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# Complementation Between Different Mutations in the *ilvA* Gene of *Escherichia coli* K-12

RENÉE FAVRE, MAURIZIO IACCARINO, AND MARK LEVINTHAL

*International Institute of Genetics and Biophysics, CNR, 80125 Naples, Italy, and Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907*

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An *ilvA* mutation carried by a $\phi$80i$^{\lambda}$*dilv* transducing phage complemented some *ilvA* mutations and did not complement others. Complementation was accompanied by appearance of threonine deaminase activity in vivo. These results divided the *ilvA* mutations into two sets which formerly appeared to define two cistrons.

In *Escherichia coli* K-12 the isoleucine and valine biosynthesis is accomplished by five enzymatic steps which are specified by a cluster of five *ilv* genes designated *A* through *E* (16). A complex system of multivalent control allows these enzymes to exhibit derepression in response to limitations of isoleucine, of valine, or of leucine (5). Recent evidence implicates threonine deaminase [L-threonine hydrolyase (deaminating) EC 4.2.1.16], the product of the *ilvA* gene, in the regulation of both the *ilv* gene cluster and the branched-chain aminoacyl transfer ribonucleic acid synthetases, in addition to its catalytic role in isoleucine biosynthesis (3, 8, 11, 14, 19).

Mutations of the *ilvA* gene resulting in the loss of catalytic activity of threonine deaminase confers an Ile$^-$ phenotype to strains bearing such lesions, although the phenotype of strains containing an *ilvC*, *D*, or *E* mutation is Ilv$^-$. We prepared a specialized defective transducing phage containing the *ilv* gene cluster of *E. coli* K-12 to facilitate studies on the mechanism of multivalent control (1). We then mutagenized strain MI199 (see Table 1 for the genotype of the strains used) with *N*-methyl-*N*-nitrosoguanidine as described elsewhere (6) and obtained a derivative with a mutation of the *ilvA* gene contained in the defective phage. The resulting Ile$^-$ strain (MI199d) was induced to produce transducing phage. Such purified phage lysates were used to transduce strain AB3590 (containing the *ilvDAC115* multisite mutation) and Val$^+$Ile$^-$ transductants were obtained at a frequency comparable to that of Ilv$^+$ transductants obtained in similar crosses using a parental $\phi$80i$^{\lambda}$*dilv*$^+$ phage lysate.

We performed crosses between various *ilv* mutant strains and a lysate of $\phi$80i$^{\lambda}$*dilvA602* phage. Table 2 shows that Ilv$^+$ transductants are obtained with strains containing *ilvE12*, *D538*, *C485*, *A201*, *A537*, *A601*, and *A2003* mutations, but not with strains bearing *ilvA451*, *A454*, *A483*, *A529*, *A534*, *A550*, *A2001*, and *A2001* mutations. For each of MI2, CU2, CU26, CU97, CU105 and CU255, we purified a single Ilv$^+$ transductant and induced and prepared a lysate. Each of these lysates contained the original $\phi$80i$^{\lambda}$*dilvA602* phage as indicated by transduction of AB3590. In all cases, Val$^+$ Ile$^-$ transductants appeared, but in no case were Ilv$^+$ transductants observed. In addition, Ile$^+$ transductants were obtained with a strain isogenic to strain AB3590, strain MI166i, which contains the *ilvA601* mutation of strain MI2. To determine whether the Ile$^+$ clones were recombinants, a transductant of strain M12 and two transductants of strain MI166i were purified and induced, and lysates were made. These lysates contained $\phi$80i$^{\lambda}$*dilv*, *ilvA602* phage as indicated by transduction of strain AB3590 to yield Val$^+$, Ile$^-$ transductants and by transduction of strain MI166i to yield Ile$^+$ transductants. Furthermore, P1 phage grown on one of the Ile$^+$ transductants of strain MI166i was used to determine if the two *ilvA* mutations had recombined. Strain AB3590 was transduced with this P1 lysate and Val$^+$transductants were selected on plates containing isoleucine. All the transductants tested (20/20) were Ile$^-$, which shows that *ilvA*$^+$ recombinants were absent. These results insure that the Ilv$^+$ transductants reported in Table 2 are the result of complementation and not recombination in the *ilv* region of the chromosome.

Table 3 shows the enzymatic activity of threonine deaminase in different strains. Threonine deaminase is undetectable in strains

TABLE 1. *Escherichia coli strains: genotype and source*

| Strain | Genotypes | Source |
|---|---|---|
| AB1255 | *thi-1 ilvA201 argH1 metB1 his-1 xyl-7 malA1 tsx-5 str-8,9, or 17 sup48* λ[r] | E. Adelberg (13) |
| AB1412 | *thi-1 ilvA44 argH1 metB1 his-1 xyl-7 malA1 gal-6 lacY1 str8,9 or 17 mtl-2* λ[r] | E. Adelberg (13) |
| AB3590 | *thi, mtl, malA str his trpC tsx lacZ ilvDAC115* | E. Adelberg (10) |
| CU2 | *ilvE453* | H. E. Umbarger (18) |
| CU8 | *ilvA451* | H. E. Umbarger (18) |
| CU24 | *ilvA483 metE200 rbs-215 gal* | Pledger (14) |
| CU26 | *ilvC485* | Pledger (14) |
| CU96 | *ilvA529 gal* | Levinthal |
| CU97 | *ilvD530 gal* | Levinthal |
| CU102 | *ilvA534 gal* | Levinthal |
| CU105 | *ilvA537 gal* | Levinthal |
| CU140 | *ilvA550 gal* | Levinthal |
| CU253 | *ilvA2001 gal* | Pledger |
| CU254 | *ilvA2002 gal* | Pledger |
| CU255 | *ilvA2003 gal* | Pledger |
| CU1008 | *ilvA451* | McGilvray |
| M12 | *argH trpA23 ilvA601* | Iaccarino (9) |
| MI166b | *thi mtl malA str his trpC tsx lacZ* Ilv$^+$ transductant of AB3590 | Iaccarino |
| MI199 | AB3590 lysogenic for φ80i$^\lambda$ CI857 t68d*ilv*$^+$/ φ80i$^\lambda$ CI857t68 | Iaccarino (1) |
| MI199d | AB3590 lysogenic for φ80i$^\lambda$ CI857 t68d*ilvA602*/ φ80i$^\lambda$ CI857 t68 | Iaccarino |

TABLE 2. *Transduction of ilv mutations with φ80i$^\lambda$dilvA602*[a]

| Recipients | | Frequency $\times 10^{-3}$ of *ilv*$^+$ transductants[b] |
|---|---|---|
| Strain no. | *ilv* allele no. | |
| CU2 | *ilvE12* | 15.1 |
| CU26 | *ilvC485* | 13.6 |
| CU97 | *ilvD530* | 12.6 |
| CU8 | *ilvA451* | <0.001 |
| CU1008 | *ilvA454* | <0.001 |
| CU24 | *ilvA483* | <0.001 |
| CU96 | *ilvA529* | <0.001 |
| CU102 | *ilvA534* | <0.001 |
| CU140 | *ilvA550* | <0.001 |
| CU253 | *ilvA2001* | <0.001 |
| CU254 | *ilvA2002* | <0.001 |
| AB1412 | *ilvA44* | 2.0 |
| AB1255 | *ilvA201* | 0.2 |
| CU105 | *ilvA537* | 20.8 |
| M12 | *ilvA601* | 0.9 |
| CU255 | *ilvA2003* | 2.3 |

[a] Transductions were performed as described by Press et al. (15). Ilv$^+$ transductants were selected by plating the transduction mixture on appropriately supplemented minimal agar plates.

[b] The frequency of Ilv$^+$ transductants is computed as the number of transductant colonies divided by the titer of plaque-forming units.

AB3590 and MI2 and in a Val$^+$ Ile$^-$ transductant of strain AB3590. Strain MI166b contains a normal level of threonine deaminase. The Ilv$^+$ transductant derived from strain MI2 shows

TABLE 3. *Intracellular concentration of threonine deaminase in different strains*

| Strain | Threonine deaminase (sp act)[a] |
|---|---|
| MI166b | 28 |
| MI2 (*ilvA601*, Ile$^-$) | <0.2 |
| AB3590 | <0.3 |
| Ile$^+$ transductant of MI2[b] | 5 |
| Val$^+$, Ile$^-$ transductant of AB3590 | <0.3 |

[a] Threonine deaminase was assayed as described in by Guardiola and Iaccarino (6). Specific activity is expressed as nanomoles of product formed per minute per milligram of protein.

[b] Transduction was performed with the φ80i$^\lambda$*dilv*, *ilvA602* phage described in this note.

some threonine deaminase activity (18% of strain MI166b). We conclude that the *ilvA* mutation carried by the transducing phage can complement some *ilvA* mutations and this complementation is accompanied by the appearance of detectable levels of intracellular threonine deaminase activity.

Our results indicate that the *ilvA* gene can be divided into two cistrons with respect to catalytic function. We assign *ilvA451, 454, 483, 529, 534, 550, 602, 2001* and *2002* to the *ilvAa* cistron and *ilvA44, 201, 537, 601,* and *2003* to the *ilvAb* cistron.

Evidence was reported (4) that threonine

deaminase is a tetramer composed of four identical subunits as indicated by ultracentrifugation and acrylamide gel electrophoresis. However, the definition of a cistron (2) does not specify any relationship between the number of complementation groups and the number of polypeptide chains (identical or non-identical) in the protein specified by the gene. Indeed, the *hisD* gene of *Salmonella typhimurium* contains two complementation groups (7) while the enzyme it specifies, histidinol dehydrogenase, is composed of two identical polypeptide chains (12). This situation can be contrasted with the *hisC* gene which contains one cistron (7) and specifies the enzyme imidazole acetol phosphate transaminase, an enzyme also composed of two identical polypeptide chains (17).

In conclusion, our discovery of two complementation groups in the *ilvA* gene might indicate the occurrence in threonine deaminase of two non-identical polypeptide chains or non-covalent interactions between identical polypeptide chains. Work is in progress in one of our laboratories which will discriminate between these two possibilities.

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## LITERATURE CITED


1. Avitabile, A., M. S. Carlomagno-Cerillo, R. Favre, and F. Blasi. 1972. Isolation of transducing bacteriophages for the histidine and isoleucine-valine operons in *Escherichia coli* K-12. J. Bacteriol. **112**:40-47.
2. Benzer, S. 1959. On the topology of the genetic fine structure. Proc. Nat. Acad. Sci. U.S.A. **45**:1607-1620.
3. Calhoun, D. H., and G. W. Hatfield. 1973. Autoregulation: a role for a biosynthetic enzyme in the control of gene expression. Proc. Nat. Acad. Sci. U.S.A. **70**:2757-2761.
4. Calhoun, D. H., A. R. Rimerman, and G. W. Hatfield. 1973. Threonine deaminase from *Escherichia coli*. Purification and properties. J. Biol. Chem. **248**:3511-3516.
5. Freundlich, M., R. O. Burns, and H. E. Umbarger. 1962. Control of isoleucine, valine and leucine biosynthesis. I. Multi-valent repression. Proc. Nat. Acad. Sci. U.S.A. **48**:1804-1808.
6. Guardiola, J., and M. Iaccarino. 1971. *Escherichia coli* K-12 mutants altered in the transport of branched-chain amino acids. J. Bacteriol. **108**:1034-1044.
7. Hartman, P. E., Z. Hartman, and D. Serman. 1960. Complementation mapping by abortive transduction of histidine-requiring *Salmonella* mutants. J. Gen. Microbiol. **22**:354-368.
8. Hatfield, G. W., and R. O. Burns. 1970. Specific binding of leucyl transfer RNA to an immature form of L-threonine deaminase: its implications in repression. Proc. Natl. Acad. Sci. U.S.A. **66**:1027-1035.
9. Iaccarino, M., and P. Berg. 1971. Isoleucine auxotrophy as a consequence of a mutationally altered isoleucyl-transfer ribonucleic acid synthetase. J. Bacteriol. **105**:527-537.
10. Kiritani, K., T. Matsuno, and Y. Ikeda. 1965. Genetic and biochemical studies on isoleucine and valine requiring mutants of *Escherichia coli*. Genetics **51**:341-349.
11. Levinthal, M., L. S. Williams, M. Levinthal, and H. E. Umbarger. 1973. Role of threonine deaminase in the regulation of isoleucine and valine biosynthesis. Nature N. Biol. **246**:65-68.
12. Loper, J. 1968. Histidinol dehydrogenase from *Salmonella typhimurium*. J. Biol. Chem. **243**:3264-3272.
13. Marsh, N. J., and D. E. Duggan. 1972. Ordering of mutant sites in the isoleucine-valine genes of *Escherichia coli* by use of merogenotes derived from F'14: a new procedure for fine-structure mapping. J. Bacteriol. **109**:730-740.
14. Pledger, W. J., and H. E. Umbarger. 1973. Isoleucine and valine metabolism in *Escherichia coli*. XXII. A pleiotropic mutation affecting induction of isomeroreductase activity. J. Bacteriol. **114**:195-207.
15. Press, R., N. Glandorff, P. Miner, J. De Vries, R. Kadner, and W. K. Mass. 1971. Isolation of transducing particles of $\phi$80 bacteriophage that carry different regions of the *Escherichia coli* genome. Proc. Nat. Acad. Sci. U.S.A. **68**:795-798.
16. Ramakrishnan, T., and E. A. Adelberg. 1965. Regulatory mechanisms in biosynthesis of isoleucine and valine. III. Map order of the structural genes and operator genes. J. Bacteriol. **89**:661-664.
17. Rechler, M. M., and C. B. Bruni. 1971. Properties of a fused protein formed by genetic manipulation. J. Biol. Chem. **246**:1806-1813.
18. Umbarger, H. E., and Adelberg, E. A. 1951. The role of $\alpha$keto-$\beta$-ethyl butyric acid in the biosynthesis of isoleucine. J. Biol. Chem. **192**:883-889.
19. Wasmuth, J., H. E. Umbarger, and W. B. Dempsey. 1973. A role for a pyridoxine derivative in the multivalent repression of the isoleucine and valine biosynthetic enzymes. Biochem. Biophys. Res. Commun. **51**:158-164.