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Mapping of a Structural Gene for Valyl-Transfer Ribonucleic Acid Synthetase in *Escherichia coli* by Transduction

MARJORIE A. TINGLE[1] AND FREDERICK C. NEIDHARDT
Department of Biological Sciences, Purdue University, Lafayette, Indiana 47907

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A structural gene, *valS*, for the valyl-transfer ribonucleic acid synthetase of *Escherichia coli* has been mapped on the clockwise side of *pyrB* and is closely linked to it.

Early investigations by interrupted-conjugation experiments had shown that the *valS* gene [a structural gene for valyl-transfer ribonucleic acid (tRNA) synthetase] lies between the *ilv* and *leu* loci at about minute 87 on the linkage map of *Escherichia coli* (1). The recent establishment of the relative order of several new markers in this region of the chromosome (Fig. 1) permitted further genetic analysis of the map position of the *valS* marker by using transduction mediated by phage Plkc (4).

To determine whether the *valS* marker was in transduction range of any of the previously mapped loci, phage grown on strain NP29, which carries a temperature-sensitive valyl-tRNA synthetase mutation (*valS*), was used to infect strains carrying *argE*, *metA*, *malB*, *fdp*, *pyrB*, *thr*, *leu*, and *ara* markers. Prototrophic transductants selected at 30 C were then screened for the unselected *valS* marker by replica plating to 40 C. Cotransduction of the *valS* marker was observed only when *fdp* (55% cotransduction) or *pyrB* (85% cotransduction) was used as the selected marker.

Mapping of the *valS* locus was achieved by the three-factor transduction cross illustrated in Table 1. In agreement with Fraenkel (3), we find that *fdp* cotransduces with *pyrB* (47% cotransduction). The *valS* and *pyrB* markers are so closely linked that the difference in the coinheritance of the unselected *fdp* marker is too small to permit ordering of the *pyrB* and *valS* genes. However, the distribution of unselected markers among the selected transductants shows that only 0.9% of the *valS*$^+$ recombinants carried *pyrB*, *fdp*, whereas 5% of the *pyrB*$^+$ recombinants carried *fdp*. Assuming that the rarest recombinant class would arise as the result of a quadruple exchange, the most probable gene order is *fdp*, *pyrB*, *valS*.

As a direct test of this gene order, the one

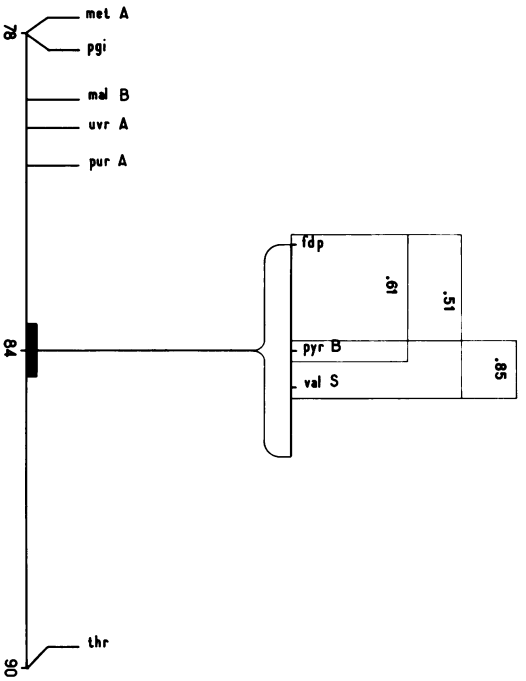

FIG. 1. *A section, from 78 to 90 min, of the genetic map of E. coli, taken from Taylor and Trotter (5) and Fraenkel (3). A segment of the map, expanded 4×, shows the relative order of fdp, pyrB, and valS. The average cotransduction frequencies given above the expanded segment were obtained from data given in Tables 1 and 2. There was no significant difference between the values for the independently isolated alleles of valS used in the two experiments.*

[1] Present address: Laboratoire d'Enzymologie, C.N.R.S. 91-Gif-sur-Yvette, France.

TABLE 1. *Ordering of fdp, pyrB, and valS by three-factor crosses*[a]

Selected marker	No. of transductants scored	Unselected markers			
		fdp$^+$, *pyrB*$^+$	*fdp*, *pyrB*$^+$	*fdp*$^+$, *pyrB*	*fdp*, *pyrB*
valS$^+$	111	35	53	22	1
		fdp$^+$, *valS*$^+$	*fdp*$^+$, *valS*	*fdp*, *valS*$^+$	*fdp*, *valS*
pyrB$^+$	155	66	16	66	7
		fdp$^+$	*fdp*		
valS$^+$, *pyrB*$^+$	37	15	22		

[a] All of the symbols for genes are those defined by Taylor and Trotter (5). Transductants were selected as follows: *valS*$^+$, by plating at 40 C on minimal glucose plates supplemented with tryptophan and uracil; *pyrB*$^+$, by plating at 30 C on minimal plates supplemented with tryptophan; *valS*$^+$, *pyrB*$^+$, by plating at 40 C on minimal plates supplemented with tryptophan. Scoring of *fdp* was done by replication to glycerol plates supplemented with uracil and tryptophan. All transductants retained the *trpA* marker. The P1 donor was *E. coli* JC411-6, *fdp pyrB*$^+$ *valS*$^+$ *trpA*$^+$; it was kindly supplied by D. G. Fraenkel. The recipient was *E. coli* NP910211, *fdp*$^+$ *pyrB valS trpA*; it was constructed in the following way. A temperature-sensitive *valS* mutation was produced in strain A-3 (a *trpA* mutant obtained from C. Yanofsky) according to the methods described by Eidlic and Neidhardt (2). This mutant was then infected with P1kc grown on strain PA200-Y (a *pyrB* mutant obtained from D. G. Fraenkel) and *valS*$^+$ transductants were selected. These transductants were screened to find one that carried the *pyrB* lesion, and this one was used to select the temperature-sensitive *valS* mutant, NP910211, of the desired genotype.

TABLE 2. *Ordering of fdp, pyrB, and valS by three-factor crosses*[a]

Selected marker	No. of transductants scored	Unselected markers			
		pyrB$^+$, *valS*	*pyrB*, *valS*$^+$	*pyrB*$^+$, *valS*	*pyrB*, *valS*
fdp$^+$	213	27	75	110	1
		fdp$^+$, *valS*$^+$	*fdp*$^+$, *valS*	*fdp*, *valS*$^+$	*fdp*, *valS*
pyrB$^+$	273	11	187	19	56
		valS$^+$	*valS*		
fdp$^+$, *pyrB*$^+$	243	30	213		

[a] Transducatnts were selected at 30 C as described in Table 1. The P1 donor was *E. coli* NP29, *fdp*$^+$ *pyrB*$^+$ *valS trpA*$^+$ which carries a temperature-sensitive *valS* lesion. The recipient was the one transductant of the given genotype isolated in the experiment described in Table 1. The recipient was NP9102111: *fdp pyrB valS*$^+$ *trpA*.

double recombinant obtained in the above experiment was infected with phage grown on strain NP29. The data in Table 2 show that the unselected donor marker, *valS*, was cotransduced at a frequency of 89% with *pyrB*$^+$ alone and 52% with *fdp*$^+$ alone. Simultaneous selection for *pyrB*$^+$, *fdp*$^+$ recombinants did not increase the coinheritance of *valS* (87% cotransduction), although it should have if the *valS* marker were on the counter-clockwise side of *pyrB*. Thus, the *valS* gene must be on the clockwise side of the *pyrB* marker at minute 84 on the *E. coli* chromosome (Fig. 1). This position is 10 min distant from the loci governing the valine biosynthetic enzymes (*ilv*) and is not near any of the other known cistrons for aminoacyl-tRNA synthetases.

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