G FACTOR MUTANTS OF ESCHERICHIA COLI: MAP LOCATION AND PROPERTIES

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SUMMARY

Factor G, the translocation factor in protein synthesis, was genetically marked by fusidic acid resistance or by a temperature sensitive lesion. All the characteristic properties of the G factor were more than 97% cotransducible with streptomycin resistance, the locus near which many ribosome mutations map.

Mutants have been derived in the G translocation factor by selecting for cells resistant to fusidic acid, a steroid antibiotic that blocks G factor-catalyzed translocation of peptidyl tRNA (1-3). In another approach, a mutant temperature-sensitive in G factor, G1, was isolated by Tocchini-Valentini and Mattoccia (3,4). Here we show that mutations to fusidic acid resistance are genetically separable from the temperature sensitive lesion in strain G1; but they are very closely linked to each other and to the streptomycin locus in E. coli.

In each type of mutant, various properties in which G factor participates--ribosome-dependent GTPase (5); protein synthesis (5); and RNase V (6); -- are modified. For example, Figure 1 shows that polyphenylalanine synthesis in extracts of a fur (fusidic acid resistant) mutant, as in those earlier reported (3), is resistant to levels of fusidic acid that severely inhibit extracts of the parental strain. Assays of GTPase and RNase V at various levels of fusidic acid gave similar results.

Using the temperature sensitivity and fusidic acid resistance as plate markers, bacterial crosses and transductions showed the linkage of the traits

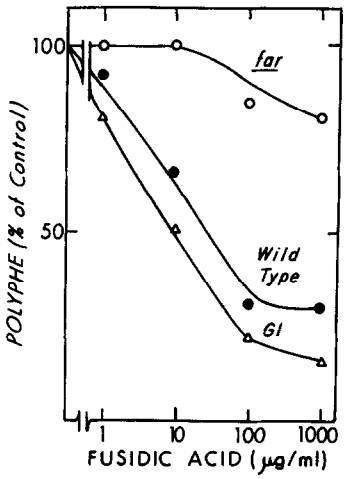

Figure 1. Response of polyphenylalanine synthesis to fusidic acid in crude extracts of wild type (D10), GI, and a *far* strain. Reaction mixtures were according to reference 15. 100% corresponds to about 8,000 counts/minute.

Table 1. Transduction Mapping of GI and *far* Mutants

	Donor	Recipient	Markers[1] selected	Transductants analyzed str-R	far	ts^+ [2]
Cross 1	D10 *ts*$^+$ *far* *str*	x D10 *ts*$^+$*far*$^+$*str*$^+$	far	185	189	-
			str-R	190	187	-
Cross 2	D10 *ts*$^+$ *far*$^+$ *str*	x GI *ts* *far*$^+$ *str*$^+$	str-R	106	-	104

Phage preparation and transductions were carried out according to the procedures of Hashimoto (13). Fusidic acid resistant (*far*) mutants were obtained by plating cultures of strain D10 (14) on freshly-prepared plates containing 500 μg/ml fusidic acid. (1) Selection was with 500-1,000 μg/ml fusidic acid or 100 μg/ml of streptomycin. (2) *ts*$^+$ indicates growth at 42° on broth agar.

near the *str* locus. Table I shows the results of two transduction experiments that clearly establish the close linkage of *str*, GI*ts*, and a *far* allele. Four

other far alleles gave essentially identical results. In a more extensive, three point cross, D10 ts$^+$far str-r phage were the donors and G1 ts far$^+$str$^+$ the recipient strain. 423 str-r transductants were selected and found to include 412 str-r far ts$^+$, 7 str-r far$^+$ts, and 4 str-r far ts colonies.

These data indicate a probable order of str-r far ts, with about 2.5% recombination between the str-r and Glts alleles. The exact linkage of str-r and and far may not be that close, because alleles can show anomalously high cotransduction with str (11). However, the linkage is certainly close. The 1% recombination between far and Glts is higher than one might expect for alleles in a single gene coding for G factor. However, G factor, while it is a single polypeptide chain (2), is a very long one (about 84,000 (7)). Since 1% recombination on the E. coli chromosome is estimated to correspond to a molecular weight of protein of the order of 60,000 (8), the far and ts alleles could easily reside in same gene.

We think that the linkage of G factor to the streptomycin locus, with many ribosomal proteins (9), is not coincidental -- and had, in fact, expressly tested for it. (Linkage of the G factor to the str locus has recently been shown by other investigators as well, including P. Leder and collaborators (personal communication) and J. Gordon (personal communication)). For some time, the question of whether G factor should be considered a "ribosomal protein" has been obscure. Employing defining criteria suggested earlier (10), we note that G factor functions bound to ribosomes (11), exists in a stoichiometric ratio with ribosomal particles in growing cells (12), and is genetically closely linked to other ribosomal proteins. We suggest that G factor can properly be termed a ribosomal protein, and that its genetic linkage to other ribosomal proteins is part of a mechanism to ensure coordinate production of these proteins.

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