δ-Aminolevulinic Acid Dehydratase Deficiency Can Cause δ-Aminolevulinate Auxotrophy in Escherichia coli

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Ethylmethane sulfonate-induced mutants of several Escherichia coli strains that required δ-aminolevulinic acid (ALA) for growth were isolated by penicillin enrichment or by selection for respiratory-defective strains resistant to the aminoglycoside antibiotic kanamycin. Three classes of mutants were obtained. Two-thirds of the strains were mutants in hemA. Representative of a third of the mutations was the hem-201 mutation. This mutation was mapped to min 8.6 to 8.7. Complementation of the auxotrophic phenotype by wild-type DNA from the corresponding phage 8F10 allowed the isolation of the gene. DNA sequence analysis revealed that the hem-201 gene encoded ALA dehydratase and was similar to a known hemB gene of E. coli. Complementation studies of hem-201 and hemB1 mutant strains with various hem-201 gene subfragments showed that hem-201 and the previously reported hemB1 mutation are in the same gene and that no other gene is required to complement the hem-201 mutant. ALA-forming activity from glutamate could not be detected by in vitro or in vivo assays. Extracts of hem-201 cells had drastically reduced ALA dehydratase levels, while cells transformed with the plasmid-encoded wild-type gene possessed highly elevated enzyme levels. The ALA requirement for growth, the lack of any ALA-forming enzymatic activity, and greatly reduced ALA dehydratase activity of the hem-201 strain suggest that a diffusible product of an enzyme in the heme biosynthetic pathway after ALA formation is involved in positive regulation of ALA biosynthesis. In contrast to the hem-201 mutant, previously isolated hemB mutants were not ALA auxotrophs and had no detectable ALA dehydratase activity. Analysis of another class of ALA-requiring mutants showed that the auxotrophy of the hem-205 mutant could be relieved by either methionine or cysteine and that the mutation maps in the cysG gene, which encodes uroporphyrinogen III methylase. The properties of these nonleaky ALA-requiring strains suggest that ALA is involved more extensively in E. coli intermediary metabolism than has been appreciated to date.

In a wide variety of eubacteria, including Escherichia coli, and in the chloroplasts of higher plants and algae, the first committed precursor of tetrapyrrole biosynthesis is δ-aminolevulinic acid (ALA) derived from glutamate (3, 5, 22, 30; reviewed in references 7, 17, and 32). The transformation of glutamate into ALA, termed the C2 pathway, can be reconstituted in vitro with purified Glu-tRNA synthetase, Glu-tRNA reductase, glutamate-1-semialdehyde (GSA) aminotransferase, tRNA\textsuperscript{Glu}, glutamate, ATP, and NADPH (9). Thus, three enzymes and tRNA\textsuperscript{Glu} are sufficient for this conversion. However, our understanding of the exact mechanism of this biotransformation and its key role in the regulation of tetrapyrrole biosynthesis is still rather sketchy (reviewed in references 7 and 32).

Analysis of the C2 pathway in E. coli has so far relied on the isolation and characterization of mutant strains auxotrophic for ALA. Such strains have been isolated from several eubacterial species that are known to use the C2 pathway, including E. coli (28, 34, 37, 39, 46), Bacillus subtilis (1, 19), and Salmonella typhimurium (13, 14, 38). The isolation of hemA mutants usually involves screening on media containing an aminoglycoside antibiotic such as neomycin (40). Slow-growing small-colony-variant (SCV) mutants or dwarf colonies grow on this selective medium (37, 40). The SCV mutant population has been shown to be enriched in respiratory-defective mutants, of which a small number are auxotrophic for ALA. The mutants are neither resistant to nor dependent on the aminoglycoside antibiotic, show a leaky growth requirement for ALA under nonfermentative growth conditions, and display wild-type growth characteristics under anaerobic conditions (14, 46). While the intriguing relationship between the resistance to aminoglycoside antibiotics and the requirement for heme allows for the enrichment of ALA auxotrophs, it is not known whether this selection technique biases the mutant population or totally excludes certain types of ALA-requiring phenotypes. Mapping studies of E. coli ALA auxotrophs of the SCV type have defined two loci, hemA and popC, at min 27 and 4, respectively (6, 34, 39, 46). The E. coli hemA gene has been cloned by complementation of the original hemA strain by several groups (11, 22, 42), and homologous hemA genes have been isolated from S. typhimurium (13) and B. subtilis (33). Although direct biochemical evidence for the function of HemA has not been reported, reconstitution experiments with extracts of mutant strains implicates HemA at the level of Glu-tRNA\textsuperscript{Glu} conversion to GSA by the Glu-tRNA reductase (3, 4). The original E. coli popC mutants have perished (27a), but the hemL locus of S. typhimurium appears to be analogous (13, 14). Recently an E. coli gene homologous to a barley gene encoding the GSA aminotransferase was cloned and physically mapped to a chromosomal position near popC (14a). However, in the absence of the original popC mutant, it is unclear whether this gene and popC are identical. The fact that a hemA hemL double mutant still was able to produce siroheme led to the suggestion that there are two pathways of ALA formation in S. typhimurium (14).

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Since the original popC mutant has been lost and no mutants have definitively been shown to result in loss of Glu- tRNA reductase or GSA aminotransferase activities, we carried out a mutant search in E. coli for additional ALA auxotrophs. The ALA auxotrophs isolated in the presence or absence of aminoglycoside antibiotics could be subdivided into three groups which mapped to three loci: the hemA locus and two new map positions, hemB and cysG, not previously associated with ALA auxotrophy. The ALA auxotrophy induced by these mutations is unusual because of their lethal, nonleaky phenotype and their occurrence in genes that encode enzymes that utilize ALA or ALA-derived products. The cysG gene in E. coli encodes an S-adenosyl- methionine-dependent uroporphyrinogen III methylase required for biosynthesis of cobalamin and siroheme (10, 15, 25, 44). The product of the hemB gene is ALA dehydratase, which condenses ALA to porphobilinogen (12, 16, 23, 24). The hemB mutation analyzed here is markedly different from previously characterized mutants at this locus since it requires ALA for growth, contains no detectable ALA-forming activity, and retains about 10% of the wild-type levels of ALA dehydratase activity. In contrast, other hemB mutants (12, 24) have no requirement for exogenous ALA and have no detectable ALA dehydratase activity.

MATERIALS AND METHODS

Bacterial strains. All bacterial strains, phages, and plasmids used are listed in Table 1. All bacterial strains used are derivatives of E. coli.

Bacterial procedures. Bacterial cultures were routinely grown in L broth or on L-agar plates. When used for growing mutants requiring ALA, this medium was supplemented with ALA (25 μg/ml). When required for selection of ALA mutants, kanamycin was added to a final concentration of 5 μg/ml. The minimal medium used, unless otherwise stated, was medium M63 (29). Glucose or glycerol was added as a carbon source at a final concentration of 2 mg/ml. Transduction with phage P1 was carried out in L broth with 2.5 × 10^{-3} M CaCl₂. Stationary-phase cells (10^{9} to 2 × 10^{9} ml) were mixed with approximately equal numbers of phage and incubated at 37°C for 10 to 15 min. The mixture was centrifuged twice and suspended in minimal salt solution before plating on selective media. Induction of mutations with ethyl methanesulfonate was done as described previously (29).

Recombinant DNA procedures. Procedures for growing phage λ and for isolation of DNA fragments were essentially as described by Maniatis et al. (26). DNA was sequenced by the dyeoxy method (36).

Enzyme assays. Bacteria were grown aerobically to a density of A_{600} = 0.8 in M9 medium (100 ml) containing 0.4% glycerol, ALA (50 μg/ml), and ampicillin (50 μg/ml) and harvested by centrifugation. To exhaust the endogenous ALA supply, the cells were resuspended for 60 min at 37°C in M9 medium containing 0.4% glycerol but not supplemented with amino acids or ALA. The cells were then resuspended in 2 ml of the appropriate assay buffer for determining either ALA-forming activity or ALA dehydratase activity. The assay buffer for the determination of in vitro and in vivo ALA-forming activity contained 0.1 M Tricine hydroxide (pH 7.9), 0.3 M glycerol, 10 mM KCl, 5 mM dithiothreitol, and 2 mM phenylmethylsulfon fluoride. The assay buffer for the determination of ALA dehydratase activity contained 0.1 M potassium phosphate (pH 6.8) and 20 mM dithiothreitol. Aliquots of the cell suspensions in buffer A were assayed directly for in vivo ALA-forming activity as described below. The remaining cells were disrupted by treatment with lysozyme and sonication in the presence of glass beads (100-μm diameter; 1 g of beads per 1 g [wet weight] of cells). The cell extracts were clarified by centrifugation at 100,000 × g for 60 min and then dialyzed against assay buffer for 90 min. The in vitro ALA-forming activity was determined (30, 31) in 1.0 ml of buffer A supplemented with 15 mM MgCl₂, 1 mM NADPH, 2 mM ATP, 10 mM levulinate, 1.0 μCi of [^{14}C]glutamate (293 mCi/mmole), E. coli trNA (5 A_{260} units), and cell extract (10 mg of protein). Following incubation of the assay mixture at 37°C for 60 min, the reaction was stopped and the [^{14}C]ALA formed was purified and quantitated exactly as described previously (30, 31). The assay mixture (0.25 ml) for the in vivo conversion of [^{14}C]glutamate to [^{14}C]ALA by intact cells (2) contained 2.5 × 10^{9} log-phase cells, 0.1 M Tricine hydroxide (pH 7.9), 10 mM KCl, 5 mM MgCl₂, 2 mM ATP.
50 mM levulinate, and 1.0 µCi of [14C]glutamate (293 mCi/mmol). The assay mixture was incubated at 37°C for 30 min, and the [14C]ALA formed was extracted, purified and quantitated as described previously (2, 30, 31). For both the in vitro and in vivo assays, ALA was converted to ALA pyrrole and identified by thin-layer chromatography (30, 31).

ALA dehydratase activity in the supernatant fraction was determined by assaying the conversion of ALA to porphobilinogen and detection of the formed porphobilinogen by using Ehrlich reagent (16). Assay conditions were as follows: 0.2 ml of cell extract (1 mg of protein per ml), 0.8 ml of 0.1 M potassium phosphate (pH 6.8) containing 20 mM dithiothreitol, and 50 µg of ALA. The assay mixture was incubated for 60 min at 30°C, and the reaction was stopped by the addition of 1.0 ml of 10% (wt/vol) trichloroacetic acid containing 0.1 M HgCl₂. The terminated assay mixtures were centrifuged (12,000 × g for 10 min), and the precipitate was discarded. The supernatant (1.5 ml) was mixed with an equal volume of Ehrlich reagent (1 g of p-dimethylamino-benzaldehyde dissolved in 42 ml of glacial acetic acid and 8 ml of 70% perchloric acid). The Ehrlich salt of porphobilinogen yields a red compound quantitated at 555 nm with an extinction coefficient of 60,200 mol/liter (16).

Nucleotide sequence accession number. The sequence reported has been assigned GenBank accession number X17417.

RESULTS AND DISCUSSION

Isolation of mutants. Mutants requiring ALA for growth (Ala−) were isolated in strains W3110, AB1157, and GE1361 after treatment with ethyl methanesulfonate, penicillin selection in glycerol minimal medium, subsequent plating on glucose minimal agar supplemented with ALA (25 µg/ml), and replica plating on glycerol minimal medium without ALA. Ala− mutants were also obtained in strain GE1361 by picking slow-growing colonies after plating ethyl methanesulfonate-treated cells on L agar with kanamycin (40). A total of 14 mutants were obtained after penicillin selection, but 38 were obtained after kanamycin selection. All mutations are referred to as hem mutations, and their phenotype is referred to as Ala−.

Characterization of mutants. Growth tests of mutants were routinely carried out by suspending cells in a minimal salt solution and streaking them on glycerol minimal and L-agar plates with and without ALA (25 µg/ml). These tests permitted division of the mutants into three main phenotypic classes.

(i) Class A. Thirty-three mutants grew normally on L agar when supplemented with ALA but either did not grow or were growth retarded (leaky phenotype) on L agar without ALA supplementation. On glycerol minimal agar, all of these strains grew normally with 0.25 µg of ALA per ml. Mutants that had a leaky phenotype on L agar showed a similar degree of leakiness on glycerol minimal medium without ALA. All seven mutants obtained after penicillin selection showed a nonleaky phenotype, whereas most mutants obtained after kanamycin selection were leaky. The phenotype of this class of mutants (apart from the leakiness observed for many mutants in this study) corresponds to the phenotype of hemA mutants described in previous studies (28, 37, 39, 46).

(ii) Class B. Sixteen mutants were retarded in growth on L agar with or without ALA supplementation (25 µg/ml); representative mutant alleles are hem-201 in strain GE1360 and hem-202 in strain GE1363. The degree of growth retardation varied among these mutants but was not affected by addition of ALA to the medium. Mutants that showed the highest degree of growth retardation (e.g., GE1360) formed colonies with diameters of about 0.6 mm upon incubation for 2 days at 37°C. Mutants of this class failed to grow (e.g., GE1360) or grew to a limited extent (leaky mutants) when streaked on glycerol minimal medium without ALA. Normal growth was restored when this medium was supplemented with ALA at 2.5 µg/ml, but 0.25 µg/ml did not stimulate growth. On glycerol minimal medium with ALA, these mutants could be distinguished by their white color from wild type and from mutants of classes A and C. In cross-streaking tests on glycerol minimal medium without ALA, mutants of class A were clearly fed by mutants of class B. Mutants of classes A and B grew anaerobically on glucose minimal medium without ALA.

(iii) Class C. One mutant of GE1361, strain GE1369 with mutant allele hem-205 obtained after penicillin selection grew normally on L agar with or without ALA supplementation. Growth on glycerol or glucose minimal medium was dependent on the supplementation by either ALA, methionine, or cysteine. On glycerol or glucose minimal agar without ALA, methionine, or cystine, this mutant had a nonleaky phenotype but reverted at a frequency of about 10−3.

Genetic mapping. Fifteen mutations of class A obtained in W3110, AB1157, and GE1361 were mapped by transduction with phage P1. All of these mutations showed cotransduction with zch-506::Tn10 present at min 27.25 in strain JW380. Six of the mutations were also tested for cotransduction with the trp-3 mutation present in strain GE1976. Cotransduction frequencies of 8 to 10% were found. It is concluded that these 15 mutations are in the hemA gene, which is located at 26.7 min (6, 39).

Mutations of class B were mapped by conjugation and P1 transduction. In interrupted mating experiments with strain CA8000, the hem-200 mutation present in a derivative of AB1157 was located near min 9 on the map. This result was confirmed by P1 transduction, which showed 20 to 25% cotransduction between lacY and hem-200. Further mapping of hem-200, hem-201, hem-202, and 11 other mutations of phenotype B showed their close linkage to the proC locus. For the fine genetic mapping, we used the hem-202 allele; for the biochemical studies, the hem-201 allele was used. Results of mapping experiments with hem-202 are shown in Table 2. Cotransduction between hem-202 and proC24 was about 80% when selection is made for proC+ but near 70% when selection was made for hem+. A cotransduction frequency of 21% was found for lacY and hem-202. Distribution of unselected markers showed the gene order lacY-hem-proC. A cotransduction frequency of 0.8 is estimated to correspond to a map distance of about 0.2 min (27, 45). With
the procC gene being located at min 8.8 to 8.9 (6), our data put hemB to min 8.6 to 8.7.

The hemB-205 mutation of class C (originally present in strain GE1309) was by conjugation found to be closely linked to the rpsL locus. Further mapping was done by PI transduction. First, hemB-205 was shown to be cotransducible at a frequency of 15% with the rpsL gene (at min 73.4). Second, strain GE319 carrying the cysG44 mutation (at min 74) and requiring cystine for growth (not responding to ALA or methionine) was used as the recipient for PI phage grown on GE1369. Selection was made for transductants on minimal medium supplemented with methionine or ALA. No transductants were obtained on medium with ALA. Transductants obtained on medium with methionine (50 tested) grew with methionine or cystine, but ALA could not support their growth. It is concluded that in these transductants cysG44 has been replaced by hemB-205 and that hemB-205 is in or very close to the cysG gene. Clearly, the ability to respond to ALA is lost by transfer of hemB-205 into GE319. This ability was unaffected after transduction of hemB-205 from a streptomycin-resistant (rpsL8 or -9) derivative of GE1369 into GE1361, showing that it is solely due to the hemB-205 mutation. The reason for this difference between GE319 and GE1369 is not known.

ALA formation from glutamate in the hemB-201 mutant. When grown aerobically, the hemB-201 mutant GE1360 displayed a strict dependence for aerobic growth on the addition of exogenous ALA, suggesting that the defect was in one of the components of the C5 pathway. The ability of GE1360 cells and extracts to transform [14C]glutamate into ALA was analyzed by using the in vivo and in vitro assays for ALA formation described in Materials and Methods (2, 30, 31). Cells and cell extracts were prepared from log-phase cultures of GE1360 grown in M9 medium (29) supplemented with 0.4% glycerol and 50 μg of ALA per ml and assayed by using the in vivo and in vitro ALA-forming tests. Conversion of [14C]glutamate into ALA could not be detected in strain GE1360 in vivo (<0.01 pmol of ALA formed in 30 min per 2.5 × 106 cells) or in cell extracts (10 mg of protein formed <0.01 pmol of ALA in 60 min at 37°C), while it was easily detected in wild-type W3110 cells (78 pmol of ALA formed in 30 min per 2.5 × 108 cells) and in cell extracts (18 pmol of ALA formed in 60 min at 37°C). These results suggested that the cause of the ALA auxotrophy is a defect in ALA-forming activity.

Cloning and DNA sequence of the hemB-201 gene. The nonleaky Alα− phenotype of strain GE1360, its lack of ALA-forming activity, and the mapping of hemB-201 to a locus other than popC or hema indicated that we had identified a new locus involved in ALA formation. To further characterize this locus, the hemB-201 gene was cloned by complementation. DNA from two recombinant λ phages, 8F10 and 6E2, covering min 8.5 to 9.3 of the E. coli chromosome (20), was inserted into pUC18 and shown to rescue the GE1360 strain to ALA prototrophy. Phage 8F10 contained a 16.6-kb insert. Subcloning experiments localized the hemB-201-complementing activity to a 7.5-kb EcoRI-EcoRI fragment that was progressively subcloned to a 3.1-kb BamHI-BamHI fragment and then a 2.5-kb BamHI-BstXI subfragment (Fig. 1). These subclones, but not subclones pUC18ALAH1.7 and pUC18ALAH1.4 (Fig. 1), still rescued hemB-201 from auxotrophy. These data indicate that the HindII site used to prepare pUC18ALAH1.7 and pUC18ALAH1.4 is located within the region conferring hemB-201-complementing activity. Nucleotide sequence analysis of the 2.5-kb BamHI-BstXI subfragment revealed that the previously characterized hemB gene was present on this fragment (12, 23). The hemB gene had previously been mapped to min 8.1 (6).

The ATG initiation codon of the hemB gene on the 2.5-kb BamHI-BstXI hemB fragment was located 629 nucleotides from the BamHI site. The ATG initiation codon of the hemB gene reported by Li et al. (23) was located 458 nucleotides from the BamHI site. Thus, our sequence of hemB, in comparison with that of Li et al. (23), contains an additional 171 nucleotides between the BamHI site and the ATG initiation codon of hemB (Fig. 2). Further examination of the hemB sequence by Li et al. (23) shows that the first 26 nucleotides of their sequence are identical to the Splh-PstI-XbaI-BamHI multiple cloning site of the sequencing vectors M13mp18 and -19 (43). The published hemB sequence (23) extends another 1,882 nucleotides from the BamHI site and is identical to our sequence of the hemB BamHI-BstXI fragment. Mapping of restriction sites predicted from the nucleotide sequence (23) to occur in the hemB gene for NruI, Sall, PstI, and HindIII also map to the exact positions in the hemB 2.5-kb BamHI-BstXI fragment. From these results we conclude that the 2.5-kb BamHI-BstXI hemB fragment derived from phage 8F10 (20) is essentially identical to the hemB gene of Li et al. (23) except that through an insertion of 171 nucleotides the BamHI site is located upstream from the previously reported BamHI site. Whether this is due to strain differences is unclear. Our sequence analysis of the 5′ and 3′ flanks of the hemB gene on the 2.5-kb BamHI-BstXI hemB fragment did not reveal any open reading frames greater than 50 amino acids. This suggests that the ability of the 2.5-kb BamHI-BstXI hemB fragment to rescue the hemB-201 mutant strain from ALA auxotrophy must be due to the expression of the hemB gene product. This is an unexpected finding since no hemB mutants are known to affect ALA biosynthesis. We have confirmed that strain SHSP1 carrying the hemB1 mutation is unable to grow on minimal media with ALA.

ALA dehydratase levels in various strains. To confirm that the hemB-201 mutation is in hemB, the gene for ALA dehydratase, and also to confirm that the 2.5-kb BamHI-BstXI hemB fragment encoded ALA dehydratase, we assayed for ALA dehydratase activity in the hemB-201 mutant, GE1360, and in GE1360 containing a plasmid carrying the 2.5-kb BamHI-BstXI hemB fragment. The results clearly showed that GE1360 containing a drastrically reduced level of ALA dehydratase activity and that plasmids carrying the 2.5-kb BamHI-BstXI hemB fragment conferred highly elevated levels of ALA dehydratase to the mutant strains (Table 3). The hemB1 mutant strain (SHSP1) could also be transformed to prototrophy by plasmids carrying various subclones of the hemB-201 gene (Fig. 1). Complementation by pUC18ALABA3 was demonstrated for hemB-202 and other hem mutations that were mapped at this locus.

Conclusions. Our search for new genetic loci to characterize ALA formation in E. coli has yielded ALA auxotrophs resulting from mutations in genes not previously associated with the regulation of ALA formation. One class of mutant strains does not have detectable levels of ALA-forming activity and has greatly reduced levels of ALA dehydratase. The mutation was mapped to the hemB locus, which encodes ALA dehydratase (EC 4.2.1.24), an enzyme in the heme biosynthetic pathway which catalyzes the condensation of two ALA molecules to form porphobilinogen. Various E. coli hemB mutants and the corresponding hemB gene have been characterized by several groups (12, 23, 28); none of the known hemB mutant E. coli strains has been reported to
show ALA auxotrophy. Because hemB mutations were not expected to display ALA auxotrophy, previous selection schemes for hemB mutants (e.g., reference 24) have incorporated a screen that eliminates ALA-requiring mutants. In contrast, our mutant search concentrated on ALA− mutants. Obviously such mutants were discarded in earlier investigations. It should be noted that the use of aminoglycoside antibiotics as a selection scheme to enrich for respiration-defective mutants may result in a biased mutant population. Indeed, in our selections for ALA auxotrophs using kanamycin, only leaky ALA auxotrophs were obtained, whereas nonleaky ALA− phenotypes and the hem-201 mutant were obtained only by using penicillin enrichment techniques. It is not clear why the newly isolated hemB mutants display an ALA requirement for aerobic growth. Possibly the product of ALA dehydratase, porphobilinogen, or another product of the heme biosynthetic pathway is

| TABLE 3. ALA-dehydratase levels in E. coli strains containing cloned hem-201-complementing fragments |
|-----------------|-----------------|-----------------|-----------------|
| Bacterial strain | Plasmid | Synthesis of porphobilinogen in cell extract* |
|                 |       | Inactivated | Experimental     |
| W3110           | pKS   | 0.001 | 0.082 |
| GE1360          | pKS  | 0.002 | 0.009 |
| GE1360          | pKSalaBX | 0.004 | 2.27 |

* Micromoles of porphobilinogen synthesized per milligram of protein of total cell extract.
required for derepression or activation of one of the C₅ enzymes in E. coli. For example, it is generally accepted that heme is a potent feedback repressor of ALA biosynthesis (reviewed in reference 7). Studies on the regulation of expression of ALA synthase in yeast cells, have shown that its regulation is a composite of activation and repression (18). In related work, it was shown that the product of the rnm-I locus is required for normal levels of ALA-forming activity in vivo (8). Furthermore, the product of ALA dehydratase, porphobilinogen, is required for the induction of porphobilinogen deaminase activity in E. coli (41). Our genetic and biochemical data do not distinguish whether the newly characterized hem-201 mutation lies within the structural gene or in a regulatory sequence. The nature of the published hemB mutations has not been determined (12, 24). It is noted that a relatively high concentration of ALA is noted that a relatively high concentration of ALA is required for aerobic growth of the new hemB mutants. Under such conditions, the mutants must have enough dehydratase activity to permit growth, which is completely inhibited in the absence of ALA. Possibly, ALA is needed to activate the complex regulation of ALA and heme biosynthesis.

Mutations of the cysG gene which respond to ALA have not been described before. On the other hand, a response to methionine has been reported for certain mutations of cysG and other cys genes (21, 35). It has been suggested that such mutations affect regulation of cysG function (35). While hem-205 maps to the cysG locus, we did not establish the regulatory activity of the cysG gene product. The response of hem-205 to ALA suggests that ALA may play a role in controlling the expression of the cysG gene.

A further search for ALA auxotrophs may reveal additional loci and provide further information on the regulation of ALA synthesis and its involvement in the intermediary metabolism of E. coli.

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