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Genetic Evidence for Subunits in the Fertility Repressor Produced by F-Like R Factors[1]

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Repressor-negative mutants derived from R100 were tested for genetic complementation. The results indicate that the active repressor is a multimer. The properties of an unusual repressor mutant are also discussed.

The F-like drug resistance transfer factors (R factors) are capable of repressing the fertility of the F factor and exhibit only a low frequency of transfer in their native state (9). Recent studies have demonstrated that the fertility of these R factors is controlled by a repressor-operator regulatory system (1, 2). An important function controlled by this regulatory system has been demonstrated to be the synthesis of F pili (5). The demonstration that nonsense-suppressor-sensitive repressor mutants can be isolated (1) supports the idea that the repressor molecule is a protein (8). Evidence of genetic complementation is presented here to demonstrate that the active fertility repressor is a multimer.

The bacterial strains, R-factor mutants of R100, media, and methods were previously described (1).

The mutants used in the complementation tests were previously characterized as being either repressor minus or operator constitutive. These mutants are independently sensitive to the F-specific phage MS-2. Since homologous R factors cannot coexist stably (3), complementation tests were possible only if selective pressure for both R factors was maintained. This was accomplished by selecting for both chloramphenicol (Cm) and tetracycline (Tc) resistance by using two R factors with the drug resistance patterns Cm^sTc^r and Cm^rTc^s. All mutants were tested for genetic complementation by forming hetero-R clones using an *Escherichia coli* K-12 strain UC61 (*proA1*, *metE2*, *gal*$^-$, *recA1*, *su*$^+$ amber) as the recipient host. When two similar R factors are forced to remain together, by selection with drugs, they will yield stable recombinants (3). The presence of the *recA1* mutation virtually eliminates recombination between R factors, and stable hetero-R clones can be constructed and maintained on the appropriate selective medium (1). Hetero-R clones that were resistant to MS-2 lysis were taken to be complementing.

A total of 294 crosses were made between 14 Cm^rTc^s repressor-minus (*pil* i^-o^+) R-factor mutants and 21 Cm^sTc^r repressor-minus R-factor mutants. The hetero-R clones isolated on selective media were tested for resistance to MS-2 phage on L agar plates containing 25 μg of chloramphenicol (a gift from Parke, Davis & Co.) per ml and 12.5 μg of tetracycline (a gift from Bristol Laboratories) per ml as previously described (1).

Table 1 lists those mutants demonstrating a positive complementation response (i.e., resistance to MS-2 phage lysis). All other hetero-R recombinants showed no complementation and remained MS-2 sensitive.

Complementation studies reported by Sadler and Novick (7) provided genetic evidence that the lactose repressor consisted of subunits. Physical studies on the lactose repressor have confirmed that the active repressor contains four identical subunits (6). The results reported here provide genetic evidence for the existence of subunits in the active fertility repressor molecule. The data suggest that the complementation is intragenic, and this can be taken as evidence that the fertility repressor contains identical subunits.

Although the R-factor mutant described below does not provide any evidence for subunit interaction, it has been included here since it may provide further information concerning the nature of the fertility repressor. The R-factor mutant UCR109, derived from UCR4 (itself a spontaneous $SMA^rTc^rCm^s$ R-factor mutant of R100) by nitrosoguanidine mutagenesis, was identified on the basis of an unusual response in the MS-2 phage cross-streak test. The growth of $(UCR109)^+$ cells appeared to be enhanced compared to that of $(UCR4)^+$ cells in the presence of MS-2 phage. The mating behavior of a $(UCR109)^+$ strain was similar to that of a

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TABLE 1. *Complementation between repressor-negative mutants*[a]

Cm^s*Tc*^{r} *pil* (*i*$^-$*o*$^+$) mutants	*Cm*^{r}*Tc*^{s} *pil* (*i*$^-$*o*$^+$) mutants						
	drd-68	*drd-70*	*drd-74*	*drd-75*	*drd-76*	*drd-77*	*drd-78*
drd-80	+	+	+	+	+	+	−
drd-81	−	+	+	−	+	+	+

[a] Symbols: +, complementation (resistance to MS-2); −, no complementation (sensitivity to MS-2).

TABLE 2. *Complementation of UCR109 with operator-constitutive mutants*[a]

UCR109	*Cm*^{r}*Tc*^{s} *pil* (*o*^{c}) mutants							
	drd-63	*drd-64*	*drd-65*	*drd-66*	*drd-95*	*drd-97*	*drd-100*	*drd-101*
Cm^s*Tc*^{r}	−	−	−	−	−	+	−	+

[a] Symbols: +, complementation (resistance to MS-2); −, no complementation (sensitivity to MS-2).

strain carrying a wild-type R factor, and its growth kinetics were indistinguishable from a strain carrying its parent R factor when they were infected with MS-2 phage at a multiplicity of infection of 10. Evidence indicating that UCR109 was a repressor mutant came from complementation studies. The enhanced growth response in the cross-streak test was dominant to either a wild-type repressor or a *pil* (*i*$^-$*o*$^+$) mutant in hetero-R clones. The results of complementation tests between UCR109 and eight operator-constitutive-like mutants (*pil o*^{c}) are shown in Table 2.

The mutant UCR109 appears to produce a *trans*-acting repressor. The behavior of this mutant could be explained in two ways. The complementation shown with the two *pil o*^{c} mutants could be the consequence of an altered repressor molecule that is now capable of recognizing the altered operator sites of *drd*-97 and *drd-101* while retaining the ability to bind to a normal operator site, or UCR109 could be a quantitative mutant similar to the *i*^{q} mutants of the lactose operon (4). If the latter case is true, *drd*-97 and *drd-101* should be sensitive to higher concentrations of repressor. Further experiments are being conducted in an attempt to distinguish these possibilities.

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