JOURNAL OF BACTERIOLOGY, Sept. 1980, p. 1519-1523
0021-9193/80/09-1519/05$02.00/0

Vol. 143, No. 3

Selection of *Escherichia coli* K-12 Chromosomal Mutants That Prevent Expression of F-Plasmid Functions

PHILIP SILVERMAN,* KARYL NAT, JOAN McEWEN, AND RICHARD BIRCHMAN

Department of Molecular Biology, Division of Biological Sciences, Albert Einstein College of Medicine, Bronx, New York 10461

Chromosomal mutants of *Escherichia coli* deficient in the expression of F-plasmid functions were selected by mutagenizing F^- cells, introducing an F′ plasmid into the mutagenized cells by conjugation, and identifying transconjugants resistant to the donor-specific bacteriophage Qβ by a simple spray test. All but 1 of 25 mutants were defective in an extracellular stage of Qβ infection, suggesting that they fail to elaborate F-pili. At least six of these were also deficient as deoxyribonucleic acid donors. More than half of the mutants appear to be altered in previously undetected chromosomal genes required for the expression of F-related cellular functions.

Escherichia coli cells containing both chromosomal and F-plasmid DNA express a variety of specialized cellular functions. Most striking is their capacity to donate plasmid and sometimes chromosomal DNA to other cells by conjugation. The contributions of plasmid gene products to these functions have been established by an extensive analysis of plasmid mutants (see reference 1 for a recent review). More recently, mutations in certain chromosomal genes have been shown to abolish them, even when the cells contain normal plasmid DNA (6, 9, 14). The number of such genes, their precise contribution to the expression of plasmid-related functions, and their contribution to other cellular processes are not known. To examine these questions, we have developed a generally applicable method for identifying chromosomal mutations that abolish or reduce plasmid-directed activities. The method is simple, rapid, and suitable for use with different bacterial genera and any conjugative plasmid with a selectable genetic marker, including all of the R-plasmids.

Mutations that affect DNA donor activity can occur in plasmid or chromosomal genes, but plasmid mutants are much more common when a plasmid-containing strain is mutagenized (2; P. Silverman, unpublished data). To avoid this ambiguity and the tedious job of identifying rare chromosomal mutants, we mutagenized an F^- strain of *E. coli* K-12, introduced an F′ plasmid into the mutagenized cells from a suitable donor, selected transconjugants of the mutangenized cells, and identified those resistant to the RNA bacteriophage Qβ. These bacteriophage adsorb to F-pili, which are filamentous organelles required for the DNA donor activity of cells containing the conjugative plasmid F (1). As we have previously shown (14), chromosomal mutants of *E. coli* selected for Qβ resistance fail to elaborate funcitonal F-pili and are deficient in DNA donor activity. However, it should be pos-

TABLE 1. *Bacterial strains*

Strain[a]	Relevant genotype	Source[b] or comment
AE2087	F^- *rpsL104 thyA23 metB1 leu-6 his-1 cpxB1*	F^- derivative of AE3087 (14)
AE2004	F^- *thyA metB1 leu-6 his-1 gyrA*	(14)
AE2005	F^- *thyA recA1 his-1 leu-6*	From JC355 (CGSC 869); *recA1* introduced by conjugation with KL16-99 (10), and *thyA* introduced by spontaneous mutation to trimethoprim resistance
AE3127	pAE4000 (*thyA*$^+$ *zzf*::Tn*10*)/AE2005	pAE4000 is described in reference 14 as F′116 *zzf*::Tn*10*
AE3134	F′116 (*thyA*$^+$)/AE2005	Source of F′116 (11); constructed by conjugation with CGSC strain 4254 as F′ donor
AE3135	F′150 (*his*$^+$)/AE2005	Source of F′150 (11); constructed by conjugation with CGSC strain 4326 as F′ donor
KLF1/AB2463	F′101 (*leu*$^+$)/AB2463 (*leu-6*)	Source of F′101 (11); same as CGSC strain 4250

[a] All strains are derivatives of *E. coli* K-12 and were constructed by standard methods (15).
[b] CGSC refers to the Coli Genetic Stock Center at Yale University, New Haven, Conn.

Downloaded from http://jb.asm.org/ on September 12, 2017 by ETH-BIBLIOTHEK ZURICH

TABLE 2. *Chromosomal mutants resistant to Qβ*

Mutant[a]	Mutagen[a]	Infectious centers[b]	Burst size
AE2087	(Cpx^+)	$(2.9 \pm 1.5) \times 10^7$ (8)[c]	268 ± 167 (5)[c]
KN1001[d]	NTG	$(5.4 \pm 3.1) \times 10^6$ (8)[c]	0.2 ± 0.36 (5)[c]
KN106	AP	$<10^5$	—
KN109	AP	$<10^5$	—
KN301	AP	3×10^5	233
KN308[d]	AP	$<10^5$	—
KN309[e]	AP	$<10^5$	—
KN402[d]	AP	$<10^5$	—
KN406	AP	9×10^5	222
KN409	AP	10^5	350
KN504[d]	AP	$<10^5$	—
KN507	AP	$<10^5$	—
KN606[e]	AP	$<10^5$	—
KN607	AP	$<10^5$	—
KN708	AP	10×10^5	160
KN803[e]	AP	$<10^5$	—
KN905[e]	AP	10^5	10
KN1004	NTG	$<10^5$	—
KN1516	EMS	$<10^5$	—
KN1702[d]	EMS	$<10^5$	—
KN1703	EMS	$<10^5$	—
KN1909[d]	EMS	$<10^5$	—
KN1911	EMS	$<10^5$	—
KN1913	EMS	1.5×10^5	130
KN2002	EMS	$<10^5$	—
KN2103[d]	EMS	$<10^5$	—

[a] Treatment of strain AE2087 with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) was as previously described (14). Ethyl methane sulfonate (EMS) and 2-aminopurine (AP) mutagenesis were as described by Miller (15). In all cases the frequency of valine-resistant mutants (3) was increased about 10^3-fold by mutagenesis. Cells mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine or ethyl methane sulfonate were stored at −20°C in 40% glycerol immediately after mutagenesis. Before mutant isolation, samples of these cells were first grown to saturation in nutrient broth at 30°C. Cells were mutagenized with 2-aminopurine in separate samples. The first digit of three-digit strain numbers and the first two digits of four-digit strain numbers identify the sample of mutagenized cells from which each mutant was derived. Mutants with strain numbers that differ in these positions are of independent origin. Mutagenized cells were grown in nutrient broth at 34°C to an optical density (660 nm) of 1.0 and mixed with an equal volume of the pAE4000 donor AE3127. Cell mixtures were incubated at 34°C for 90 min without agitation to allow formation of transconjugants, agitated on a Vortex mixer to disrupt mating aggregates, and chilled. The number of transconjugants was determined for each sample of mutagenized cells by plating 10^{-2} and 10^{-3} dilutions of the mating mixture on EMBO plates containing streptomycin (100 μg/ml) and tetracycline (15 μg/ml). After this determination, samples of a suitable dilution of the mating mixture (stored at 4°C) were plated on each of 50 EMBO plates containing both drugs. The dilution and size of the sample plated were chosen to give 50 to 100 transconjugants on each plate. The identification of Qβ-resistant mutants among these transconjugants is described in the text.

[b] Mutant cells containing the conjugative plasmid F'116 were grown at 41°C to an optical density of 1. $CaCl_2$ (1 M) was added to a final concentration of 5 mM, and Qβ was added at a multiplicity of infection of 0.1. Incubation at 41°C was continued for 20 min with aeration. At this time, 0.1 ml of a 10^{-1} dilution of anti-Qβ antiserum (K = 800) was added. After 10 min, the cells were diluted 10^4-fold into nutrient broth warmed to 41°C. A sample (1 ml) was removed and assayed for plaque-forming units resistant to anti-Qβ antiserum (infectious centers). The remaining cells were incubated at 41°C for an additional 60 min (90 min postinfection). At this time the cells were lysed by addition of 0.2 ml of $CHCl_3$, and phage yields were assayed as total plaque-forming units. Burst sizes are expressed as yield per infectious center. For both assays plaque-forming units were measured by plating suitable dilutions on Hfr strain Q13 at 37°C.

[c] These entries include the standard deviation of data from the number of experiments indicated in parentheses.

[d] These mutants were complemented by F'144 and F'101 (see text).

[e] These mutants were complemented by F'144; they were not tested for complementation by F'101.

sible with replica mating techniques (12) to select directly mutants that are deficient in donor activity.

We mutagenized the Str^r F^- strain AE2087 (see Table 1) with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, 2-aminopurine, or ethyl methane sulfonate and then used mutagenized cells as recipients in a cross with AE3127, a Str^s strain

Downloaded from http://jb.asm.org/ on September 12, 2017 by ETH-BIBLIOTHEK ZURICH

containing pAE4000. This derivative of F'116 (*thyA*$^+$) contains the Tn*10* transposon at a locus that does not affect the *thyA*$^+$ allele or the transfer properties of pAE4000 (14). Tn*10* includes genes that confer tetracycline resistance (8); AE2087 cells that have received pAE4000 were therefore selected in the presence of both streptomycin and tetracycline. To detect Qβ-resistant mutants, we plated the mating mixture on EMBO medium containing both drugs. *E. coli* produces white colonies on this medium (16). When sprayed with an aerosol of a donor-specific RNA bacteriophage, such as Qβ, colonies containing sensitive cells turn red or form a red ring around their periphery, whereas colonies containing resistant cells remain white (13, 16). To accommodate temperature-sensitive mutants whose growth at 41°C is inhibited, we incubated the plates at 32°C for 36 to 48 h to allow colony formation, shifted them to 41°C for 2 h, sprayed them with an aerosol of Qβ, and returned them to 41°C for an additional 4 to 5 h. Colonies that remained white were then purified by two cycles of single-colony isolation on minimal medium at 32°C and tested for Qβ resistance at 41°C by standard plaque assay; approximately 50% of the isolates were Qβ resistant by this test and were examined further, as described below.

TABLE 3. *Chromosomal genes required for the expression of F-plasmid functions*

Gene[a]	Map position[b] (min)	Reference
cpxA	88	14
cpxB	41	14
fex (sfrA)[c]	100/0	6, 9
sfrB	85	6

[a] Mnemonics are explained in the references.
[b] Map positions are according to reference 5.
[c] *fex* and *sfrA* mutations are allelic (T. Lerner, personal communication).

The frequencies of Qβ-resistant mutants after mutagenesis of F$^-$ strain AE2087 with ethyl methane sulfonate, 2-aminopurine, or *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine were 0.03%, 0.07%, and 0.02%, respectively, of the Str^r Tc^r transconjugant colonies. In contrast, we found no mutants among 36,000 transconjugants derived from unmutagenized cells, setting an upper limit to the frequency of spontaneous Qβ-resistant mutants at ~0.003%. Hence, at least 90% of the mutants were the result of mutagenesis and are therefore chromosomal, since plasmid DNA was not present during mutagenesis.

We previously described two classes of *E. coli* chromosomal mutants resistant to Qβ. Mutants in the first class were unable to express cellular properties normally associated with donor strains, i.e., capacity to transfer DNA to other cells by conjugation and poor activity as conjugal recipients (surface exclusion); we designated this phenotype Cpx$^-$ (14). These mutants are presumably defective in cellular components that interact with plasmid genes or gene products. Mutants in the second class failed to replicate bacteriophage RNA inside the cell (13). These mutants presumably contain altered bacterial proteins required for the replication reaction (7). To compare the frequency of mutants in each class, we examined 73 Qβ-resistant mutants obtained by screening about 216,000 Str^r Tc^r colonies. We measured completion of the extracellular stages of bacteriophage infection, which is defective in Cpx$^-$ mutants (14), by the formation of Qβ infectious centers, and completion of the intracellular stages of bacteriophage

TABLE 4. *Complementation analysis of selected mutants*[a]

Strain	Qβ sensitivity[b] in the presence of:		Donor activity[c] in the presence of:		
	F'116	F'101	F'116 (*thyA*$^+$)	F'150 (*his*$^+$)	F'101 (*leu*$^+$)
AE2087	2.2×10^{10}	6.5×10^{10}	4.0×10^6	1.8×10^7	5.1×10^7
KN308	$<10^8$	6.1×10^{10}	$<10^2$	$<10^3$	6.5×10^7
KN402	$<10^8$	5.6×10^{10}	$<10^2$	$<10^3$	5.1×10^7
KN504	$<10^8$	4.6×10^{10}	$<10^2$	$<10^3$	7.0×10^7
KN1702	$<10^8$	2.8×10^{10}	$<10^2$	$<10^3$	3.1×10^7
KN1909	$<10^8$	1.2×10^{10}	$<10^2$	$<10^3$	3.0×10^7
KN2103	$<10^8$	1.5×10^{10}	$<10^2$	$<10^3$	3.3×10^7

[a] To replace pAE4000 with a different F' plasmids, mutant cells spontaneously cured of pAE4000 were isolated as trimethoprim-resistant colonies (15). These F$^-$ mutants were mated in liquid to various F' donors (Table 1), and transconjugants were selected on appropriate media. These were purified twice on selective media by single-colony isolation. The DNA donor activities and Qβ sensitivity of the transconjugants were measured as described below.
[b] Plaque-forming units per milliliter.
[c] DNA donor activity (transconjugants per milliliter) was measured as we previously described (14). The recipient strain was AE2004. Selection was for Thy$^+$, His$^+$, or Leu$^+$ transconjugants according to the F' being tested, and counter-selection was with nalidixic acid.

Downloaded from http://jb.asm.org/ on September 12, 2017 by ETH-BIBLIOTHEK ZURICH

replication, which is defective in RNA replication mutants (13), was measured by the burst size of progeny Qβ per infectious center (Table 2). Twenty-five mutants could be placed in one or the other category by these assays. At least 14 of these were of independent origin. The remaining 48 mutants were defective in one or both assays, but the defects were of insufficient magnitude to permit an unambiguous classification.

Twenty-four of the 25 mutants showed severely reduced capacity to form Qβ infectious centers, only 6 at a frequency high enough to be detected by the assay (Table 2). Of these six, five showed burst sizes comparable to parental cells, suggesting that once Qβ RNA enters these mutants the replication and assembly stages of infection proceed normally. These 24 mutants are presumptive Cpx$^-$ mutants. In contrast, one temperature-sensitive mutant, KN1001, formed Qβ infectious centers at 20% the frequency of parental cells at the nonpermissive temperature, but the burst size was reduced by three orders of magnitude to less than one plaque-forming unit per infectious center (Table 2). This mutant may be defective in an intracellular stage of Qβ replication, probably the replication of viral RNA (McEwen, Sambucetti, and Silverman, unpublished data). Since a ratio of progeny bacteriophage (measured 90 min after infection) to infectious centers (measured 20 min after infection) of less than 1 suggests a time-dependent loss of intact, parental bacteriophage RNA inside the cell, the infectious-center assay provides a minimum estimate for the completion of the extracellular stages of infection which may be normal in mutant KN1001.

With the exception of KN1001, all of the mutants that we could classify are presumptive Cpx$^-$ mutants. Their mutations should be in chromosomal genes required for the elaboration of functional F-pili and possibly for the expression of other plasmid-related functions. Four such genes have previously been identified (Table 3). Mutations in at least three of these, *cpxA*, *fex* (*sfrA*), and *sfrB*, are recessive, since mutant cells containing an F′ with the corresponding wild-type allele were sensitive to donor-specific bacteriophages and exhibited normal levels of F′ transfer. Mutatations in the *fex* (*sfrA*) gene are complemented by the *fex*$^+$ allele of F′101 (6, 9) or F′144 (McEwen and Silverman, unpublished data), and mutations in the *cpxA* or *sfrB* genes are complemented by the respective wild-type alleles of F′133 (6; McEwen and Silverman, unpublished data). The fourth gene, *cpxB*, was not examined. Mutations in the *cpxB* gene exacerbate the effect of *cpxA* mutations, but they are cryptic in a *cpxA*$^+$ cell (14). Strain AE2087, which we mutagenized, is a *cpxB* mutant (14). We chose this strain so a single mutational event in the *cpxA* gene would produce a full mutant phenotype.

Of the 24 presumptive Cpx$^-$ mutants, 10 became sensitive to Qβ when F′144 replaced pAE4000; 6 of these were also Qβ sensitive in the presence of F′101 (the other 4 were not tested) (Table 4). These six mutants were also deficient in F′ transfer to a degree suggesting complete loss of activity; this activity was specifically restored in the presence of F′101 (Table 4). None of the mutants became Qβ sensitive when F′133 replaced pAE4000. This preliminary survey suggests that at least 6 and as many as 10 of the 24 mutants are *fex* (*sfrA*) mutants, whereas none appears to be a *cpxA* or an *sfrB* mutant. This is consistent with our earlier finding that *cpxA* mutants are rare (14). Of greater interest, more than half the mutants obtained by our selection method appear to be altered in previously undetected chromosomal genes required for the expression of F-plasmid functions. The number of these genes has not been determined.

We are indebted to Barbara Bachmann, curator of the Coli Genetic Stock Center, for providing bacterial strains, and to Margaret Clarke for critically reading the manuscript.

This work was supported by Public Health Service grants GM-11301, Ca-13330-08, and GM-07491-03 from the National Institutes of Health and by grant 75-144 from the American Heart Association. P.S. is an Established Investigator of the American Heart Association.

LITERATURE CITED

1. **Achtman, M., and R. Skurray.** 1977. A redefinition of the mating phenomenon in bacteria, p. 233–279. *In* J. L. Reisig (ed.), Microbial interactions (Receptors and recognition, series B), vol. 3. Chapman and Hall, London.
2. **Achtman, M., N. Willetts, and A. J. Clark.** 1971. Beginning a genetic analysis of conjugational transfer determined by the F-factor of *Escherichia coli* by isolation and characterization of transfer-deficient mutants. J. Bacteriol. **106**:529–538.
3. **Adelberg, E., M. Mandel, and G. Chen.** 1965. Optimal conditions for mutagenesis by *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine in *E. coli*. Biochem. Biophys. Res. Commun. **18**:788–795.
4. **Bachmann, B.** 1972. Pedigrees of some mutant strains of *Escherichia coli* K-12. Bacteriol. Rev. **36**:525–557.
5. **Bachmann, B., and K. B. Low.** 1980. Linkage map of *Escherichia coli* K-12, edition 6. Bacteriol. Rev. **40**,116–167.
6. **Beutin, L., and M. Achtman.** 1979. Two *Escherichia coli* chromosomal cistrons, *sfrA* and *sfrB*, which are needed for expression of F factor *tra* functions. J. Bacteriol. **139**:730–737.
7. **Blumenthal, T., and G. Carmichael.** 1979. RNA replication: function and structure of the Qβ replicase. Annu. Rev. Biochem. **48**:525–548.
8. **Kleckner, N., R. K. Chan, B.-K. Tye, and D. Botstein.** 1975. Mutagenesis by insertion of a drug-resistance element carrying an inverted repetition. J. Mol. Biol.

Downloaded from http://jb.asm.org/ on September 12, 2017 by ETH-BIBLIOTHEK ZURICH

97:561–575.

9. **Lerner, T., and N. D. Zinder.** 1979. Chromosomal regulation of sexual expression in *Escherichia coli*. J. Bacteriol. **137**:1063–1065.
10. **Low, K. B.** 1968. Formation of merodiploids in matings with a class of Rec⁻ recipient strains of *E. coli* K12. Proc. Natl. Acad. Sci. U.S.A. **60**:160–167.
11. **Low, K. B.** 1972. *Escherichia coli* K-12 F-prime factors, old and new. Bacteriol. Rev. **36**:587–607.
12. **Low, K. B.** 1973. Rapid mapping of conditional and auxotrophic mutations in *Escherichia coli* K-12. J. Bacteriol. **113**:798–812.
13. **Mandal, N. C., and P. Silverman.** 1977. An *E. coli* mutant with a temperature-sensitive function affecting bacteriophage Qβ RNA replication. Proc. Natl. Acad. Sci. U.S.A. **74**:1657–1661.
14. **McEwen, J., and P. Silverman.** 1978. Chromosomal mutations of *E. coli* that alter the expression of conjugative plasmid functions. Proc. Natl. Acad. Sci. U.S.A. **76**:513–517.
15. **Miller, H. J.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **Silverman, P., H. Mobach, and R. C. Valentine.** 1967. Sex hair (F-pili) mutants of *E. coli*. Biochem. Biophys. Res. Commun. **27**:412–416.

Downloaded from http://jb.asm.org/ on September 12, 2017 by ETH-BIBLIOTHEK ZURICH