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Regulation of the Lysine Biosynthetic Pathway in *Escherichia coli* K-12: Isolation of a *cis*-Dominant Constitutive Mutant for AK III Synthesis

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A method for isolating regulatory mutants for the synthesis of lysine biosynthetic enzymes in *Escherichia coli* is described. One of them is identified as a *cis*-dominant constitutive mutant for the synthesis of the lysine-sensitive asportokinase AK III (*lysC* gene).

To the best of our knowledge no mutation impairing regulation of the synthesis of one or several enzymes of the lysine biosynthetic pathway has been described in *Escherichia coli*. The following features may account for the inability to isolate such mutants. (i) The first two reactions are the common part of the branched biosynthetic pathway of the aspartate family of amino acids in which isofunctional and multifunctional enzymes have been described (14) (Fig. 1); (ii) the genes known to specify eight of the nine enzymes of the lysine pathway are scattered along the chromosome (20, 21); and (iii) feedback inhibition seems to play a prominent role in the regulation of this pathway. We have been able to select many mutants that excrete lysine in the growth medium, thus overcoming the growth inhibition of the wild-type strain exerted by lysine analogues. Most of them appeared to have an aspartokinase III (AK III; EC 2.7.2.4) activity which was partially or totally desensitized towards lysine; the mutations were localized in the *lysC* gene specifying this enzyme (2, 21).

In a general study of the overall regulation of this pathway, we have recently isolated regulatory mutants by a procedure taking the three above-mentioned features into account. In this paper the isolation procedure and the characterization of one of the mutants, a *cis*-dominant constitutive mutant of the *lysC* gene, are described.

MATERIAL AND METHODS

Bacterial strains. All strains were derived from *E. coli* K-12. Phenotypes and genotypes are given in Table 1. Allele numbers were allocated to the Service de Biochimie Cellulaire, Institut Pasteur, by the *E. coli* Genetic Stock Center of Yale University.

Transduction. Transduction with phage P1*kc* was performed according to the method of Lennox (9).

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

Preparation of recA derivatives. This involved the following steps: (i) preparation of Thy$^-$ derivatives, selecting for trimethoprim resistance (19); (ii) selection of Thy$^+$ recombinants after 30 min of mating with Hfr KL 16-6; (iii) identification of *recA* recombinants by comparing their ultraviolet sensitivity (4) with that of *rec*$^+$ and *recA* control strains.

Preparation of stable merodiploïds. All merodiploïds described in Table 1 were prepared by mating F' donor strains (carrying episomes F_{110}, F_{112}, or F_{14}) with the *recA* derivatives OR 161 or OR 162. Diploïds were examined for their *recA* property by the ultraviolet sensitivity test (4) and for their diploidy by allowing segregation of the episome to take place in broth.

Media. Minimal medium (5) was supplemented with 1 μg of thiamine hydrochloride per ml and 2 mg of glucose per ml. The auxotrophic requirements of the strains were met by the use of the required amino acids at 0.3 mM of the L isomer (DL-threonine was 1 mM). Lysine inhibits the growth of many strains under study (see below); therefore, the following conditions were used, in all cases, as the "repressed conditions": 4 mM L-lysine; 0.05 mM DL-*meso*-diaminopimelate (Sigma); 5 mM DL-threonine; 2 mM L-isoleucine; 2 mM DL-methionine.

Enzyme assays. Aspartokinase, aspartic semialdehyde dehydrogenase, and diaminopimelate decarboxylase activities were measured as previously described (6, 13, 16).

RESULTS

Isolation of regulatory mutants. To overcome presumed difficulties, the following procedure was used. The parental strain, Gif 106, lacking AK I and AK II activities, is able to grow in minimal medium. Lysine addition inhibits growth, however, owing to inhibition and repression of AK III, the only remaining enzyme that is able to catalyze aspartylphosphate syn-

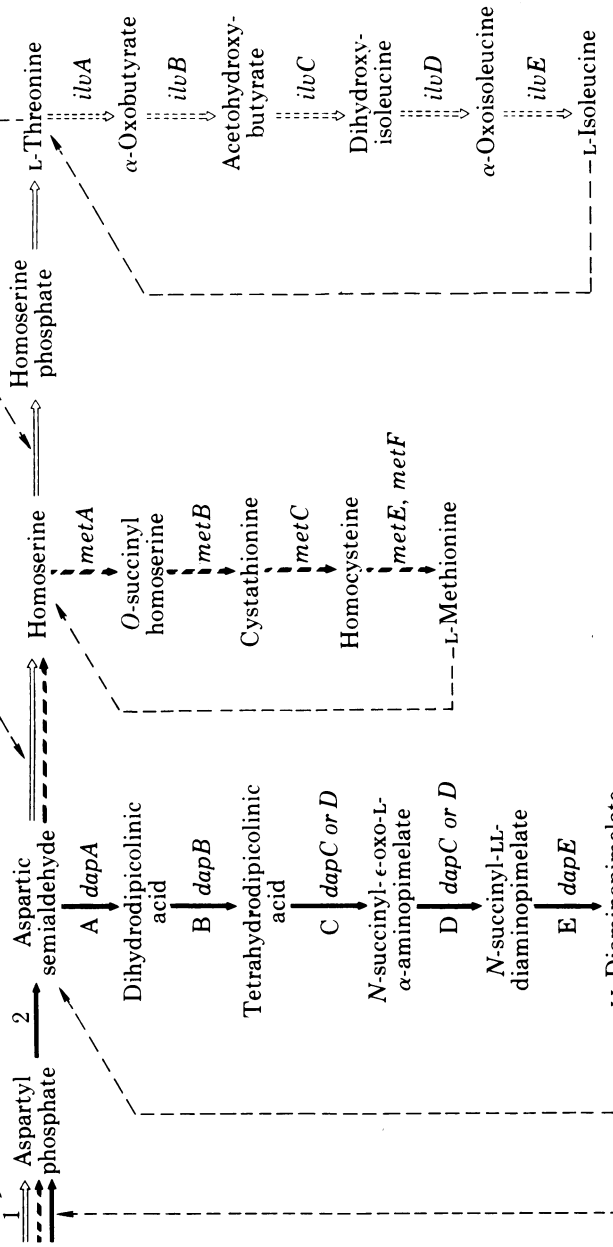

FIG. 1. *Biosynthetic pathway for the amino acids deriving from aspartate. Reaction 1: Aspartokinase I (EC 2.7.24) (AKI), thrA; aspartokinase II (AKII), metL; aspartokinase III (AKIII), lysC. Reaction 2: aspartic semialdehyde dehydrogenase (EC 1.2.1.10) (ASA-dehydrogenase), asd. Reaction G: diaminopimelate decarboxylase (EC 4.1.1.20) (DAP-decarboxylase), lysA. Symbols: solid arrow, enzymes repressed by lysine; open arrow, enzymes repressed by threonine plus isoleucine; solid dashed arrow, enzymes repressed by methionine; open dashed arrow, enzymes repressed by isoleucine, leucine, and valine. The dashed lines point to the reactions subject to feedback inhibition.*

TABLE 1. *List of strains used*

Strain	Concerned loci	Other loci	Genotype	Origin
Hfr KL 16-6		*recA*		G. Buttin (*recA* derivative of Hfr KL 16, ref. 7)
476-22	*asd1101*			J. C. Patte and G. N. Cohen (unpublished data)
Gif 106		*thrA1101, metLM1000, ilvA, arg-1000*		(2)
106 M1	*lysC1001*[a]	idem		(2)
106 G21	*lysC1002*[b]	idem		(2)
G21 M4	*lysC1002, asd1100*[c]	idem		*asd* derivative of 106 G21 obtained after mutagenesis
G21 M22	*lysC1002, asd1101*	idem		Asd$^+$ transductant of G21 M4; P1*kc* grown on 476-22
G21 M111	*lysC1002, lysC1010*[a] *asd1101*	idem		*lysC* derivative of G21 M22 obtained after mutagenesis
ORA 11	*lys-1100, lysC1002, asd1101*	idem		*lys-1100* derivative of G21 M22 (see text)
ORK 20	*lys-1101, lysC1002, asd1101*	idem		*lys-1101* derivative of G21 M22 (see text)
ORA 101	*lys-1100*	idem		LysC$^+$ transductant of 106 M1; P1*kc* grown on ORA 11
ORA 103	*lys-1100, lysC1002*	idem		LysC$^+$ transductant of 106 M1; P1*kc* grown on ORA 11
OR 160	*lys-1100*	idem; *thyA1100*		Thy$^-$ derivative of ORA 101 (see Materials and Methods)
OR 161	*lys-1100,*	idem; *recA*		Thy$^+$ conjuguant of OR 160 with Hfr KL 16-6
OR 162	*lys-1100, lysC1012*[b]	idem; *recA*		*lysC* derivative of OR 161 (see text)
DO 6	*lys-1100*	*thrA1101, metLM1000, ilvA*		Arg$^+$ transductant of ORA 101; P1*kc* grown on Hfr H
Merodiploid strains				
KLF 10/JC 1553			F_{110}/F$^-$ *argG leu$^-$ his$^-$ metB recA1*	E. Adelberg
KLF 12/PA 505 MB 10143			F_{112}/F$^-$ *argH metA malB recA*	C. Babinet
9884			F_{14}/F$^-$ *argH ilvA pro$^-$ his$^-$ recA*	M. Hofnung
OR 161-10			F_{110}/F$^-$ OR 161	See text
OR 162-10			F_{110}/F$^-$ OR 162	See text
OR 161-12			F_{112}/F$^-$ OR 161	See text
OR 162-12			F_{112}/F$^-$ OR 162	See text
OR 161-14			F_{14}/F$^-$ OR 161	See text
OR 162-14			F_{14}/F$^-$ OR 162	See text

[a] This mutation leads, in this strain, to a growth requirement for homoserine plus diaminopimelate.
[b] These mutations lead to desensitization of AK III activity towards lysine.
[c] The phenotype of *asd* strains is a growth requirement for homoserine plus diaminopimelate.

thesis (2). Strain 106 G21 was isolated as a lysine-resistant mutant from strain Gif 106 and shown to have an AK III activity partially desensitized towards lysine inhibition (2). From this strain we prepared strain G21 M22 (see Table 1), in which the *asd* wild-type allele was replaced by the *asd1101* allele, which specifies a highly leaky aspartic semialdehyde (ASA)-dehydrogenase. Again, growth of G21 M22 (*lysC1002, asd1101*) is inhibited by lysine, which represses the synthesis of AK III and ASA-dehydrogenase (leaky in this strain); though AK III activity is feedback resistant to lysine, this repression phenomenon decreases ASA synthesis to such a low level that diaminopimelate, threonine, and methionine are required for growth in these conditions. Addition of these amino acids to the culture medium restores normal growth (Table 2).

Clones no longer sensitive to growth inhibition were isolated from this strain by plating 10^9 bacteria on agar medium containing 50 mM L-lysine. Colonies appeared in 48 h at a frequency of 10^{-7} against a thin background of confluent growth. Over 200 mutants have thus been obtained and reisolated on minimal medium. The specific activity of AK III has been measured in the cells grown either in the absence or in the presence of 4 mM L-lysine to detect repression. The values obtained with

TABLE 2. *Inhibition of the growth of different mutant strains by lysine*[a]

Addition to culture medium	Generation time (min)				
	Hfr	Gif 106	106 G21	G21 M22	ORA 11
None	50	85	90	95	70
+ 10 mM L-lysine	50	—[b]	120	—[b]	73
+ 10 mM L-lysine + 10 mM DL-threonine + 0.5 mM DL-methionine + 0.05 mM diamino-pimelate	50	59	58	60	57

[a] Growth was performed in synthetic medium at 37 C with vigorous aeration. In all cases isoleucine and arginine were added to the medium for the auxotrophic requirements. Growth was estimated by measure of absorbance at 420 nm.

[b] Does not grow exponentially in these conditions.

most strains were identical to those observed with the parental strain G21 M22 (Table 3). It must be noted that the AK III specific activity of strain G21 M22 is much higher than that of the parental strain 106 G21; this may be explained by a lower internal pool of lysine, owing to the leaky ASA-dehydrogenase activity. The AK III specific activity of 20 of the strains was significantly higher than with strain G21 M22 when growth occurred in the absence of lysine, but a normal repression pattern was observed when 4 mM L-lysine was added to the growth medium (strain OR K20 is given as an example). The two other strains (ORA 11 and ORA 19) led to different results (Table 3): AK III specific activity was highly derepressed when cells were grown in minimal medium, and a high level of activity was also observed when growth was effected in the repressed conditions, indicating a partially constitutive synthesis of AK III in these strains. Similar results were obtained when the lysine concentration was raised to 100 mM (instead of 4 mM). Results with strain ORA 19 were identical to those observed with strain ORA 11.

Characterization of the mutation. These preliminary observations (apparent partial constitutivity) were compatible with the mutation in strain ORA 11 (*lys-1100*) being a *cis*-dominant constitutive (operator-type) or a promoter-up mutation for AK III synthesis. Its co-transducibility with mutations in the *lysC* gene was therefore investigated. Transduction was performed using ORA 11 (*lys-1100, lysC1002*) as donor and 106 M1 (*lysC1001*) as recipient (in this strain, *lysC1001* mutation leads to an auxotrophic requirement for homoserine plus diaminopimelate). Transductants growing in the absence of these amino acids (LysC$^+$ phenotype) were selected. After reisolation of 50 clones, the inhibition of growth in the presence of lysine was tested. Inhibition occurred for two of 50 strains tested. These two strains and five other transductants (among the clones resistant to lysine inhibition of growth) were grown in the repressed conditions, and their AK III specific activity was measured in crude extracts. In all cases a not completely repressible synthesis of AK III was observed, indicating that all strains have received the *lys-1100* mutation from strain ORA 11. In the case of the five colonies resistant to lysine inhibition, the AK III activity was desensitized towards lysine inhibition (owing to the presence of the *lysC1002* mutation); strain ORA 103 was one of them.

In the two strains whose growth was inhibited by lysine, the AK III activity was sensitive to lysine inhibition. These strains have the *lysC*$^+$ gene. Thus, both mutations *lysC1001* and *lysC1002* were lost during the recombination events. The presence of lysine in the growth medium is sufficient to inhibit AK III activity and prevent aspartylphosphate synthesis, even though the synthesis of this enzyme is not completely repressed. These two strains are thus isogenic to the parental strain Gif 106, except for the *lys-1100* mutation. One of them, strain ORA 101, was studied in detail.

TABLE 3. *AK III specific activity of mutants derived from G21 M22*

Strain	AK III sp act (nmol/min/mg)[a]	
	Minimal medium	Repressed conditions
Gif 106	33.5	<1.5
106 G21 (*lysC1002*)	14.8	<1.5
G21 M22 (*lysC1002, asd1101*)	65	<1.5
ORK 20 (*lys-1101, asd1101*)	111	<1.5
ORA 11 (*lys-1100, lysC1002, asd1101*)	131	24

[a] Minimal medium indicates synthetic medium plus arginine and isoleucine (added for the auxotrophic requirements). For repressed conditions see Materials and Methods (arginine and isoleucine were also added).

Table 4 indicates that normal levels of ASA-dehydrogenase and diaminopimelate decarboxylase, enzymes under the repressive control by lysine (2, 15), are obtained with strain ORA 101 grown in minimal medium; AK III synthesis is highly derepressed in minimal medium and partially constitutive in the repressed conditions.

The fact that the *lys-1100* mutation is co-transducible with *lysC* mutations and that the synthesis of two other enzymes of the pathway appear unmodified in strains carrying this mutation is in agreement with the hypothesis that the *lys-1100* mutation could be either a *cis*-dominant constitutive or a promoter-up mutation.

Localization of the mutation. A more precise localization of the *lys-1100* mutation required a higher number of transductants. Therefore, the following experiment, which takes into account the phenotype (resistance to growth inhibition by lysine) exhibited by strains carrying the *lys-1100* mutation in the presence of the *asd1101* and *lysC1002* mutations, was performed. Phage grown on strain ORA 11 (*lys-1100*, *lysC1002*, *asd1101*) was used as a donor in a transduction in which the recipient was G21 M111 (*lysC1010*, *lysC1002*, *asd1101*). LysC$^+$ transductants (growing in the absence of homoserine plus diaminopimelate) were selected. The inhibition of growth in the presence of lysine was tested by the replica plating on agar medium containing 10 mM L-lysine of master plates taken after only 10 h of growth. Complete resistance to lysine inhibition (normal growth after 16-h incubation) was observed for 98% of the transductants (over 450); for the other 2% (nine "sensitive" colonies) growth was delayed for 24 h. To ensure the correlation between this phenotypic test and the constitutive synthesis of AK III, all nine sensitive clones and 50 resistant clones (chosen at random) were grown in the presence of 4 mM L-lysine (repressed conditions), and their AK III specific activity was measured in crude extracts. In all nine sensitive colonies AK III synthesis was completely repressed; the level of the parental strain ORA 11 was obtained for all 50 lysine-resistant clones.

TABLE 4. *Specific activities of some lysine enzymes in strain ORA 101*[a]

Strain	Growth conditions	Sp act (nmol/min/mg)		
		AK III	ASA-dehydrogenase	DAP-decarboxylase
Gif 106	Minimal medium	32	980	6.3
	Repressed conditions	<1.5	330	<0.3
ORA 101 (*lys-1100*)	Minimal medium	125	950	6.7
	Repressed conditions	22	315	<0.3

[a] Growth conditions are the same as in Table 3. DAP, Diaminopimelate.

It thus appears that the *lys-1100* mutation is 98% co-transducible with the *lysC1010* mutation of strain G21 M111, confirming the high co-transducibility with the *lysC1001* mutation of strain 106 M1 observed in the preliminary experiment.

Dominance tests. The possibility that the *lys-1100* mutation is a *cis*-dominant constitutive one was tested by dominance studies using episomes carrying part of the bacterial chromosome with a *lysC*$^+$ gene.

Strain ORA 161 (*recA* derivative of ORA 101) was used to select a *lysC* mutant in which AK III was desensitized towards lysine inhibition. The selective procedure which employed resistance to lysine-mediated growth inhibition (2) resulted in the isolation of strain OR 162 (mutation *lysC1012*). Merodiploïds were then prepared by conjugation of F$^-$ strains OR 161 and OR 162 with F′ strains that carry episomes F_{110} or F_{112} (see Table 1), selecting for Arg$^+$ conjuguants (see Fig. 2). These merodiploïds (OR 161-10 and OR 162-10 with F_{110}, and OR 161-12 and OR 162-12 with F_{112}) were grown either in the absence or in the presence of lysine and without arginine, which is the selective marker for the episome; AK III and ASA-dehydrogenase specific activities were measured. Results are given in Table 5. AK III specific activity is given either as "lysine-sensitive activity" (specified by *lysC* genes of strain OR 161 and of episomes) or "lysine-desensitized activity" (specified by the *lysC* gene of strain OR 162). Though F′ strains carry the *metLM* gene, which specifies AK II enzyme (21), this aspartokinase activity was not detectable in our experimental conditions (14); moreover, similar results were obtained with strains grown in minimal medium with methionine. Results of the strains carrying episome F_{112} are not given, since they were identical to those obtained with strains carrying episome F_{110}.

It is evident that the *lys-1100* mutation is dominant over the wild-type allele, the AK III specific activity being derepressed in strains OR 161-10 and 162-10 grown in minimal medium. A dominant effect also is indicated by the par-

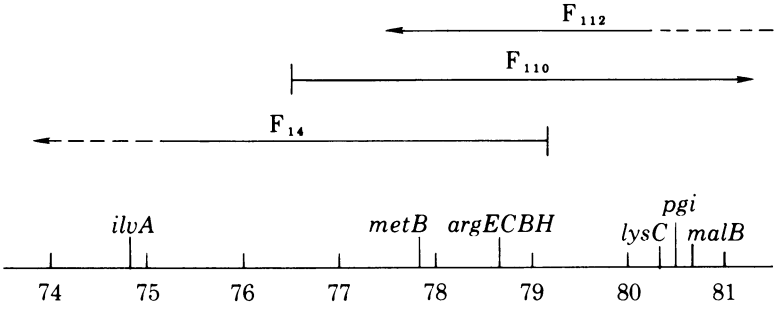

FIG. 2. *Map of the episomes used for constructing diploid strains (11).*

TABLE 5. *Repression of AK III in different diploid strains*

Strains	Growth conditions[a]	AK III sp act (nmol/min/mg)		ASA-dehydrogenase sp act (nmol/min/mg)
		Inhibited by lysine[b]	Desensitized towards lysine[c]	
OR 161 (*lys-1100, ilvA, arg-1000*)	M + Arg + Ile	111		1,090
	R + Arg + Ile	22		320
OR 162 (*lys-1100, lysC1002, ilvA, arg-1000*)	M + Arg + Ile		50	450
	R + Arg + Ile		21.5	310
OR 161-10 (F_{110}/F⁻ OR 161)	M + Ile	120		865
	R + Ile	11		330
OR 162-10 (F_{110}/F⁻ OR 162)	M + Ile	<1.5	26	402
	R + Ile	<1.5	11	325
OR 161-14 (F_{14}/F⁻ OR 161)	R	11		-
	R + Arg	21		-
OR 162-14 (F_{14}/F⁻ OR 162)	R		12	-
	R + Arg		21	-
DO 6 (*lys-1100, ilvA*)	M + Ile	138		-
	M + Ile + Arg	125		-
	R + Ile	10.5		-
	R + Ile + Arg	23		-

[a] M, Minimal synthetic medium; R, repressed conditions (see Material and Methods).

[b] Activity inhibited by lysine corresponds to the difference between total aspartokinase activity (assay in the absence of inhibitors) and activity assayed in the presence of lysine.

[c] Activity desensitized towards lysine corresponds to aspartokinase activity assayed in the presence of lysine (no difference between total activity and activity assayed in the presence of lysine).

tially derepressed level of enzyme when the merodiploïds are cultured under repressed conditions; however, this level is significantly lower (approximately one-half) than found in the parental strain OR 161.

To see if this effect is *cis* or *trans* dominant, we have used strains OR 162-10 and OR 162-12, in which the episome carries a *lysC*$^+$ gene, and the chromosome carries the *lys-1100* mutation and a *lysC1012* mutation that specifies a desensitized AK III (the use of a desensitized mutation for dominance studied has been already proposed by Mattern and Pittard [12]). It can be seen in Table 5 that only desensitized AK III activity is observed in repressed conditions; thus, the gene in *trans* that specifies an inhibitable AK III is normally repressed. In fact, it is also repressed even in minimal medium; this is ex-

plained by the existence of a high lysine internal pool, as usually is observed in strains possessing a desensitized AK III (2). This repression phenomenon in minimal medium also is observed for the desensitized AK III activity (24 units instead of 120 for the non-desensitized diploïd) and ASA-dehydrogenase activity (402 units instead of 865). It must be noted that, in this case also (growth being always effected in the absence of arginine), the partially repressed level was lower than with the parental strain OR 162. It has been verified that the episome was always present in all these strains, by subsequent conjugation with an F^- *arg*$^-$ *lysC*$^-$ strain and selection of Arg^+ $LysC^+$ conjugants.

The fact that the partially repressed level was lower in the diploïds than with the parental strain was further studied. We have shown that it is not due to the presence of a *lysC*$^+$ allele on the episome, by the use of episome F_{14} which does not carry this gene (Fig. 2), but is Ile^+ and Arg^+, allowing an Ile^+ selection. Merodiploïds OR 161-14 and OR 162-14 were prepared as described above. When these strains were grown in the absence of arginine, the repressed level of the other diploïds was obtained, though when grown in the presence of arginine the level of the parental strains OR 161 or OR 162 was again observed, indicating a possible role of arginine.

To ensure that the presence of the episome is not involved in the phenomenon, strain DO 6, an Arg^+ derivative of strain ORA 101, was prepared (Table 1). AK III specific activity was determined on cultures grown in repressed conditions in the presence or absence of arginine. With this strain also, a more repressed level was observed in the absence of arginine (Table 5), confirming that this amino acid is involved in the repression phenomenon.

DISCUSSION

To select regulatory mutants in the lysine biosynthetic pathway, a parental strain has been prepared that has the following characteristics: AK I and AK II activities are absent; AK III activity is no longer sensitive to lysine inhibition; ASA-dehydrogenase is leaky (Fig. 1). Owing to this last mutation, growth of this strain is inhibited by adding lysine to the medium at a high concentration, thus allowing the selection of mutants resistant to this lysine inhibition of growth; similar procedures have already been used by Roth et al. (18) in the case of the histidine pathway and Jacoby and Gorini (7) for the arginine pathway. This resistance could not be due to the desensitization of AK III, the main regulatory mechanism (2) in this system, since such a mutation is already present in the strain.

Among the different possibilities leading to resistance to lysine, one may think either of reversion to a normal ASA-dehydrogenase activity or of mutants with defects in lysine permeation or regulatory mutants. This last class may comprise: (i) constitutive mutants for lysine-decarboxylase synthesis (which will rapidly destroy lysine); (ii) constitutive mutants for ASA-dehydrogenase synthesis; (iii) constitutive mutants for AK III synthesis; (iv) derepressed mutants for the synthesis of all the genes of the regulon. In approximately 10% of the 200 mutants tested, a derepressed synthesis of AK III was observed in minimal medium; but this effect of derepression appeared phenotypic, because normal repression could be obtained. These mutants are not yet identified. A possible role of lysyl-transfer ribonucleic acid, the concentration of which may be lowered in these strains, appears unlikely; recent results in our laboratory on a lysyl-transfer ribonucleic acid synthetase mutant indicate that modification in the percentage of lysyl-transfer ribonucleic acid charged in vivo does not affect the synthesis of AK III (unpublished data).

Only strain ORA 11 (ORA 19 appeared to be identical) showed a phenotype that could be genetically identified. The *lys-1100* mutation in this strain appears to be a *cis*-dominant constitutive one for AK III synthesis, for the following reasons. (i) The derepression of synthesis, maximal in minimal medium, is not total when the strain is grown in the presence of an excess of lysine (Table 3), as is frequently the case for *cis*-dominant constitutive mutants identified as operator constitutive. (ii) This mutation has no effect on the synthesis of two other enzymes of the pathway, ASA-dehydrogenase and diaminopimelate decarboxylase (Table 4). In fact, an effect of this mutation on the synthesis of the diaminopimelate decarboxylase can be observed, but only in the simultaneous presence of a mutation leading to the desensitization of AK III towards lysine; this phenomenon is explained by an inducible role of the AK III protein itself on the diaminopimelate decarboxylase synthesis (13). (iii) The mutation is dominant over the wild-type allele (derepression of AK III in merodiploïds) but only in the *cis* position: in a merodiploïd in which a *lysC* desensitized mutation is in *cis* position with *lys-1100* and in which the episome carries the two wild-type alleles only the desensitized activity is derepressed (Table 5). (iv) These results could also be explained by a promoter-up mutation. This is unlikely. We have prepared

(data not shown) lysine auxotrophs of strains ORA 101 and Gif 106; when the growth of these auxotrophic strains was limited by lysine supply in the chemostat, similar derepressed AK III specific activity was obtained in both strains and no increase was observed in the derepressed level of the mutant, as would be expected for a promoter-up mutation.

Some additional points should be emphasized. The AK III protein purified from strains carrying the *lys-1100* mutation is identical by all criteria tested, to the enzyme purified from the wild-type strain; strain DO 6 was thus already used as a source of bacteria in order to purify the AK III protein, allowing the preparation of 400 mg of pure AK III from 1 kg of frozen cells (18).

The fact that the growth of strain ORA 101, which carries this constitutive mutation with a *lysC*$^+$ gene, is inhibited by lysine supports the idea (2) that lysine inhibition of AK III activity appears to be the essential point of regulation in the pathway.

In addition, we have shown that arginine in the medium affects the maximal level of repression obtained; the effect of repression by lysine is less important in the presence of this amino acid than in its absence (Table 5). Preliminary experiments indicate that an effect on permeation through the common permease (3) is not involved and that the phenomenon appears rather specific for arginine. A direct role of arginine in the repression by lysine may thus occur and may represent another example of the "metabolism interlock" as defined by Jensen (8). This possibility is currently under study.

We have made numerous attempts to select mutants in which regulation in the lysine pathway was altered, using different standard procedures (such as the use of lysine analogues); all were unsuccessful until now. The *cis*-dominant constitutive mutant described here is the first mutant with altered regulation of synthesis identified in this pathway in *E. coli* K-12. However, here again, among the numerous clones tested, none appeared to be affected in a common repressor molecule (R type). These observations, though negative, may lead to different possibilities. Either the methods of selection are not sufficient (this appears not to be true in the present case); or no R molecule exists; or the R molecule plays an essential role and its alteration is lethal; or different molecules are involved for the different genes. The recent observation that AK III protein acts as an inducer of the diaminopimelate decarboxylase synthesis (13) indicates that the latter two possibilities are likely to be involved.

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