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Regulatory Mechanisms in the Biosynthesis of Isoleucine and Valine

III. Map Order of the Structural Genes and Operator Genes

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ABSTRACT

RAMAKRISHNAN, T. (Yale University, New Haven, Conn.), AND EDWARD A. ADELBERG. Regulatory mechanisms in the biosynthesis of isoleucine and valine. III. Map order of the structural genes and operator genes. J. Bacteriol. **89**:661-664. 1965.—A new method has been employed to determine the map order of the structural genes and operator genes governing the enzymes of the isoleucine-valine biosynthetic pathway. This method relies on the observation that phage transduction of markers carried on an F-genote leads to the establishment in the recipient of F-genotes of various lengths. Using this method, we have established that the order of loci is the following: *F/ilvE ilvD ilvA oprA/ilvC/ilvB oprB*. The operator locus, *oprA*, regulates the activity of structural genes *ilvE* (transaminase B), *ilvD* (dehydrase), and *ilvA* (threonine deaminase). The operator locus, *oprB*, regulates the activity of *ilvB* (condensing enzyme). An operator for *ilvC* (reductoisomerase) can be inferred to exist, but has not yet been detected genetically. The loci *ilvB* and *oprB* have been shown to be at the extreme right end of the sequence, but their positions relative to each other remain to be established.

In the preceding papers of this series (Ramakrishnan and Adelberg, 1964, 1965), we have shown that in *Escherichia coli* K-12 the enzymes of isoleucine and valine biosynthesis are controlled by a cluster of five structural genes comprising three distinct operons. Operon I consists of the loci *ilvE*, *ilvD*, *ilvA*, and the operator locus, *oprA*, which regulates their activity. Operon II includes the locus *ilvC*; its operator locus has not yet been detected genetically, but is inferred to be present on the basis of end-product repression of the *ilvC* locus. Operon III consists of the locus *ilvB* together with the operator locus controlling it, *oprB*. The meaning of the above genetic symbols is given in Table 2 of the preceding paper; the structures of the biosynthetic intermediates and the sequence of enzymatic reactions are diagrammed in Fig. 1 of the preceding paper (Ramakrishnan and Adelberg, 1965).

In the present paper, we report experiments which have permitted us to assign relative map positions to the five structural genes and two operator genes listed above. The mapping procedure we have used is a rather novel one; it takes advantage of the fact that, when phage P1kc is used to transduce markers from the F^- genote of an F′ strain into an F^- recipient, many of the transductants are found to have become F′ donors carrying F-genotes of varying lengths (Pittard and Adelberg, 1963). For example, if the F-genote carries the hypothetical loci X^+, Y^+, and Z^+, phage P1kc can be grown on the F′ strain and used to transduce marker X^+ into an F^- recipient having an X^- mutation. Many of the X^+ transductants obtained in this way are found to be F′ donors; these are tested for their ability to transfer markers by conjugation to a set of F^- recipients carrying the mutations X^-, Y^-, and Z^-. Such tests show that some of the transduced F′ donors carry $F\text{-}X^+$, some $F\text{-}X^+Y^+$, and some $F\text{-}X^+Y^+Z^+$. These results establish that the order of genes on the original F-genote is $F\text{-}X,Y,Z$, the different F-genotes found among the transductants having arisen by breakage at some point in the transduction process.

By experiments of the type described above, we have ascertained that the order of genes for isoleucine-valine biosynthesis is: *F ilvE ilvD ilvA oprA ilvC* (*ilvB oprB*). The relative positions of loci within parentheses have not yet been determined.

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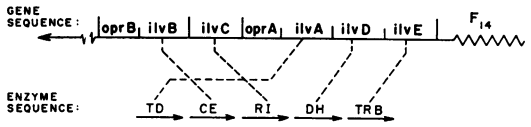

FIG. 1. *Sequence of genes controlling the formation of enzymes in the isoleucine-valine biosynthetic pathway. TD, threonine deaminase; CE, condensing enzyme; RI, reductoisomerase; DH, dehydrase; TRB, transaminase B. It is not known whether oprA and oprB are distinct loci, or whether they are segments of the structural genes ilvA and ilvB, respectively. (Note: the sequence of loci on F_{14} is consistent with the operator being at the clockwise end of the operon when the sequence forms part of the circular chromosome of Escherichia coli.)*

MATERIALS AND METHODS

Organisms. Table 1 summarizes the genotypes of strains not already described in the preceding paper (Ramakrishnan and Adelberg, 1965).

Media, culture methods, and genetic procedures. All media, methods, and procedures were as described in the previous paper (Ramakrishnan and Adelberg, 1965).

RESULTS

Mapping of operons I and II. The donor strain used for transduction was AB1005, a valine-resistant (*oprA1*) mutant of F′ strain AB1206. The F-genote carried by this strain, F_{14}, bears all of the loci for isoleucine and valine biosynthesis, as well as a number of loci concerned with methionine and arginine biosynthesis. The isoleucine-valine loci are closest to F (Pittard, Loutit, and Adelberg, 1963). Strain AB1206 has a chromosomal deletion corresponding to the entire F_{14} genote, and is thus haploid for all markers (Pittard and Ramakrishnan, 1964).

The recipient strain used for transduction was F^- strain AB1514. This strain lacks transaminase B activity, as a result of a mutation in the *ilvE* structural gene; it requires isoleucine and valine for rapid growth. Cells of the F^- strain were infected with phage P1kc grown on the F′ donor AB1206, and were plated on minimal medium to select for isoleucine-valine independent transductants. Two hundred transductants were isolated and purified by two successive replatings from single colonies; they were then tested by replica plating for their ability to transfer markers by conjugation to various F^- recipients. The results (Table 2) show that many of the transductants were high-frequency donors of isoleucine-valine markers, and that five types of F-genotes were represented: *F-ilvE*; *F-ilvE, ilvD*; *F-ilvE, ilvD, ilvA*; *F-ilvE, ilvD, ilvA, oprA*; and *F-ilvE, ilvD, ilvA, oprA, ilvC*. The order of the genes on the original F_{14} of strain AB1206 is inferred to be the order shown for the longest F-genote.

The mechanism by which the shorter F-genotes are generated is not clear. It is not a matter of the transducing phage picking up different lengths of deoxyribonucleic acid (DNA) from the F′ donor, since the same spectrum of types is found regardless of the marker selected in the transduction. That is, when we select separately for X^+, Y^+, and Z^+ transductants, in each selection we recover the same proportion of F-genotes of the types $F\text{-}X^+$, $F\text{-}X^+Y^+$, and $F\text{-}X^+Y^+Z^+$. Since, in the Z^+ selection, the phage had to carry the Z^+ gene which is stably integrated in the transductant, the $F\text{-}X^+$ and $F\text{-}X^+Y^+$ genotes must have been formed by the breakage of an $F\text{-}X^+Y^+Z^+$ genote in the Z^+ transductant. The reason for this breakage is not apparent; experiments are now in progress to test the possibility that the break occurs during a cross over event.

Mapping of operon III. As reported in the previous paper (Ramakrishnan and Adelberg, 1965), we have obtained a mutant deficient in condensing enzyme by selecting for an aminobutyric acid (ABA)-sensitive derivative of an ABA-resistant strain. ABA resistance had been shown to be a consequence of a mutation in *oprB*, derepressing the formation of the *p*H 8.0 condensing enzyme. The ABA-sensitive mutant, F^- strain AB1052, lacks the *p*H 8.0 enzyme but retains the *p*H 6.0 enzyme which *E. coli* K-12 also possesses (Radhakrishnan and Snell, 1960).

The locus controlling the formation of condensing enzyme has been designated *ilvB*. F′

TABLE 1. *Genotypes of strains*

Strain no.	*ilv* mutations*	Other mutations	Sex
AB1203	*ilvC7*	*lac-1,4(?), gal-2, tfr-3, xyl-5, arg-3, thi-1, T6r-6*	F^-
AB1264	*ilvB197*	*pro-2, lac-1, gal-2, his-4, str-8, thi-1*	F_{14}
AB1472	*ilvD16*	*str-17,9,8(?), mal-1, met-1, arg-1, thi-1,2(?)*	F^-
AB1514	*ilvE12*		F^-
AB1255	*ilvA201*	*thi-1, arg-1, met-1, his-1, xyl-1, mal-1*	F^-

* Phenotypes associated with *ilv* mutations: *ilvC7*, loss of reductoisomerase activity; *ilvB197*, resistance of condensing enzyme to feedback inhibition by valine; *ilvD16*, loss of dehydrase activity; *ilvE12*, loss of transaminase B activity; *ilvA201*, loss of threonine deaminase activity.

TABLE 2. *Marker sequences on F-genotes formed by transduction from an F_{14} male*

F-genote formed in transductant[a]	Transduction donor[b]		
	AB1005	AB1264	AB1048
F ilvE$^+$	20	20	24
F ilvE$^+$ *ilvD*$^+$	3	3	4
F ilvE$^+$ *ilvD*$^+$ *ilvA*$^+$	2	3	2
F ilvE$^+$ *ilvD*$^+$ *ilvA*$^+$ *oprA1*	2[c]	—	—
F ilvE$^+$ *ilvD*$^+$ *ilvA*$^+$ *oprA1* *ilvC*$^+$	4[c]	—	—
F ilvE$^+$ *ilvD*$^+$ *ilvA*$^+$ (*oprA*$^+$) *ilvC*$^+$	—	3	3
F ilvE$^+$ *ilvD*$^+$ *ilvA*$^+$ (*oprA*$^+$) *ilvC*$^+$ *ilvB*$^+$ *oprB1*	—	—	2[d]
F ilvE$^+$ *ilvD*$^+$ *ilvA*$^+$ (*oprA*$^+$) *ilvC*$^+$ *ilvB197* (*oprB*$^+$)	—	2[e]	—
F ilvE$^+$ *ilvD*$^+$ *ilvA*$^+$ (*oprA*$^+$) *ilvC*$^+$ *ilvB*$^+$ *oprB1*	—	—	2[f]

[a] The presence of the alleles *ilvE*$^+$, *ilvD*$^+$, *ilvA*$^+$, and *ilvC*$^+$ on F-genotes was recognized by replica plating transductants onto lawns of appropriate F$^-$ recipients, and selecting for isoleucine-valine independent recombinants. The F$^-$ recipients used were, respectively: AB1514 (*ilvE12*); AB1472 (*ilvD16*); AB1255 (*ilvA201*); and AB1203 (*ilvC7*). The loci which appear in the table in parentheses could not be tested but are assumed to be present.

[b] The numbers refer to the number of transductants found to carry the F-genote shown.

[c] *oprA1* on F-genotes recognized by replica plating transductants onto F$^-$ strain AB1254 which carries *oprA*$^+$, and selecting for valine resistance.

[d] *ilvB*$^+$ *oprB1* on F-genotes recognized by replica plating transductants onto F$^-$ strain AB1052 which carried *ilvB196 oprB1*, and selecting for valine resistance.

[e] *ilvB197* on F-genotes recognized by replica plating transductants onto F$^-$ strain AB1254 which carries *ilvB*$^+$, and selecting for valine resistance.

[f] *oprB1* on F-genotes recognized by replica plating transductants onto F$^-$ strain AB1254 which carries *oprB*$^+$, and selecting for valine resistance.

strain AB1048, which has the genotype *ilvB*$^+$ *oprB1*, is resistant to ABA and is also resistant to low levels (2×10^{-4} M) of valine.

To map the position of the *ilvB* locus relative to the other structural genes of isoleucine-valine synthesis, phage P1kc was grown on F′ strain AB1048 and used to transduce the *ilvB*$^+$ marker into cells of F$^-$ strain AB1514. Two hundred of the transductants were examined for the presence of F-genotes; the results (Table 2) show that *ilvB* is at the extreme right end of the sequence of structural genes.

Another means of mapping the *ilvB* locus has been made possible by the isolation of a valine-resistant mutant of AB1206 which owes its resistance to a complete lack of sensitivity of condensing enzyme to feedback inhibition by valine (Pittard et al., 1963). The properties of this mutant, AB1264, are compared with those of related strains in Table 3. The mutation conferring resistance to feedback inhibition, formerly referred to as *val-r12*, has been redesignated *ilvB197*.

When AB1264 is used as the donor for the transduction of *ilvE*$^+$ into F$^-$ strain AB1514, the transductants are again found to contain a variety of F-genotes. As shown in Table 2, the sequences of markers found among the different F-genotes again indicate that *ilvB* is at the extreme right end of the series.

The mapping of *oprB* is based on the same principles. F′ strain AB1048 has the genotype *ilvB*$^+$ *oprB1* and is resistant to low levels of valine. When AB1048 is used as the donor for the transduction of *ilvE*$^+$ into F$^-$ strain AB1514, the transductants are found to contain a variety of F-genotes in which *oprB* is only found at the extreme right end of the marker series (Table 2).

DISCUSSION

The mapping method described above, based on recognizing F-genotes of different lengths, permits the unequivocal ordering of loci but does not give any indication of their relative distances apart. The order of the five structural genes and of the two operators discovered so far is shown in Fig. 1. The mapping data confirm the conclusion of the preceding paper (Rama-

TABLE 3. *Feedback inhibition of condensing enzyme*

Strain no.	Condensing enzyme activity		Per cent inhibition by valine
	Valine absent	Valine (10^{-3} M)	
AB1206	0.50	0.30	40
AB1048	9.80	5.80	40
AB1264	0.50	0.49	2

krishnan and Adelberg, 1965) that the enzymes of the isoleucine-valine biosynthetic pathway are controlled by a group of genes which together form a tightly clustered set of three operons. The polarity mutation in the *ilvD* locus which lowers the activity of *ilvE* (see Table 3 of the preceding paper) adds further evidence for the relative positions of the structural genes and operator locus of operon I.

The advantage to the cell (if any) of such complexity is not apparent, but may be related to the fact that the enzymes concerned are involved in the production of four different end products: isoleucine, valine, leucine, and pantothenic acid. The last two compounds are formed by pathways which start with the keto acid precursor of valine, α-ketoisovaleric acid, as shown in Fig. 1 of the preceding paper (Ramakrishnan and Adelberg, 1965). We have already shown that the rate-limiting enzyme for isoleucine biosynthesis is threonine deaminase, and for valine biosynthesis is the condensing enzyme. Thus, feedback regulation demands two points of control, rather than one. Furthermore, we have found one regulator locus which can be mutated to derepress only the condensing enzyme, showing that the different operons must respond to different repressors. We have not yet discovered whether the four end products are effectors for the same repressor or for different repressors; experiments designed to answer his question are in progress.

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