ESCHERICHIA COLI: THE K12 RIBOSOMAL PROTEIN AND THE STREPTOMYCIN REGION OF THE CHROMOSOME

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A number of mutations affecting the 30S ribosomal subunit of Escherichia coli map in the same region of the chromosome (see Table 8, Apirion, 1967). Here we discuss three of them:

str^r (streptomycin resistance); spc^r (spectinomycin resistance, Davies, Anderson and Davis, 1965); and the K12 band, a ribosomal protein in K12 strains with an electrophoretic mobility different from that of the corresponding band in B strains (Leboy, Cox and Flaks, 1964). We show here that the K12 band is separable from a mutation to streptomycin resistance, and is most likely determined by a gene that is located on one side of the str gene, with the spc gene on the other side.

Results. Transduction experiments were carried out, using strain N321 (K12) (spc^r str^r) as donor and strains B148 (B) and N2 (K12), (both spc^s str^s) as recipients. Streptomycin resistant or spectinomycin resistant transductants were selected, and the selected colonies were tested to find out whether or not they had received the unselected marker. It can be seen (Table 1) that the co-transduction values for the K12, K12 crosses (about 40%) is twice as high as for the K12, B crosses (about 20%), suggesting that there is some chromosomal non-homology between strains B and K12 in this region.

TABLE 1

Transduction Experiments

Recipient	$\underline{str^r}$	$\underline{spc^r}$	% Cotransduction
B148	<u>116</u>	25	21.5
B148	27	<u>157</u>	17.2
N2	<u>111</u>	40	36.0
N2	74	<u>142</u>	52.1

Two experiments are summarized for each transduction; the donor strain used is the K12 strain N321. In each experiment the selection was carried out only for either streptomycin or spectinomycin resistant transductants. The selected transductants are underlined.

Strain B148 is an E. coli B strain from this laboratory requiring uracil, and sensitive to streptomycin and spectinomycin. N321 is an E. coli K12 strain, resistant to spectinomycin and streptomycin, derived from JC12, which requires adenine and methionine (JC12 was obtained from B. Low, New York University). N2 is a K12 strain, sensitive to both drugs and requiring only adenine; it was derived from JC12 by transducing into it a met^+ gene from another K12 strain. Transduction experiments were carried out according to Luzzatto, et al., (1968); using transducing phage P1Kc.

The ribosomal proteins of transductants from the K12, B cross were analyzed by electrophoresis in polyacrylamide gels. 10 of each of the three possible classes, $\underline{str^r}$, $\underline{spc^r}$ and $\underline{str^r}$ $\underline{spc^r}$ were analyzed. The ten spectinomycin resistant transductants had the pattern of the recipient strain B, but only a single transductant from each of the other two classes had the pattern of the recipient B strain; all the other eighteen strains had the pattern of the donor K12 strain. Plate I shows an analysis of the 30S ribosomal proteins of both parental strains, together with two $\underline{str^r}$ transductants, N266 having the B pattern and N267 having the K12 pattern.

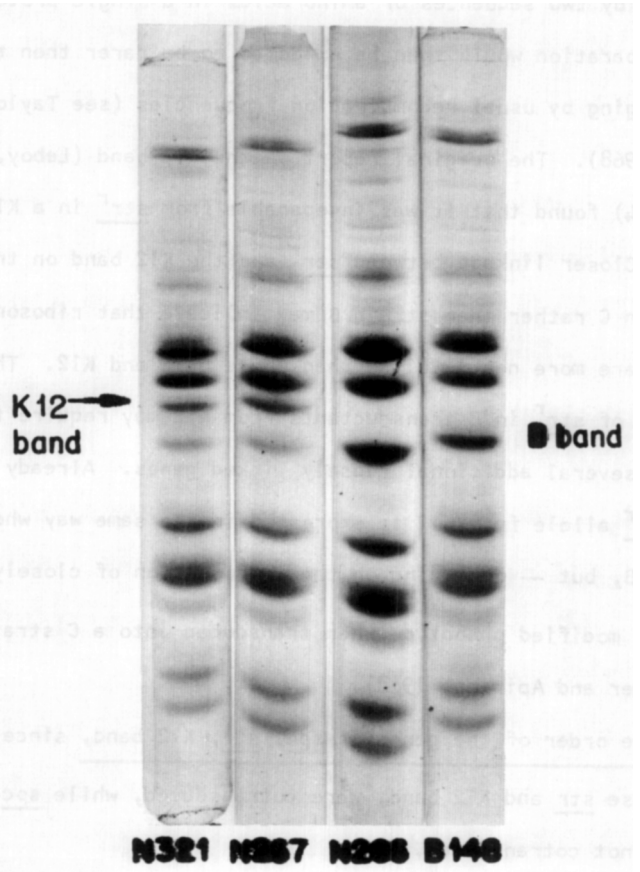

Plate I. Electrophoresis on polyacrylamide gels of 30S ribosomal proteins from the two parental strains, N321 (K12) and B148 (B), and two str^r transductants, N266 and N267. Preparation of proteins and electrophoresis according to Krembel and Apirion (1968), except that the washed ribosomes were not further treated with ammonium sulfate. The arrow indicates the position of the K12 band. Notice that in the run shown here there is a band which is common to both K12 and B strains which runs together with the B band. It is separable from the B band under other electrophoretic conditions. Electrophoresis was carried out for 5 hrs. with 3mA applied per gel.

Discussion. We infer from these results that:

1) The K12 band does not itself contain the mutational change that leads to str^r. Since the K12 band and str^r were separable, they are unlikely to be in the same protein. The two characteristics might be

determined by two sequences of amino acids in a single protein chain, but the separation would then be expected to be rarer then the 10% found, judging by usual recombination frequencies (see Taylor and Trotter, 1968). The original report on the K12 band (Leboy, Cox and Flaks, 1964) found that it was inseparable from str^r in a K12, C transduction. Closer linkage between str^r and the K12 band on transduction into strain C rather than strain B may indicate that ribosomes of strains B and K12 are more nearly alike than those of C and K12. Thus, the expression of str^r in C transductants from K12 may require the introduction of one or several additional closely linked genes. Already it is known that a str^r allele from K12 is expressed in the same way when transferred to strain B, but — depending on the introduction of closely linked genes — can give a modified phenotype when transduced into a C strain (Luzzatto, Schlessinger and Apirion, 1968).

2) The order of the genes is spc, str, K12 band, since spc and str and likewise str and K12 band, were cotransduced, while spc and the K12 band were not cotransduced.

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