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*INTERACTION OF STREPTOMYCIN AND A SUPPRESSOR FOR GALACTOSE FERMENTATION IN E. COLI K-12**

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An observation was made some time ago on a suppressor of a galactose-negative mutation in *E. coli*. The suppressor action was found to be overcome by a mutation to streptomycin resistance. Furthermore, in some of the streptomycin-resistant stocks, the action of the suppressor was partially restored by the addition of streptomycin (Table 1). Although the situation was not analyzed in much detail, this brief report may be relevant to current interest in the effect of suppressor genes,[1–3] and particularly in the light of Gorini's suggestion[4] that streptomycin may act as a phenotypic suppressor altering the reading of the genetic code.

The Suppressor Mutation.—As summarized in Table 1, a Gal_4^- mutant, blocked in the capacity to produce UDP galactose transferase,[5] was isolated after ultraviolet irradiation of *E. coli* strain Y-87,[11] a derivation of K-12. A reversion from it, selected on EMB galactose medium, was indicated to be the result of a suppressor mutation, as lambda phage prepared from it by UV induction transduced Gal^+ to all mutants tested, except to Gal_4^-. The recovery of Gal^- recombinants in crosses between the suppressed strain and a Gal^+ strain is also observed in Table 3, cross 2.

On closer examination, the suppressed strain, W-1802, can actually be distinguished from wild-type Gal^+ by virtue of slower fermentation on EMB indicator

TABLE 1

HISTORY OF THE STRAINS

Name	Genotype	Galactose transferase phenotype		Strain no.
Wild-type	*Gal*$^+$	Normal (+)		Y-87
Gal mutant	*Gal*$_1^-$	Absent (−)		W-518*
Revertant (suppressed)	*Gal*$_4^-$*su*(*Gal*)†	Partially restored		W-1801 and W-1802
Streptomycin-resistant	*Gal*$_4^-$*su*(*Gal*)*Sm*^{r}	Streptomycin		
		Present	Absent	
		+	− ‡	W-4903
		− ‡	+ ‡	W-4904

* Intermediate mutations not relevant to the history of these strains[11] are omitted here as well as other markers not directly concerned.
† The phenotype of this strain will be described here as *Gal*^{s}, for *slow* fermentation.
‡ The phenotype of these strains is usually negative on the first day of observation and may show some weak fermentation on the second day. It is therefore called here *Gal*^{vs} (*very slow*).

TABLE 2

CROSS 1*

Time (min)	Percentages of Male Alleles for Markers†					
	su$^+$	*Xyl*$^-$	*Mtl*$^-$	*M*$^+$	*Lac*$_4^-$	*Ade*$^-$
20	77	4	2	0	0	0
40	28	19	16	0	0	0
60	32	32	24	0	0	0
90	52	54	48	7	2	0

* Cross 1, between strains:
Mal$_1^+$ *Xyl*$^-$ *Mtl*$^-$ *M*$^+$ *Th*$^-$ *Lac*$_4^-$ (*Gal*$_4^+$*Gal*$_2^-$) *Ade*$^-$ (W-4884, also: *su*$^+$)
Mal$_1^-$ *Xyl*$^+$ *Mtl*$^+$ *M*$^-$ *Th*$^+$ *Lac*$_4^+$ (*Gal*$_4^-$*Gal*$_2^+$) *Ade*$^+$ (W-4878, also: *su*(*Gal*))
† Selection for *Mal*$_1^+$ recombinants was carried out after interruption of the mating at the times indicated. Percentages obtained from 40–50 recombinants each.

media, as might be expected of a suppressed strain. A direct assay for transferase carried out by R. L. Soffer (unpublished) confirmed the re-establishment of transferase activity in W-1802. The specificity of the suppressor, *su*(*Gal*)$_4$ with respect to other mutants in the same cistron, and other cistrons, has not been determined.

Streptomycin-resistant Mutants and Streptomycin-dependent Fermentation (sdf).—When a streptomycin-resistant mutant was selected from the suppressed, galactose-fermenting strain W-1802, it was found that the capacity to ferment had been lost again if the fermentation test was carried out in the absence of streptomycin, but was similar to that of the parental W-1802 strain in the presence of streptomycin. Ten independent streptomycin-resistant mutants were then selected from W-1802 to test for possible differences. All of them had lost the capacity to ferment galactose, at least by the EMB test, although three of them were still capable of fermenting at a very low rate. This behavior had not been encountered before in streptomycin-resistant mutants from normal, galactose-fermenting strains. When, however, the fermentation test was carried out on EMB media supplemented with streptomycin in concentrations toxic to sensitive strains, it was found that four of the ten resistant strains were capable of fermenting at a rate similar to that of W-1802. In other words, some of the strains had become streptomycin-dependent for the fermentation of galactose (*sdf*), though not for growth. Genetic instability was a remarkable feature of most of the suppressor-carrying streptomycin-resistant mutants.

Mapping the Suppressor Locus.—The strain carrying the *su*(*Gal*)$_4$ gene is a female, methionine auxotroph (F$^-$M$^-$). A *Mal*$_1^-$ marker was added by selecting for resistance to a virulent phage mutant of lambda,[6] thus obtaining strain W-4878. The latter was crossed to male W-4884 (*Vfr*, or very high frequency of recombina-

tion[7]). This male injects the *Mal* locus at about 20 min. The order of entry of the other markers is presented in Table 2. Both male and female are streptomycin-sensitive so that the segregation of the suppressor, $su(Gal)_4$ carried by the female, can thus be scored directly. The data in Table 2 show clearly that $su^+(Gal)$ enters earlier than any of the other loci tested. Therefore, it is closely linked to *Mal* and probably enters earlier than *Mal*.

Crosses using a streptomycin-resistant marker were carried out with streptomycin-resistant *Vfr* males (like W-4884 provided by E. A. Adelberg). W-4882 (AB-312) used in cross 2, Table 3, is believed to have the same order of entry as the male used in Table 2, while W-4883 (AB-313) used in cross 3, Table 3, has the

TABLE 3

CROSSES 2 AND 3

Cross	Time (min)	No. Mal^+ recombinants	Sm^s		Sm^r		
			Gal^s*	Gal^-	Gal^s	Gal^{rs}*	Gal^-
2	20–60	314	46	0	0	261	7
3	20	56	55	0	0	0	1
	30	42	28	0	0	14	0
	40–90	136	71	0	5	90	0

* See second footnote, Table 1.

Crosses 2 and 3 were interrupted at various intervals; only time intervals showing differences are reported separately. Only Mal^+ recombinants tested.

reverse order of entry, with *Mal* entering at 15 min. The appearance of Gal^- segregants in a $Gal^s \times Gal^+$ mating is shown by cross 2, Table 3. Because the segregations of *Gal* and of *Sm* are independent of time, both markers are believed to be located on the same side with respect to *Mal* to suggest the order: *O...su...-Sm...Mal.* Cross 3 shows that *Sm* enters after *Mal* with this male, but su^+, expected to enter after *Sm* on the basis of the order given above (reversed with this male as *O...Mal...Sm...su...*) does not seem to enter to any significant extent. The appearance of a few Gal^s and one $Gal\text{-}Sm^r$ is not readily explained. Apart from this inconsistency, which may be due to chance, to the existence of modifiers in this male, or other peculiarities of the chromosomal region under investigation, it would seem that the suppressor may be mapped not far from *Sm*, away from *Mal*.

Discussion.—Our findings may be summarized as follows: in a particular strain of *E. coli* K-12, mutation to streptomycin resistance was found to affect the enzyme galactose-transferase, whose production then becomes streptomycin-dependent. In several other streptomycin-resistant mutants, however, there is an almost complete elimination of enzymatic action, both in the presence and absence of streptomycin.

The strain showing this peculiar behavior was capable of producing enzyme at a subnormal rate, thanks to the presence of a gene suppressing the action of another mutation, which had in turn inhibited the formation of the enzyme. Streptomycin resistance made the action of the suppressor gene streptomycin-dependent.

The physiology of the suppressor in question seems to merit further investigation. Genetic studies are incomplete, but a chromosomal or mapping location not far from the streptomycin gene seems reasonable on the basis of the data summarized above.

According to present views, many suppressors act by perturbing the code of specific amino acids, at least partially, in such a way that a mutant making an

altered and inactive protein because of an amino acid change, under the action of the suppressor can make some normal protein.[1-3] On the other hand, streptomycin resistance is believed to be a property of the ribosomes.[8] This may seem at first sight to conflict with the view that streptomycin resistance is recessive, at least in *E. coli*.[9] In heterozygotes both types of ribosomes, streptomycin-sensitive and resistant, should be produced; if, in the presence of streptomycin, only the former do not function, protein synthesis would be reduced to one half, presumably compatible with life, and making resistance dominant. The aggregation of ribosomes into polysomes,[10] however, coupled with the hypothesis that streptomycin may prevent the progression of the messenger by jamming the mechanism of advancement, may explain the dominance of sensitivity. Under such a hypothesis, in fact, it would be enough if one ribosome in a polysome chain were of the sensitive type, to prevent the formation of protein by all the ribosomes of the chain. The residual activity would then be only 1 in 2^n, if n is the number of elements in the polysome. The drastic reduction of the rate of protein synthesis thus determined might therefore explain the dominance of streptomycin sensitivity in cells heterozygous for resistance sensitivity.

In its simplest form, the hypothesis would assert that streptomycin tends to displace messenger RNA from the ribosome thus perturbing its transcription and eventually jamming its passage. Some mutations, by altering ribosome structure, also disturb the messenger-ribosome complex, and because they perturb transcription, act as suppressors. According to this lemma, neighboring codons could influence the extent of perturbations and allow some discrimination in the occurrence of transcription noise.[3] The mutation for streptomycin resistance (Sm^r) modifies the ribosome in the opposite sense so as to increase its affinity for typical messengers to mitigate the perturbations resulting from the presence either of streptomycin or of certain suppressor mutations. The mitigation may, however, fail to cope with both disturbances simultaneously, and streptomycin-dependent suppression may result. Finally, without regard to suppressors, the altered ribosome may bind the messenger too tightly for normal function, in this case producing the phenotype of streptomycin dependence for growth.

There is a close relationship between our studies and those of Gorini,[4] who found mutants for several amino acids, such that the requirements of one amino acid in a given mutant could be dispensed with by the addition of streptomycin. This "conditional streptomycin dependence" is quite analogous in that it seems that streptomycin restores the production of a specific enzyme, albeit a different one in each strain. Gorini suggests, on the basis of these results, that streptomycin acts by increasing the ambiguity of the code, thus leading to the synthesis of wrong protein. The argument of dominance of sensitivity mentioned above, which could now be tested directly *in vitro*, would specify that streptomycin jams the advancement of the messenger in the polysome chain, perhaps by linking it to ribosomal RNA.

Summary.—An *E. coli* K-12 strain which has lost its capacity to produce galactose transferase carries a suppressor mutation (mapping not far from streptomycin resistance) which has partially restored the capacity to ferment. Some mutations to streptomycin resistance in this suppressed strain make galactose fermentation streptomycin-dependent. The implications of the similarity between suppressor genes and streptomycin drug action are discussed. It is possible that some sup-

pressors act by altering the ribosome and that streptomycin acts by jamming the mechanism of advancement of the messenger, and it is suggested that this might explain the dominance of streptomycin sensitivity in heterozygotes.

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*REPLICATION OF VIRAL RNA, III. DOUBLE-STRANDED REPLICATIVE FORM OF MS2 PHAGE RNA**

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The multiplication of viruses which carry the genetic information in a single strand of RNA[1] has been the subject of detailed investigations in several laboratories. It has been established that the replication of viral RNA does not involve host DNA[2–5] and that the RNA itself can function as a messenger in a system of protein synthesis *in vitro*, directing the synthesis of coat protein[6] and possibly of other proteins[6, 7] required for virus multiplication. Infection with an RNA virus leads to the appearance of a new RNA-synthesizing enzyme in both animal cells[8, 9] and bacteria.[10–13] The enzyme induced by infection of *Escherichia coli* with phage MS2 was partially purified, and circumstantial evidence was presented that this enzyme (RNA synthetase) is involved in the replication of the viral RNA.[10, 11]

Further insight into the replication mechanism of viral RNA was provided by the observation that infection of Krebs II ascites cells with the RNA-containing encephalomyocarditis virus leads to the accumulation of virus specific double-stranded RNA (replicative form) at the end of the infection cycle.[14] The significance of this finding was underlined by our subsequent observation[15] that part of the radioactivity incorporated into acid-insoluble products on incubation of RNA synthetase with C^{14}-labeled ribonucleoside triphosphates was present in double-stranded RNA. We further reported on preliminary experiments indicating that, upon infection of *E. coli* with P^{32}-labeled MS2 phage, the infecting P^{32}-RNA strand was converted into a double-stranded form.[15] These results clearly supported the earlier suggestion[11] that replication of RNA-containing viruses involves the forma-