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Cloning, Characterization, and Expression of the *dapE* Gene of *Escherichia coli*

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The *dapE* gene of *Escherichia coli* encodes *N*-succinyl-L-diaminopimelic acid desuccinylase, an enzyme that catalyzes the synthesis of LL-diaminopimelic acid, one of the last steps in the diaminopimelic acid-lysine pathway. The *dapE* gene region was previously purified from a lambda bacteriophage transducing the neighboring *purC* gene (J. Parker, J. Bacteriol. 157:712–717, 1984). Various subcloning steps led to the identification of a 2.3-kb fragment that complemented several *dapE* mutants and allowed more than 400-fold overexpression of *N*-succinyl-L-diaminopimelic acid desuccinylase. Sequencing of this fragment revealed the presence of two closely linked open reading frames. The second one encodes a 375-residue, 41,129-M_r polypeptide that was identified as *N*-succinyl-L-diaminopimelic acid desuccinylase. The first one encodes a 118-residue polypeptide that is not required for diaminopimelic acid biosynthesis, as judged by the wild-type phenotype of a strain in which this gene was disrupted. Expression of the *dapE* gene was studied by monitoring amylomaltase activity in strains in which the *malPQ* operon was under the control of various fragments located upstream of the *dapE* gene. The major promoter governing *dapE* transcription was found to be located in the adjacent *orf118* gene, while a minor promoter allowed the transcription of both *orf118* and *dapE*. Neither of these two promoters is regulated by the lysine concentration in the growth medium.

Diaminopimelic acid (DAP) plays a dual essential role in most bacteria, such as *Escherichia coli*. It is the precursor of lysine and a structural component of peptidoglycan. The conversion of *meso*-DAP to lysine is catalyzed by DAP decarboxylase, the product of the *lysA* gene (30). Transcription of *lysA* requires the product of the adjacent regulatory gene *lysR* and is repressed by lysine and induced by DAP (31, 32). *meso*-DAP is synthesized from aspartic acid by the successive action of eight enzymes. The first two steps are catalyzed by the products of the *lysC* and *asd* genes and are part of the common pathway leading to lysine, threonine, and methionine. Transcription of both *lysC* (26) and *asd* (11) is repressed by lysine, but the molecular basis of this regulation is not understood (8, 12).

Six genes belong to the DAP pathway. The first gene of this pathway, *dapA*, is constitutively expressed, but the activity of its product, dihydrodipicolinate synthetase, is inhibited by lysine (28, 38). Transcription of both *dapB* and *dapD* is repressed by lysine, and there is some indication that this regulation could be mediated by the interference of lysine with a protein activating *dapB* and *dapD* transcription (4, 25). The *dapC* gene has not yet been cloned, and nothing is known about the regulation of its expression by lysine. The region containing the *dapE* gene has been cloned from a lambda bacteriophage transducing the neighboring *purC* gene (20). Conflicting results have been reported on the regulation of expression of the *dapE* gene, based on direct measurements of the activity of its product, *N*-succinyl-L-diaminopimelic acid desuccinylase (SDAP-deacylase; EC 3.5.1.18) (9, 15). The last step in the DAP pathway, the isomerization of LL-DAP to *meso*-DAP, is controlled by the *dapF* gene, which is constitutively expressed (23). Disruption of the *dapF* gene does not lead to a DAP^- phenotype, suggesting that another enzyme can fulfill the same function, at least under these conditions (24).

In the course of our analysis of the DAP-lysine pathway, we decided to characterize the *dapE* gene and to study its regulation by constructing gene fusions to circumvent the difficulty of the SDAP-deacylase assay. Cloning of the *dapE* gene was also a prerequisite for the overproduction of SDAP-deacylase and a study of inhibitors of its activity that could be used as efficient and selective antibacterial agents.

MATERIALS AND METHODS

Bacterial strains and media. The presence of the *dapE* gene on recombinant plasmids was checked with several *E. coli* K-12 strains: AT978 (Hfr KL16 *dapE9*) and AT984 (F^+ *dap-6*) (7), obtained from B. Bachmann, and M35 (a *dapE1101* [previously *dap-335*; 9] derivative of strain MC4100 [*araD139 ΔlacU169 rpsL thiA relA1*]) and RDE51 (a *dapE*::Mucts derivative of strain RM4102 [MC4100 *relA*$^+$] [27]), from our laboratory. The genetic linkage with the *purC* gene was checked with strain H624 (F^- *thr-1 leuB6 metB1 purC60 rpsL126 tsx-71* [20]), provided by J. Parker. Recombination with linear DNA was carried out with strain JC7623 (AB1133 *tsx-33 recB21 recC22 sbcB15* [35]), obtained from H. de Reuse. The insertion of DNA fragments upstream of the chromosomal *malPQ* operon was performed with strain pop2239 (C600 *ΔmalA510* [22]), provided by O. Raibaud. Strains JM83 and JM109 (37) were used for identifying recombinant plasmids and producing DNA for sequencing.

E. coli strains were grown at 37°C (except for strain RDE51, which was grown at 30°C) in LB medium (19) or in M63 minimal medium (19) with 0.4% glucose as a carbon source, in the presence of the appropriate antibiotics (50 μg of ampicillin per ml, 10 μg of tetracycline per ml, and 25 μg

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of kanamycin per ml). In the case of *dapE* mutants, DAP (a racemic mixture of all three DAP isomers) was added at a final concentration of 0.5 mM (minimal medium) or 50 μM (rich medium). For regulatory studies, cells were grown in glucose-supplemented M63 minimal medium in the presence of 4 mM lysine.

DNA manipulations. Restriction analysis, gel electrophoresis, plasmid construction, and hybridization were done by standard procedures (29). The nucleotide sequence was determined by the chemical procedure of Maxam and Gilbert (16). Restriction fragments were ^{32}P labeled at their 5′ ends with T4 polynucleotide kinase or at their 3′ ends with the Klenow fragment of DNA polymerase. Uniquely end-labeled DNA fragments were purified after secondary cleavage or after DNA strand separation. The complete sequence of the 2,270-bp *Sph*I-*Sal*I fragment (see Fig. 2) was obtained for both strands, and all restriction sites used for labeling were cross-checked.

Construction of *malPQ* fusions. The 850-bp *Hae*III DNA fragment (coordinates 40 to 890; see Fig. 2) carrying the proximal part of the *orf118* gene and its upstream region, the 693-bp *Taq*I DNA fragment (coordinates 763 to 1456; see Fig. 2) carrying the proximal part of the *dapE* gene and part of the upstream *orf118* gene, and the 1,935-bp *Sph*I-*Hpa*I DNA fragment (coordinates 1 to 1935; see Fig. 2) carrying the proximal part of the *dapE* gene and the entire *orf118* gene and its upstream region were cloned in the polylinker of plasmid pSB118 (3), a step that introduced *Eco*RI sites on both sides of the inserts. The resulting *Eco*RI fragments were cloned into pOM41 (34) and recombined onto the chromosome of strain pop2239 upstream of the *malPQ* operon by selection for growth on M63 agar plates supplemented with maltose as the sole carbon source (22). The Mal^+ clones were reisolated twice on LB agar in the absence of antibiotics, a procedure that led to the loss of the plasmid. Amylomaltase activity in sonicated extracts was measured as described previously (22) and expressed as nanomoles of glucose produced per minute per milligram of protein.

Disruption of the chromosomal genes flanking *dapE*. The 850-bp *Hae*III fragment carrying the proximal part of the *orf118* gene and upstream sequences was cloned in the *Sma*I site of pUC18. The flanking 1,154-bp *Hae*III fragment containing the distal part of *orf118* and downstream sequences was cloned in the *Hin*cII site of the plasmid obtained in the first step. This strategy introduced in the middle of *orf118* a unique *Bam*HI site that was used for inserting a *Bam*HI cartridge containing a kanamycin resistance marker issued from plasmid pUC4-KSAC (2). The resulting plasmids, pDE21 and pDE22, differing only in the orientation of the *kan* marker, contained upstream and downstream of the kanamycin resistance marker stretches of adjacent DNA that allowed homologous recombination into the chromosome of exonuclease V-deficient strain JC7623 (35). Plasmids pDE21 and pDE22 were linearized with *Hin*cII (which cuts at the distal end of the downstream *E. coli* insert) and used to transform competent JC7623 cells. Transformants were selected for kanamycin resistance and screened for ampicillin sensitivity. The absence of free plasmids was checked by making plasmid minipreparations from a few clones and transforming strain JM83 to kanamycin resistance. Half of the clones were found to be devoid of plasmids and appeared to be chromosomal recombinants. Two clones issued from either pDE21 or pDE22 were kept for further studies. A P1 lysate was grown on these clones and used to transduce strain H624 to purine auxotrophy. Seventy percent of the transductants were kanamycin resistant, confirming the genetic linkage between the *purC* gene and the *kan* marker.

A similar strategy was used for inactivating the gene located downstream of *dapE*. A 4.3-kb *Pvu*II fragment overlapping the *dapE* region (see Fig. 1) was cloned into the *Sma*I site of pUC18. The *Bam*HI cartridge from pUC4-KSAC containing the *kan* marker was cloned into the unique *Bgl*II site present at the beginning of the open reading frame located immediately downstream of *dapE*. A plasmid in which the *kan* gene is transcribed in the same direction as the genes of the *dapE* region was linearized with *Bam*HI and used to transform strain JC7623. Chromosomal recombinants were selected, and the genetic linkage between the *kan* marker and the *purC* gene was determined by transduction of strain H624 as described above. Seventy-eight percent of the purine auxotrophs were kanamycin resistant.

Preparation of SDAP. DAP (a racemic mixture of all three DAP isomers; 190 mg) was dissolved in 5 ml of water and adjusted to pH 10 with 2 N KOH. Succinic anhydride (120 mg) dissolved in 5 ml of dimethylformamide was added slowly with stirring, and sufficient 2 N KOH solution was added to maintain pH 10. After base consumption ceased, the pH was adjusted to 7 and the solution was desalted by applying the total flask content to a column of AG50-x4 (1 by 5 cm). A mixture of SDAP and DAP was obtained by step gradient elution with water and 1 N HCl. This mixture was reduced to dryness at 45°C on a rotary evaporator, dissolved in 2 ml of water, and applied to a second column of AG50-x4 (1.7 by 9 cm). SDAP was separated from DAP by elution with a step gradient (8 ml of water, 10 ml of 0.2 N HCl, 15 ml of 0.5 N HCl, 15 ml of 0.8 N HCl, and 30 ml 1 N HCl), and 5-ml fractions were collected and analyzed by chromatography on cellulose F plates (developing solvent: methanol-H_2O-10 N HCl-pyridine [80:17.5:2.5:10]). SDAP (R_f, 0.62) and DAP (R_f, 0.28) were detected with ninhidrin.

Enzymatic assays. SDAP-deacylase was conveniently assayed by measuring the time-dependent appearance of DAP by high-pressure liquid chromatography (HPLC). The chromatography system consisted of a Chromegabond SCX column equilibrated at 40°C. Eluant A contained 80% trifluoroacetic acid (0.05 N) and 20% acetonitrile adjusted to pH 1.8 with NaOH. Eluant B contained 75% trifluoroacetic acid (0.2 N) and 25% acetonitrile adjusted to pH 2.5. At a flow rate of 1 ml/min, the program for the separation of SDAP from DAP was 20% eluant B–80% eluant A for 2 min, a linear gradient of 20 to 60% eluant B for 16 min, and 20% eluant B for 9 min for equilibration.

The SDAP-deacylase assay was performed with a reaction mixture containing buffer C (20 mM Tris-HCl [pH 8], 1 μM cobalt chloride) and 0.69 M SDAP. Incubation was done at 37°C for 20 min, with the removal of fractions at regular intervals. The reaction mixture was quenched by the addition of perchloric acid (final concentration, 0.2 N), vortexed, and placed on ice. Protein precipitates were removed by centrifugation in a microcentrifuge for 2 min, and fractions were applied to the HPLC system.

SDAP-transaminase was assayed by a rough method that we derived from the backward reaction previously described (21). Crude extracts were incubated in 200 mM Tris-HCl (pH 8) in the presence of 2 mM SDAP and 2 mM α-ketoglutarate. The disappearance of SDAP and the appearance of glutamate were monitored on an amino acid analyzer as already described (18).

Purification of SDAP-deacylase. About 2 to 3 g of RM4102 cells containing plasmid pDE10 and overproducing the *dapE* gene product were harvested at the mid-log phase after

growth in M63 minimal medium, suspended in 30 ml of buffer C, passed through a French pressure cell, and centrifuged at 30,000 × g for 20 min. This crude extract was treated for 45 min with manganese chloride (40 mM final concentration) added dropwise with constant stirring. After centrifugation of the DNA precipitate (30,000 × g for 20 min), the supernatant was made 45% in ammonium sulfate and kept at 4°C for 35 min. After centrifugation (30,000 × g for 20 min), the precipitate was resuspended in 1 ml of buffer C and dialyzed against 2 liters of the same buffer for 18 h with two changes of buffer. The dialyzed enzyme solution (1.8 ml) was injected in 500-μl fractions onto a Mono-Q 5/5 column equilibrated with buffer C. Elution was done at a flow rate of 1 ml/min with a gradient of NaCl (0 to 0.4 M for 35 min and 0.4 to 1 M for 5 min) in buffer C. Fractions were analyzed on a sodium dodecyl sulfate-polyacrylamide gel (14).

Nucleotide sequence accession number. The sequence shown in Fig. 2 has been deposited in the EMBL sequence data library under accession number X57403.

TABLE 1. SDAP-deacylase activity and excretion of metabolite intermediates in various strains

Strain	SDAP-deacylase activity[a]	SDAP excretion[b]	Glutamate excretion[b]
MC4100	26	ND	ND
RM4102	25	ND	ND
M35	14	0.8	0.8
RDE51	6	0.45	0.3
AT978	5	2	0.8
AT984	3	0.3	0.11
RM4102(pDE10)	11,700	NT	NT
RDH1	19	NT	NT
RDH2	28	NT	NT

[a] Expressed in nanomoles of DAP produced per minute per milligram of protein. Bacteria were grown as described in Materials and Methods. Two independent experiments yielded similar results.

[b] Expressed in nanomoles of metabolite excreted per milliliter of growth medium after overnight culturing in M63-glucose liquid medium supplemented as required. Assays were done by amino acid analysis as described previously (18). ND, not detectable. NT, not tested.

RESULTS AND DISCUSSION

Cloning of the *dapE* gene. The first mutation to be isolated in the *dapE* gene was described to lead to excretion into the growth medium of SDAP, a chromogenic substance (10). We used a similar test to determine the step of blockage created by several *dap* mutations that had been mapped in the 53-min region of the *E. coli* chromosome (Table 1). We confirmed by amino acid analysis of culture supernatants that strain AT978 (7) is blocked in the *dapE*-controlled step. Similarly, strain AT984, which had been reported to be mutated in the *dapA* gene (7), a mutation that could not be corrected by a plasmid carrying *dapA* (28), was also found to accumulate SDAP in the medium, as well as glutamate, the substrate of the preceding transaminase reaction. Strain M35, which carries the *dap-335* allele previously described (9), as well as strain RDE51, a Mu-generated *dap* mutant (27), were also characterized as being mutated in the *dapE* gene by the same criteria.

SDAP-deacylase was assayed in sonicated extracts from these mutants. As shown in Table 1, very low levels of SDAP-deacylase were found in strain AT984, AT978, and RDE51. However, significant residual activity (about 50% the wild-type level) was present in strain M35. This result was unexpected, because strain M35 is more severely affected in DAP biosynthesis than strain AT978 since, unlike that strain, it cannot grow in LB medium without the addition of exogenous DAP. Presumably, the SDAP-deacylase activity measured in vitro does not reflect accurately the in vivo situation, in which additional parameters might modulate the activity of the enzyme. In this regard, it is interesting that the overproduction of SDAP-deacylase is able to suppress a mutation in heat shock gene *grpE* (36), suggesting that chaperone proteins may interact with SDAP-deacylase in vivo.

Plasmid pSIU111 carrying a large insert (ca. 13 kb) from a

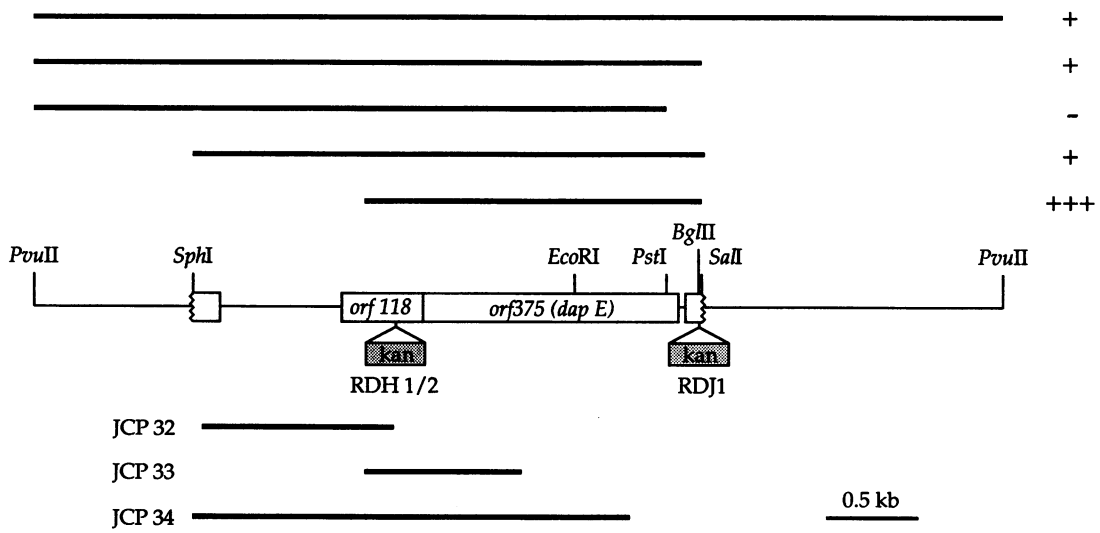

FIG. 1. Simplified physical map of the *E. coli dapE* region. Above the map are shown plasmid inserts, with their ability to complement *dapE* mutants indicated on the right. The fragment used for the overproduction of SDAP-deacylase is indicated by +++. The sites of disruption of the genes flanking *dapE* with a kanamycin resistance marker are shown by the shaded *kan* boxes (not to scale), and the names of the corresponding strains in the RM4102 background are indicated. The bars under the map show the fragments that were used for the construction of *malPQ* fusions, and the names of the corresponding strains are indicated.

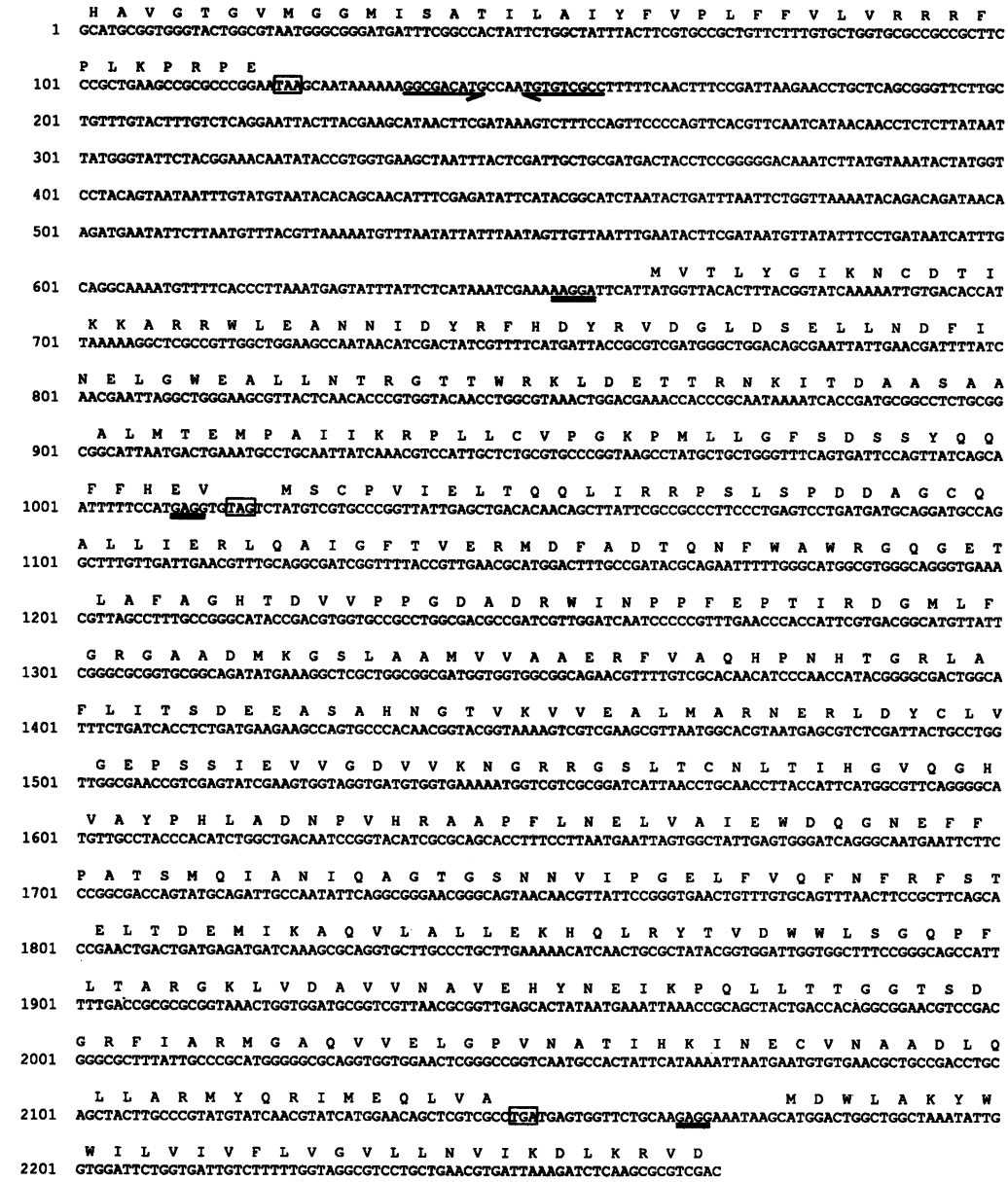

FIG. 2. Nucleotide sequence of the *dapE* locus. The sequence of the 2,270-bp *Sph*I-*Sal*I region indicated in Fig. 1 is shown, as is the predicted sequence of the encoded polypeptides. Termination codons are boxed. The ribosome binding sequences are underlined twice. A potential transcription termination sequence is indicated by convergent arrows.

bacteriophage transducing the *purC* gene has been described to correct the *dap* defect of strains AT978 and AT984 (20). This plasmid, kindly provided by J. Parker, was found to complement strains M35 and RDE51 as well. A 4.3-kb *Pvu*II fragment from the pSIU111 insert was also able to complement the four *dapE* mutants. Successive subcloning steps allowed us to reduce the *dapE*-complementing region to a 2.3-kb *Sph*I-*Bgl*II fragment (Fig. 1). One extremity of the *dapE* gene was localized between a *Pst*I site and a *Bgl*II site.

Sequencing of the *dapE* locus. The sequence of a 2,270-bp *Sph*I-*Sal*I fragment was determined (Fig. 2). All the potential open reading frames were found to be on the same strand. Two complete open reading frames are present in the insert, as are what appear to be the end and the beginning of adjacent open reading frames extending outside the *Sph*I-*Sal*I fragment. The larger open reading frame contains 375 codons and encodes a 41,129-M_r polypeptide. This open reading frame, *orf375*, overlaps the *Pst*I site, which was shown previously to be internal to the *dapE* gene, and stops before the *Bgl*II site. Moreover, recombination with plasmids carrying truncated parts of *orf375* indicated that the four *dapE* mutations were located either upstream (RDE51, AT978, and AT984) or downstream (M35) of the *Eco*RI site internal to this open reading frame. From these data, we conclude that *orf375* is part of the *dapE* locus.

The other open reading frame contains 118 codons and encodes a 13,600-M_r polypeptide. This open reading frame, *orf118*, is separated by only 3 bp from *orf375*. Such a genetic organization suggested that the products of these two genes could be functionally related, encoding, for instance, two

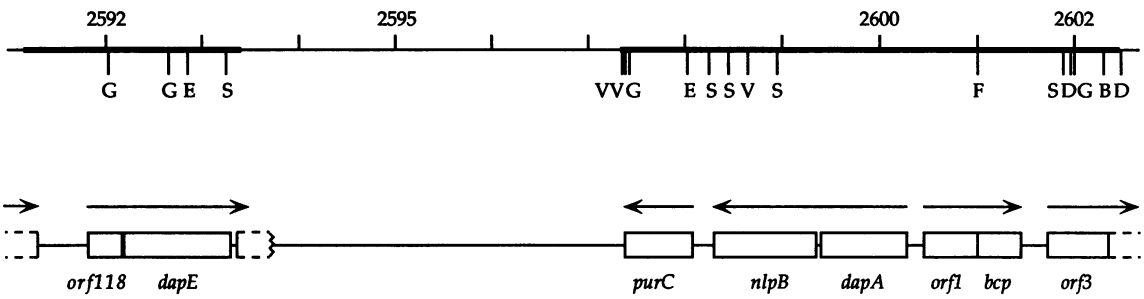

FIG. 3. Genetic organization of the *dapE-dapA* region of the *E. coli* chromosome. The sequencing data reported in this paper and others (1, 3, 28, 33) have been correlated (thick bars) with the updated physical map of this region (17). Only the restriction sites present in the sequenced regions are shown. The corresponding coordinates (in kilobases) on the *E. coli* chromosome are indicated above the physical map. The abbreviations used for restriction enzymes are the same as those used in reference 17:B (*Bam*HI), D (*Hin*dIII), E (*Eco*RI), F (*Eco*RV), G (*Bgl*I), S (*Pst*I), and V (*Pvu*II). The arrows indicate the orientation of transcription of the genes in this region.

subunits of SDAP-deacylase or controlling two successive steps in the DAP pathway, catalyzed by the products of *dapC* (for which no mutant is known) and *dapE*. Therefore, we disrupted the chromosomal *orf118* gene as detailed in Materials and Methods. The resulting strains, RDH1 and RDH2 (differing only in the orientation of the kanamycin resistance cartridge inserted into *orf118*), were found to grow normally on M63 minimal medium without DAP and to contain normal levels of SDAP-deacylase (Table 1). Therefore, the *orf118* gene product is not required for DAP biosynthesis. This result suggested that *orf375* was sufficient for providing *dapE* activity. A pUC19 derivative in which only the 375-codon open reading frame was intact (from the *Taq*I site at position 763 to the *Bgl*II site at position 2252) was constructed. As indicated in Table 1, the presence of this plasmid, pDE10, led to a large increase in SDAP-deacylase activity (more than 400-fold), confirming that *orf375* is the *dapE* gene.

SDAP-deacylase was easily purified from the overproducing strain (see Materials and Methods). Its amino-terminal sequence was determined (Ser-X-Pro-Val-Ile) (6) and found to fit perfectly with the nucleotide sequence, the amino-terminal methionine being removed posttranslationally. Purified SDAP-deacylase migrated as a single band of 40 kDa on denaturing polyacrylamide gels (data not shown), in accordance with the prediction of the nucleotide sequence and with other observations concerning the purified enzyme (15). The sequence of SDAP-deacylase is significantly similar throughout its whole length to that of acetylornithine deacetylase, the *E. coli argE* product (5), a result that probably reflects a common evolutionary origin for these two functionally related enzymes (data not shown).

The presence of another open reading frame starting only 27 bp downstream of *dapE* suggested that this gene could be cotranscribed with *dapE*. To investigate its function, we disrupted the chromosomal copy of this gene as described in Materials and Methods. The resulting strain, RDJ1, was found to grow normally without the addition of exogenous DAP. In the absence of any discernible phenotype for strain RDJ1 and of any sequence similarity with the EMBL nucleotide data base (release 29), the role of this gene, as well as the role of *orf118*, remains unknown.

Another open reading frame is present in the first 120 bp of the sequence shown in Fig. 2. The carboxy terminus of its putative product is highly similar to the carboxy terminus of the *E. coli envD* gene product (13), with 24 identical and 6 similar residues out of 35 (data not shown). These sequencing data have been combined with results obtained for the neighboring *dapA* region (1, 3, 28, 33) and correlated with the physical map of the *E. coli* chromosome (17) (Fig. 3). The *dapA* and *dapE* genes are 6 kb apart and transcribed in opposite directions.

Expression of *dapE*. Insertion of the kanamycin resistance cartridge into the *orf118* gene has no significant polar effect on the expression of the downstream *dapE* gene, as judged by the levels of SDAP-deacylase found in strains RDH1 and RDH2 (Table 1) and by their Dap$^+$ phenotype. This result suggests that a promoter directing the transcription of *dapE* is present in *orf118*, downstream of the site of insertion of the *kan* marker. However, if the *orf118* gene is actually expressed, another promoter should be present upstream of the 118-codon open reading frame and should allow the transcription of both *orf118* and *dapE* because of the proximity of these two genes. Several attempts to map these putative promoters by primer extension were unsuccessful, presumably because of the low abundance of the mRNAs covering that region of the chromosome. To obtain functional evidence for the existence of these two promoters, we used the strategy devised by Raibaud and coworkers (22, 34), which allows promoter activity in a cloned DNA fragment to be assessed by integration into the chromosome upstream of the *malPQ* operon and assay of amylomaltase, the *malQ* product. As detailed in Materials and Methods, three DNA fragments that covered various regions of the *orf118-dapE* locus were used (Fig. 1). Three strains that contained the postulated *orf118* promoter (JCP32), the internal *dapE* promoter (JCP33), or both promoters (JCP34) were constructed. These three strains were grown in M63-glucose liquid medium, and amylomaltase assays were performed. Amylomaltase activity was found in each case. Strain JCP32 synthesized only a low level of amylomaltase (specific activity, 6 U; in comparison, a background level of 1 nmol of glucose is produced per min per mg of protein in the absence of any insert cloned upstream of *malPQ*). Strain JCP33, which contains the *dapE* internal promoter fused to *malPQ*, synthesized a significantly higher level of amylomaltase (specific activity, 23 U), while strain JCP34 (containing both promoters) produced about 37 U of amylomaltase. These results confirm the presence upstream of *orf118* of a weak promoter that allows the transcription of both *orf118* and *dapE* (several putative σ^{70} recognition sequences are present upstream of *orf118*) and the presence in the *orf118* coding sequence of a promoter that is the major promoter directing the transcription of *dapE* (a good candidate is the TTGCTC–17-bp–TATGCT sequence located at positions 942 to 970). The possibility that the open reading frame located down-

stream of *dapE* is cotranscribed with *orf118* and *dapE* was not investigated, although this appears likely, because of the very short region separating this gene from *dapE*.

The availability of *dapE-malPQ* fusions allowed us to study more precisely the putative repression of *dapE* transcription by lysine. Such a regulation has been proposed (9), but the difficulties inherent in the SDAP-deacylase assay made that result uncertain, and more recent measurements suggested that SDAP-deacylase synthesis was not affected by lysine (15). Strains JCP32, JCP33, and JCP34 were grown in M63-glucose liquid medium in the presence of 4 mM lysine. No significant variation in the level of amylomaltase synthesis was found in comparison with that in the same strains grown in the absence of lysine (data not shown). We conclude that the expression of *dapE* is not controlled by the level of lysine in the growth medium. In the absence of any data on the *dapC* gene, it appears that only *dapB* and *dapD*, which control the second and third steps of the DAP pathway, respectively, are transcriptionally regulated (4, 25). The identity of the corresponding regulatory gene(s) remains to be elucidated.

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