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Mapping of the Structural Genes of the Three Aspartokinases and of the Two Homoserine Dehydrogenases of *Escherichia coli* K-12

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Mutants requiring threonine plus methionine (or homoserine), or threonine plus methionine plus diaminopimelate (or homoserine plus diaminopimelate) have been isolated from strains possessing only one of the three isofunctional aspartokinases. They have been classified in several groups according to their enzymatic defects. Their mapping is described. Several regions of the chromosome are concerned: *thrA* (aspartokinase I-homoserine dehydrogenase I) is mapped in the same region as *thrB* and *thrC* (0 min). *lysC* (aspartokinase III) is mapped at 80 min, far from the other genes coding for diaminopimelate synthesis. *metLM* (aspartokinase II-homoserine dehydrogenase II) lies at 78 min closely linked to *metB*, *metJ*, and *metF*.

The main features of regulation in the branched biosynthetic pathway of the aspartate family of amino acids in *Escherichia coli* K-12 (see review 5) are shown in Fig. 1. The first reaction that leads to the synthesis of aspartylphosphate is catalyzed by three isofunctional aspartokinases (EC 2.7.2.4), the inhibition and repression of which respond to different end products: the activity of aspartokinase I (AKI) is inhibited by threonine and its synthesis is repressed by threonine plus isoleucine; aspartokinase II (AKII) is repressed by methionine; aspartokinase III (AKIII) is inhibited and repressed by lysine. The synthesis of homoserine from aspartic semialdehyde is catalyzed by two different homoserine dehydrogenases (EC 1.1.13): homoserine dehydrogenase I (HDHI) activity is inhibited by threonine and its synthesis is repressed by threonine plus isoleucine; homoserine dehydrogenase II (HDHII) is repressed by methionine. AKI and HDHI activities are carried by a single polypeptide chain (9); AKII and HDHII activities are also carried by a multifunctional protein (10).

This investigation describes the positions of the corresponding genes on the chromosome of *E. coli*. The nomenclature used in this investigation is described in Fig. 1 and is commented on in the Discussion.

Figure 2 summarizes the results and gives the position on the genetic map of *E. coli* of the different genes coding for the enzymes of the pathway, as well as the position of the genes used for mapping, as given by Taylor (24).

This investigation has been possible only after construction of strains possessing only one or the other of the three isofunctional enzymes, whose loss leads to a recognizable phenotype.

MATERIALS AND METHODS

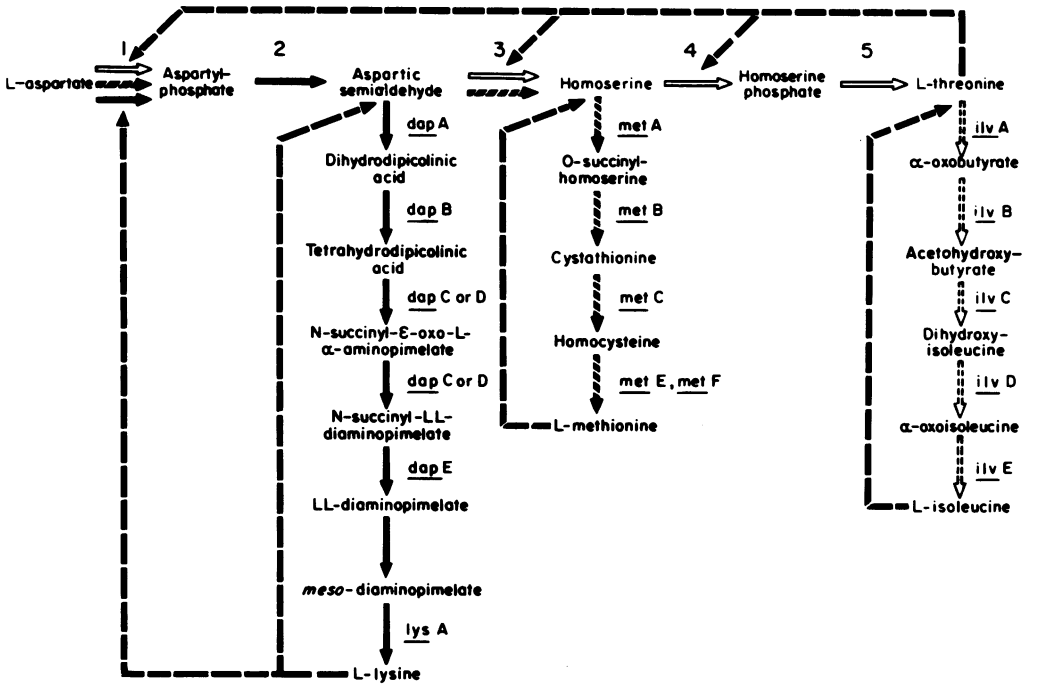
Bacterial strains. All strains were derived from *Escherichia coli* K-12. Phenotypes and genotypes are given in Tables 1, 4, and 8. The allele numbers used were allocated to the Service de Biochimie Cellulaire by the *E. coli* Genetic Stock Center, Yale University.

Transduction. Transductions with phage P1*kc* were performed according to the method of Lennox (16). Phage P1 *vir* was kindly given by M. Yarmolinsky and was used exclusively for mapping *thr* genes.

Mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and penicillin enrichment are described in reference 1.

Media. Selective plates contained minimal medium (8) supplemented with 1 μg of thiamine hydrochloride per ml, 2% agar, and the carbon source (glucose or fructose, 4 mg/ml). Except when specified, amino acids were used at 3×10^{-4} M of the L-isomer; DL-threonine was used at 2×10^{-3} M. The *pgi* character (phosphoglucose isomerase) was checked by use of media supplemented with eosine Y (40 mg/100 ml) and methylene blue (6.5 mg/100 ml) on which Pgi^+ strains give red colonies and Pgi^- strains give white colonies.

Enzyme measurements. Aspartokinase, homoserine, dehydrogenase, and aspartate semialdehyde dehydrogenase (EC 1.2.1.11) activities were measured as previously described (14, 20). Homoserine kinase (EC 2.7.1.39) activity was measured by coupling homoserine-dependent adenosine 5'-diphosphate (ADP) pro-



Reaction	Name of the enzyme	Abreviation	Name of the corresponding genes	Regulation of biosynthesis by:	Regulation of activity by:	References
1	Aspartokinase I	AK I	*thr* A[a]	thr and ile	thr	(22) (12)
1	Aspartokinase II	AK II	*met* L[b]	met	0	(18)
1	Aspartokinase III	AK III	*lys* C	lys	lys	(22)
2	Aspartic semialdehyde dehydrogenase	ASADH	*asd*	lys, thr, met	0	(3)
3	Homoserine dehydrogenase I	HDHI	*thr* A[a]	thr and ile	thr	(19) (12)
3	Homoserine dehydrogenase II	HDH II	*met* M[b]	met	0	(18)
4	Homoserine kinase	HSK	*thr* B	thr and ileu	thr	(26)
5	Threonine synthetase	TS	*thr* C	thr and ileu	n.d.	

FIG. 1. *Biosynthetic pathway for the amino acids deriving from aspartate. Symbols: →, enzymes repressed by lysine; ⟹, enzymes repressed by threonine plus isoleucine; ▪▪▪▶, enzymes repressed by methionine; ▪▪▪▶, enzymes repressed by isoleucine, leucine, and valine. The dashed lines point to the reactions subject to feed-back inhibition. The symbols in italics represent the genes corresponding to each reaction when this correspondence is known. The symbols for the genes corresponding to reaction 1 to 5 are detailed below. (a/For thrA see Discussion; b/for metL and MetM see Discussion.)*

duction to pyruvate kinase (EC 2.7.1.40) and lactate dehydrogenase (EC 1.1.1.27) (L. Kleidman and J. Thèze, manuscript in preparation).

MAPPING OF THE THR GENES

Isolation and classification of thr mutants (see Table 1). For selecting *thrA* mutants, it was necessary to construct a strain possessing only AKI-HDHI in order to select mutants devoid of one of the two activities of the enzyme on the basis of their auxotrophy.

Strain Gif 54, lacking AKIII, had already been obtained. Its growth is inhibited by threonine plus methionine (17). Starting from Gif 54 the direct selection of a strain having lost AKII-HDHII and unable to grow in the presence of threonine proved unsuccessful. Therefore, we had to resort to an indirect method. Using a derivative of Gif 54, Gif 101 (15), which has lost HDHI and retained AKI activity, it was easy to select an auxotroph for homoserine that had lost HDHII. In this strain, Gif 102, AKII activity was simultaneously lost (15).

From Gif 102 we have constructed a strain that has recovered HDHI activity. This strain is able to grow in minimal medium and unable to grow on threonine or threonine plus isoleucine. A strain having recovered HDHII would also be able to grow in minimal medium but methionine would inhibit its growth. Hence the following selection: Gif 102 was transduced by phage P1 *vir* grown on GC19 and prototrophs were selected

on a medium containing 10^2 M DL-methionine. One of the transductants was kept (GT1) and it was verified that its growth is inhibited by threonine plus isoleucine or threonine alone. Direct measurements in the crude extracts of GT1 confirm that only AKI-HDHI is present at the exclusion of AKII-HDHII and AKIII. From this strain *thrA* mutants have been selected after nitrosoguanidine mutagenesis.

One *thrA* mutant obtained from GT1 is used in this investigation: GT3 (*thrA1016*). It has a specific requirement for homoserine and diaminopimelate (Dpm) since it has lost AKI (but retained HDHI activity and is designated AK^-—HDH^+). It is distinguished from *asd* mutants by enzymatic assay.

Two other *thrA* mutants have already been described: Gif 106M1 (*thrA1101*) (3) has the same enzyme activities and nutritional requirements as GT3. Gif 102 has lost HDHI activity and retained AKI activity (AK^+—HDH^-). It requires threonine plus methionine, or homoserine, for growth (15).

thrB and *thrC* mutants have been obtained from Hfr H. Both types require threonine and must lack homoserine kinase or threonine synthetase (EC 4.2.99.2). Mutants lacking homoserine kinase are classified as *thrB*. Mutants possessing homoserine kinase are assumed to lack threonine synthetase and are classified as *thrC*. In mapping experiments we have used the *thrB1000* and *thrC1001* alleles carried respectively by strains YA73 and Gif 41.

When *thrB* or *thrC* alleles (that normally lead to an auxotrophy for threonine) are introduced in a strain which possesses only AKI-HDHI the repression and feedback inhibition caused by threonine creates a phenotypic additional requirement for homoserine and Dpm (e.g., GT12 and GT13, see Table 1).

Localization of the thrA gene. Mutations leading to desensitization of AKI-HDHI co-transduce with the usual *thr* marker (20). It was of particular interest to check independently that *thrA* mutants leading to inactivation of one of the two activities are linked to *thrB* and *thrC*. The distance of *thr* mutants of each type to *serB* and *pyrA*, markers on each side of the *thr* locus, has been estimated. A phage grown on MI 154 (*pyrA53*) is used to transduce GT15, GT12, and GT13 (*serB* strains that are, respectively, *thrA*, *thrB*, and *thrC*). Table 2 gives the results of such transductions, selecting for Ser^+ or Thr^+ characters. The distance of *thrA* to *pyrA* and *serB* is of the same order as that of *thrB* and *thrC* to the same markers. The cotransduction frequencies vary from 15 to 20% between *thr* genes and *pyrA53* and 60 to 66% between *thr* genes and *serB22*.

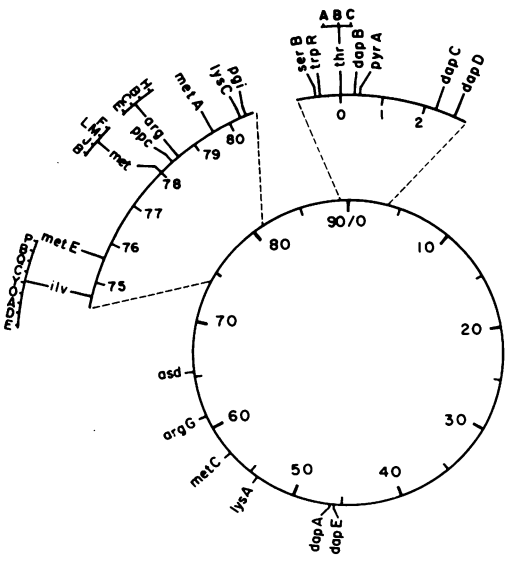


FIG. 2. *Location of the structural genes involved in the biosynthesis of threonine, methionine, diaminopimelate, lysine, and isoleucine. Genes used in the present work as genetic markers are also indicated (24).*

Table 3 gives the list and the results of the crosses carried out. The percentage of Ser^+ among Thr^+ transductants in each cross is shown. The frequency of Ser^+ is consistently different in each reciprocal cross.

The results of the crosses 1 and 2 performed between *thrB* and *thrC* give the order: *serB22 thrB1000 thrC1001*. For *thrA* mutants, we have used three different mutations of this gene, including two AK^--HDH^+ and one AK^+-HDH^- mutants. All three give unambiguous results in reciprocal crosses with the *thrB1000* allele (see in particular crosses 5, 6, 7, and 8). From these results, we can derive the following order: *...serB...thrA thrB thrC... pyrA....*

MAPPING OF LYSC

Isolation of lysC mutants (Table 4). For mapping of *lysC*, mutants were derived from strain Gif 106 previously described (3). This strain has lost by successive mutations (*thrA1101* and *metLM1000*) AKI, AKII, and HDHII activities; AKIII and HDHI are conserved. Strain Gif 106 may thus grow in minimal medium, but lysine inhibits its growth by inhibition and repression of AKIII, the only enzyme left that can catalyze aspartylphosphate synthesis. By mutagenesis of Gif 106, mutants auxotrophic for homoserine plus Dpm were selected; such a phenotype may correspond to mutations either for AKIII activity or for aspartate semialdehyde dehydrogenase activity. By measuring these two enzymes in crude extracts, strain 106 M1 was verified to lack AKIII activity. As growth of Gif 106 is inhibited by lysine,

TABLE 1. *List of the strains used for the mapping of the thr genes*

Genotype / Strain no.	*thrA*	*thrB*	*thrC*	*metLM*	*lysC*	Other loci	Origin
AT2459	+	+	+	+	+	*serB22, thi-1*	M. Iaccarino
GC19	+	+	+	+	+	*trpR 1000*	(6)
MI154	+	+	+	+	+	*pyrA53, thi-1*	M. Iaccarino
YA73	+	1000	+	+	+		F. Jacob
Gif 41	+	+	1001	+	+		(7)
Gif 54	+	+	+	+	1004		(17)
Gif 102	1015[a]	+	+	+	1004		(15)
Gif 106M1	1101[b]	+	+	1000	1001	*ilvA, arg-1000*	(3)
GT1	+	+	+	1005	1004		See text
GT2	+	+	+	1005	1004	*pro-1001*	Pro⁻ derivative of GT1 obtained after mutagenesis
GT3	1016[b]	+	+	1005	1004		See text
GT5	1017[c]			1005	1004	*pro-1001*	Thr⁻ derivative of GT2 obtained after mutagenesis
GT6	+	+	+	1005	1004	*pro-1001, pyrA53*	Thr⁺ transductant of GT5; P1 *vir* grown on MI154
GT9	1018[c]			1005	1004	*pro-1001, pyrA53*	Thr⁻ derivative of GT6 obtained after mutagenesis
GT10	+	+	+	1005	1004	*pro-1001, pyrA53, serB22*	Thr⁺ transductant of GT9; P1 *vir* grown on AT2459
GT12	+	1000	+	1005	1004	*pro-1001, serB22*	PyrA⁺ transductant of GT10; P1 *vir* grown on YA73
GT13	+	+	1001	1005	1004	*pro-1001, serB22*	PyrA⁺ transductant of GT10; P1 *vir* grown on Gif 41
GT14	1101[b]	+	+	1005	1004	*pro-1001, serB22*	PyrA⁺ transductant of GT10; P1 *vir* grown on Gif 106M1
GT15	1015[a]	+	+	1005	1004	*pro-1001, serB22*	PyrA⁺ transductant of GT10; P1 *vir* grown on Gif 102
GT16	1016[b]	+	+	1005	1004	*pro-1001, serB22*	PyrA⁺ transductant of GT10; P1 *vir* grown on GT3

[a] This mutation, AKI⁺-HDHI⁻, leads in this strain to a growth requirement for homoserine.
[b] This mutation, AKI⁻-HDHI⁺, leads in this strain to a growth requirement for homoserine *plus* Dpm.
[c] For strain GT5 and GT9, the *thr* mutation is not identified.

TABLE 2. *Frequency of cotransduction of thrA, thrB, and thrC mutations with serB22 and pyrA53 alleles, phage grown on MI154 (pyrA 53)*

Recipient	Selected marker	No. of colonies examined	Unselected markers (%)			
			Ser⁺	Thr⁺	PyrA⁻	Ser⁺ PyrA⁻
GT15 (*serB22 thrA1015*)	*ser*⁺	397		66	6	
	thr⁺	355	67		18	9
GT12 (*serB22 thrB1000*)	*ser*⁺	399		60	7	
	thr⁺	400	62		15	10
GT13 (*serB22 thrC1001*)	*ser*⁺	400		60	6	
	thr⁺	436	64		20	9

mutants resistant to this inhibition were isolated: in the case of 106 G21, 106 G61, and 106 R46, it was shown that AKIII is less sensitive to feedback inhibition by lysine (3). By mutagenesis of each of these strains, mutants lacking AKIII auxotrophic for homoserine plus Dpm were prepared. They were called G21 M8, G61 M17, and R46 M2.

Localization of lysC. As preliminary matings had shown that *lysC* gene may be linked to *arg-1000*, (see legend of Table 4) a transduction was carried out with Hfr H (wild type) as donor and 106 M1 (*thrA, metLM, lysC, arg-1000*) as recipient. Among Arg⁺ transductants, about 25% could grow in the absence of homoserine and Dpm; but two phenotypes were

observed: 10% of the transductants grew normally in minimal medium but did not grow in 10^{-2} M L-lysine; they had recovered AKIII activity, and only this one; the other 15% gave very small colonies in minimal medium; a better growth was observed in the presence of 10^{-3} M DL-threonine; growth was totally inhibited by 2×10^{-3} M DL-methionine. This phenotype corresponds to the presence of AKII alone (see below); the corresponding gene must also be cotransducible with *arg-1000*. No clone appeared to have gained both AKIII and AKII activities: the genes *lysC* and *metLM* must thus be located each on one side of *arg-1000*.

Reciprocal transductions were performed between strains OR 11 (*metA*) and 106 M1 (*lysC, arg-1000*) (Table 5). The results clearly demonstrate the order *arg-1000, metA, lysC* (see for instance absence of Met^+ Arg^+ recombinants in the first cross). The frequencies of cotransduction observed between *arg-1000* and *metA* are similar to ones found by Fraenkel (11). The frequency between *metA* and *lysC* is about 30%, a value very close to that reported (26%) for the co-transduction of *metA* and *pgi* (11).

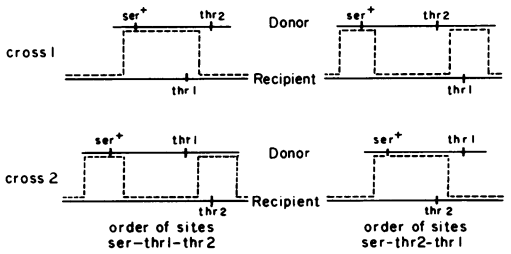


FIG. 3. *Three-point mapping method with reciprocal cross. A, When the order is ser thr1 thr2, two crossovers are required to give Ser^+ Thr^+ transductants in cross 1 and four crossovers in cross 2. The percentage of Ser^+ among Thr^+ transductants is higher in cross 1. B, If the order is ser thr2 thr1 the situation is exactly opposite: four crossovers are required to give Ser^+ Thr^+ transductants in cross 1 and two crossovers in cross 2; the percentage of Ser^+ among Thr^+ is in the case higher in cross 2. Therefore, the comparison of the percentage of Ser^+ among Thr^+ transductants in the cross 1 and 2 will permit us to decide if the order is A or B.*

Results of Table 6 demonstrate that *lysC* is highly linked (about 95% co-transduction) with *pgi*. Furthermore, they suggest the order *metA lysC pgi* (for instance Lys^- Pgi^- recombinants in the first cross must correspond to four crossing-over events) though differences in the observed frequencies are not highly significant, due to the high linkage between *lysC* and *pgi*. However, as appears from the linkage map (24) no known genes exist near enough *pgi* to confirm this localization.

Other mutations affecting gene *lysC* were also mapped by using strains G21 M8 (*lysC1002, lysC1007*), G61 M17 (*lysC1005, lysC1008*), R46 M2 (*lysC1006, lysC1009*). Each of these strains carries two mutations in *lysC* leading, respectively, to desensitization and loss of function in addition to the *arg-1000* mutation. They were transduced by phage grown on OR11 (*metA*). $LysC^+$ transductants were selected; among them we scored *metA* and *arg-1000* markers and the marker leading to desensitization. The results of Table 7 demonstrate that the other *lysC* mutations are located about the same distance from *arg-1000* and *metA* as was the *lysC* mutation of 106 M1. In all three cases, the mutations leading to desensitization are very tightly linked (100, 99, 95%) to the mutations corresponding to the absence of enzyme activity.

The seven mutations we have studied that affect LysC wild-type phenotype are thus clustered in the same region of the *E. coli* chromosome.

MAPPING OF METLM AND METM

Isolation of metL and metM mutants. All strains are derived from Gif 88, that carries the mutation *thrA1000* leading to absence of AKI-HDHI activities (18). This strain grows slowly in minimal medium; the growth is enhanced by threonine and is completely abolished by methionine, which represses the synthesis of HDHII, the only remaining activity for homoserine synthesis. From this strain, mutant Gif 881-L was selected (18) as resistant to methionine inhibition; in this mutant (mutation *met-1001*) the synthesis of AKII-HDHII is constitutive. Mutants auxotrophic for homoserine were selected after mutagenesis of either Gif 88, or of strain OR30 derived from Gif 881-L (see

TABLE 3. *Three-factor crosses between different pairs of thr mutants*

Cross no.[a]	Donor	Recipient	No. of Thr^+ analyzed	Ser^+ (%)
1	YA73 (*thrB1000*)	GT13 (*thrC1001*)	318	18
2	Gif 41 (*thrC1001*)	GT12 (*thrB1000*)	645	38
3	Gif 102 (*thrA1015*)	GT12 (*thrB1000*)	347	25
4	YA73 (*thrB1000*)	GT15 (*thrA1015*)	376	38
5	Gif 106M1 (*thrA1101*)	GT12 (*thrB1000*)	280	16
6	YA73 (*thrB1000*)	GT14 (*thrA1101*)	407	51
7	GT3 (*thrA1016*)	GT12 (*thrB1000*)	206	16
8	YA73 (*thrB1000*)	GT16 (*thrA1016*)	446	60

[a] Selection was carried out for Thr^+ transductants on petri dishes containing serine and small quantities of complete media varying from 10 to 50 μliters.

TABLE 4. *List of the strains used for the mapping of the lysC gene*[a]

Genotype / Strain no.	*thrA*	*metLM*	*lysC*	Other loci	Origin
DF40	+	+	+	*pgi-2*	D. Fraenkel
PA505MBΔ101C	+	+	+	*metA, argH*	M. Schwartz
Gif 106	1101	1000	+	*arg-1000*[b], *ilvA*	(3)
106M1	1101	1000	1001[c]	*arg-1000, ilvA*	(3)
106G21	1101	1000	1002[d]	*arg-1000, ilvA*	(3)
106G61	1101	1000	1005[d]	*arg-1000, ilvA*	(3)
106R46	1101	1000	1006[d]	*arg-1000, ilvA*	(3)
G21M8	1101	1000	1002, 1007	*arg-1000, ilvA*	*lysC* derivative of 106G21 obtained after mutagenesis
G61M17	1101	1000	1005, 1008	*arg-1000, ilvA*	*lysC* derivative of 106G61 obtained after mutagenesis
R46M2	1101	1000	1006, 1009	*arg-1000, ilvA*	*lysC* derivative of 106R46 obtained after mutagenesis
OR10	+	+	+	*metA*	Arg^+ transductant of PA505MBΔ101C; P1*kc* grown on HfrH
OR11	1101	1000	+	*metA, ilvA*	Arg^+ transductant of Gif 106; P1*kc* grown on OR10
ORE1	1101	1000	1001[c]	*metA, ilvA*	Arg^+ transductant of 106M1; P1*kc* grown on OR11
OR66	1101	1000	+	*pgi-2, ilvA*	$LysC^+$ transductant of ORE1; P1*kc* grown on DF40[e]
OR713	1101	1000	+	*metA, pgi-2, ilvA*	$LysC^+$ transductant of ORE1; P1*kc* grown on DF40[e]
OR150	1101	1000	1001[c]	*pgi-2, arg-1000, ilvA*	Met^+ transductant of OR713; P1*kc* grown on 106M1

[a] All strains were constructed to be isogenic for mutations *thrA1101* and *metLM1000* of Gif 106 in order to avoid recombinations that could allow the appearance of AKI or AKII activities during transduction.

[b] *arg-1000* is an *arg* mutation mapping in the *arg ECBH* cluster.

[c] This mutation leads in this strain to a growth requirement for homoserine plus Dpm.

[d] These mutations lead to desensitization of AKIII activity towards lysine. The corresponding phenotype is the growth in the presence of 10^{-2} M L-lysine.

[e] In this experiment, transductants growing in the absence of homoserine plus Dpm were selected. Among bacteria transduced for AKI or AKII activities, $LysC^+$ strains were recognized by their growth inhibition in the presence of 10^{-2} M L-lysine (AKIII activity was also measured in crude extracts).

TABLE 5. *Ordering of arg-1000, metA, and lysC*

Donor	Recipient	Selected phenotype	No. of transductants scored	Unselected phenotypes				Co-transduction frequencies		
				Met^+ Arg^+	Met^+ Arg^-	Met Arg^+	Met Arg^-	*arg-1000 metA*	*arg-1000 lysC*	*metA lysC*
OR11 (*arg*$^+$-*metA*$^-$ *lysC*$^+$)	106 M1 (*arg-1000 metA*$^+$-*lysC*$^-$)	$LysC^+$	361	0	244	34	83		9.5	32.3
				$LysC^+$ Arg^+	$LysC^+$ Arg^-	$LysC^-$ Arg^+	$LysC^-$ Arg^-			
106 M1 (*arg-1000 metA*$^+$-*lysC*$^-$)	OR11 (*arg*$^+$-*metA*$^-$ *lysC*$^+$)	$MetA^+$	362	172	92	84	14	29.2		27

Table 8). Two different phenotypes occured: *metM* mutants (Gif 96, OR105) had lost HDHII activity alone, whereas AKII activity remained (phenotype $MetM^-$); *metLM* mutants (Gif 99, OR30 M2) had lost both AKII and HDHII activities (phenotype $MetLM^-$) (see Discussion).

Localization of metLM and metM. As described above, the gene(s) coding for AKII-HDHII activities is co-transducible with *arg-1000*, but on the side away from *lysC*. Experiments were then performed by

using as external markers *metB*, *metF*, *arg-1000*, and *ppc*. Table 9 gives the results of transduction experiments by using three markers: *metB* (or *F*), *metLM* (or *M*), and *arg-1000* (or *ppc*). It can be seen that *metLM* and *metM* are co-transducible at 87% with *metB* and 97% with *metF*. Similar results, not given here, were obtained by using strains Gif 96 or OR30 M2. Co-transduction frequencies observed between *metB* or *metF* and *arg-1000* or *ppc* are in agreement with the position of these markers on the reference map (24). The three-point test experiments summarized in Table 10 demonstrate the order *metB metLM arg-1000* (the MetB$^+$ MetLM$^+$ Arg$^-$ recombinants must occur after four crossing-overs). The same type of experiments were performed with strain OR35 (*metF*). Due to the very high linkage of *metLM* with *metF*, the results do not allow to draw definite localization of *metLM* clockwise or counterclockwise with respect to *metF*.

To obtain more precise mapping, transductions were performed with both *metB* and *metF* as external markers on different chromosomes, using the fact that MetB$^-$ clones may grow on cystathionine or methionine, whereas MetF$^-$ clones are absolute methionine auxotrophs. Table 11 gives the results of two types of experiments: either selecting for MetB$^+$ MetF$^+$ at the same time; or selecting for MetF$^+$ (in the presence of cystathionine) and using *metB* as an unselected marker. We can observe that in the case of experiment 2 no transductant of the phenotype MetF$^+$ MetB$^-$ MetM$^+$ is obtained. This should correspond to four crossing-overs as expected if *metM* lies between *metB*

TABLE 6. *Ordering of metA, lysC, and pgi*[a]

Donor	Recipient	Selected phenotype	No. scored	Unselected phenotypes				Co-transduction frequencies (%)		
				LysC$^+$ Pgi$^+$	LysC$^+$ Pgi$^-$	LysC$^-$ Pgi$^+$	LysC$^-$ Pgi$^-$	*metA lysC*	*metA pgi*	*lysC pgi*
OR66 (*met*$^+$-*lysC*$^+$ *pgi*$^-$)	OR E1 (*metA*$^-$-*lysC*$^-$ *pgi*$^+$)	Met$^+$	1645	18	325	1295	7	20.8	20.1	
OR150 (*metA*$^+$-*lysC*$^-$ *pgi*$^-$)	OR11 (*metA*$^-$-*lysC*$^+$ *pgi*$^+$)	Met$^+$	384	290	3	5	86	23.7	23.1	
106 M1 (*metA*$^+$-*lysC*$^-$ *pgi*$^+$)	OR713 (*metA*$^-$-*lysC*$^+$ *pgi*$^-$)	Met$^+$	633	9	416	194	14	32.8	32	
				Met$^+$ Pgi$^+$	Met$^+$ Pgi$^-$	Met$^-$ Pgi$^+$	Met$^-$ Pgi$^-$			
OR66 (*metA*$^+$-*lysC*$^+$ *pgi*$^-$)	OR E1 (*metA*$^-$-*lysC*$^-$ *pgi*$^+$)	LysC$^+$	1131	17	326	45	743	30.3		94.5
OR11 *metA*$^-$-*lysC*$^+$ *pgi*$^+$)	OR150 (*metA*$^+$-*lysC*$^-$ *pgi*$^-$)	LysC$^+$	630	405	45	173	7	28.5		91.7

[a] In most of the experiments, a few percent of the colonies gave, on glucose indicator plates, a phenotype intermediate between Pgi$^+$ and Pgi$^-$ parent strains. Therefore, only those Pgi$^+$ colonies that retained their phenotype after purification were scored.

TABLE 7. *Mapping of mutations leading to desensitization of AKIII activity*[a]

Donor	Recipient	Selected phenotype	No. of transductants scored	Unselected phenotypes (%)		
				Met$^-$	Arg$^+$	Inhibited by lysine (%)
OR11 (*metA*$^-$-*arg*$^+$-*lysC*$^+$)	G21 M8 (*metA*$^+$, *arg-1000*, *lysC1002*, *lysC1007*)	LysC$^+$	348	26	2	100
OR11	R46 M2 (*metA*$^+$, *arg-1000*, *lysC1005*, *lysC1008*)	LysC$^+$	208	27.5	4.5	95
OR11	G61 M17 (*metA*$^+$, *arg-1000*, *lysC1006*, *lysC1009*)	LysC$^+$	387	21	7	99

[a] The phenotypes of the three receptor strains are identical, but the three *lysC* mutations that lead to auxotrophy for homoserine plus Dpm and the mutations leading to desensitization are independent mutations. The desensitization of AKIII allow the growth of bacteria in the presence of 10^{-2} M L-lysine. Transductants are thus replicated on a plate containing this amino acid to score for the desensitized character.

TABLE 8. *List of the strains used for the mapping of the metLM genes; all strains were constructed to carry thrA1000 mutation*

Genotype / Strain no.	*thrA*	*metLM*	Derepression of *metL* and *metM*[a]	*lysC*	Other loci	Origin
B-512-65	+	+		+	*metF64*	Glansdorff
342-167	+	+		+	*ppc-1, argC, his, leu, thr*	Glansdorff
R47	+	+		+	*metB, argG, leu*	Babinet
Gif 88	1100	+		+	*leu*	(18)
Gif 881-L	1100	+	*met-1001*	+		(18)
Gif 96	1100	M1002[b]		+	*arg-1000, ilvA*	MetM$^-$ derivative of Gif 88 obtained after mutagenesis (18)
Gif 99	1100	LM1000[c]		+	*arg-1000, ilvA*	MetLM$^-$ derivative of Gif 88 obtained after mutagenesis (18)
OR30	1100	+	*met-1001*	+	*arg-1000, ilvA*	MetLM$^+$ transductant of Gif 99, P1*kc* grown on Gif 881-L[d]
OR30 M2	1100	LM1003[c]	*met-1001*	+	*arg-1000, ilvA*	MetLM$^-$ derivative of OR30 obtained after mutagenesis
OR32	1100	+	*met-1001*	+	*metB, ilvA*	Arg$^+$ transductant of OR30, P1*kc* grown on R47
OR35	1100	+	*met-1001*	+	*metF64, ilvA*	Arg$^+$ transductant of OR30, P1*kc* grown on B-512-65
OR75	1100	LM1000		+	*ilvA*	Arg$^+$ transductant of Gif 99, P1*kc* grown on HfrH
OR80	1100	+		+	*ppc-1, ilvA*	MetLM$^+$ transductant of OR75, P1*kc* grown on 342-167
OR85	1100	LM1000		+	*ppc-1, ilvA*	Arg$^+$ transductant of Gif 99, P1*kc* grown on OR80
OR105	1100	M1004[b]	*met-1001*	+	*arg-1000, ilvA*	MetM$^-$ derivative of OR30 obtained after mutagenesis
OR110	1100	M1004[b]	*met-1001*	+	*metB, ilvA*	Arg$^+$ transductant of OR105, P1*kc* grown on OR32
OR115	1100	+		+	*arg-1000, metF64, ilvA*	MetM$^+$ transductant of OR105, P1*kc* grown on OR35

[a] The mutation *met-1001* leads to the constitutive synthesis of AKII-HDHII enzyme (18). Strains carrying this mutation are resistant to growth inhibition by methionine.

[b] *metM* mutation (AKII$^+$-HDHII$^-$) leads to a growth requirement for homoserine in this strain.

[c] *metLM* mutation (AKII$^-$-HDHII$^-$) leads to a growth requirement for homoserine plus Dpm in this strain.

[d] The result of this transduction indicates that mutation *met-1001* is co-transducible with *metLM* and may be identical to *metJ* mutations described in *S. typhimurium* (2) and in *E. coli* (23) as regulatory mutants for the synthesis of methionine enzymes and mapped in this region of the chromosome.

and *metF*. This was confirmed when selecting at the same time for MetB$^+$ and metF$^+$: the frequency of co-transduction of *metM* with *metF*, instead of being 97% as previously, decreases to 74% in experiment 3 and 84.5% in the reciprocal cross (experiment 4) (in experiment 2 also, among the few MetB$^+$ MetF$^+$ transductants, 83% are MetM$^-$). *metM* and *metLM* must thus be located between *metB* and *metF* (Fig. 4).

DISCUSSION

A branched biosynthetic pathway leads from aspartate to diaminopimelate, lysine, methionine, and threonine. A complete picture of this pathway cannot be attained without the knowledge of the chromosomal location of the corresponding genes and of their possible organization into discrete operons. Almost all the genes of the branches leading to diaminopimelate and lysine, to methionine and to isoleucine have been previously mapped, as well as the gene coding for aspartate semialdehyde dehydrogenase. Due to the multiplicity of aspartokinases and homoserine dehydrogenases, the mapping of the corresponding genes was greatly delayed. The present work fills this gap.

The new nomenclature used here for the *thr* genes takes into account the knowledge of the threonine biosynthetic pathway, and is thus different from the one previously used in *Salmonella typhimurium* (13). *thrA* is a single gene corresponding to aspartokinase I-homoserine dehydrogenase I since these two activities are carried by a single polypeptide chain (9). However, the two activities are rather independent since it is possible to obtain AKI$^-$-HDHI$^+$ (21; this investigation) and AKI$^+$-HDHI$^-$ mutants

(15). From mutant Gif 102, a nonsense mutant, it is possible to extract a fragment containing the NH2-terminus of AKI-HDHI, able to carry out the AKI activity, whereas by limited proteolysis of the wild-type protein, a fragment carrying the carboxy terminus and the HDH activity can be obtained (25). It thus appears that *thrA* codes sequentially for AKI and HDHI activities in this order. *thrB* corresponds to homoserine kinase and *thrC* to threonine synthetase. (*thrB* and *thrC* correspond to the classical Thr$^-$ locus.) In this investigation we have shown that *thrA* is closely linked to *thrB* and *thrC*. The order of these three genes has been established: *serB ... thrA thrB thrC ... pyrA*. They are in the same order as in *Salmonella typhimurium* (13). Evidence will be presented in a forthcoming investigation showing that they belong to the same operon transcribed from *thrA* to *thrC* (J. Thèze and I. Saint-Girons, manuscript in preparation).

The present results show that *lysC* is located at 80 min, closely linked to *pgi* (and not at 66 min as was previously suggested, ref. 17). Three mutations leading to desensitization of the enzyme activity towards lysine also were mapped in this region. Since the AKIII protein appears to be a dimer of two identical subunits, its catalytic and regulatory sites must be carried by the same polypeptide chain (C. Richaud and J. C. Patte, manuscript in preparation); consequently, in agreement with these results, muta-

TABLE 9. *Co-transduction frequencies of metLM mutations with metB, metF, arg-1000, and ppc*

Donor	Recipient	Selected phenotype	No. of transductants scored	Co-transduction frequencies (%)			
				metB-metLM (or *M*)	*metB-arg*	*metM-arg*	*metB-ppc*
Gif 99 (*metLM*$^-$, *metB*$^+$, *arg-1000*)	OR32 (*metLM*$^+$, *metB*$^-$, *arg*$^+$, *met-1001*)	MetB$^+$ [a]	623	87.5	27.6		
OR105 (*metM*$^-$ *metB*$^+$, *arg-1000*, *met-1001*)	OR32 (*metLM*$^+$, *metB*$^-$, *arg*$^+$, *met-1001*)	MetB$^+$ [b]	208	86.2	28.8		
OR32	OR105	Arg$^+$ [b]	220		34.5	33.1	
OR32	OR105	MetM$^+$ [b]	149	87.9			
OR85 (*metLM*$^-$, *metB*$^+$, *ppc*$^-$)	OR32	MetB$^+$ [a]	156	90.5			44.2
				metF-metLM (or *M*)	*metF-arg*	*metM-arg*	*metF-ppc*
Gif 99	OR35 (*metLM*$^+$, *metF*$^-$, *arg*$^+$, *met-1001*)	MetF$^+$ [a]	724	96.6	35.6		
OR 105	OR35 (*metLM*$^+$, *metF*$^-$, *arg*$^+$, *met-1001*)	MetF$^+$ [b]	311	97.5	31.2		
OR35	OR105	Arg$^+$ [b]	288		38.8	38.1	
OR35	OR 105	MetB$^+$ [b]	401	95.8			
OR85	OR35	MetF$^+$ [a]	156	97.5			46.1

[a] In these experiments using Gif 99 or OR85 (not carrying the *met-1001* mutation), most of the transductants do not carry this mutation and grow very slowly in minimal medium; when scoring for *metLM*, examination of the replica plates must thus be made 24 h later than usual.

[b] When donor and recipient strains carry the *met-1001* mutation, the Met$^+$ transductants excrete methionine in the medium and allow the satellite growth of Met$^-$ colonies around them. Similarly *met-1001* Met$^-$ clones excrete a quantity of homoserine large enough to allow a satellite growth of MetLM$^-$ or M$^-$ colonies. Therefore replica plates were examined early (15 h) after incubation.

TABLE 10. *Ordering of metLM, metB, and arg-1000*

Donor	Recipient	Selected phenotype	No. of transductants scored	Unselected phenotypes			
				Arg$^+$ MetLM$^+$	Arg$^+$ MetLM$^-$	Arg$^-$ MetLM$^+$	Arg$^-$ MetLM$^-$
Gif 99 (*metLM*$^-$, *metB*$^+$, *arg-1000*)	OR32 (*metLM*$^+$, *metB*$^-$, *arg*$^+$, *met-1001*)	MetB$^+$	623	75	374	2	172
OR105 (*metM*$^-$, *metB*$^+$, *arg-1000*, *met-1001*)	OR32	MetB$^+$	208	28	120	1	59

TABLE 11. *Ordering of metLM, metB, and MetF*[a]

No.	Donor	Recipient	Selected phenotype	No. of transductants scored	Unselected markers				Co-transduction frequencies (%)	
									metB-metF	*metF-metM*
1	OR32 (*metB*⁻, *metF*⁺, *met-1001*)	OR35 (*metB*⁺, *metF*⁻ *met-1001*)	MetF⁺	309	MetB⁻ 270				87.5	
					MetB⁺ MetM⁺	MetB⁺ MetM⁻	MetB⁻ MetM⁺	MetB⁻ MetM⁻		
2	OR110 (*metB*⁻, *metM*⁻ *metF*⁺, *met-1001*)	OR35 (*metB*⁺, *metM*⁺ *metF*⁻, *met-1001*)	MetF⁺	456	10	48	0	398	87.2	97.8
					MetLM⁻					
3	OR110	OR35	MetB⁺ MetF⁺	383	247					74.1
4	OR35	OR110	MetB⁺ MetF⁺	189	160					84.6

[a] The first experiment using OR32 and OR35 gives the co-transduction frequency between *metB* and *metF* as a control of the other transductions where *metLM* is inferred.

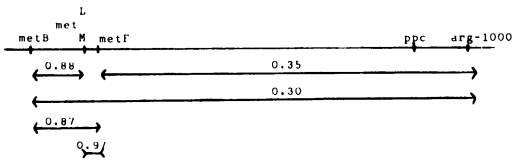


FIG. 4. *Relative map position of metB, metLM, metF, and arg. Some co-transduction frequencies are given (e.g., 30% co-transduction between metB and arg-1000).*

tions leading to the lack or the desensitization of AKIII activity are expected to be in the same cistron. The known genes coding for the enzymes of lysine biosynthesis are scattered on the chromosome. Only two genes, *dapA* and *dapE*, are linked with one another. However, since the synthesis of dihydrodipicolinate synthetase (coded by *dapA*) does not appear to be repressed by lysine (J. C. Patte, unpublished results) there is no evidence for the existence of an operon in the lysine regulon.

Campbell et al. (4) suggested that the gene coding for homoserine dehydrogenase II activity maps near *metB*. We show here that mutations affecting AKII-HDHII activities indeed map between *metB* and *metF*, close to *metF*. Though the results of Falcoz-Kelly (Thèse de doctorat ès sciences naturelles, Paris VII, 1971) favor the hypothesis that the two activities are carried by a single polypeptide chain, we followed the suggestion of Taylor (personal communication) to call *metL* the gene for aspartokinase II activity and *metM* the gene for HDHII activity (the mutants that have lost both activities are classified as *metLM*). If new results definitely show that a single polypeptide chain carries the two activities, only *metL* will be used. The fact that *metB*, *metF*, and *metLM*, as *metJ* (23), are closely linked suggests that an operon for some of these genes may exist in this region. Experiments are in progress to see if this is the case.

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