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NOTES

Development of Auxotrophy by Streptomycin-Resistant Mutation

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Several streptomycin-resistant mutants of *Escherichia coli* have been isolated which require exogenous isoleucine for growth. The majority of these strains were of streptomycin-dependent phenotype. If grown in the absence of streptomycin, these streptomycin-dependent auxotrophs ($Sm^{d\text{-}aux}$) strains were unable to produce β-galactosidase and aldolase activities and also failed to exhibit donor properties in conjugation. Genetic analysis indicated that the isoleucine requirement of these strains could be caused by a mutation at the *strA* locus.

In streptomycin-sensitive *Escherichia coli* all mutations conferring high-level resistance to streptomycin are located at the *strA* locus (3). The *strA* locus determines the structure of the ribosomal protein P10 (3). Some streptomycin-resistant mutations when grown in the presence of streptomycin or in association with a second ribosomal mutation, namely *ram*, can suppress mutant phenotypes of many nonsense mutations (2). In the present report we describe a phenomenon in which a mutation that confers high-level resistance to streptomycin also suppresses some of the wild-type characters of the streptomycin-sensitive parent.

A large number of spontaneous streptomycin-resistant derivatives of the prototroph parent E3003 (Hfr *pgi glp* Sm^S) were screened for auxotrophy. Thirteen isolates out of a total of 438 streptomycin-resistant derivatives of strain E3003 were found to require exogenous isoleucine for growth. Ten out of the 13 isoleucine auxotrophs also needed addition of streptomycin for full growth; these were termed $Sm^{d\text{-}aux}$ mutants. Growth of 3 of the 13 isoleucine auxotrophs were insensitive to the presence of streptomycin; these mutants were termed the $Sm^{r\text{-}aux}$ mutants.

Table 1 summarizes some of the properties of the $Sm^{d\text{-}aux}$ and $Sm^{r\text{-}aux}$ strains compared with those of their parent E3003. It may be noted that if grown in the absence of streptomycin, the $Sm^{d\text{-}aux}$ strains were unable to produce β-galactosidase and aldolase activities and were also unable to act as donors in conjugational crosses. Growth in the presence of the drug retrieved these deficiencies, showing that these disturbances were phenotypic rather than genotypic. The β-galactosidase and aldolase activities of the $Sm^{r\text{-}aux}$ strains were not influenced by the presence of streptomycin; nor could the loss of donor ability be recovered by growth in the presence of the drug.

It was, however, found that the $Sm^{r\text{-}aux}$ strains and the $Sm^{d\text{-}aux}$ strains would function as recipients in conjugational crosses if these strains were pregrown in absence of streptomycin. This property of the strains was exploited to determine the separability of the isoleucine requirement from streptomycin-resistance and also to determine the linkage between the mutation causing streptomycin resistance and isoleucine auxotrophy with the *strA* locus. Conjugation experiments were carried out under the conditions indicated to maximize the yield of recombinants (1), and the results are summarized in Table 2.

It should be noted that when a streptomycin-sensitive donor (*strA*$^+$) was used, recombinants of the (isoleucine)$^+$ Sm^R phenotype could not be obtained (see Table 2, crosses 1 and 2). Even when 200 colonies of the (isoleucine)$^+$ phenotype from each of cross 1 and 2 were screened on media containing streptomycin, none was found to be resistant to the drug. When a streptomycin-resistant (*strA*) donor was used instead (see crosses 3 and 4), recombinants of the (isoleucine)$^+$ Sm^R phenotype were readily obtained. Moreover, none of the 200 recombinant

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TABLE 1. *Comparison of the properties of E3003 and its streptomycin-resistant auxotroph derivatives*

Strain	Streptomycin (100 μg/ml) during growth	Growth in absence of exogenous isoleucine	β-Galactosidase and aldolase activities	Mating type
E3003	Absent	+	+	Hfr
$Sm^{d\text{-}aux}$	Absent	−	−	F⁻
	Present	−	+	Hfr
$SM^{r\text{-}aux}$	Absent	−	+	F⁻
	Present	−	+	F⁻

TABLE 2. *Lack of segregation of isoleucine requirement from streptomycin resistance in str ($Sm^{r\text{-}aux}$) and str ($Sm^{d\text{-}aux}$) derivatives of E3003*

Cross	Selected phenotype (no. of recombinants/10^8 recipient cells)	
	(isoleucine)$^{+a}$	(isoleucine)$^{+}$ Sm^{Rb}
1. HfrC-*met strA*$^+$ × *str* ($Sm^{r\text{-}aux}$)	8×10^2	0
2. HfrC-*met strA*$^+$ × *str* ($Sm^{d\text{-}aux}$)	10^3	0
3. HfrC-*met strA* × *str* ($Sm^{r\text{-}aux}$)	10^3	10^3
4. HfrC-*met strA* × *str* ($Sm^{d\text{-}aux}$)	10^3	10^3

[a] (isoleucine)$^+$ recombinants were selected on minimal medium-agar-glucose.

[b] (isoleucine)$^+$ Sm^R recombinants were selected on minimal medium-agar-glucose containing streptomycin (100 μg/ml).

colonies of the (isoleucine)$^+$ phenotype obtained from each of the cross 3 and 4 was streptomycin sensitive when screened on streptomycin-containing media. It is indicated therefore that streptomycin resistance and isoleucine auxotrophy in these strains were due to a single mutation very closely linked to or at the *strA* locus itself.

The data presented here suggest that certain mutations affecting the structure of ribosomal protein can suppress expression of one or more genes determining biosynthesis of isoleucine. These genes determining biosynthesis of isoleucine are normally active in the wild-type streptomycin-sensitive parent. The phenotypic disturbances of the $Sm^{d\text{-}aux}$ strains (Table 1) would indicate that disruption of ribosomal function by the environmental stimulus, namely growth in presence of streptomycin, could also suppress expression of some genes, e.g., those of β-galactosidase and aldolase. It is conceivable that certain ribosomal changes could suppress expression of genes which are active in the wild-type strain either by erroneously translating the corresponding message to yield nonfunctional protein or by being unable, for some unknown reason, to translate the corresponding messages. Experiments are in progress to distinguish between the two possibilities.

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