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Derepression of F-*lac* in *Salmonella typhimurium* by a determinant for kanamycin resistance

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SUMMARY

When a determinant for neomycin-kanamycin resistance (K) was transferred by an F-*lac* factor into *Salmonella typhimurium*, the resulting KF-*lac* strain was sensitive to the male-specific phage μ_2 and F-*lac* was derepressed. However, F-*lac* alone is repressed in *S. typhimurium*. When kanamycin resistance is spontaneously lost from *S. typhimurium* KF-*lac* an element persists which derepresses F-*lac* in *S. typhimurium*. The results are consistent with the hypothesis that a locus *der*, for derepression of F-*lac* in *S. typhimurium*, lies on the K plasmid. The R factor R1*drd*19 is derepressed in K12 but is repressed in *S. typhimurium*. It also is derepressed by *der*. In contrast to F-*lac* and R1, another R factor, R136*drd*H8, is derepressed in both K12 and *S. typhimurium*, so that the intervention of *der* is unnecessary for its derepression in the salmonella host.

A determinant for resistance to neomycin and kanamycin (K) was described recently by Anderson, Mayhew & Grindley (1969). This determinant was isolated from a strain of *S. typhimurium* phage type 29 in which it was associated with an *fi*$^+$ transfer factor. The association between K and the transfer factor is characteristic of the class of R factors first observed in *S. typhimurium*, in which the transfer factor and the resistance determinant regularly segregate in transfer, and are independent of each other in the host cell (Anderson & Lewis, 1965*a*, *b*; Smith, Anderson & Clowes, 1970).

For the experiments described in this paper, the K determinant, without its original transfer factor, was isolated in *Escherichia coli* K12F$^-$ (= K12). It was then mobilized with an F-*lac* factor, using the triparental cross for determinant mobilization (Anderson, 1965), the final recipient being *S. typhimurium*, phage type 36 (= *S. typhimurium*). Except for the presence of *lac*, which was simply used as a marker for F transfer to *S. typhimurium*, the resulting R factor, KF-*lac*, was indistinguishable from the KF resistance factor described previously (Anderson *et al.* 1969; Grindley, Grindley & Anderson, 1970). The effects of K on F-*lac*, described below, are thus identical with its effects on the F factor alone.

Transfer of F-*lac* from *S. typhimurium* KF-*lac* and *S. typhimurium* F-*lac* to K12 and *S. typhimurium* is shown in Table 1.

This table shows that *S. typhimurium* KF-*lac* transfers F-*lac* to both K12 and *S. typhimurium* at a frequency a 100-fold higher than that from *S. typhimurium* F-*lac* to the same recipients. All of 148 *S. typhimurium* KF-*lac* recombinants tested were sensitive to the male-specific phage μ_2 (Dettori, Maccacaro & Piccinin, 1961). By contrast, 337 *S. typhimurium* clones that had received F-*lac* alone were resistant to μ_2, although K12 carrying F-*lac* only is sensitive to μ_2. Thus, the F-*lac* factor, which is derepressed in K12,

is ordinarily repressed in *S. typhimurium*, and its derepression in this system seems to be effected by the K determinant. The reactions of *S. typhimurium* and K12 lines with phage μ_2 are summarized in Table 2.

Table 1. *Transfer of F*-lac *from* Salmonella typhimurium *KF*-lac *and* S. typhimurium *F*-lac *in 2 h crosses*

Cross: Donor	Cross: Recipient	Frequency of F-*lac* transfer	Sensitivity of donor to phage μ_2	Proportion of donor cells carrying F fimbriae (electron microscopy)
S. typhimurium KF-*lac*	× K12	1×10^{-3}	+	18/28 = 64 %
	× *S. typhimurium*	2×10^{-3}		
S. typhimurium F-*lac*	× K12	2×10^{-5}	−	1/36 = 2·8 %
	× *S. typhimurium*	2×10^{-5}		
S. typhimurium (K)⁻ F-*lac**	× K12	1×10^{-2}	+	21/47 = 44·7 %

* Resulting from spontaneous loss of K from *S. typhimurium* KF-*lac*.
\+ = Visible lysis in spot tests with phage μ_2 on surface culture (Grindley *et al.* 1970);
− = No visible lysis with μ_2.

Table 2. *Reactions of* S. typhimurium *and K*12 *strains with male-specific phage* μ_2

Strains	Reaction with μ_2
S. typhimurium F-*lac*	−
S. typhimurium KF-*lac*	+
S. typhimurium (K)⁻ F-*lac**	+
S. typhimurium (KF-*lac*)⁻†	−
K12 F-*lac*	+
S. typhimurium	−
K12 F⁻	−

\+ = Visible lysis with μ_2; − = No visible lysis with μ_2.
* Spontaneous loss of K.
† Spontaneous loss of both K and F-*lac*.

The state of repression of *S. typhimurium* KF-*lac* and *S. typhimurium* F-*lac* was also investigated by determining the degree of F fimbriation in the electron miscroscope (see Table 1). Specific adsorption of phage μ_2, followed by negative staining with sodium silicotungstate, was used for the identification of sex fimbriae. Of 28 *S. typhimurium* KF-*lac* cells observed, 18 (64 %) carried sex fimbriae, while *S. typhimurium* F-*lac* showed only one sex-fimbriated cell out of 36 examined (2·8 %).

Other workers have also observed the repression of an F factor in *S. typhimurium* (Mäkelä, Lederberg & Lederberg, 1962; Easterling *et al.* 1969), although no explanation has been suggested and derepression does not seem to have been described.

Lines of *S. typhimurium* KF-*lac* were then examined for spontaneous loss of K, and it was found that when such loss occurred the derepression persisted, as shown in Table 1. This was also confirmed by observation of sex-fimbriation by electron microscopy.

Further experiments with *S. typhimurium* carrying the derepressed F-*lac* factor without K showed that, although the derepressed state was stable in that host strain,

subsequent transfer of this F-*lac* to *S. typhimurium* produced a majority of recombinants in which F-*lac* was repressed. However, about 4% of 220 recombinant clones tested retained the derepression. As F-*lac* in this experiment transferred at 2×10^{-1} in an overnight cross, the frequency of transfer of derepression was 4% of 2×10^{-1}, that is, 8×10^{-3}. This frequency is very similar to that of K transfer from *S. typhimurium* KF-*lac* in overnight crosses to *S. typhimurium*, about 10^{-2}.

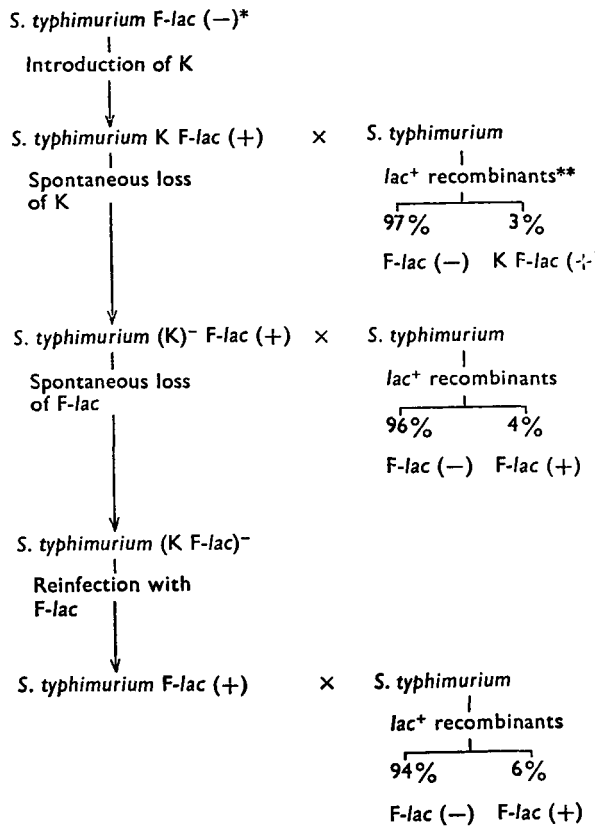

Fig. 1. Properties of F-*lac* and K in *S. typhimurium*. * (+); (−): reactions with male-specific phage μ_2. ** F-*lac* was transferred at a frequency of *c.* 2×10^{-1} in overnight crosses in all cases.

It should be noted that whether F-*lac* is in the repressed or derepressed state in *S. typhimurium*, it is derepressed in K12.

An *S. typhimurium* line that had spontaneously lost both K and F-*lac* (see Table 2) was then investigated. When a (repressed) F-*lac* factor was transferred from *S. typhimurium* into this strain, sensitivity to μ_2 was regained. The recipient strain had therefore retained the element for derepression of the F-*lac* factor, in spite of the loss of K and F-*lac*. Crosses from the resultant derepressed *S. typhimurium* F-*lac* strain into *S. typhimurium* established that F-*lac* was transferred at the same frequency as before, that is, $1{\cdot}8 \times 10^{-1}$, and that it was derepressed in 6·5% of *S. typhimurium* F-*lac* recipients. Thus, the frequency of transfer of the derepression of F-*lac* was unchanged (*c.* 1×10^{-2}).

These observations are summarized in Fig. 1.

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The results suggest that a derepressor locus, which we designate *der*, is associated with the K plasmid. This hypothesis is supported by the fact that the transfer frequency of *der* by *S. typhimurium der* F-*lac* is the same as that of the original K plasmid. Spontaneous loss of kanamycin resistance may result from mutation in, or deletion of, the resistance locus, and retention of *der* may indicate that the remainder of the plasmid is intact. This residuum, carrying *der*, would be transferable by F-*lac* in the same way as the original K plasmid.

Alternatively, *der* could be on a separate plasmid from K, in which case the plasmids should be lost independently of each other. However, if this hypothesis is correct the two plasmids must be postulated to be very closely associated during transfer in spite of their independence, as we have been unable to separate the kanamycin resistance from the derepression property by conjugation.

If *der* is effectively part of the K plasmid, the properties of derepression and kanamycin resistance should be readily co-transduced, whereas if the two markers are on independent plasmids, co-transduction should be a very rare event. Bacteriophage P22 was grown on *S. typhimurium* KF-*lac*. The resulting phage preparation (titre *c.* 8×10^{10} p.f.u./ml) was sterilized with toluene, and 1 ml was mixed with an equal quantity of a culture of *S. typhimurium* F-*lac* (*c.* 5×10^8 organisms/ml). After 30 min at 37 °C the bacteria were washed and plated with selection for kanamycin resistance. Of 150 kanamycin-resistant transductant colonies tested, 16 (10·7 %) were sensitive to the male-specific phage μ_2. This co-transduction supports the suggestion that K and *der* are closely linked.

The possibility that *der* might affect derepressed factors other than F was explored. Two derepressed fi^+ R factors, R1*drd*19 and R136*drd*H8 (Meynell & Datta, 1967) were investigated. These will be referred to as R1 and R136 hereafter. R1 carries resistance to ampicillin, kanamycin, streptomycin and sulphonamides, and R136 resistance to tetracyclines only. R1 and R136 were originally identified in strains of *S. paratyphi B* phage type 3a var 4 and *S. typhimurium* phage type 29 respectively (E. S. Anderson & N. Datta, unpublished).

K12 carrying R1 or R136 is sensitive to μ_2. K12 R1 was crossed with *S. typhimurium* and *S. typhimurium der*, to yield *S. typhimurium* R1 and *S. typhimurium der* R1 progeny respectively. Forty-nine of 50 colonies of *S. typhimurium* R1 gave no visible lysis with μ_2, while all of 40 *S. typhimurium der* R1 colonies gave good lysis with the phage. In the colony of *S. typhimurium* R1 which gave lysis with μ_2, this lysis was more turbid than that on *S. typhimurium der* R1.

Transfer of R1 from μ_2-insensitive *S. typhimurium* R1 to *S. typhimurium* occurred at a repressed frequency of $2{\cdot}5 \times 10^{-4}$ in 2 h, and $1{\cdot}6 \times 10^{-2}$ overnight. The μ_2-sensitive line of *S. typhimurium* R1, in contrast, transferred R1 at a derepressed frequency of 10^{-1} in 2 h and $4{\cdot}5 \times 10^{-1}$ overnight. The derepression was not transferred, however, since all of 100 progeny tested from the overnight cross were insensitive to μ_2.

S. typhimurium der R1 transferred R1 to *S. typhimurium* at a frequency of $1{\cdot}5 \times 10^{-1}$ in a 2 h cross, and 3×10^{-1} overnight. Ten of 100 colonies from the overnight cross were sensitive to μ_2. Thus, *der* was transferred by R1 at a frequency of about 10^{-2}, similar to that of its transfer by F-*lac*.

When R136 was transferred from K12 to *S. typhimurium* and *S. typhimurium der*, all the *S. typhimurium* R136 and *S. typhimurium der* R136 progeny tested (20 of each) gave good lysis with μ_2.

The presence of *der* in *S. typhimurium* therefore potentiated derepression of R1*drd*19, as it did of F-*lac* in that host. Since R136*drd*H8 is already derepressed in *S. typhimurium*, there was no evidence that its state of derepression was affected by *der*.

It may be significant that F and R1*drd*19 are i^- mutants which do not synthesize repressor, but are sensitive to repressor in K12, while R136*drd*H8 is an o^c mutant which

is insensitive to repressor in K12 (Frydman & Meynell, 1969; Meynell & Cooke, 1969). The possibility that *S. typhimurium* actively represses both F and R1*drd*19 must therefore be explored.

Alternatively, *S. typhimurium* may be (passively) unable to express the derepressed state of F-*lac* and R1*drd*19 without the intervention of an element such as *der*. In any case, it is reasonable to suppose that the derepressing effect of *der* is the same in *S. typhimurium* R1*drd*19 as it is in *S. typhimurium* F-*lac*.

On the basis of the above evidence we conclude that the K plasmid, which apparently possesses no transfer factor, carries a locus *der*, which derepresses F-*lac* and R1*drd*19 in *S. typhimurium*.

These phenomena are under further examination and our findings will be reported later.

[*Note added in proof.*] Recent experiments support the hypothesis that *S. typhimurium* represses i^- but not o^c mutants of F-like plasmids. This work is being prepared for publication.

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