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Mapping of a Locus for Unsaturated Fatty Acid Biosynthesis in *Escherichia coli*

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A mutation affecting unsaturated fatty acid biosynthesis in *Escherichia coli* has been mapped. This mutation, which is in the *fabB* locus, lies between the *aroC* and *purF* loci.

Bacterial mutants blocked in the biosynthesis of fatty acids have been isolated in a number of laboratories. We report here the mapping of the locus for a mutation affecting the synthesis of unsaturated fatty acids in *Escherichia coli* K-12. The mutant strain studied, 30-, was derived from strain MO (F^- *strA*^{r}) and has an absolute requirement for an unsaturated fatty acid (6). In a recent study by Cronan, Birge, and Vagelos (1), mutants of *E. coli* that require unsaturated fatty acids were found to fall into two complementation groups. The abbreviation *fab* was proposed for this type of mutant. Strain 30- belongs to the *fabB* group (1).

The media and techniques used were those described elsewhere (3) except for the changes mentioned here. The medium used in mating, transduction, and in growing cells to prepare P1*kc* transducing lysates contained: tryptone, 10 g/liter; NaCl, 7 g/liter; and, in the case of solid medium, agar, 15 g/liter. All media were supplemented with 0.25% Tween 40 and 0.02% oleic acid. Because strain 30- is rather sensitive to osmotic shock, the cells were suspended for phage absorption in a solution of higher osmolality than that usually used. The resuspension solution contained: NaCl, 120 mM; $CaCl_2$, 5 mM; and $MgSO_4$, 1 mM.

In mating strain 30- with a number of Hfr strains, it was found that the *fabB*+ marker was brought in early by strain KL98, an Hfr that injects markers in the order: *dsdA-aroC-purF--his-*(5). Transduction of 30- to prototrophy for the fatty acid character with a lysate of strain EM3003 (pertinent markers: *dsdA aroC purF*) showed that the *fabB* locus was cotransduced at a frequency of 78% with *aroC*, 47% with *purF*, and 20% with *dsdA*.

The relative order of *aroC* and *fabB* was investigated by analyzing the frequencies of the different types of recombinants obtained when a lysate of strain 30- was used to transduce strain EM3003 to *purF*+, or to *aroC*+. When *purF*+ is the selected marker, the rarest type of recombinant obtained is the *aroC*+ *fabB*+ class (line A of Table 1). This result implies that the order is *aroC*−*fabB*−*purF*, because this order requires four crossovers in order to obtain *aroC*+ *fabB*+ recombinants in this cross. If *fabB* were to the left of *aroC*, the type of recombinant that would require four crossovers and thus would be expected to be least frequent is the *aroC-fabB-* class. When *aroC*+ is selected (line B in Table 1), the least common class is *purF*+ *fabB*+. Again, the order indicated is *aroC-fabB-purF*, since this order requires four crossovers to obtain this class of recombinant. If *fabB* were to the left of *aroC*, all four types of recombinants could be obtained by two crossovers, and one would not expect the *purF*+ *fabB*+ recombinants to be significantly less frequent than the *purF*− *fabB*+ recombinants.

TABLE 1. *Ordering of fabB by three-factor crosses*

P1*kc* donor: 30- (*fabB*)	No. scored	Recipient: EM3003 (*aroC purF*) (no. of recombinants with indicated genotype)			
A. *purF*+ selected	226	*aroC*+ *fabB*+ (10)	*aroC*+ *fabB*− (77)	*aroC*− *fabB*+ (117)	*aroC*− *fabB*− (22)
B. *aroC*+ selected	227	*purF*+ *fabB*+ (14)	*purF*+ *fabB*− (95)	*purF*− *fabB*+ (56)	*purF*− *fabB*− (62)

Schairer and Overath reported studies on a mutant of *E. coli* which requires unsaturated fatty acids (4). These authors report that the mutation in their strain is near the origin of Hfr strain KL98 and is 15% cotransduced with the *dsd* marker. Since this description is entirely consistent with the location of *fabB*, it is reasonable to assume that the mutation in their strain is in or very near the *fabB* locus. An additional marker in the vicinity of *fabB* is the pyridoxine marker *pdxB* which Dempsey has recently mapped as lving between *aroC* and *purF* (2).

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