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Indirect Selection for Plasmid Mutants: Isolation of ColVB*trp* Mutants Defective in Self-Maintenance in *Escherichia coli*

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An efficient method for isolation of a large number of plasmid mutants is described. It is based on the fact that *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine induces a number of closely linked mutations within a short segment of the bacterial chromosome. Thus, selection for reversions of an auxotrophic marker located on the ColVB*trp* plasmid yielded a large fraction (more than 50%) of mutants defective in some plasmid functions, including its own maintenance in the host bacteria. The results of preliminary characterization of strains carrying these mutated plasmids are presented.

One approach to the problems of chromosomal replication and cell division in bacteria is to study the mechanisms of replication and segregation of a certain class of plasmid deoxyribonucleic acid (DNA) in the cell cycle of the host bacteria. The F factor and the related plasmids of *Escherichia coli* K-12 can replicate autonomously as an independent replicon, as does the host chromosome (9). However, the replication and subsequent segregation of such plasmids into daughter cells must be under the strict control of the host cell, since the host bacteria harboring such plasmids can maintain basically a single copy of the plasmid per chromosome (3). In fact, it has been shown that the F factor replicates at a certain stage of the host cell cycle (13) and usually cosegregates with the host chromosome into daughter cells during cell division (8, 10).

The ColVB*trp* plasmid discovered in a certain strain of *E. coli* by Fredericq (4) is similar to the F factor in many respects and used to be erroneously referred to as an F*trp* factor. It has a circular double-stranded DNA of 107×10^6 daltons, corresponding to about 5% of the host chromosome (7). Strains carrying this plasmid produce three different colicins (V, B, and M), form the F-type pili, and transfer the plasmid to female Col$^-$ cells at high frequency (4, 5; P. Fredericq, personal communication). They contain one to two copies of the plasmid per chromosome at log phase (7, 16) and exclude other F-type plasmids such as F*lac* and R_{222} when present in the same cell (Wada, Koyama, and Yura, unpublished results).

In this report, we describe a very efficient method for isolation, by means of indirect selection, of plasmid mutants particularly those defective in self-maintenance. It is known that *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NG) induces a number of closely linked mutations within a short segment of the *E. coli* chromosome (2, 6). Because of the small size and circular structure of the ColVB*trp* plasmid, selection for any mutation of the plasmid would be expected to produce strains carrying a number of simultaneous mutations on the whole plasmid genome. Thus, we have selected NG-induced Trp$^+$ revertants from a strain carrying a missense *trpA* mutation on the plasmid and a *trpA-E* deletion on the host chromosome. As expected, a considerable fraction of the revertants was found to carry additional mutations affecting various plasmid functions. Such an indirect method of selection may be useful for isolating various mutants that cannot be selected directly. It should also be applicable to other plasmids, provided that they carry a certain marker(s) that can conveniently be used for such a selection.

MATERIALS AND METHODS

Bacterial and phage strains. The bacterial strains used are all derivatives of *E. coli* K-12 and are listed in Table 1. Strain KY7509, used as a parental strain in this study, carries a *trpA-E* deletion on the chromosome and a missense *trpA24* mutation on the ColVB*trp* (hereafter designated simply as Col*trp*) plasmid. The *trpA24* mutation (12) was introduced into Col*trp* plasmid by homogenote formation. Mutant strains designated as KY7601 through KY7617 are the Trp$^+$ revertants that are also defective in plasmid maintenance, and were isolated from strain KY7509 after treatment with NG. The Col$^-$ strains derived from each of these Col$^+$ mutants were numbered as

TABLE 1. *Bacterial strains*

Strain	Plasmid	Genetic characters or derivation
KY7500[a]		*arg met his pro ppc* (*tonB-trpA-E*) del[b] *thy supE*
KY7502		Spontaneous Arg$^+$ Ppc$^+$ Thy$^+$ revertant of KY7500
KY7507		Spontaneous Arg$^+$ His$^+$ Thy$^+$ revertant of KY7500
KY7509	Col*trpA24*[c]	Col$^+$ derivative of KY7500, carrying Col*trpA24*
KY7520		*gal lac*(*tonB-trp*) del (derived from W3350)
KY7521	Col*trp*	*trpA24*/Col*trp* (The original plasmid of P. Fredericq was transferred into an F$^-$ derivative of Y *mel trpA24*)
KY7600	Col*trp*	Spontaneous Trp$^+$ revertant of KY7509
KY7601 to KY7617	Col*trp*	Trp$^+$ revertants that are defective in maintenance of Col*trp* plasmid obtained from KY7509
KY7700 to KY7717		Spontaneous Col$^-$ segregants of strains KY7600 to 7617, respectively
KY7800 to KY7817	Col*trp*	Col$^+$ (KYCT0) derivatives of strains KY7700 to 7717, respectively

[a] Strain KY7500 was derived from strain 2-4 *argE-15* of N. Glansdorff.
[b] del, Deletion.
[c] Col*trp* plasmid that carries a missense *trpA24* mutation in the *trp* operon.

KY7701 derived from strains KY7601, KY7702 derived from strain KY7602, and so on.

Each plasmid in these Trp$^+$ revertants was also assigned a unique number of the type KYCTn, as suggested by Achtman et al. (1), where KY indicates the laboratory in which the mutants were isolated and CT stands for a colicinogenic factor carrying the *trp* genes. A spontaneous Trp$^+$ revertant of strain KY7509, designated KY7600, was used as a control strain in most experiments involving characterization of the mutants harboring a mutant Col*trp* plasmid. The Col$^-$ strain and the parental Col*trp* plasmid derived from strain KY7600 will be called KY7700 and KYCT0, respectively.

Phages M13, Qβ, and MS2 were used to determine the capacity of bacteria to grow male-specific phages, and $\lambda vir h^{80}$ was used for isolation of *tonB-trp* deletion mutants. Phage M13 was kindly provided by V. N. Iyer.

Media. Medium E (14) with 0.5% glucose was used as a minimal medium. Medium EA was medium E supplemented with 0.2% Difco Casamino Acids and 1 μg of thiamine per ml. Peptone-glucose medium (PG broth) consisted of 20 g of polypeptone (Wako Drug Co.), 5 g of NaCl, and 2 g of glucose per liter; pH was adjusted to 7.4. All solid media contained 15 g of agar per liter. Segregation of Col$^-$ cells from strains carrying Col*trp* was scored on medium EA agar supplemented with a limited amount (0.5 μg/ml) of L-tryptophan. To test the sensitivity to male-specific phages, P hard agar (PG agar containing no glucose) and λ top agar containing 10 g of polypeptone, 2.5 g of NaCl, and 5 g of agar per liter were used.

Treatment by NG. A single colony of strain KY7509 was used to inoculate medium E supplemented with appropriate amino acids and thymine and the culture was grown to a log phase at 30 C. NG (Aldrich Chemical Co.) was added at a final concentration of 15 μg/ml, and the culture was kept standing at 30 C for 15 min; it was then washed thoroughly by repeated centrifugations, and the cells were plated directly on medium EA.

Sensitivity to male-specific phages. All male-specific phages were grown on a nonlysogenic derivative of strain A19 lacking ribonuclease I, and were sterilized with chloroform (for Qβ and MS2) or with ether (for M13). Bacterial strains to be tested were grown overnight in medium EA. Samples (0.02 ml) of the cultures were mixed with 3 ml of λ top agar containing 50 mM $CaCl_2$ and were poured onto P hard agar plates. About 5 μl of each phage lysate was then spotted on the plates and incubated at 34 C. The results were scored after 2 to 7 days. Control Col$^+$ strains (KY7509 and KY7600) always showed a clear zone of lysis after 2 days of incubation, but some of the mutant strains showed zone of lysis only after incubation for more than 4 days.

Mating procedure. Transfer of plasmids was usually carried out by incubating the mixture of the donor and recipient cultures (in Penassay broth) at 30 C for 90 min and plating samples on an appropriate selective medium. For mutant plasmids with low transfer efficiencies, 0.05 ml each of donor and recipient culture was inoculated together into 5 ml of Penassay broth and incubated overnight at 30 C. The mixture was centrifuged and washed, and appropriate dilutions were plated on a selective medium. Cells that received the Col*trp* factor from the donor formed colonies after incubation at 30 C for 3 to 5 days. At least several Col$^+$ (Trp$^+$) colonies from each cross were purified and examined for their capacity to segregate Col$^-$ clones.

Determination of frequency of Col$^-$ segregants. For method I, several independent colonies of a Col$^+$ (Trp$^+$) strain on medium EA were used to inoculate PG broth, and the cultures were grown overnight at 30 C. One loopful of each culture was applied to small areas on duplicate PG agar plates that were then incubated overnight at 30 or 42 C. Cell suspensions were made from each growth area, and appropriate dilutions were spread on medium EA with limited tryptophan. After incubation for 4 days at 30 C, cells carrying a Col*trp* plasmid formed yellowish, thick colonies, whereas Col$^-$ segregants requiring trypto-

phan for growth formed pale, flat colonies. At least 100 to 200 colonies were scored for each patch.

For method II, several independent Col$^+$ colonies on medium EA were used to inoculate liquid medium EA; the cultures were grown overnight at 30 C, diluted 10^{-4} into duplicate PG broth, and incubated further at 30 or 42 C. When the cultures reached maximal growth, they were appropriately diluted and plated on medium EA with limited tryptophan, and segregation of Col$^-$ (Trp$^-$) clones was determined as in method I.

RESULTS

Isolation of plasmid mutants. As the parental strain for isolation of plasmid mutants, a tryptophan-requiring mutant carrying a *trpA-E* deletion on the host chromosome and the missense *trpA24* mutation on the Col*trp* plasmid was constructed as follows. Strain KY7521, carrying *trpA24* on the chromosome and *trp*$^+$ *cysB*$^+$ on the Col*trp* plasmid, was mated with a female Col$^-$ strain carrying *cysB* and the *trpA-E* deletion, and Cys$^+$ Col-ductants were selected. Of 1,500 Col-ductants thus obtained, two were found to require tryptophan or indole for growth. Thus, we concluded that they arose by transfer of the Col*trpA24* that was formed by homogenote formation in the donor strain KY7521. The Col*trpA24* plasmid from one of the Trp$^-$ Col-donor ductants was then transferred into strain KY7500 carrying *trpA-E* deletion and several other markers on the chromosome. The resulting Col$^+$ strain (KY7509) was used as the parent for isolation of plasmid mutants.

A number of Trp$^+$ revertants were isolated independently after cells of the parental strain KY7509 were treated with NG. The Trp$^+$ revertants were selected at 30 C so as to retain possible mutants that were temperature sensitive for plasmid maintenance. After incubation for 5 to 7 days at this temperature, revertant colonies appeared at the frequency of 10^{-5} to 10^{-6} per survivor. The survival fraction varied between 4 and 30% depending on the particular experiment. Most of the Trp$^+$ reversions were expected to occur at the *trpA* gene on the plasmid rather than on the chromosome, because the plasmid carried a *trpA* point mutation, but the chromosome carried a *trpA-E* deletion. The following results showed that this was indeed the case.

In the first series of experiments, only fast-growing Trp$^+$ revertants were isolated. They were purified on PG agar and examined for possible alterations in some plasmid functions. About one-third of the revertants (37/101) showed a reduced ability to transfer the plasmid, and more than half (53/101) exhibited an altered pattern of sensitivity to the three male-specific phages tested (Table 2). There were four different types of the sensitivity pattern and, in each type, both transfer-proficient and -deficient mutants were obtained. The nonuniform response to male-specific phages has also been observed with strains carrying certain mutant F factors (1). These results clearly indicate the potential usefulness of the method for isolation of various plasmid mutants. Moreover, since cells were plated on selective medium immediately after the NG treatment, all revertants obtained may be considered as independent in origin from one another.

Plasmid maintenance mutants. The present method was then to search for plasmid mutants defective in self-maintenance during cell growth. Altogether 109 Trp$^+$ revertants, including both fast and slow growers, were isolated from strain KY7509. They were tested for their ability to maintain the plasmid during growth at 30 or 42 C in PG broth (method I was used for rapid screening of these revertants). Among them, 17 strains were found to segregate Col$^-$ clones at frequencies higher than that of the control strain KY7600, a spontaneous Trp$^+$ revertant obtained from the same parent (Table 3). The majority of these mutants segregated Col$^-$ clones at both temperatures, although the segregation frequency was usually higher

TABLE 2. *Alterations of plasmid functions in the mutants*

Type	Response to male-specific phages[a]			Transfer ability[b]	No. obtained
	M13	Qβ	MS2		
Parent	S	S	S	+	
Mutant I	S	S	S	+	41
	S	S	S	−	7
Mutant II	S	S	R	+	2
	S	S	R	−	1
Mutant III	S	R	R	+	5
	S	R	R	−	
Mutant IV	R	R	R	+	16
	R	R	R	−	27

[a] S, Sensitive; R, resistant.

[b] Transfer ability was examined by cross-streaking cells of the mutants (Trp$^+$ revertants) against those of Col$^-$ *trp*-del strain (KY7520) on medium E agar. After incubation at 30 C for 3 to 5 days, the number of Trp$^+$ colonies that appeared in the intersection was scored. +, >20 colonies; −, <10 colonies.

TABLE 3. *Frequencies of Col⁻ segregants from the mutant Col⁺ strains*[a]

Strain	Frequency (%) at:	
	30 C	42 C
KY7600	<0.4	<0.4
KY7601	10.3	99.9
KY7602	7.9	19.3
KY7603	33.3	61.4
KY7604	10.8	96.6
KY7605	9.8	98.6
KY7606	23.5	67.8
KY7607	0.5	3.7
KY7608	14.7	39.9
KY7609	4.1	70.3
KY7610	76.3	98.5
KY7611	27.1	54.3
KY7612	28.0	22.1
KY7613	6.0	8.9
KY7614	6.7	42.9
KY7615	13.1	97.2
KY7616	57.3	97.5
KY7617	1.8	5.6

[a] Frequencies of Col⁻ segregants shown in this table are those obtained by method II, except for KY7601 and 7610 where the values obtained by method I are presented.

at 42 C than at 30 C. Possible reinfection of the plasmid into Col⁻ segregant cells should not affect segregation frequencies appreciably, since all plasmids in these mutants except KYCT1 and KYCT17 were found to be transfer defective. It was also confirmed that Trp⁻ segregants obtained were always Col⁻ when tested for colicine production on appropriate indicator bacteria.

Plasmid nature of the maintenance mutations. To determine whether these mutations affecting plasmid maintenance occurred on the plasmid or on the chromosome, segregation of Col⁻ clones was examined with a pair of Col⁺ strains constructed from each mutant as follows. (i) Col⁻ strains (KY7700 through KY7717) were obtained as spontaneous Trp⁻ segregants at 30 C from each mutant, and the wild-type plasmid KYCT0 was introduced into each of them by crossing with the donor strain KY7521. (ii) The Col*trp* factor of each mutant was transferred into the Col⁻ strains isogenic with the parent (KY7502 and KY7507).

The results obtained with the first series of Col⁺ strains are shown in Table 4. It can be seen that all Col⁺ strains tested, except the one derived from strain KY7601 (KY7801), failed to segregate Col⁻ clones at high frequency. This suggested that all but strain KY7601 were defective in plasmid maintenance due to a mutation or mutations on the plasmid rather than on the chromosome.

Table 5 presents the results of experiments with the second series of Col⁺ strains. Not all of the mutants transferred their plasmid into either of the isogenic Col⁻ strains used, but where the mutants did transfer the plasmid the resulting Col⁺ strains were always found to segregate Col⁻ clones at high frequencies. The frequencies of Col⁻ segregants were somewhat variable between the two isogenic Col⁺ strains examined (Col⁺ derivatives of KY7502 and KY7507), presumably due to variation in number of generations that underwent during incubation in PG broth and in the frequency of Col⁻ cells in the inoculum employed. In spite of these minor variabilities, the results are generally in good agreement with those presented above (Tables 3 and 4), including the temperature-sensitive segregation of Col⁻ clones associated with many of the mutant plasmids. It may be concluded, therefore, that one or more mutations affecting stable maintenance of the Col*trp* plasmid occurred on the plasmid rather than on the chromosome in these mutants with one exception, strain KY7601.

In the case of strain KY7601, it is apparent that a chromosomal mutation is largely responsible for the defect in plasmid maintenance. It

TABLE 4. *Frequencies of Col⁻ segregants from Col⁺ strains, series I*[a]

Strain	Frequency (%) at:	
	30 C	42 C
KY7800	<0.2	<0.4
KY7801	<0.7	74.8
KY7802	<0.3	<0.7
KY7803	0.7	<0.6
KY7804	<0.2	<0.3
KY7805	<0.3	<0.5
KY7806	0.2	<0.8
KY7807	<0.3	<1.0
KY7808	<0.4	<0.5
KY7809	<0.7	<0.8
KY7810	0.7	<0.6
KY7811	<0.3	<0.3
KY7812	<0.3	<0.4
KY7813	<0.4	<0.7
KY7814	0.4	0.3
KY7815	0.2	<0.6
KY7816	—[b]	—[b]
KY7817	<0.3	<0.3

[a] (KYCT0)⁺ derivatives of strains KY7700 through KY7717 were used in this experiment. See text for construction of Col⁺ strains.

[b] Not determined owing to the extremely slow growth rate of this strain.

TABLE 5. *Frequencies of Col⁻ segregants from Col⁺ strains, series 2*[a]

Plasmid (KYCTn)	Frequency (%)			
	KY7502		KY7507	
	30 C	42 C	30 C	42 C
0	<0.4	<0.6	<0.3	<0.2
1[b]	1.2	0.7	4.5	0.3
2	11.4	19.7	20.7	14.9
4			12.7	81.0
5			9.6	74.9
7	2.1	13.3	0.8	43.4
8	25.0	19.8	32.0	10.7
9	12.0	58.9		
10	99.6	>99.6	98.9	>99.8
11	1.4	8.9	19.9	15.6
14	10.0	48.0		
16	11.0	96.3	4.3	83.8
17[b]			4.3	0.4

[a] The Col*trp* plasmid of each mutant (KY7601–KY7617) was transferred to strains KY7502 and KY7507 by conjugation, selecting for Col-ductants that are Trp⁺ Arg⁺ Thy⁺ Ppc⁺ and Trp⁺ Arg⁺ Thy⁺ His⁺, respectively. Frequencies of Col⁻ segregants from these Col⁺ strains were determined by method II. KYCT0 is a control Col*trp* plasmid carried by a spontaneous Trp⁺ revertant (KY7600) of KY7509.

[b] The low frequencies of Col⁻ segregants observed at 42 C may be related to the fact that these are the only mutants that retain the normal ability to transfer the plasmid; re-infection of Col⁻ segregant cells by Col⁺ plasmid occurs much more efficiently at 42 C than at 30 C.

remains possible, however, that a mutation on the plasmid also contributes to the plasmid instability in this strain, in view of the slightly higher frequency of Col⁻ segregants observed with the strains carrying KYCT1 as compared to those carrying KYCT0 (Table 5).

Simultaneous alteration of other plasmid functions. As was expected from the method of selection employed, many mutants defective in plasmid maintenance were also found to be altered with respect to other plasmid functions, such as the ability to transfer the plasmid to other strains and the capacity to support growth of male-specific phages (Table 6). When these mutated plasmids were transferred into the isogenic Col⁻ strains KY7502 or KY7507, the resulting Col⁺ strains inherited the altered phenotypes, in most instances (data not shown). Although some of the characteristics of the mutants observed may be the results of pleiotropic effects of certain mutations involved, it remains possible that a number of simultaneous mutations in the neighboring region of the plasmid DNA are responsible for such multiple alterations.

Growth characteristics of the mutants. During isolation of these mutants, it was noticed that many of them were slow growers as compared to the parental strain either in liquid or solid medium at 30 C. Furthermore, at least two mutants (KY7607 and KY7610) were found to be clearly temperature sensitive; they showed little or no growth at 42 C when tested by streaking dilute cell suspensions on solid medium (medium EA or PG agar). In addition, three other mutants (KY7604, KY7605, and KY7616) exhibited significantly reduced growth rates at high temperatures as compared to the strain carrying KYCT0.

It was also found that the number of colony formers per optical density unit for some of the mutants is significantly lower (up to 100-fold) than that of the parental Col⁺ strain. When overnight cultures of these mutants grown at 30 C were diluted in fresh medium and aerated at 42 C for 4 to 5 h, significant although variable fractions of cells were found to form long filaments. Two temperature-sensitive mutants (KY7607 and KY7610) produced filaments even

TABLE 6. *Simultaneous alterations of the plasmid functions in the maintenance defective Coltrp mutants*

Strain	Transfer ability[a]	Response to male specific phages[b]		
		M13	Qβ	MS2
KY7600	+	S	S	S
KY7601	+	S	S	S
KY7602	±	R	R	R
KY7603	−	R	R	R
KY7604	±	R	PR	PR
KY7605	±	S	R	R
KY7606	−	—[c]	—	—
KY7607	±	R	R	R
KY7608	±	R	R	R
KY7609	±	S	S	S
KY7610	±	R	R	R
KY7611	±	PR	R	R
KY7612	−	R	R	R
KY7613	−	R	R	R
KY7614	±	R	R	R
KY7615	−	—	—	—
KY7616	±	R	R	R
KY7617	+	S	S	S

[a] Transfer ability was determined by the overnight mating procedure. +, 10^{0} to 10^{-1}; ±, 10^{-4} to 10^{-6}; −, $<10^{-8}$ of the wild-type control.

[b] S, Sensitive; R, resistant; PR, partially resistant.

[c] Not tested because of the extremely slow growth of these strains.

at 30 C, although the extent of filamentation was much less than at 42 C. This would at least partially account for the reduction of colony formers per optical density unit observed with these mutants.

DISCUSSION

Guerola *et al.* (6) estimated that more than 50% of the *E. coli* mutants induced by NG under nongrowing conditions contained second mutations in the genes located within 1.6 min on either side of the particular mutation selected; this region corresponds to about 3.5% of the whole chromosome. Since the Col*trp* plasmid has a double-stranded DNA the size of 5% of the chromosome, a specific mutation of the plasmid induced by NG should frequently be accompained by a second mutation elsewhere in the structure. Thus, we have selected reversions of an auxotrophic marker *trpA24*, expecting that most reversions should occur at or near *trpA24* on the plasmid and that such a selection should minimize restrictions on the type of plasmid mutants that may be obtained as secondary mutations. This expectation seemed to be borne out, since the Trp^+ character of many of the revertants, except for those completely defective in the transfer, could be transferred into a female Col^- strain albeit at variable frequencies.

About 60% of the Trp^+ revertants (fast growers) obtained by the present selection turned out to carry a mutation or mutations affecting some of the plasmid characters examined (Table 2). Transfer-deficient mutants, for example, represented 37% of the revertants examined. In contrast, Achtman et al. (1) found that only 0.16% of the survivors after NG treatment represented transfer mutants of the F*lac* factor. By our screening method, about 16% of the Trp^+ revertants obtained (both fast and slow growers included) were mutants defective in maintenance of the plasmid. These included many temperature-sensitive plasmid mutants (at least 6% of the total Trp^+ revertants), similar to those described by Jacob et al. (9). They obtained such mutants from the F*lac* factor at a frequency of 0.01% among survivors after NG mutagenesis. These comparisons illustrate the extremely high efficiency of the indirect screening method reported here. It may also be noted that the selection of only fast-growing Trp^+ revertants yielded a much smaller fraction of mutants defective in plasmid maintenance than did the selection, as in the later experiments, of both slow- and fast-growing revertants. This is quite consistent with our finding that many of the mutants defective in plasmid maintenance are slow growers at low, as well as at high, temperatures.

Mutagenesis with NG in the present experiments was carried out under conditions that allowed growth; cells were exposed to a low concentration of NG for a short period (15 min, which corresponds to one-eighth of the generation time at 30 C). These conditions may allow mutagenesis of a larger region of DNA while reducing the number of mutations per unit length of DNA, as compared to the usual procedure employing higher drug concentrations under nongrowing conditions. We have made no attempt to compare the two methods.

The advantages of the present method for selection of plasmid mutants are as follows. (i) A wide variety of plasmid mutants can be obtained because of the indirect nature of the selection. (ii) Many plasmid mutants for which no screening method is available can be collected as Trp^+ revertants. (iii) All revertants obtained in single NG treatment can be considered as independent in origin, since mutagenized cells are immediately plated on selective medium without postmutagenization incubation. (iv) The method is applicable to other plasmids provided that they carry a certain marker(s) that can conveniently be used for direct selection.

On the other hand, the inherent disadvantage in NG mutagenesis is that the mutant phenotype cannot safely be attributed to a single mutation. Some of the plasmid mutants we have obtained may well contain multiple mutations, besides a Trp^+ reversion, affecting one or more plasmid characteristics studied. In fact, many of the mutants defective in plasmid maintenance were also deficient in transfer and in response to male-specific phages (Table 6). It is possible that the latter two properties are the results of single plasmid mutations affecting the structure of the F-type pili. In this connection, it is noteworthy that the transfer deficiency of the classical replication mutant of F*lac* (9) has recently been shown to be due to a second mutation on the F factor simutaneously induced by the NG treatment (15).

Preliminary characterization of strains carrying mutated plasmids defective in self-maintenance has revealed that they are often slow growers, and some of them are temperature sensitive in that they grow extremely slowly at 42 C but almost as fast as the wild-type strain at 30 C. Some mutants also contained varying fractions of filamentous cells even at low temperatures. Most interestingly, many of these mutant

properties are due to a mutation on the Col*trp* plasmid rather than on the chromosome, at least in some cases (11). The relationships between plasmid mutations and the host growth characteristics are examined in more detail in the accompanying paper (11).

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