Genet. Res., Camb. (1971), **17**, *pp.* 89–93
Printed in Great Britain

SHORT PAPER

The effects of *Salmonella typhimurium* on derepressed mutants of F-like factors

By N. D. F. GRINDLEY, E. S. ANDERSON, H. R. SMITH, and JUNE N. GRINDLEY

Enteric Reference Laboratory, Public Health Laboratory Service, Colindale Avenue, London, N.W.9

(*Received* 3 *December* 1970)

SUMMARY

Derepressed mutants of F-like transfer factors, isolated by mutagenesis, were characterized as repressor-minus (i^-) or operator-constitutive (o^c). Mutants of the i^- class are derepressed in K12 but repressed in *Salmonella typhimurium*. They are derepressed in *S. typhimurium* by a kanamycin resistance determinant carrying a locus *der*, described previously. Most o^c mutants of F-like factors are derepressed in both K12 and *S. typhimurium*. However, one mutant of F-*lac* was o^c in K12 but was repressed in *S. typhimurium*. It was derepressed by *der*. Repression by *S. typhimurium* is different from that by fi^+ factors, since *der* reverses the former but does not affect the latter. Possible interpretations of these findings are discussed.

In a previous article (Smith *et al.* 1970) we described the effect of *S. typhimurium* phage type 36 (= *S. typhimurium*) on an F-*lac* factor and on the derepressed F-like R factors R1*drd*19 and R136*drd*H8. *S. typhimurium* carrying either F-*lac* or R1*drd*19 showed poor donor ability and was insensitive to the male-specific phage μ_2; that is, the factors were repressed in the *S. typhimurium* host. In contrast, R136*drd*H8 remained derepressed in *S. typhimurium*. The fact that F and R1*drd*19 are repressor-minus (i^-) mutants while R136*drd*H8 is an operator-constitutive (o^c) mutant (Meynell & Cooke, 1969) was felt to be possibly significant. Repressor-minus mutants do not produce an active repressor but remain sensitive to the repressor, while o^c mutants are insensitive to the repressor although they still code for its synthesis.

An earlier paper described the transfer of a kanamycin resistance determinant (K) by the F factor (Anderson, Mayhew & Grindley, 1969). Later work showed that the K determinant derepressed *S. typhimurium* strains carrying F-*lac* and R1*drd*19 (Smith *et al.* 1970). K could also be transferred by either F-*lac* or R1*drd*19 from such strains to *S. typhimurium*. On the basis of these and other results, we postulated the existence of a locus on the K plasmid responsible for the derepression of F-*lac* and R1*drd*19 in *S. typhimurium*. This locus was designated *der*.

In this paper we describe the effect of *S. typhimurium* on further derepressed mutants of F-like R factors and on o^c mutants of F-*lac*. It should be borne in mind that F-*lac* is i^-, so that o^c mutants of F-*lac* are both i^- and o^c. Table 1 shows the F-like factors studied and their derivation.

Derepressed mutants of the R factors were obtained by the selection procedure of Edwards & Meynell (1968) after treatment of the R factor in *Escherichia coli* K12 (= K12) with ethyl methane sulphonate (EMS) or *N*-methyl-*N'*-nitro-*N*-nitroso-

guanidine (NG). EMS was used at 0·2, 0·3 or 0·4 M in broth, and NG at 50, 250 or 500 μg/ml in 0·1 M citrate buffer, pH 5·5. A standard exposure time of 30 min to each mutagen was used. Derepressed colonies were detected by their sensitivity to phage μ_2. To determine whether the derepressed R factors were i^- or o^c, they were transferred into a K12 strain carrying the factor 782, which also confers resistance to kanamycin. Factor 782 is incompatible with the fi^- I-like factor Δ of Anderson & Lewis (1965). Strains carrying factor 782 propagate the I-specific phage If1. However, 782 represses the fertility and male-specific phage sensitivity of strains carrying the F factor; that is, it is fi^+, in spite of being I-like; it is the prototype of a group of factors shortly to be described (J. N. Grindley and E. S. Anderson, in the press). Since 782 is compatible with both F and F-like R factors, it is useful for testing the repressor sensitivity of derepressed F-like factors. Derepressed mutants which were repressed by factor 782 were designated i^-, while those that remained derepressed were designated o^c. The results were confirmed by examining the effect of the derepressed mutants on the fertility of HfrH. Although direct selection for derepression after mutagenesis yields mainly o^c mutants, we also obtained i^- mutants in this way.

Table 1. *F-like factors and their derepressed mutants*

		Derepressed mutants	
Ref. no.	Resistance*	i^-	o^c
334†	ACSSu	.	334o^c1
R1†	ACKSSu	R1*drd*19	.
240‡	T	240i^-1 and 2	240o^c1 to 8
F-*lac*§	.	F-*lac*	F-*lac*o^c1 to 3

* A, Ampicillin; C, chloramphenicol; K, neomycin–kanamycin; S, streptomycin; Su, sulphonamides; T, tetracyclines.

† R factors 334 and R1 were isolated from a strain of *S. paratyphi B* BB7268. Factor 334 is a kanamycin-sensitive segregant of the original R factor which carried the resistances ACKSSu (see Pitton & Anderson, 1970).

‡ R factor 240 was freshly isolated from its wild host strain of *S. typhimurium* 3M4466. An earlier isolation (E. S. Anderson and N. Datta, unpublished) was designated R136 (Meynell & Datta, 1966).

§ The F-*lac* factor of Jacob and Adelberg (1959), supplied by Professor W. Hayes.

The o^c mutants of F-*lac* were isolated by a modification of the method of Frydman *et al.* (1970). K12F-*lac* carrying 782 was treated with EMS or NG as described above. The treated strain was then incubated overnight in nutrient broth and crossed in a ratio of 20:1, with a K12F$^-$-*lac*-Str^r* recipient carrying 782. Mating was interrupted at 30 min, when 1 ml samples of the mating mixtures were diluted into 100 ml of broth containing 500 μg/ml of streptomycin. After 3 h incubation at 37 °C, 1 ml quantities were subcultured to 100 ml of M9 minimal medium containing 500 μg streptomycin/ml and with lactose (0·2 %) as the sole carbon source. The mixture was allowed to grow overnight. The resultant cultures were then crossed in a ratio of 20:1 with a K12F$^-$-*lac*-Nal^r recipient strain carrying 782. After 30 min, suitable dilutions were plated on MacConkey plates containing 20 μg/ml of nalidixic acid; the plates were incubated overnight at 37 °C. Lactose-fermenting colonies were then purified and tested with male-specific phage μ_2. The majority of colonies tested were sensitive to phage μ_2 in spite of the presence of the fi^+ factor 782, and therefore carried F-*lac* o^c mutants. The three F-*lac*o^c mutants studied were isolated in independent derepression experiments. They were separated from factor 782 by interrupted crosses into K12F$^-$-*lac*-Str^r.

* Str^r = streptomycin-resistant mutant; Nal^r = nalidixic acid resistant mutant.

With the exception of R1*drd*19, all the derepressed mutants listed in Table 1 were obtained by these methods. Each number refers to an independent isolation.

Table 2 shows the effect of 782 on the phage μ_2 sensitivity of the K12 strains carrying the derepressed mutants, and the effect of these mutants on the fertility of HfrH.

It is evident from Table 2 that the i^- mutants R1*drd*19 and 240i^-1 and 2 confer sensitivity to phage μ_2 on K12F$^-$, and are repressed by factor 782 in the same host. They do not significantly reduce the fertility of HfrH. The o^c mutants, in contrast, while conferring μ_2 sensitivity on K12, are not repressed by factor 782, but reduce the fertility of HfrH, presumably because they still produce repressor.

Table 2. *Characterization of derepressed mutants*

Derepressed factor	Sensitivity to μ_2 of K12 carrying the factor	Sensitivity to μ_2 of K12 carrying the factor & 782	Transfer frequency of *pro* from HfrH + factor (HfrH = 1)
334o^c1	+	+	0·001
R1*drd*19	+	−	0·5
240o^c1 to 8	+	+	0·07
240i^-1 and 2*	+	−	0·5–1
F-*lac*o^c1 to 3	+	+	.
F-*lac*	+	−	.

+, Visible lysis with μ_2; −, no visible lysis with μ_2.

* The derepressed mutants 240i^-1 and 2 gave good lysis with μ_2 only when grown without shaking. Consequently all μ_2 phage sensitivity tests of strains carrying these factors were carried out on unshaken cultures.

Table 3. *Sensitivity to phage μ_2 of* Salmonella typhimurium *and* S. typhimurium *K* der *carrying derepressed factors*

		Sensitivity to μ_2	
Derepressed factor	Type in K12	In *S. typhimurium*	In *S. typhimurium* K *der*
334o^c1	o^c	+	NT
R1*drd*19	i^-	−	+
240o^c1 to 8	o^c	+	NT
240i^-1 and 2	i^-	−	+
F-*lac*o^c1	o^c	−	+
F-*lac*o^c2 and 3	o^c	+	NT
F-*lac*	i^-	−	+

NT, Not tested; +, visible lysis with μ_2; −, no visible lysis with μ_2.

All the derepressed mutants were transferred into *S. typhimurium* and the resulting progeny were tested with phage μ_2. The factors repressed in *S. typhimurium* were also transferred to *S. typhimurium* K *der*. Table 3 shows the results of these experiments.

This table shows that all the i^- factors were repressed by *S. typhimurium*. In contrast, all the o^c R factor mutants and two of the three F-*lac*o^c mutants were derepressed in *S. typhimurium*, although one mutant of F-*lac* which was o^c in K12 was repressed in *S. typhimurium*. All the factors repressed in *S. typhimurium* alone were derepressed when that host carried *der*.

When the *fi*+ R factor 334 was transferred to *S. typhimurium* F-*lac*K*der*, the F-*lac* became repressed, and the strain was no longer sensitive to μ_2. Thus, *der* has no influence on the repressor activity of an *fi*+ R factor. The repressor activity of *S. typhimurium* on *i*− derepressed mutants, which is reversed by *der*, is therefore different from that of the *fi*+ R factors.

The Jacob & Monod (1961) model of regulation was first applied by Egawa & Hirota (1962) to the control of F fertility and sex fimbrial synthesis. Using the same model, we suggest the following as a plausible explanation of our observations. *S. typhimurium* produces a repressor which binds to the operator of F or F-like transfer factors. This repressor is different from the *fi*+ repressors encoded by 334 and 782, as shown above. The locus *der* reverses the repression of *S. typhimurium*, perhaps by producing an antirepressor which inactivates the *S. typhimurium* repressor, or by blocking the synthesis of this repressor.

As the *fi*+ R factor repressors tested were not affected by *der*, it must be postulated that they cannot be bound by this antirepressor, or alternatively that their synthesis is not blocked by the *der* product.

We have found that, when F-*lac* or R1*drd*19 is transferred to *S. typhimurium*, although the majority of recombinant colonies are resistant to μ_2, rare recombinant clones are sensitive to the phage. This spontaneous derepression cannot be transferred and is therefore not due to mutation in the transfer factor. When lines which had lost their transfer factor were isolated from these clones, reinfection with F-*lac* or R1*drd*19 gave only derepressed progeny. These clones could be spontaneous repressor-minus mutants of *S. typhimurium*, the existence of which is predictable on the hypothesis that the repression of *i*− mutants of F-like factors by *S. typhimurium* is caused by a host-synthesized repressor.

The F-*lac o*^{c}1 mutant is repressed by *S. typhimurium*, although it remains derepressed in K12 carrying 334 or 782. On the above hypothesis it could therefore be suggested that F-*lac o*^{c}1 has a mutation in the operator rendering it insensitive to the *fi*+ repressors of 334 and 782 but not affecting its sensitivity to the *S. typhimurium* repressor. The effect of the *S. typhimurium* repressor on F-*lac o*^{c}1 is reversed by *der*, as would be expected. These results suggest that *o*^{c} mutants of F, and presumably of F-like transfer factors, can be divided into two classes, depending on whether they are repressed or derepressed in *S. typhimurium*. The *o*^{c} property can thus be defined, not only in terms of the transferable plasmid itself, but also in terms of the host organism, because, as we have shown, a mutant which is *o*^{c} in one host, may be repressed by another.

REFERENCES

ANDERSON, E. S. & LEWIS, M. J. (1965). Characterization of a transfer factor associated with drug resistance in *Salmonella typhimurium*. *Nature, London* **208**, 843–849.

ANDERSON, E. S., MAYHEW, J. N. & GRINDLEY, N. D. F. (1969). Transfer of a neomycin–kanamycin resistance determinant by the F factor of *Escherichia coli* K-12. *Nature, London* **222**, 349–351.

EDWARDS, S. & MEYNELL, G. G. (1968). General method for isolating de-repressed bacterial sex factors. *Nature, London* **219**, 869–870.

EGAWA, R. & HIROTA, Y. (1962). Inhibition of fertility by multiple drug resistance factor (R) in *Escherichia coli* K-12. *Japanese Journal of Genetics* **37**, 66–69.

FRYDMAN, A., COOKE, M., MEYNELL, E. & MEYNELL, G. G. (1970). Repressor-insensitive mutants of the F sex factor. *Journal of Molecular Biology* **48**, 177–179.

JACOB, F. & ADELBERG, E. A. (1959). Transfert de caractères génétiques par incorporation au facteur sexuel d'*Escherichia coli*. *Comptes rendus des séances de l'Académie des sciences* **249**, 189–191.

JACOB, F. & MONOD, J. (1961). Genetic regulatory mechanisms in the synthesis of proteins. *Journal of Molecular Biology* **3**, 318–356.

MEYNELL, E. & COOKE, M. (1969). Repressor-minus and operator-constitutive de-repressed mutants of F-like R factors: their effect on chromosomal transfer by HfrC. *Genetical Research, Cambridge* **14**, 309–313.

MEYNELL, E. & DATTA, N. (1966). The relation of resistance transfer factors to the F-factor (sex-factor) of *Escherichia coli* K12. *Genetical Research, Cambridge* **7**, 134–140.

PITTON, J. S. & ANDERSON, E. S. (1970). The inhibitory action of transfer factors on lysis of *Escherichia coli* K12 by phages μ_2 and ϕ_2. *Genetical Research, Cambridge* **16**, 215–224.

SMITH, H. R., GRINDLEY, J. N., GRINDLEY, N. D. F. & ANDERSON, E. S. (1970). Derepression of F-*lac* in *Salmonella typhimurium* by a determinant for kanamycin resistance. *Genetical Research, Cambridge* **16**, 349–353.