HIGH PLEIOTROPY OF STREPTOMYCIN MUTATIONS IN ESCHERICHIA COLI.

M. Couturier, L. Desmet and R. Thomas

Laboratory of Genetics, Faculty of Sciences, University of Brussels, Belgium.

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Bacterial mutants selected for their resistance to streptomycin (resistant Sm^r, or dependent Sm^d) frequently manifest characters which have no obvious relation with the Sm^r or Sm^d character itself (Lederberg,1957 ; Thomas and Lambert,1962 ; Rosenkranz,1964).

We wish to report here about the highly pleiotropic character of many Sm^r mutation in E. Coli K 12, strain C600 (Appleyard,1954). This pleiotropy is in some ways similar to that of allele-specific "supersuppressors".

As shown in table I, most Sm^r (or Sm^d) derivatives of C600 express three new characters and there is a complete correlation in that each strain expresses either all three or none. These mutations :

1. Partially suppress (more specifically, shift to higher temperatures) the temperature-sensitivity of the regulatory mechanism of λc_{72} (this is known as "temperator" character of the Sm^r mutants : see Thomas and Lambert, 1962).
2. Reduce the rate of growth.
3. Suppress the restriction imposed by prophage P1 to the growth of phage λ previously grown on a strain non lysogenic for P1. The fact that some Sm^r mutations suppress this restriction was observed by Lederberg (1957). Arber made all our Sm^r derivatives of C600 lysogenic for P1 and discovered the remarquable correlation between the suppression of restriction and the temperator character (personal communication). We extended this result to phages 434 and 82.
   Table II gives numerical data on the temperator character and the suppression of restriction by some of the Sm^r mutants.

Table I

		"Temperator" character	Abnormally slow growth	Suppression of restriction of λ by prophage P1
R4	Sm^r	+	+	+
R6	"	+	+	+
R7	"	+	+	+
R8	"	+	+	+
R9	"	–	–	–
R10	"	–	–	–
R11	Sm^r, probably a double mutant Sm^d-su	+	+	+
R12	Sm^r	+	+	+
R13	"	+	+	+
R14	"	+	+	+
R15	"	+	+	+
R16	"	+	+	+
R17	"	–	–	–
R18	"	+	+	+
R19	"	+	+	+
R20	"	+	+	+
R21	Sm^d	+	+	+

Table II.

Bacterial mutant	Characteristics	Frequency of lysogenization by λc_{72} at 37° *	Efficiency of plating (e.o.p.) of λ, 434hy, 434 and 82 on the strain made lysogenic by P1**
C600	Sm^s	0.01	10^{-4} - 10^{-5}
C600 # R4	Sm^r, "temperator"	10.0	10^{-1}
C600 # R9	Sm^r, non-temperator	0.01	10^{-4} - 10^{-5}
C600 # R10	"	0.03	10^{-4} - 10^{-5}

*Expressed as a fraction of the λ-resistant mutants

**As compared with C600 non lysogenic

4. It has been found in addition that the Sm^r mutation of strain R4 (a representative of the "temperator" type) strongly interferes with the correction by pm^+, of some, but not all sus mutations of λ. For instance, the efficiency of plating (e.o.p.) of λsus_5 on C600 # R4 is only 10^{-6} (Table III).

A similar situation has been found independently with our Sm^r mutants of C600, among amber mutants of T4 (Epstein, personal communication). The Sm^r mutants of the non temperator type also tend to depress the e.o.p., but this effect is not striking and it is found even for λsus^+.

The discovery by Gorini (1964) of the effect of the streptomycin prompted us to compare the e.o.p. of sus mutants of λ on different strains with (200 μg/ml) and without Sm. In every case the presence of the antibiotic was found to improve the e.o.p. but usually within a factor of 10.

Table III.

Efficiencies of plating of sus mutants of λ on different strains.

	sus_7	sus_5	sus_{201}	sus_{203}	sus^+
C600 (pm^+)	1	1	1	1	1
112-12 su(pm^+)	0.6	0.5_6	0.2_8	0.5_8	0.4_6
112-12 (pm^-)	1.9×10^{-6}	8.7×10^{-6}	2.5×10^{-6}	5.2×10^{-7}	0.5_5
3110 (pm^-)	6.7×10^{-7}	5.2×10^{-6}	3.9×10^{-6}	4.5×10^{-7}	0.6_8
C600 # R4 (pm^+, Sm^r of the "temperator" type)	0.08	5.10^{-6}	4.10^{-6}	6.9×10^{-3}	0.5_3
idem, with Sm (200μg/ml) present	0.8_3	8.7×10^{-6}	4.1×10^{-3}	6.9×10^{-2}	1.0
C600 # R9 (pm^+, Sm^r of the non-temperator type)	0.8_3	0.16	8.10^{-2}	0.4_8	
idem, with Sm (200μg/ml) present	1.2_5	0.8	0.5_2	1.0	0.9
C600 # R10 (pm^+, Sm^r of the non-temperator type)	1.0	0.2	0.1_3	0.5_8	0.6_6
idem, with Sm (200μg/ml)	1.0_8	0.7_6	0.8_4	0.9_3	1.0

5. Finally the expression of virulence by the mutant $\lambda c_{17}c_{90}$ (Silva and Jacob, personal communication) is prevented by the bacterial mutation Sm^r # R4, since $\lambda c_{17}c_{90}$, which grows on C600 (λ) does not grow on R4 (λ).

Obviously, Sm^r mutations interfere with the expression of a wide variety of functions. As already suggested (Thomas et Lambert, 1962) this pleiotropy may be ascribed to the fact (suggested by Spotts and Stanier (1961) and confirmed since in many laboratories) that Sm mutations affect the structure of ribosomes, which take part in the synthesis of all proteins and thus to the expression of almost every function. One has to assume that the ribosomes, as well as the transfer RNAs and activation enzymes, take part in the specificity of the reading process. Our working hypothesis during this work was essentially as follows. In "Polar" mutations, the alteration in the nucleotide sequence of the messenger RNA is supposed to modify the interaction between the polycistronic messenger and the ribosome in such a way that the reading is interrupted : the ribosome "falls" from the messenger (Hartman, personal communication) or is blocked at the level of the mutational site. Similarly, we feel that changes in the structure of the ribosome (either in a polyribonucleotide sequence or in a ribosomal protein) may change the interaction between the ribosome and some nucleotide sequences in messenger RNA. Sequences that in other strains interrupt the normal progression of the ribosome are now read but in counterpart some normal sequences may become difficult or impossible to read. Following this view the thermosensibility of the regulatory mechanism of λc_{72} would mean that during the synthesis of the repressor the ribosome tends to fall from the messenger at the level corresponding to the site of the mutation, when the temperature is high. This situation would be shifted to higher temperatures when the structure of the ribosome is modified in the proper way, as in C600 Sm^r # R4.

However, the same change in the structure of the ribosome would on the other hand hinder the synthesis of several other proteins and this would result in the abnormality of bacterial growth and in the low efficiencies of the restriction by prophage P1 and of the correction of some sus and amber mutants by the factor pm^+.

The behaviour of C600 is rather unusual in that the Sm^r derivatives of a majority of the other K 12 strains tested are not "temperator". The factor present in C600 and responsible for this difference is not its pm^+, since there is no

correlation, among a number of K 12 strains, between the pm^+ or pm^- character and the behaviour of the Sm^r derivatives.

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