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Cloning of Genes That Suppress an *Escherichia coli* K-12 Alanine Auxotroph When Present in Multicopy Plasmids

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To facilitate molecular analyses of a previously uncharacterized gene involved in alanine synthesis, attempts were made to clone the wild-type allele of this gene, *alaA*, with a mini-Mu plasmid element used for in vivo cloning. Seventy-six independent Ala$^+$ plasmids were isolated and characterized. Physiological, enzymological, and restriction endonuclease analyses indicated that three different genes, none of them *alaA*, were cloned. These genes were *avtA*$^+$, which encodes the alanine-valine transaminase (transaminase C); *tyrB*$^+$, which encodes the tyrosine-repressible transaminase (transaminase D); and a previously undescribed gene, called *alaB*, which encodes an alanine-glutamate transaminase.

Traditionally, genetic and physiological analyses of null-activity mutations have been used to help elucidate biosynthetic pathways in microorganisms. When more than one enzyme catalyzes any particular step, however, such mutations are difficult but not impossible to obtain (see, for example, references 7, 14, 16, 19, and 20). Despite the power of physiological genetics, no tight L-alanine auxotrophs have been reported, probably because this simple amino acid can be synthesized from pyruvate by a number of different transaminases. Mutations responsible for a leaky requirement for either alanine or valine, called *alaA*, have, however, been isolated by transposon mutagenesis in both *Escherichia coli* and *Salmonella typhimurium* (3; W. Whalen, Ph.D. thesis, The University of Connecticut, Storrs, 1984).

To identify the enzyme encoded by *alaA* and to begin a molecular genetic analysis of this apparently new gene, we attempted to clone *alaA*$^+$ on a multicopy plasmid by using a mini-Mu plasmid element (8) as the in vivo cloning vector. In the results presented below, 76 independent Ala$^+$ plasmids were characterized, but none of these carried *alaA*$^+$. Instead, three transaminase genes were cloned. One of these genes, *alaB*, has not been described previously.

MATERIALS AND METHODS

Chemicals and media. Lennox (L) complex medium and Vogel and Bonner glucose-medium E salts were used as described elsewhere (2). Medium E was supplemented at the following levels where required: L-alanine (0.23 mM), L-aspartate (0.13 mM), L-isoleucine (0.15 mM), L-leucine (0.15 mM), L-phenylalanine (0.24 mM), L-proline (0.26 mM), thiamine (6 μM), thymine (0.079 mM), L-tryptophan (0.10 mM), L-tyrosine (0.11 mM), and L-valine (0.17 mM). Chloramphenicol (20 μg/ml) or 3-chloro-L-alanine (3-CA) (1 mM) was added where indicated. Specialized chemicals were purchased from Sigma Chemical Co., St. Louis, Mo. Restriction endonucleases and the DNA size markers were purchased from Bethesda Research Laboratories, Gaithersburg, Md., and agarose was purchased from FMC Corp., Rockland, Maine.

Enzyme assays. Cells were grown in appropriately supplemented medium E, and crude extracts were prepared and assayed as described previously (20), except that chloramphenicol was added to the medium to maintain the mini-Mu plasmid. Isoleucine-glutamate transaminase activity (transaminase B, encoded by *ilvE*) was measured by using isoleucine as the amino donor in 2-keto-3-methylvalerate production (2). Alanine-valine transaminase activity (transaminase C, encoded by *avtA*) was measured by using alanine as the amino donor in pyruvate production (12, 20). Tyrosine-glutamate transaminase activity (principally transaminase D, encoded by *tyrB*) was measured by using tyrosine as the amino donor in *p*-hydroxyphenylpyruvate production (5). Aspartate-glutamate transaminase activity (principally transaminase A, encoded by *aspC*) and alanine-glutamate transaminase activity (encoded by *alaB*, described below, and possibly other genes) were measured in a coupled reaction by using glutamate as the amino donor and the appropriate keto acid as the amino receptor (7).

Bacterial strains, phage, and plasmids. Table 1 lists the strains of *E. coli* K-12 used. Cultures containing mini-Mu or Mu *cts* were grown at 30°C to prevent phage induction. Other cultures were grown at 37°C.

alaA20::Mu dI1 had been isolated following Mu dI1 mutagenesis and penicillin enrichment for valine-requiring (*avtA*) mutants of an *ilvE* (isoleucine-requiring) strain that did not grow on 2-ketoisovalerate (the precursor of valine), but crossfed *ilvD* mutants (which grow on 2-ketoisovalerate). Unlike *ilvE avtA* strains, *ilvE alaA* mutants have normal transaminase C activity and a partial requirement for either alanine or valine (3). The physiological basis for this phenotype is not yet understood, but preliminary evidence suggests that *alaA* encodes a regulatory product. *alaA* is located in the 40-min region of the *E. coli* map (B. Wang, L. Liu, and C. M. Berg, unpublished data).

Mini-Mu-Mu *cts* double lysogens were constructed as described elsewhere (17). Cloning of quasi-random DNA fragments was carried out in vivo with a mini-Mu phage, Mu dII4042 (8), as depicted in Fig. 1 and described in greater detail elsewhere (8, 17, 18). Mu dII4042 is a short (16.7-kilobase [kb]) derivative of phage Mu (37.5 kb) that has Mu genes A and B, the two Mu ends needed for replication and transposition, the replication origin of the multicopy plasmid P15A, and the chloramphenicol (*cam*) resistance gene of Tn*9* (8). Chromosomal segments up to about 20 kb in length can be cloned with Mu dII4042, but most segments are 5 to 13 kb (8, 18; this study). Because Mu dII4042 has a multicopy

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TABLE 1. *E. coli* K-12 strains, phages, and plasmids

Strain, phage, or plasmid	Genotype	Relevant phenotype or trait(s) conferred	Derivation, reference, or source
E. coli strains			
CBK700	Δ(*proB-lac*) *thyA ilvE720*::Tn*5*	Ile$^-$	20
CBK717	CBK700 *tyrB17*::Mu dI1	Ile$^-$ Leu$^-$	Mu dI mutagenesis[a]
CBK740	Δ(*proB-lac*) *thyA ilvE12*	Ile$^-$	21
CBK741	CBK740 *avtA23*::Tn*5*	Ile$^-$ Val$^-$	21
CBK759	CBK700 *alaA20*::Mu dI1	Ile$^-$ Ala/Val$^-$	Mu dI mutagenesis[a]
CBK760	CBK740 *alaA20*::Mu dI1	Ile$^-$ Ala/Val$^-$	Transduction to Amp^r by using P1 · CBK759
CBK801	XPh43 *ilvE720*::Tn*5*	Ile$^-$	Transduction to Kan^r, by using P1 · CBK717
CBK802	CBK717 *aspC25* Δ(*zbc2011*::Tn*10*)275	Ile$^-$ Leu$^-$ Asp$^-$ Phe$^-$ Tyr$^-$	Spontaneous *aspC* mutation[b]
XPh43	Δ(*argF lacIPOZYA*)U169 *trp* Δ(*hrnQ phoA proC phoB phoR*)*24*		4
Phages			
Mu *cts*	*cts*62		9
Mu dII4042	*cts*62 *cam rep*P154A *lac*('*ZYA*)*931*	Cam^r	8
Plasmids			
pEG109	Mu dII4042-*phoA*$^+$ *proC*$^+$		8
pIF001	Mu dII4042-*avtA*$^+$	Ile$^+$ Val$^+$ 3-CA^r	Val$^+$ transductant of CBK741
pIF023	Mu dII4042-*avtA*$^+$	Ile$^+$ Ala/Val$^+$ 3-CA^r	Ala/Val$^+$ 3-CA^r transductant of CBK760
pIF036	Mu dII4042-*avtA*$^+$	Ile$^+$ Ala/Val$^+$ 3-CA^r	Ala/Val$^+$ transductant of CBK760
pIF039	Mu dII4042-*ilvE*$^+$	Ile$^+$ Ala/Val$^+$ 3-CA^r	Ala/Val$^+$ 3-CA^r transductant of CBK760
pIF048	Mu dII4042-*tyrB*$^+$	Ile$^-$ Leu$^+$ 3-CA^r	Leu$^+$ transductant of CBK717
pIF056	Mu dII4042-*tyrB*$^+$	Ile$^-$ Ala/Val$^+$ 3-CA^r	Ala/Val$^+$ transductant of CBK760
pIF059–pIF062	Mu dII4042-*alaB*$^+$[c]	Ile$^-$ Ala/Val$^+$	Ala/Val$^+$ transductant of CBK760
pIF063	Mu dII4042-*tyrB*$^+$	Ile$^-$ Ala/Val$^+$ 3-CA^r	Ala/Val$^+$ 3-CA^r transductant of CBK760

[a] W. A. Whalen, Ph.D. thesis, The University of Connecticut, Storrs, 1984.
[b] Isolated as *aspC* Tet^r following Tn*10* mutagenesis. Tn*10* was about 20% linked to *aspC*. A Tet^s derivative was selected.
[c] See Results for a description of *alaB*.

plasmid replication origin, it is not normally found inserted into the chromosome (8, 17).

Isolation and characterization of Mu dII4042 plasmids carrying chromosomal genes. Lysates were prepared in the Mu *cts*-Mu dII4042 double lysogen XPh43 Mu *cts*/pEG109 or CBK801 Mu *cts*/pEG109 and used to infect CBK760Mu*cts* as described elsewhere (17). Cells were plated at appropriate dilutions on medium E plates containing thymine, thiamine, proline, isoleucine, and chloramphenicol (selecting Cam^r Ala/Val$^+$ transductants) or on the same medium plus 3-CA (selecting Cam^r Ala/Val$^+$ 3-CA^r transductants). After incubation at 30°C for 3 to 5 days, isolated colonies were picked and purified on the same medium.

Ala/Val$^+$ revertants arise in CBK760 (which has a partial alanine or valine requirement) at a frequency of about 10^{-5}, whereas mini-Mu plasmids carrying a given gene can be found at a frequency of 10^{-8} to 10^{-5} (8, 17). The recovery of unwanted chromosomal mutations was reduced by adding to the medium either chloramphenicol, which killed recipients that lacked an established mini-Mu plasmid, or 3-CA, which reduced background growth.

Mini-Mu plasmids are rapidly lost if not maintained by selection (8, 17). To determine whether the selected phenotype was due to a plasmid-borne gene or to either generalized transduction or a chromosomal mutation (with the independent acquisition of a mini-Mu plasmid), cells from Cam^r colonies were streaked on L agar lacking chloramphenicol, and the resulting colonies were screened for loss of resistance and for growth requirements: colonies in which the Ala/Val$^+$ determinant was plasmid-borne gave rise to Cam^s segregants that had regained the alanine requirement.

Restriction analysis of mini-Mu plasmids. Cells were grown with aeration overnight in L broth plus chloramphenicol to prevent plasmid loss. Plasmid DNA was obtained by a rapid alkaline extraction procedure (11) and digested with restriction endonucleases as specified by the supplier. The digests were analyzed by electrophoresis in 0.75% agarose. Fragment sizes were estimated by comparison with fragments of the DNA gel marker (1-kb ladder) and, where available, with published data.

RESULTS

Preliminary results. Phage were prepared by induction of XPh43 Mu *cts*/pEG109, and Ala/Val$^+$ 3-CA^r transductants of CBK760 Mu *cts* (*ilvE alaA*) were selected. Each of 15 transductants tested regained the ability to synthesize isoleucine and had 30- to 40-fold elevated transaminase B activity (Table 2; data not shown). These results indicate that the *ilvE*$^+$ gene rather than the *alaA*$^+$ gene of XPh43 was cloned in each case. *ilvE*$^+$ plasmids were one of the classes expected because haploid *ilvE*$^+$ *alaA* strains were prototrophs (W. Whalen, Ph.D. thesis, The University of Connecticut, Storrs, 1984) and transaminase B is one of the enzymes inhibited by 3-CA (1), so that overproduction of transaminase B would result in increased resistance. The absence of other classes in this experiment led us to use an *ilvE* donor subsequently.

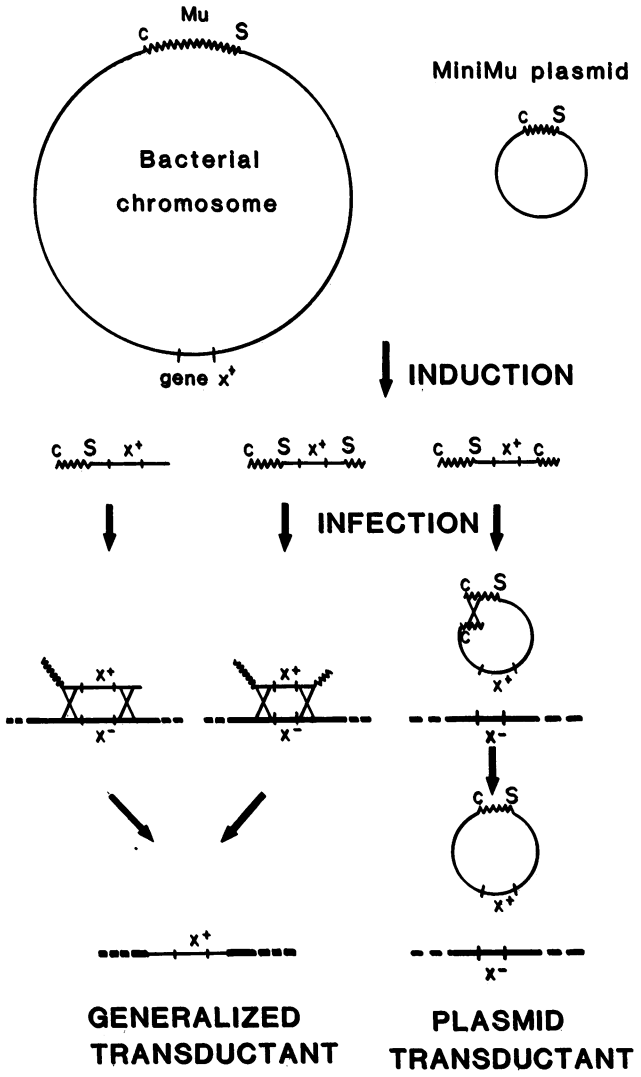

FIG. 1. In vivo generation of generalized transductants and plasmids by mini-Mu. Zigzag line indicates Mu or mini-Mu DNA. Packaging is initiated at the left (*c*) end of mini-Mu and occurs by a headful mechanism. Induction of a Mu-mini-Mu double lysogen produces mini-Mu-containing phage of the types depicted. If only one mini-Mu genome or one genome plus a portion of a second genome in the opposite orientation is packaged, a generalized transductant can be formed in the recipient, as shown on the left. If one mini-Mu genome plus a portion of a second in the same orientation is packaged, a plasmid transductant can be formed in the recipient, as shown on the right.

Selection of plasmids that complement or suppress *alaA*. To clone *alaA*$^+$ Mu-mini-Mu lysates were prepared on CBK801 (*ilvE*), and Cam^r Ala/Val$^+$ plasmid transductants of CBK760 Mu *cts* were isolated. Large colony transductants were found on medium containing 3-CA, and both large and small colony transductants were found on medium lacking 3-CA. Seventy of 72 large-colony transductants were found to be isoleucine independent, whereas two retained the isoleucine requirement of the recipient strain. All large-colony transductants were 3-CA^r regardless of which medium they were isolated on. All of 16 small-colony transductants required isoleucine, were 3-CA^s, and made large colonies on alanine- or valine-supplemented medium. Cam^s (plasmid-free) segregants of 12 large-colony transductants and of 4 of 16 small-colony transductants were found to regain the parental phenotype, indicating that their properties were due to plasmid-borne genes.

(i) Characterization of plasmids from isoleucine-independent transductants. Because *ilvE* encodes transaminase B, the only transaminase previously known to catalyze isoleucine biosynthesis in vivo (15), and both the donor and recipient have *ilvE* mutations, the isoleucine independence of most large-colony Ala/Val$^+$ plasmid transductants indicated the recruitment of an enzyme not previously implicated in isoleucine synthesis. All of 26 isoleucine-independent transductants tested had elevated transaminase C activity, but no transaminase B activity (Table 2; data not shown), like a strain with an *avtA*$^+$ plasmid (Table 2). In confirmation, restriction mapping showed overlaps between these plasmids and bona fide *avtA*$^+$ plasmids (Fig. 2A) (18). These data suggested that the increased levels of transaminase C obtained when *avtA*$^+$ was on a multicopy plasmid resulted in synthesis of physiologically significant levels of isoleucine.

(ii) Characterization of plasmids from large isoleucine-requiring transductants. Neither transductant [CBK760 (pIF056) or CBK760(pIF063)] had detectable transaminase B activity or elevated transaminase C activity, although both had a significant elevation in tyrosine-glutamate transaminase activity and a small elevation in aspartate-glutamate transaminase activity (Table 2). Since tyrosine is the preferred substrate for transaminase D (encoded by *tyrB*), and aspartate is the preferred substrate for transaminase A (encoded by *aspC*) (7), it was likely that *tyrB* had been cloned even though neither transaminase had previously been implicated in either alanine or valine synthesis.

The restriction maps of these presumptive *tyrB*$^+$ plasmids (pIF056 and pIF063) overlapped those of plasmids that had been isolated by complementation of *tyrB* mutants (Fig. 2B) (6, 10).

(iii) Characterization of plasmids from small isoleucine-requiring plasmid transductants. The four transductants that carried the plasmid-borne Ala/Val$^+$ determinant had 5- to 10-fold elevated levels of alanine-glutamate transaminase activity and no significant elevation in transaminase A, B, C, or D activities (Table 2; data not shown). Therefore, the cloned gene in these four transductants encodes an alanine-glutamate transaminase. The restriction maps of these plasmids are shown in Fig. 2C.

The slow growth of these plasmid-containing strains suggested that a different gene, which can only partially suppress *alaA*, was cloned. The possibility that *alaA*$^+$ was cloned but that it or a linked gene was deleterious was excluded because strains containing these plasmids grew normally if alanine or valine was added to the medium. Therefore, the cloned gene encoded an enzyme that can participate in alanine synthesis, but only suboptimally. This gene was named *alaB*. The *alaB*$^+$ plasmid transductants were 3-CA^s, indicating that the enzyme encoded by *alaB*, unlike the enzymes encoded by *ilvE*, *avtA*, and *tyrB*, does not confer 3-CA resistance when overproduced.

To determine whether the alanine-glutamate transaminase encoded by *alaB* catalyzes either valine or leucine synthesis when overproduced, pIF061 was transformed into CBK741 (which cannot transaminate valine or isoleucine) and into CBK717 (which cannot transaminate leucine or isoleucine). Both plasmid-containing strains retained their original re-

TABLE 2. Transaminase activities

Strain[a]	Relevant genotype/plasmid genotype	Sp act[b] (nmol/min per mg of protein)				
		Asp-Glu (TrA)	Ile-Glu (TrB)	Ala-Val (TrC)	Tyr-Glu (TrD)	Ala-Glu
XPh43(pEG109)		—[c]	34.8	29.1	—	—
CBK801(pEG109)	*ilvE*	256.3	<1.8	25.3	89.4	25.9
CBK741	*ilvE avtA*	—	<1.8	<0.5	—	27.5
CBK717	*ilvE tyrB*	332.0	<1.8	19.0	4.7	—
CBK760	*ilvE alaA*	237.6	<1.8	21.5	83.2	26.3
CBK760(pIF039)	*ilvE alaA/ilvE*$^+$	—	1,175.5	—	—	25.3
CBK741(pIF001)[d]	*ilvE avtA/avtA*$^+$	—	<1.8	903.7	—	23.4
CBK760(pIF023)	*ilvE alaA/avtA*$^+$	—	<1.8	806.4	—	—
CBK760(pIF036)	*ilvE avtA/avtA*$^+$	—	<1.8	847.2	—	—
CBK717(pIF048)	*ilvE tyrB/tyrB*$^+$	372.1	—	18.0	1,072	29.6
CBK760(pIF056)	*ilvE alaA/tyrB*$^+$	481.1	—	19.3	1,259	21.7
CBK760(pIF063)	*ilvE alaA/tyrB*$^+$	371.3	—	17.2	1,352	28.0
CBK760(pIF061)	*ilvE alaA/alaB*$^+$	308.9	—	16.0	129.3	170.5

[a] All are Mu *cts* lysogens except CBK717, which is *tyrB*:: Mu dI1.

[b] The major transaminase that catalyzes each reaction is indicated. For each assay, the amino donor and the product assayed were respectively: transaminase A (TrA), glutamate and NADP; TrB, isoleucine and 2-keto-3-methylvalerate; TrC, alanine and pyruvate; TrD, tyrosine and *p*-hydroxyphenylpyruvate; alanine-glutamate transaminase, glutamate and NADP.

[c] —, Not tested.

[d] Previously designated pIL001 (21).

quirements and formed normal-sized colonies on appropriately supplemented medium, confirming that pIF061 does not encode an inhibitory gene product and showing that the overproduced *alaB*-encoded transaminase functioned to increase the alanine pool specifically.

DISCUSSION

The present study was initially undertaken to clone *alaA*$^+$ on a multicopy plasmid for molecular genetic analyses of this gene. This goal was not achieved because *alaA*$^+$ was not found among 76 independent Ala$^+$ plasmids tested. Instead, three other genes were cloned. The presence of Ala$^+$ plasmids carrying different genes showed that other gene products could compensate for the auxotrophy of *alaA* mutants, while the absence of *alaA*$^+$ plasmids raised the possibility that either *alaA*$^+$ or a gene linked to *alaA* is harmful when present in multiple copies. A seemingly analogous case of multicopy suppression coupled with the absence of true complementing plasmids involves *glnP*, which was lethal on a multicopy plasmid (13). We recently obtained *alaA*$^+$ on a high-copy-number plasmid by cloning from a strain in which a Tn*10* (Tet^r) transposon was tightly linked to *alaA*, indicating that *alaA*$^+$ is not a multicopy-lethal gene. The *tetA*$^+$ *alaA*$^+$ plasmid strains constituted only about 2% of the Ala$^+$ plasmid transductants (B. Wang, L. Liu, and C. M. Berg, manuscript in preparation). Thus, the absence of *alaA*$^+$ clones in the present study was due to the cloning of other genes, which also conferred an Ala$^+$ phenotype. Because there is no obvious physiological reason why *avtA*$^+$, *tyrB*$^+$, and *alaA*$^+$ were cloned at such unequal frequencies (70:2:0, respectively), this bias probably reflects the relative frequencies with which appropriately spaced pairs of mini-Mu insertions occur (Fig. 1). This deviation from random Mu insertion appears to be regional rather than site specific, since for any one gene the endpoints of cloned fragments are quite random (18) (Fig. 2). The nonrandomness in mini-Mu cloning detected here does not, however, seriously compromise the value of mini-Mu for routine cloning of bacterial DNA, since in vitro restriction endonuclease cloning is also far from random.

The three genes cloned in this study by suppression of the *alaA* mutation on CBK760 encoded enzymes which, when overproduced, catalyzed either directly or indirectly the synthesis of physiologically significant levels of alanine or valine. Two of them (*avtA* and *tyrB*) were cloned both in the presence and in the absence of the transaminase inhibitor 3-CA. The third gene (named *alaB*) was cloned only in the absence of 3-CA and did not confer increased 3-CA resistance when in a multicopy plasmid. *alaB* encoded an alanine-glutamate transaminase (Table 2). To our knowledge, this is the first description of an alanine-glutamate transaminase gene from *E. coli*.

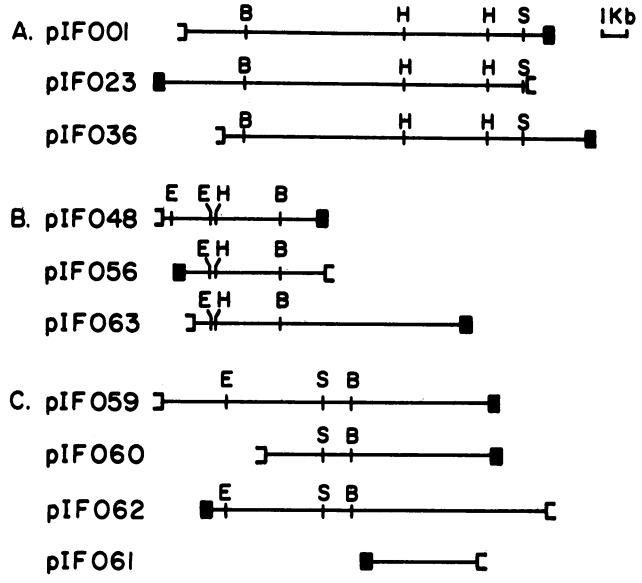

FIG. 2. Restriction map of the chromosomal portion of representative mini-Mu plasmids. Symbols: open bracket, the left (*c*) end of mini-Mu; closed block, the right (*S*) end of mini-Mu. Restriction endonucleases: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII S, *Sal*I; Kb, Kilobase. (A) *avtA*$^+$ plasmids. Restriction maps of additional plasmids are depicted elsewhere (18). (B) *tyrB*$^+$ plasmids. (C) *alaB*$^+$ plasmids. The orientation and exact position of the cloned fragment in pIF061 have not been determined.

The cloning of three genes other than *alaA* illustrates the ability of an excess of the product of one gene to compensate for a defect in a nonallelic gene and provides a valuable approach for analyzing metabolic pathways. Overproduction of an enzyme in vivo, for example, by cloning the corresponding gene, can lead to significant levels of a normally minor enzyme activity. (i) Suppression of the isoleucine requirement of *ilvE* mutants (see Results) suggests that at high levels, transaminase C can recruit isoleucine as an amino donor in alanine and valine biosynthesis. (ii) In *tyrB*$^+$ plasmid strains, excess transaminase D may function in alanine or valine synthesis directly or indirectly by increasing the pool of leucine or another amino acid, which then serves as the amino donor in transaminase C-catalyzed alanine biosynthesis. (iii) Since the alanine or valine requirement of CBK760 was only partially suppressed in *alaB*$^+$ plasmid strains despite their high alanine-glutamate transaminase activity in vitro (Table 2), the alanine-glutamate transaminase encoded by *alaB* may require the *alaA* product to function efficiently in vivo.

The present results, showing that a cloned wild-type gene which suppresses the mutant phenotype may not be allelic to the original lesion, illustrates the need for caution in cloning studies. Since most large-colony plasmid transductants carried *avtA*$^+$, one might have concluded that they actually carried *alaA*$^+$. This possibility was excluded here because bona fide *avtA*$^+$ plasmids had been characterized previously (18) and because nonoverlapping plasmids that conferred three distinct phenotypes were obtained. In most cloning studies, however, only one or a few plasmids are isolated, and different phenotypic classes cannot be so sensitively distinguished.

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ADDENDUM IN PROOF

Since on a multicopy plasmid, *avtA*$^+$ suppresses the leucine requirement of *ilvE tyrB* mutants (C. M. Berg, M.-D. Wang, N. B. Vartak, and L. Liu, submitted for publication), leucine, as well as isoleucine (see Results), can serve as an amino donor in alanine and valine synthesis.

LITERATURE CITED

1. **Arfin, S. M., and D. A. Koziell.** 1971. Inhibition of growth of *Salmonella typhimurium* and of threonine deaminase and transaminase B by β-chloralanine. J. Bacteriol. **105**:519–522.
2. **Berg, C. M., K. J. Shaw, J. Vender, and M. Borucka-Mankiewicz.** 1979. Physiological characterization of polar Tn*5*-induced isoleucine-valine auxotrophs in *Escherichia coli* K12: evidence for an internal promoter in the *ilvOGEDA* operon. Genetics **93**:309–319.
3. **Berg, C. M., W. A. Whalen, and L. B. Archambault.** 1983. Role of alanine-valine transaminase in *Salmonella typhimurium* and analysis of an *avtA*::Tn*5* mutant. J. Bacteriol. **155**:1009–1014.
4. **Brickman, E., and J. Beckwith.** 1975. Analysis of the regulation of *Escherichia coli* alkaline phosphatase synthesis using deletions and φ80 transducing phages. J. Mol. Biol. **96**:307–316.
5. **Diamondstone, T.** 1966. Assay of tyrosine transaminase activity by conversion of *p*-hydroxyphenylpyruvate to *p*-hydroxybenzaldehyde. Anal. Biochem. **16**:395–401.
6. **Fotheringham, I. G., S. A. Dacey, P. P. Taylor, T. J. Smith, M. G. Hunter, M. E. Finlay, S. B. Primrose, D. M. Parker, and R. M. Edwards.** 1986. The cloning and sequence analysis of the *aspC* and *tyrB* genes from *Escherichia coli* K12. Biochem. J. **234**:593–604.
7. **Gelfand, D. H., and R. A. Steinberg.** 1977. *Escherichia coli* mutants deficient in the aspartate and aromatic acid aminotransferases. J. Bacteriol. **130**:429–440.
8. **Groisman, E. A., B. A. Castilho, and M. J. Casadaban.** 1984. *In vivo* DNA cloning and adjacent gene fusing with a mini-Mu-*lac* bacteriophage containing a plasmid replicon. Proc. Natl. Acad. Sci. USA **81**:1480–1483.
9. **Howe, M. M.** 1973. Prophage deletion mapping of bacteriophage Mu-1. Virology **54**:93–101.
10. **Kuramitsu, S., K. Inoue, T. Ogawa, H. Ogawa, and H. Kagamiyama.** 1985. Aromatic amino acid aminotransferase of *Escherichia coli*: nucleotide sequence of the *tyrB* gene. Biochem. Biophys. Res. Commun. **133**:134–139.
11. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
12. **McGilvray, D., and H. E. Umbarger.** 1974. Regulation of transaminase C synthesis in *Escherichia coli*: conditional leucine auxotrophy. J. Bacteriol. **120**:715–723.
13. **Nohno, T., T. Saito, and J.-S. Hong.** 1986. Cloning and complete nucleotide sequence of the *Escherichia coli* glutamine permease operon (*glnHPQ*). Mol. Gen. Genet. **205**:260–269.
14. **Primerano, D. A., and R. O. Burns.** 1983. Role of acetohydroxy acid isomeroreductase in biosynthesis of pantothenic acid in *Salmonella typhimurium*. J. Bacteriol. **153**:259–269.
15. **Rudman, D., and A. Meister.** 1953. Transamination in *Escherichia coli*. J. Biol. Chem. **200**:591–604.
16. **Shaw, K. J., C. M. Berg, and T. J. Sobol.** 1980. *Salmonella typhimurium* mutants defective in acetohydroxy acid synthetases I and II. J. Bacteriol. **141**:1258–1263.
17. **Wang, B., L. Liu, E. A. Groisman, M. J. Casadaban, and C. M. Berg.** 1987. High frequency generalized transduction by miniMu plasmid phage. Genetics **116**:201–206.
18. **Wang, M.-D., L. Liu, B. Wang, and C. M. Berg.** 1987. Cloning and characterization of the *Escherichia coli* K-12 alanine-valine transaminase (*avtA*) gene. J. Bacteriol. **169**:4228-4234.
19. **Wasserman, S. A., C. T. Walsh, and D. Botstein.** 1983. Two alanine racemase genes in *Salmonella typhimurium* that differ in structure and function. J. Bacteriol. **153**:1439–1450.
20. **Whalen, W. A., and C. M. Berg.** 1982. Analysis of an *avtA*::Mu*d* 1 (Ap *lac*) mutant: metabolic role of transaminase C. J. Bacteriol. **150**:739–746.
21. **Whalen, W. A., M.-D. Wang, and C. M. Berg.** 1985. β-Chloro-L-alanine inhibition of the *Escherichia coli* alanine-valine transaminase. J. Bacteriol. **164**:1350–1352.