutation on medium containing lactose as the sole carbon source. The system has been extensively characterized, but none of the proposed models explains all of the observations (*6*).

The central issue in the controversy concerning adaptive mutability is whether a mechanism has evolved to improve survival by varying mutability in response to stress. We propose that the Cairns experimental system involves selected amplification of a mutant *lac* locus with some residual function. The growth of cells with multiple copies of the *lac* region increases the yield of detected mutations by increasing the number of targets; this can occur without any change in the mutation rate per gene copy. The model (Fig. 1) requires that the mutant *lac* region produces a small amount of β-galactosidase activity. Spontaneous duplication of the mutant *lac* operon increases β-galactosidase in proportion to copy number. Cells with a *lac* duplication initiate slow-growing microcolonies in which further amplification (to the order of 100 copies) is selected. Selection need not cause or stimulate formation of the amplification, but only favor growth of cells with the amplification. The initial *lac* duplications may arise during nonselective growth or may be generated in starved cells by occasional replication of the plasmid that carries the mutant *lac* region. The microcolonies contain many replicating cells, each with multiple copies of the *lac* operon. This enhances the probability that some copy of the *lac* operon will acquire a *lac*$^+$ reversion event (–1 frameshift mutation). The initial *lac*$^+$ allele is in a tandem array of mutant copies and is therefore unstable. However, ultimately haploid segregant types arise that retain only the revertant copy. These stably Lac$^+$ cells rapidly overgrow the microclone because they have no tendency to lose their Lac$^+$ phenotype and they no longer carry the extra DNA present in the unstable types with the amplification. An amplification model was suggested previously by Foster and Cairns (*4*), but it did not include a requirement for residual function or for growth of microclones as a result of selective amplification.

The *lac* frameshift allele used produces about 1% of the β-galactosidase made by a revertant gene. Using an F′ plasmid–borne *lac* operon in *Salmonella typhimurium*, we performed the reversion experiment (*7*) under several conditions that reduce the carbon and energy that can be derived from a given amount of β-galactosidase (Fig. 2A). A *galKT* mutation reduces by about half the amount of carbon and energy that can be derived from lactose by preventing use of the galactose moiety. This reduction substantially reduced the yield of late mutations. A similar reduction in reversion was caused by the competitive inhibitor of β-galactosidase (PETG). Under anaerobic conditions without an electron acceptor, cells must ferment lactose and forgo the energy obtained from respiration. This causes a great reduction in the carbon and energy obtainable from a fixed amount of lactose and essentially eliminates the occurrence of late revertants. All of these treatments reduced the yield of late revertants without reducing the number of preexisting mutations detected as Lac$^+$ revertant colonies on day 2. There was very little growth of the lawn with or without these treatments (*8*).

The residual level of β-galactosidase enzyme produced by a frameshift mutant allele is dictated by the frequency of spontaneous frameshifting that occurs during translation (*9*). Certain ribosomal mutations (*10*) can increase (*rpsD*) or decrease (*rpsL*) spontaneous readthrough of the frameshift mutation. By correlating residual β-galactosidase with the number of late revertants observed, one can demonstrate the effect of residual *lac* function on apparent adaptive mutability (Fig. 2B).

According to the amplification model (Fig. 1), each late Lac$^+$ revertant colony

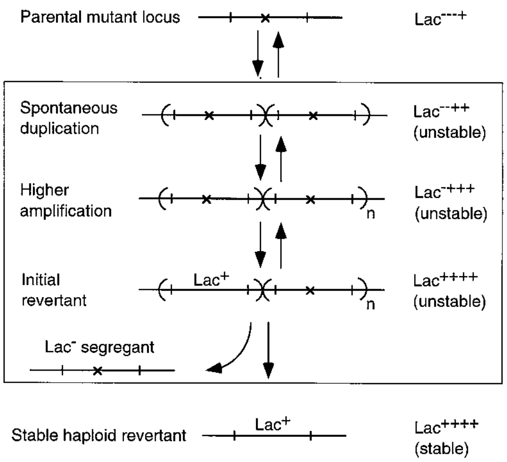

Fig. 1. A selective amplification model to explain adaptive mutability of the *lac* operon. Without being aware of the boxed events proposed by the model, a single mutant *lac* operon appears to be specifically accumulating reversion events in the absence of chromosome replication. The model provides for replication and explains the enhanced mutability by an increase in target copy number. The model was described earlier without experimental support (*13*).

Department of Biology, University of Utah, Salt Lake City, UT 84112, USA.

*Permanent address: Swedish Institute for Infectious Disease Control, S-10521 Stockholm, and Department of Microbiology, University of Uppsala, S-75123 Uppsala, Sweden.

†To whom correspondence should be addressed. E-mail: roth@bioscience.utah.edu

arises from a microclone of slowly growing cells carrying an amplified array of mutant *lac* alleles. Cells with these arrays are expected to be unstably Lac$^+$ and form sectored colonies when plated on nonselective medium containing the chromogenic LacZ substrate X-gal (*11*). Cells with this phenotype represented several percent (range 0.5 to 8%) of the cells in late-appearing Lac$^+$ revertant colonies (Fig. 3A). Such unstably Lac$^+$ cells were rarely found in day 2 colonies initiated by preexisting haploid *lac*$^+$ cells (Fig. 3C). Cells that were unstably Lac$^+$ were also found in areas of the lawn without visible revertant colonies. Samples taken on day 2 included very few unstably Lac$^+$ cells. Day 5 samples included more of the unstably Lac$^+$ type. At both time points, a roughly equal fraction of total lawn cells were stably Lac$^+$. The variance in the frequency of unstable (and stable) Lac$^+$ types in different plugs taken on day 5 was high, suggesting that some, but not all, samples included invisible microclones with amplified arrays and that some of these included stable revertants (Fig. 3C).

Elimination of recombination would be expected to prevent formation and loss of the tandem amplifications predicted by the model (*12, 13*). Introduction of a *recA* mutation into an unstably Lac$^+$ revertant resulted in stabilization (Fig. 3B). A *recA* mutation in the parent strain reduced the number of late Lac$^+$ revertants as expected (*4*); neither the few revertant colonies nor the lawn of these *recA* strains (day 2 or day 5) showed any of the unstably Lac$^+$ amplification types ($<0.03\%$). This reversion experiment was repeated with RecA$^+$ cells on medium that contained no carbon source. This condition subjected the Lac$^-$ cells to stress but did not select for *lac* amplification and no unstably Lac$^+$ cells were seen in the lawn on either day 2 or day 5 ($<0.03\%$). Thus, as predicted by the model, the unstably Lac$^+$ cell type was seen only in Rec$^+$ cells subjected to selection on lactose medium, and the frequency of this cell type increased with time on selective medium.

The predicted amplification of the *lac* region in unstably Lac$^+$ revertants was tested by quantitative Southern (DNA) hybridizations (*14*) (Fig. 3D). The unstably Lac$^+$ isolates (TT20562 through TT20565) showed (21- to 35-fold) more *lac* DNA than the unselected parent strain TT18302. The highest level of amplification observed was 50-fold (*8*). The copy number of a control gene in the chromosome (*cheY*) was unchanged in the unstable revertants. The assayed *lac* copy number is a minimal estimate of the amplification on the selection plate because revertants were purified and grown for DNA preparation with no selection to maintain the unstable amplified state. By using probes outside of the *lac* operon, we found that the amplified segment was a 20- to 40-kbp region including the *lac* operon.

To show that the unstably and stably Lac$^+$ cells in a colony are members of the same clone, we performed a reversion experiment with two genetically marked (Cm^R and Cm^S) parental strains, mixed at a ratio of 1:50. The reversion test was performed on minimal lactose medium without chloramphenicol (Cm). Day 5 Lac$^+$ revertants were replica-printed to minimal lactose containing Cm; this allowed identification of late Lac$^+$ clones that had arisen in the minority Cm^R parent type (2% of total). These clones were picked from the master plate that had not been exposed to antibiotic and scored for the frequency of Lac$^-$, Lac$^+$ (unstable), and Lac$^+$ cells; all were tested for Cm resistance. If cells from a single colony are clonal derivatives of a single plated cell, they should all be Cm^R. If the unstable clones arise independently and are not immediate ancestors of the Lac$^+$ clones, most should have the majority Cm^S phenotype. In each of five rare Cm^R Lac$^+$ colonies tested, all of the stably Lac$^+$ cells (68/68) and all of the unstably Lac$^+$ cells (37/37) tested were Cm^R, showing that the unstably and stably Lac$^+$ cells present in each Lac$^+$ colony were clonally related.

The evidence presented here supports two predictions of the model—the importance of residual function of the gene under selection and the existence of *lac* operon amplification. Although the need for residual function fits amplification during growth under selection, it does not eliminate some other models (for example, hypermutable states). However, the observed amplification is not predicted by the other models. J. Miller and co-workers previously showed selective high amplifica-

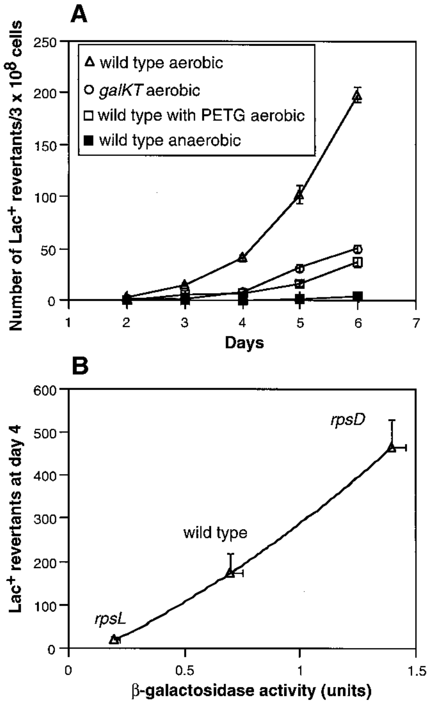

Fig. 2. (**A**) Lac$^+$ reversion under different growth conditions in the wild type (TT18302) and in a *galKT* mutant strain (TT20566). (**B**) Correlation between leakiness of the *lac* allele (β-galactosidase activity) and Lac$^+$ reversion in the wild-type (TT18302) and ribosomal mutants (TT20567 and TT20568) with an altered frequency of frameshift readthrough. Standard errors are indicated (in some cases error bars are obscured by the symbols).

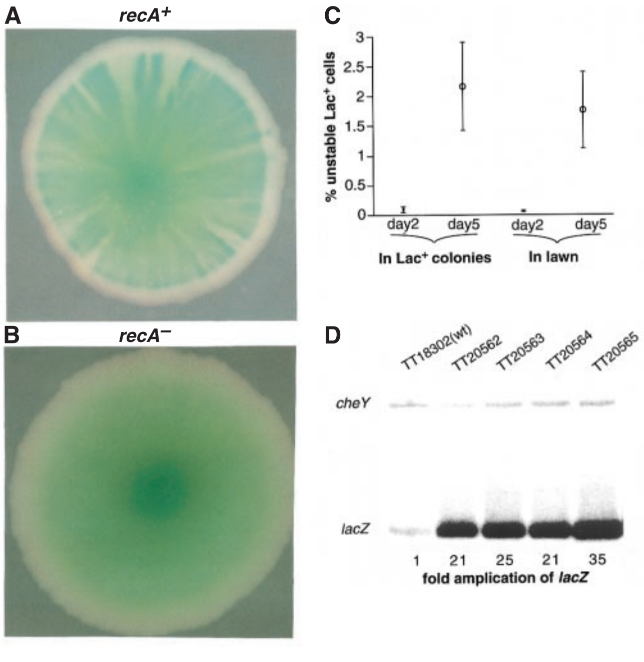

Fig. 3. (**A**) Colony of an unstably Lac$^+$ cell derived from a late revertant of the *recA*$^+$ strain (TT18302). (**B**) Colony formed by the same strain as above with an introduced *recA* mutation (TT20569); instability reappeared after reintroduction of the *recA*$^+$ allele. The photos in (A) and (B) were taken 3 days after cells were plated on X-gal indicator plates. (**C**) Frequency of unstably Lac$^+$/total cells in Lac$^+$ colonies and the lawn from day 2 and 5. Standard errors are indicated (in some cases they are obscured by the symbols). (**D**) Southern hybridization analysis of total DNA isolated from unstably Lac$^+$ cells. Analysis was performed on DNA isolated from the nonselectively grown wild-type parent strain (TT18302) and different unstably Lac$^+$ derivatives (TT20562 through TT20565), isolated from the lawn and Lac$^+$ colonies from lactose plates on day 5. The labels to the left indicate the probe target.

tion of the *lac* locus using two selection systems remarkably similar to that used by Cairns (*15, 16*). All the systems use an F′ *lac* plasmid carrying a *lacI-lacZ* hybrid gene that produces a small amount of active β-galactosidase. In all cases, the Lac$^+$ revertants were 10- to 100-fold more frequent than might be expected for correction of a point mutation. In the amplification experiments, about 60% of the frequent Lac$^+$ revertants had a highly unstable array (40 to 200 copies) of a 5- to 37-kbp sequence including the *lac* region; the stable revertants, whose frequency was also higher than expected, may have been derived from microclones with the amplification (*15, 16*). Thus, the earlier amplification experiments show the same increased mutability observed in the Cairns experiment. We propose that in both systems, the enhanced frequency of revertants can be explained, in principle, without an increase in intrinsic mutability (*17*).

The phenomenon described by Cairns as "adaptive mutability" requires recombination proficiency (*18*). This is surprising for simple reversion of a frameshift mutation but is consistent with the amplification model because both formation and segregation of duplications are processes known to be recombination dependent (*12*). However, several properties of the Cairns system are not obviously explained by the model proposed here; these can be accommodated by slight extensions of the model.

1) The apparent adaptive mutability requires involvement of a plasmid; the phenomenon is not seen if the locus under selection is in the chromosome (*19, 20*). The phenomenon is more striking if the plasmid expresses a functional *tra* operon, which encodes proteins required for conjugational transfer and replication (*20, 21*). The high amplification required by the model may be more common on F′ plasmids, perhaps because of double-strand ends generated by occasional internal firing of the transfer replication origin.

2) The reversion events (sequence changes) occurring during selection are a subset of the larger spectrum of reversion events observed during unselected growth (*22*). This difference may reflect the mechanism by which gene amplification occurs or the behavior of the F′ transfer replication complex or both.

3) The selected revertant clones have an increased probability of carrying unselected mutations at a variety of sites in the genome. This general mutagenesis (*5*) may be a secondary consequence of gene amplification that is not essential to the enhanced Lac$^+$ reversion. The amplified array or its formation may cause the induction of repair functions (SOS) that is known to occur in the course of the selection experiment (*4, 6*). This SOS induction may cause "damage-independent SOS mutagenesis" (*23*). Alternatively, the increase in genome size may titrate repair enzymes and reduce repair efficiency.

In summary, we consider that the reversion phenomenon seen in the Cairns system (and very rarely in more conventional selection systems) reflects a complex conjunction of factors that conspire to give the appearance of adaptive changes in mutability. We suggest that the standard genetic events proposed here may explain this phenomenology without requiring any evolved mechanism dedicated to regulation of mutability. If so, the phenomenon will not require revision of basic assumptions about the randomness or constancy of mutability.

References and Notes

1. J. Cairns, J. Overbaugh, S. Miller, *Nature* **335**, 142 (1988).
2. L. Boe, *Molec. Microbiol.* **4**, 597 (1990); F. W. Stahl, *Nature* **335**, 112 (1988).
3. B. G. Hall, *Genetics* **126**, 5 (1990).
4. P. L. Foster and J. Cairns, *ibid.* **131**, 783 (1992).
5. J. Torkelson *et al.*, *EMBO J.* **16**, 3303 (1997); P. L. Foster, *J. Bacteriol.* **179**, 1550 (1997).
6. ———, *Genetics* **148**, 1453 (1998); S. M. Rosenberg, C. Thulin, R. S. Harris, *ibid.*, p. 1559.
7. Bacteria were grown overnight in NCE glycerol medium with amino acid supplements at standard concentrations [T. Galitski and J. R. Roth, *Genetics* **143**, 645 (1996)]. Cells were centrifuged and washed in NCE and plated (3×10^8) on the Lac$^+$ reversion plates [NCE, lactose, X-gal (5-bromo-4-chloro-3-indolyl β-D-galactoside), leucine plates] and incubated at 37°C for 1 to 6 days. On each plate, $\sim 1 \times 10^9$ scavenger cells (wild-type LT2, Lac$^-$) were also included to consume any undefined carbon sources present in the agar (*1, 18*). Similar results were seen without scavenger cells. Each day the number of Lac$^+$ revertants was scored. Each data point in Fig. 2, A and B, and Fig. 3C represents the mean and standard error of at least five independent measurements. When present, phenylethyl β-D-thiogalactoside (PETG, Sigma) was used at 0.2 μg/ml. For anaerobic growth, cells were incubated in jars with an anaerobic atmosphere generated by a BBL Gas Pak (Becton Dickinson). Strains used were all derivatives of *S. typhimurium* (LT2) carrying the FC40 plasmid used originally in *Escherichia coli* by Cairns *et al.* (*1*). Genotypes were as follows: TT18302—*proB1657*::Tn*10 leuD21*/F′ pro$^+$ *lacI33ΩlacZ*; TT20566—*proB1661*::Tn*5 galKT*::Tn*10* dtet/F′ pro$^+$ *lacI33ΩlacZ*; TT20567—*proB1657*::Tn*10 rpsL106*/F′ pro$^+$ *lacI33ΩlacZ*; TT20568—*proB1657*::Tn*10 rpsD* (K205T)/F′ pro$^+$ *lacI33ΩlacZ*; and TT20569—*srl-203*::Tn*10*d-cam *recA1 proB1657*::Tn*10*/F′ pro$^+$ *lacI33ΩlacZ*. Activity of β-galactosidase (mean and standard error of four independent assays) was determined as described (*24*).
8. D. I. Andersson, E. S. Slechta, J. R. Roth, unpublished results.
9. J. F. Atkins, D. Elseviers, L. Gorini, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1192 (1972).
10. C. G. Kurland, D. Hughes, M. Ehrenberg, in Escherichia coli *and* Salmonella*: Cellular and Molecular Biology*, F. C. Neidhardt, Ed. (ASM Press, Washington, DC, 1996), pp. 979–1004.
11. To score the frequency of unstably Lac$^+$ cells in Lac$^+$ colonies and the lawn, we removed agar plugs with either the thick end (lawn) or the thin end (Lac$^+$ colonies) of a Pasteur pipette, and bacteria were resuspended by vigorous vortex mixing for 1 min. These cells were then diluted and plated for single colonies on nonselective rich medium (NB) with glucose, X-gal, tetracycline plates to allow scoring of unstable sectored colonies. Plates were incubated for 2 days at 37°C followed by 1 day at room temperature to develop optimal color. About 3000 colonies were screened for each plug. The sectored phenotype shown by unstably Lac$^+$ cells becomes stable after addition of a *recA* mutation. The RecA dependence of the color sectoring phenotype used to identify an unstably Lac$^+$ cell indicates a recombination requirement; this phenotype is not RecA dependent in situations for which loss of *lac*$^+$ genes occurs without recombination (for example, loss of a plasmid).
12. R. P. Anderson and J. R. Roth, *Ann. Rev. Microbiol.* **31**, 473 (1977).
13. J. R. Roth *et al.*, in (*10*), pp. 2256–2276.
14. Total DNA was isolated with a Wizard Total DNA Preparation Kit (Promega). About 1 μg of DNA was cut with Hinc II and then separated on a 0.8% agarose gel. The DNA was blotted to nylon membranes (Hybond-N, Amersham) and then probed with deoxycytidine 5′-triphosphate (α-^{33}P)–labeled polymerase chain reaction fragments intrinsic to the *lacZ* gene and the *cheY* gene. The 249-bp *lacZ* probe was internal to a 0.9-kb Hinc II fragment in the *lacZ* gene (*16*). The 400-bp control gene probe was internal to a Hinc II fragment that included the complete *cheY* gene. When determining the approximate size of the amplified region, we used four probes separated by $\sim$10 kb extending on both the upstream and downstream side of the *lac* operon. The two probes nearest the *lac* operon gave an amplified signal similar to the *lac* operon probe, whereas the two most distal probes gave the same signal as the *cheY* chromosomal control, indicating that the amplified region was at least 20 kb but less than 40 kb (*8*). Quantitation of the radioactivity in bands was performed on a Molecular Dynamics PhosphorImager, and the *lacZ* bands were normalized to the *cheY* intrinsic controls.
15. T. Tlsty, A. Albertini, J. H. Miller, *Cell* **37**, 217 (1984).
16. S. Whoriskey, V.-H. Nghiem, P.-M. Leong, J.-M. Masson, J. H. Miller, *Genes Dev.* **1**, 227 (1987).
17. Over the course of an experiment, $\sim$100 new Lac$^+$ revertants arise. Assuming no increase in the unselected reversion rate for the *lac* frameshift mutation (10^{-8}), it would require 10^{10} acts of *lac* region replication. This could be achieved with 10^5 microclones, each with 10^3 cells and 10^2 *lac* copies per cell. The estimated frequency of microclones is based on the frequency of chromosomal duplications (*12*). This would add 10^8 cells to the number of viable cells on the plate, about a 30% increase over the number of plated cells; we argue that even without some cell death this increase would be hard to demonstrate.
18. J. Cairns and P. L. Foster, *Genetics* **128**, 695 (1991); P. L. Foster, J. M. Trimarchi, R. A. Maurer, *ibid.* **142**, 25 (1996); R. S. Harris, S. Longerich, S. M. Rosenberg, *Science* **264**, 258 (1994); R. S. Harris, K. J. Ross, S. M. Rosenberg, *Genetics* **142**, 681 (1996).
19. J. P. Radicella, P. U. Park, M. S. Fox, *Science* **268**, 418 (1995).
20. E. S. Slechta, D. I. Andersson, J. R. Roth, manuscript in preparation.
21. J. E. Peters, I. M. Bartoszyk, S. Dheer, S. A. Benson, *J. Bacteriol.* **178**, 3037 (1996); J. E. Peters and S. A. Benson, *ibid.* **177**, 847 (1995); T. Galitski and J. R. Roth, *Science* **268**, 421 (1995); P. L. Foster and J. M. Trimarchi, *J. Bacteriol.* **177**, 6670 (1995); P. L. Foster and J. M. Trimarchi, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 5487 (1995).
22. P. L. Foster and J. M. Trimarchi, *Science* **265**, 407 (1994); S. M. Rosenberg, S. Longerich, P. Gee, R. S. Harris, *ibid.*, p. 405.
23. E. M. Witkin, *Bacteriol. Rev.* **40**, 869 (1976); G. C. Walker, *Microbiol. Rev.* **48**, 60 (1984).
24. J. H. Miller, *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1972).
25. Supported by NIH grant GM27068 (to J.R.R.). The Swedish Institute for Infectious Disease Control and the Swedish Natural Science Research Council partly supported D.I.A.'s sabbatical leave. We thank S. Rosenberg for suggesting the experiment to demonstrate clonal relatedness of stably and unstably Lac$^+$ cells in a revertant colony.

30 June 1998; accepted 6 October 1998