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Three-Factor Reciprocal Cross Mapping of a Gene that Causes Expression of Feedback-Resistant Acetohydroxy Acid Synthase in *Escherichia coli* K-12

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Summary. The *ilv-662* allele was previously identified as a mutation that caused acetohydroxy acid synthase activity to be resistant to feedback inhibition by valine (Davis et al. 1977). This allele was mapped between *thr* and *leu* by cotransduction analysis and labeled ilvJ. This report describes the mapping of *ilvJ* relative to genes that lie between *thr* and *leu* (*ara*, *carA* and *pdxA*) by three factor reciprocal cross analyses. We find that the probable gene order is *thr-carA-pdxA-ilvJ-ara-leu*. Although the phenotypic properties of *ilvJ662* appear to be quite distinct from *brnS*, a gene reported to involve branched chain amino acid transport (Guardiola et al. 1974), we do not rule out possible allelism because of the uncertainty of the map position of *brnS*.

Acetohyroxy acid synthase (EC 4.1.3.18) isozymes are encoded by *ilvB*, *ilvI*, and *ilvG* in *Escherichia coli* K-12, and this activity is essential for the biosynthesis of both isoleucine and valine (Ramakrishnan and Adelberg 1965; DeFelice et al. 1974; Guardiola et al. 1974a). Only *ilvB* and *ilvI* are normally expressed in K-12 since *ilvG* is cryptic (Guardiola et al. 1977; Favre et al. 1976). The products of *ilvB* and *ilvI*, acetohydroxy acid synthases I and III respectively, are sensitive to feedback inhibition by valine (Leavitt and Umbarger 1962; DeFelice et al. 1974; Guardiola et al. 1977). The cryptic *ilvG* gene significantly expresses a feedback-resistant isozyme, acetohydroxy acid synthase II, only in the presence of a mutation in the region designated *ilvO* or *ilvR* (Smith et al. 1979). In the absence of a mutation in *ilvO*, growth of the K-12 strain is inhibited by valine in a minimal medium. Valine presumably causes an isoleucine restriction by repression of synthesis of the acetohydroxy acid synthases I and III, and by inhibition of their isoleucine-forming activity. In a previous study, spontaneous mutants were selected which displayed acetohydroxy acid synthase activity with various levels of resistance to valine inhibition (Davis et al. 1977). The mutants were resistant to growth inhibition by valine as a consequence of lesions which caused acetohydroxy acid synthase activity to become resistant to feedback inhibition by valine. Therefore, valine resistance (Val^R) was useful as a genetic marker of chromosome sites which caused the expression of valine-resistant acetohydroxy acid synthase activity. One such allele, *ilvJ662*, was located in the gene order *thr-ilvJ662-ara-leu-ilvHI* by two-factor and three-factor cotransduction analyses (Davis et al. 1977). Some uncertainty remained about the position of *ilv662* relative to *ara* and other genes between *thr* and *leu*. This study was undertaken to position *ilvJ662* relative to each gene in the immediate vicinity of *ara* by three-factor reciprocal cross analyses.

All crosses were done by transduction with the Plkc (Lennox 1955) or PlCMclrlOOts coliphage (Rosner 1972). Phage lysates were routinely prepared by the confluent lysis method on media described elsewhere (Rosner 1972). A minimal medium, previously described (Szentirmai et al. 1968), was used for selection and screening of transductants. This medium was supplemented with amino acids, vitamins, and carbohydrates as indicated. All chemicals were of the highest purity available. The strains used are listed in Table 1.

Three factor reciprocal crosses were designed to map *ilvJ662* relative to *ara*. Strains MJ84 and MJ56 are shown in two possible arrangements of *ilvJ662* and *ara*; arrangement A and arrangement B (Fig. 1). For these crosses, arrangement A depicts *ilvJ*

Table 1. Strains used

Strain	Genotype	Use and derivation	Source
CU1126	*rbs, ilvB, ilvHI, ara, thi, Δ (pro-lac)*	Strain construction	H.E. Umbarger
MJ10	*thr, leu*	Strain construction	H.E. Umbarger
MJ43	*ilvJ662, rbs*-215	Strain construction	Davis et al. (1977)
MJ56	*ara, leu, his, thi, pyrB, rbs*	Strain construction and Mapping	Davis et al. (1977)
MJ84	*ilvJ662, carA, thi, his, pyrB*	Strain construction and Mapping	This study
MJ85	*ilvJ662, carA, ara, thi, his, pyrB*	Strain construction and Mapping	This study
MJ86	*carA, thi, his, pyrB*	Strain construction and Mapping	This study
MJ99	*carB, pdxA, leu, proA*	Strain construction and Mapping	This study
MJ101	*ilvJ662, ara*	Mapping	This study
MJ130	*ilvJ662, leu*	Strain construction	This study
PCO291	*carB, pdxA, ara, leu, proA*	Strain construction	B. Bachmann

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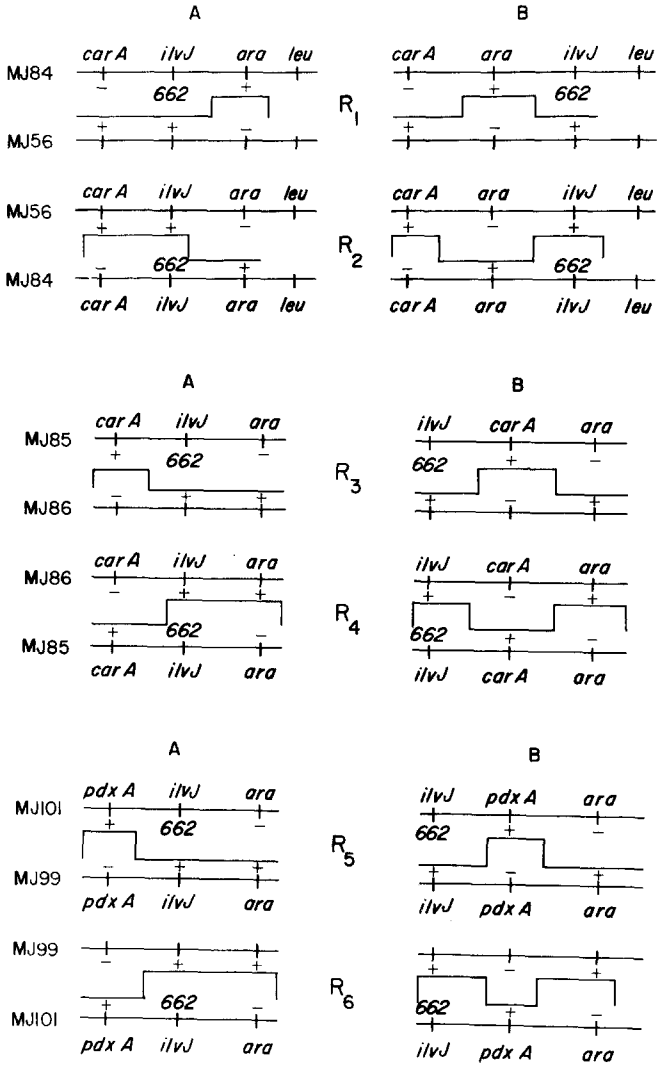

Fig. 1. Schemes for reciprocal cross analyses. Crosses between two strains are represented by R_1 through R_6. Column A shows one possible gene order and column B shows an alternate possible gene order for each cross designated by R_i. Crosses are grouped in pairs to denote the forward cross, e.g. R_j, and the reciprocal cross, e.g. R_2

to the *carA* side of *ara*, i.e. to the left of *ara*, whereas arrangement B depicts *ilvJ* to the *leu* side of *ara*, i.e. to the right of *ara*. R_1 denotes the forward cross MJ84 × MJ56, and R_2 denotes the reciprocal cross. In both crosses a recombination event was forced between *carA* and *ara* by the selection for $carA^+$, ara^+ transductants. Since *ilvJ662* causes a valine-resistant (Val^r) phenotype, $ilvJ^+$ is represented by the valine-sensitive (Val^s) phenotype typical of the normal K-12. If arrangement A correctly describes the gene order for the R_1 and R_2 crosses, where R_1 is the frequency of unselected marker (Val^s) appearance among $carA^+$ ara^+ transductants,

$$R_i = \frac{Val^s}{Ara^+ Leu^+} = \frac{ilvJ^+}{ara^+ leu^+},$$

then the unselected marker would appear as a result of a single recombinational event in each cross. The expected frequency R_i for each cross would be approximately equivalent, therefore, $R_1 \simeq R_2$. If arrangement B represents the correct gene order then $R_1 \gg R_2$, since the unselected marker would appear as a result of a single recombination in the R_1 cross and a double recombination in the reciprocal R_2 cross. The results from this experiment suggest that the correct gene order is *carA-ilvJ-ara-leu* since $R_1 \simeq R_2$ (Table 2). Alternatively, the gene order might be *ilvJ-carA-ara-leu* since the R_1 and R_2 crosses distinguish only the relative positions of *ilvJ* and *ara* but not *ilvJ* relative to *carA*.

Using a similar rationale, *ilvJ* was mapped relative to *carA*. Arrangement A shows the expected recombination events for the R_3 and R_4 crosses if *ilvJ* lies to the left of *carA* and *ara* (Fig. 1). From the results in Table 3, the values of R_3 and R_4 are consistent with the requirement for a single recombination event in both crosses, i.e. $R_3 \simeq R_4$. Therefore, the probable gene order is *carA-ilvJ-ara*.

We then mapped the *ilvJ* allele relative to *pdxA* and *ara*. If the correct gene order were *pdxA-ilvJ-ara* as shown in arrangement A of Fig. 1, the R_5 and R_6 crosses should yield similar frequencies of appearance of the unselected $ilvJ^+$ among $pdxA^+$ ara^+ recombinants. However, if the gene order were *ilvJ-pdxA-ara* as shown in arrangement B, then cross R_5 would be expected to yield a considerably higher frequency of $ilvJ^+$, $pdxA^+$, ara^+ transductants than the reciprocal cross R_6. The results in Table 2 show approximately equivalent frequencies of $ilvJ^+$ $pdxA^+$ ara^+ recombinants. All of these data are consistent with the gene order *carA-pdxA-ilvJ-ara-leu*, which is shown in Fig. 2, where the map distances are expressed in minutes based upon calculations from cotransduction frequencies with *leu* by the method of Wu (1966). The *brnS* gene, reported to encode a component of the transport system for isoleucine, leucine and valine, was previously mapped near *pdxA* and the *ara* gene cluster by Guardiola et al. (1974). Although uncertainty remains about the position of *brnS* relative to *pdxA* and nearby markers, it is tentatively placed between *pdxA* and *ara* on the current *E. coli* linkage map (Bachmann and Low 1980). In a preliminary report to the American Society for Microbiology (Abst. Ann. Mtg. Am. Soc. Microbiol. K19, pg. 129, 1980) we presented evidence that *ilvJ* is a silent or cryptic gene for an acetohydroxy acid synthase. A complete study of *ilvJ* function will be published elsewhere. One, as yet unexplained, consequence of the *ilvJ662* mutation is an apparent effect to elevate one of the branched-chain amino acid transport enzymes (unpublished observation). Prior work from this laboratory suggests the possibility of a loose association or interaction of acetohydroxy acid synthases with the cell membrane (Blatt and Jackson 1978). While we have clearly established the genetic map position for *ilvJ* and have previously shown that the *ilvJ662* mutation accounts for acetohydroxy acid synthase activity resistant to feedback inhibition by 1.0 mM valine (Davis et al. 1977), the proximity of *ilvJ* to *brnS* is intriguing. In consideration of our previously cited evidence for possible acetohydroxy acid synthase-cell membrane interaction (Blatt and Jackson 1978), we do not exclude the possibility that *ilvJ* and *brnS* are allelic. However, at present, based upon physiological evidence of function, *ilvJ* and *brnS* must be considered as distinct genes which lie close to each other on the *E. coli* chromosome.

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Table 2. Three factor reciprocal crosses with *ilvJ662*

Cross R_i	Donor (Genotype)[a] × Recipient (Genotype)	Selected markers	Unselected markers	Transductants selected	Unselected marker appearance	Frequency of appearance
R_1	MJ84 (*ilvJ662, carA*) × MJ56 (*ara*)	$carA^+$, ara^+	Val^s	394	179	0.45
R_2	MJ56 (*ara*) × MJ84 (*ilvJ662, carA*)	$carA^+$, ara^+	Val^s	465	137	0.29
R_3	MJ85 (*ilv662, ara*) × MJ86 (*carA*)	$carA^+$, ara^+	Val^s	397	94	0.24
R_4	MJ86 (*carA*) × MJ85 (*ilvJ662, ara*)	$carA^+$, ara^+	Val^s	482	328	0.68
R_5	MJ101 (*ilvJ662, ara*) × MJ99 (*carB, pdxA, ara, proA*)	$carA^+$, ara^+	Val^s	207	154	0.74
R_6	MJ99 (*carB, pdxA, ara, proA*) × MJ101 (*ilvJ662, ara*)	$carA^+$, ara^+	Val^s	471	149	0.32

[a] Donor and recipient genotypes shown in this table are the genotypes relative to the crosses shown. For some strains, with numerous markers, several markers are not listed in this table, but the complete genotype can be found in Table 1

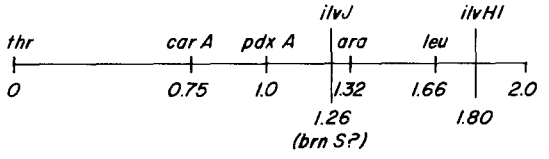

Fig. 2. Map of the chromosome region containing ilvJ. Numbers represent the map distance in minutes determined by an analysis of cotransduction frequency described by Wu (1966)

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