JOURNAL OF BACTERIOLOGY, June 1971, p. 1040-1042

Vol. 106, No. 3
Printed in U.S.A.

Non-Essentiality of the *recA*$^-$ Mutation in the Phenomenon of Bacteriophage M13-Induced Elimination of F′ Factors

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Received for publication 5 March 1971

The elimination of F′ factors promoted by coliphage M13 infection can occur in *recA*$^+$ as well as *recA*$^-$ merodiploid strains of *Escherichia coli* K-12.

From an earlier study (7), we concluded that the infection by and multiplication of the filamentous F-specific coliphage M13 in a F′ merodiploid strain of *Escherichia coli* K-12 resulted in a physical elimination of the F′ factor from the infected cells. The design of those experiments involved the use of the genetic markers *malA*$^-$ *str*^{r} *argG*$^-$/F′*mal*$^+$ *str*^{s} *arg*$^+$ in the diploid region and loss of the F′ factor being followed by scoring for Mal$^-$ Sm^r colonies. We showed that scoring for Mal$^-$ Sm^r colonies provided a reliable measure of F′ to F$^-$ conversion. In addition, the strain in question bore the *recA1*$^-$ mutation on the chromosome outside the limits of the diploid region. This was intended to prevent or reduce the integration of the F′ factor into the chromosome, an event which could have complicated interpretations based on the scoring of phenotypes. In fact, the results obtained with this strain indicated that we need not have been concerned about the influence of integration events. The rate of M13-induced conversion to the Mal$^-$ Sm^r Arg$^-$ phenotype was so high as to suggest that F′ integration in a Rec$^+$ strain would neither seriously complicate the interpretation of results nor make such an analysis tedious. Therefore, in principle and in practice, the efficacy and usefulness of this procedure can be examined in all strains of bacteria which harbor autonomous extrachromosomal elements and are susceptible to infection by this phage. As a first step in this direction and before extending this study to other strains bearing bacterial plasmids, we sought to determine whether the presence of the recA$^-$ mutation in this strain is mandatory to M13-induced F′ elimination. In addition to reducing chromosomal recombination and F or F′ integration, mutations in the *recA* gene are known to have several other physiological effects, the basis of which is not understood (2, 4, 6, 8).

An ultraviolet (UV)-resistant spontaneous mutant of the parental merodiploid strain K41 (=JC 1553 *leu*$^-$ *his*$^-$ *met*$^-$ *lac*$^-$ *xyl*$^-$ *recA1*$^-$ *malA*$^-$ *str*^{r} *argG*$^-$/F′ *mal*$^+$ *str*^{s} *arg*$^+$) was isolated. This strain is referred to as K41RP. By the associated criteria of UV sensitivity, relative resistance to spontaneous and UV-induced degradation of deoxyribonucleic acid, and chromosome mobilization (4), K41RP was inferred to be recombination-proficient. The effect of M13 infection on the viability and haploidization of this strain and the parental K41 strain was studied in parallel. The procedures used have been previously described in detail (7).

The frequency of Mal$^-$ Sm^r colonies from these two strains after 4 hr of incubation with or without M13 is indicated in Table 1. During this period, there was no observable alteration in the viable counts of the phage-infected cells, whereas the uninfected cells were estimated to have undergone about four divisions. On further examination of 200 to 300 colonies, 100% of those arising from the Rec$^-$ strain proved to be also Arg$^-$. This confirms our previous observation (6). The experiment has now been repeated several times and we find that in different experiments the efficiency of curing varies from 40 to 100% at 4 hr after infection. In those experiments in which the curing efficiency was relatively low, it was increased on further incubation of the infected cultures. For efficient curing, we find that it is desirable to synchronize the infection by previous starvation of the cells in buffer, a procedure that we have described previously (7), and to ensure that the parental strain bears the F′ episome. (Note: selection by growth in arginine-free medium can sometimes result in very stable lines that are Mal$^+$ Sm^s; such stability presumably

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TABLE 1. *Frequency of Mal⁻ Sm^r after incubation of K41 and K41RP without or with added coliphage M13*[a]

Strain	M13	Frequency (%) of Mal⁻ Sm^r colonies	Mal⁻ Sm^r colonies that were also Arg⁻ (%)
K41	−	0.005	100
	+	81.0	100
K41RP	−	0.04	15
	+	2.8	96

[a] Incubation was for 4 hr at 37 C. Multiplicity of infection: 10.

arises by rare integration of part or all of the F′ episome into the chromosome, despite the demonstrated presence of the *recA*⁻ mutation.) It is also necessary to use phage stocks that do not kill host cells; otherwise, the inferred curing effect could be a spurious one, resulting from the preferential death of F′ cells.

K41RP, unlike K41, segregated Arg⁺ derivatives that were no longer Sm^s or Mal⁺, or both. The frequency of these classes of spontaneous segregants was high for K41RP and very low for K41. For example, of 100 independent spontaneous segregants from K41RP isolated on the basis of streptomycin resistance, 82 were Arg⁺ Mal⁻, 5 were Arg⁺ Mal⁺, and only 15 were Arg⁻ Mal⁻. In a comparable analysis of segregants from K41, 100% of Sm^r segregants were also Arg⁻ Mal⁻. For the following reasons we believe, that in the recombination-proficient K41RP, phenotypes other than Sm^r Arg⁻ Mal⁻ usually arise by recombination events between the F′ episome and the chromosome. (i) F⁻ derivatives of K41RP have been isolated. These are Arg⁻ Sm^r Mal⁻. When such derivatives are grown and approximately 10^8 cells are plated out on a minimal medium selective for the Arg⁺ Sm^r phenotype, no colonies appear. The appearance of SM^r Arg⁺ derivatives from K41RP is, therefore, unlikely to be due to mutations at the *argG* and *str* loci but is rather a consequence of events promoted by the presence of the F′ factor in K41RP. (ii) From four of the Sm^r Arg⁺ K41RP segregants radioactively labeled in their DNA with ^{3}H-thymidine, lysates were prepared as previously described (7). These lysates were sedimented in 5.0 to 20.0% alkaline sucrose gradients (3). The sedimentation pattern, when compared with that of the parental K41RP, indicated that the segregants had lost the fast sedimenting DNA component that is characteristic of the presence of the F′ factor. The absence of covalent circular DNA that is associated with the F′ factor was also confirmed by equilibrium sedimentation in cesium chloride-ethidium bromide gradients (7). Extensive quantitative analyses of all possible types of segregants that may spontaneously emerge from K41 and K41RP have not been undertaken. The evidence obtained, however, indicates that under the experimental conditions used populations of the recombination-proficient K41RP are probably made up of a greater number of segregants than the recombination-deficient K41. Furthermore, a fraction of the segregants from K41RP are sensitive to the F-specific phage R17; under similar conditions, the segregants from K41 were all R17-resistant. This suggested that the population of K41RP includes cells in which the F′ episome or part of it has been integrated into the chromosome, whereas such cells are rare in K41. Thus, when K41RP is grown under the conditions described and infected with phage M13, only those cells in the population that still bear the autonomous F′ episome may be subject to the M13-induced elimination. The assumption is being made that M13 infection does not promote the loss or change of chromosomal mutations. This may serve to explain the observation (Table 1) that the M13-induced increase in frequency of Mal⁻ Sm^r colonies is less with the Rec⁺ strain than with the Rec⁻ strain and that a fraction (4%) of the Mal⁻ SM^r colonies from K41RP were Arg⁺. Exponentially growing populations of six tested Mal⁻ Sm^r Arg⁻ derivatives from the M13-exposed K41RP strain have all been found to be susceptible to conjugal F′ reinfection. They must therefore have initially suffered an M13-induced loss of the F′ factor, as previously demonstrated for the Rec⁻ strain (7).

We conclude that the *recA*⁻ mutation is not directly involved in the M13-induced F′ curing phenomenon and that an isogenic *recA*⁺ strain is susceptible to this curing effect. It seems possible, therefore, that the curing phenomenon we observe may be generally valid and useful for most F or F′ strains and possibly also for strains bearing other episomes. An example of this is strain AB2463 carrying the KLF1 factor (F′ *thr*⁺ *leu*⁺). This strain is resistant to curing by acridine orange (1; *unpublished data*). The F′ factor can, however, be eliminated from this strain by infection with M13. Similar observations have been made with an Hfr strain bearing the R factor KR9 (Russell and Iyer, *unpublished data*) from which the R factor is eliminated on M13 infection.

This research was supported by Canada National Research Council grant A4429.

LITERATURE CITED

1. Bastarrachea, F., and N. S. Willetts. 1968. The elimination by acridine orange of F30 from recombination-deficient strains of *Escherichia coli* K12. Genetics **59**:153–166.
2. Clark, A. J. and A. D. Marguiles. 1965. Isolation and characterization of recombination deficient mutants of *E. coli* K12. Proc. Nat. Acad. Sci. U.S.A. **53**:451–459.
3. Freifelder, D. 1968. Studies in *E. coli* sex factors IV. Molecular weights of the DNA of several F′ elements. J. Mol. Biol. **35**:95.
4. Haefner, K. 1968. Spontaneous lethal sectoring, a further feature of *Escherichia coli* strains deficient in the function of *rec* and *uvr* genes. J. Bacteriol. **96**:652–659.
5. Howard-Flanders, P. 1968. DNA repair. Annu. Rev. Biochem. **37**:175–200.
6. Miura, A., and J. Tomizawa. 1968. Studies on radiation-sensitive mutants of *E. coli*. III. Participation of the *rec* system in induction of mutation by ultraviolet irradiation. Mol. Gen. Genet. **103**:1–10.
7. Palchoudhury, S. R., and V. N. Iyer. 1969. Loss of an episomal fertility factor following the multiplication of coliphage M13. Mol. Gen. Genet. **105**:131–139.
8. Tomizawa, J., and H. Ogawa. 1968. Breakage of DNA in *Rec*$^+$ and *Rec*$^-$ bacteria by disintegration of radiophosphorous atoms in DNA and possible causes of pleiotropic effects of RecA mutation. Cold Spring Harb. Symp. Quant. Biol. **33**:243–252.