JOURNAL OF BACTERIOLOGY, May 1994, p. 2743–2746
0021-9193/94/$04.00+0

Vol. 176, No. 9

5-Aminolevulinic Acid Synthesis in *Escherichia coli* Requires Expression of *hemA*

WEI CHEN,[1] C. S. RUSSELL,[1]* Y. MUROOKA,[2] AND S. D. COSLOY[3]

Departments of Biochemistry[1] and Biology,[3] City College of City University of New York, New York, New York 10031, and Department of Fermentation Technology, Faculty of Engineering, Hiroshima University, Kagamiyama-1, Higashi-Hiroshima 724, Japan[2]

Received 13 December 1993/Returned for modification 8 February 1994/Accepted 28 February 1994

***hemA* and *hemM*, which are 213 bp apart and divergently transcribed, were separately cloned. We found that *hemA* is required for 5-aminolevulinic acid (ALA) synthesis in two ALA$^-$ auxotrophs. Overexpression of *hemM* alone did not produce ALA. More ALA was produced by strains harboring a plasmid with both *hemA* and *hemM* than by those with *hemA* alone. We conclude that *hemA* alone is required for ALA synthesis but *hemA* and *hemM* are required for maximal ALA synthesis.**

The pathway of tetrapyrrole biosynthesis in *Escherichia coli* is highly conserved, except for the first step. The first committed intermediate, 5-aminolevulinic acid (ALA), can be produced by two pathways, C_4 and C_5 (3). It has been shown that *E. coli* uses the C_5 pathway (13, 14). A sequence of three steps is involved: synthesis of glutamyl-tRNA by glutamyl-tRNA synthetase (1, 7, 9, 16–18, 22, 23, 26, 27), reduction of glutamyl-tRNA to glutamic semialdehyde by glutamyl-tRNA reductase (GTR) (4), and transamination of glutamic semialdehyde by aminotransferase to form ALA (8, 12). *hemA*, which is the structural gene for GTR (2), has been cloned and sequenced (5, 6, 15, 24). Söll and coworkers (11, 25) isolated two GTR activities from *E. coli*, GTR45 and GTR85, with molecular weights, under denaturing and nondenaturing conditions, of 45 and 85 kDa, respectively. The former is the product of the *hemA* gene.

When *hemA* of *E. coli* was cloned (15), an open reading frame (ORF) upstream from it and transcribed divergently was observed. The maxicell technique showed that the product of the *hemA* gene is a 49-kDa protein. The size of the ORF suggested that its gene product was much smaller. Murooka and coworkers (10) found three ALA-requiring mutants which were complemented in a heterologous fashion (small and normal colonies) by this ORF. A plasmid containing *hemA* but not the ORF gave only small colonies. The ORF, encoding a 23-kDa protein, was designated *hemM*. Only a plasmid with both *hemA* and the ORF gave normal colonies. The investigators suggested that the *hemM* gene product is the major GTR enzyme and that *hemA* encodes a protein involved in a minor pathway for ALA synthesis.

We separated *hemA* from *hemM* and subcloned each of the genes into separate plasmids (pJL69 and pWC15, respectively) (Table 1 and Fig. 1 and 2). pJL68 (15), a plasmid harboring *hemA* and *hemM*, as well as a part of *prfA*, the structural gene for release factor 1, and an ORF with an unknown function, was partially digested with *Sal*I. The partial digest generated 0.68-, 0.80-, 1.48-, 4.31-, 4.99-, and 5.79-kb fragments. Religation and transformation of JM101 yielded many white colonies. JM101 is a strain which produces blue colonies when grown on X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) medium if it has been transformed with a plasmid harboring an intact fragment of *lacZ*. If there is an insert into this *lacZ* locus, the colonies will be white. DNAs from many of the white colonies were prepared, and one strain was identified which contained a plasmid, pJL69, which when digested with *Sal*I showed 0.7- and 4.2-kb bands after electrophoresis and ethidium bromide visualization. Southern hybridization with

TABLE 1. Strains used in this study

Organism or plasmid	Relevant genotype or phenotype[a]	Source
E. coli strains		
JM101	*supE thi* Δ(*lac-proAB*) F′[*traD36 proAB*$^+$ *lacI*^{q} *lacZ*ΔM15]	U.S. Biochemical Corp.
HU227	*hemA*[b]; hemin permeable	This laboratory
JL1268	HU227(pJL68)	This laboratory
JL1269	HU227(pJL69)	This laboratory
WC1215	HU227(pWC15)	This laboratory
WC1214	HU227(pWC14)	This laboratory
WC1208	HU227(pWC08)	This laboratory
I14	C600 *hemM*	Ikemi et al. (10)
WC1216	I14(pJL68)	This laboratory
WC1217	I14(pJL69)	This laboratory
WC1218	I14(pWC15)	This laboratory
WC1219	HfrCavalli(pJL68)	This laboratory
WC1220	HfrCavalli(pJL69)	This laboratory
WC1221	HfrCavalli(pWC15)	This laboratory
WC1222	SASX41B(pJL68)	This laboratory
WC1223	SASX41B(pJL69)	This laboratory
WC1224	SASX41B(pWC15)	This laboratory
Plasmids[c]		
pJL68	pTZ19U (*hemA hemM*)	This laboratory
pJL69	pTZ19U (*hemA*)	This laboratory
pWC15	pUC19 (*hemM*)	This laboratory
pWC14	pUC19 (partial *hemA* [*hemA′*])	This laboratory
pWC08	pUC19 (partial *hemM* [*hemM′*])	This laboratory
pUC19		U.S. Biochemical Corp.

[a] Other genetic markers are as follows: HU227 and its derivatives, Hfr Cavalli *metB*; for C600 and its derivatives, *supE44 hsdR thi-1 thr-1 leuB6 lacY1 tonA21*.
[b] Strain HU227 was derived from SASX41B, the Sasarman *hemA* mutant, and Hfr Cavalli was obtained from the *E. coli* Genetic Stock Center, Yale University, New Haven, Conn.
[c] For descriptions of these plasmids and how they were constructed, see Fig. 1 and 2 and the text.

* Corresponding author. Mailing address: Department of Chemistry, City College, CUNY, 138th Street & Convent Avenue, New York, NY 10031. Phone: (212) 650-6681. Fax: (212) 650-6657.

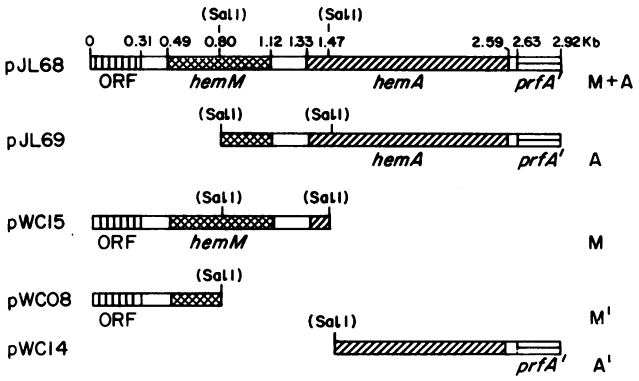

FIG. 1. Diagrams showing various inserts derived from pJL68.

the digoxigenin (DIG)-labelled 0.68-kb *Sal*I restriction fragment from pJL68 as the probe was positive. The blot was stripped and reprobed with the DIG-labelled 0.80-kb *Sal*I restriction fragment from pJL68 and was negative. Thus, pJL69 has an intact *hemA* gene, but because a 0.80-kb piece has been excised, only a part of *hemM* remains. This method of construction retains the original orientation.

To make a plasmid without *hemA* but with an intact *hemM* gene, pJL68 was digested with *Pst*I and partially digested with *Sal*I (Fig. 1 and 2). Shotgun cloning of the digestion mixture into pUC19 and transformation of JM101 yielded many white colonies. Colony hybridization with the DIG-labelled 0.68-kb *Sal*I restriction fragment from pJL68 as the probe gave two positive colonies. DNAs from these colonies were prepared and tested again by slot blot hybridization with the same probe (results not shown). One of the positive plasmids was pWC15. Slot blot hybridization of plasmid DNA with a 0.8-kb DIG-labelled *Sal*I restriction fragment from pJL68 was also positive. *Sal*I digestion of plasmid DNA gave 0.7-, 0.8-, and 2.7-kb fragments, confirming that pWC15 contained *hemM* intact. In addition, a maxicell experiment (20) showed the synthesis of a 23-kDa protein, which proves that the gene is expressed (Fig. 3).

Two other plasmids, pWC08 and pWC14, were constructed. pWC08 was obtained from the same restriction digest used to make pWC15 by using the same isolation procedures (Fig. 1 and 2). However, Southern hybridization was negative when the 0.68-kb probe was used and positive when the 0.80-kb probe was used. Digestion of this plasmid DNA with *Sal*I gave two bands, 0.8 and 2.7 kb, confirming a structure containing a piece of *hemM* (*hemM'*). pWC14 was constructed by digesting pJL68 with *Bam*HI, partially digesting it with *Sal*I, and then cloning it into pUC19. Transformation of JM101 gave many white colonies. DNA from one of these colonies gave a plasmid which, when digested with *Sal*I or *Pst*I, gave one 4.2-kb band, confirming that this plasmid contains a piece of *hemA* (*hemA'*).

HU227 is a hemin-permeable *hemA* mutant whose parent strain is SASX41B, which was isolated by Sasarman and coworkers (21). It requires hemin or ALA for growth. When HU227 was transformed with pJL69, the plasmid harboring *hemA*, or with pJL68, the plasmid which contains both *hemA* and *hemM*, the resultant strains, JL1269 and JL1268 (15), respectively, no longer required ALA or hemin for growth. When HU227 was transformed with pWC15, the plasmid

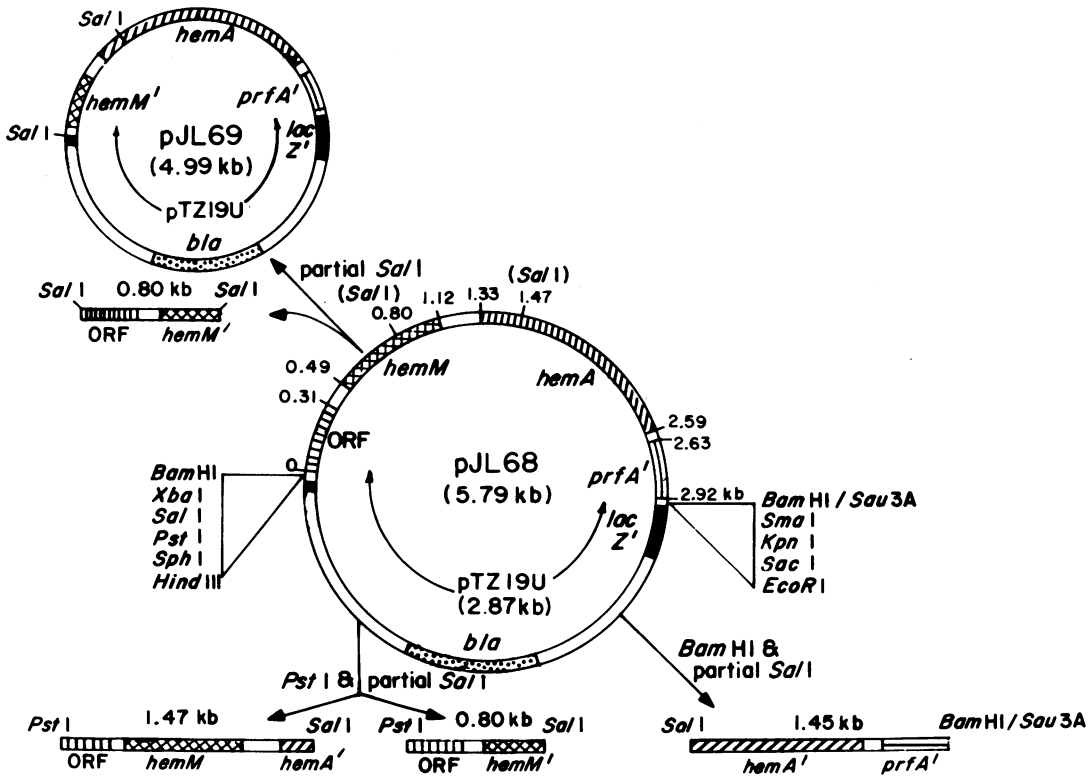

FIG. 2. Strategy used to construct a plasmid (pJL69) with *hemA* as the only intact ORF and inserts for plasmids pWC15, pWC08, and pWC14.

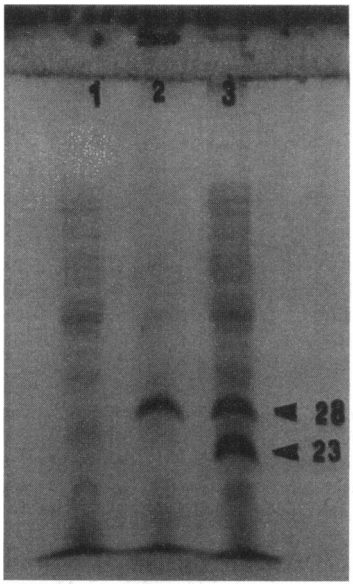

FIG. 3. Expression of *hemM*. An autoradiogram of proteins produced by pWC15 in the maxicell procedure in the presence of [^{35}S]methionine is shown. The proteins were analyzed on sodium dodecyl sulfate–10% polyacrylamide disc gels. Lanes: 1, CSR603; 2, CSR603/pUC19; 3, CSR603/pWC15. In Lane 3, the band at 28 kDa is β-lactamase and the one at 23 kDa is the *hemM* gene product.

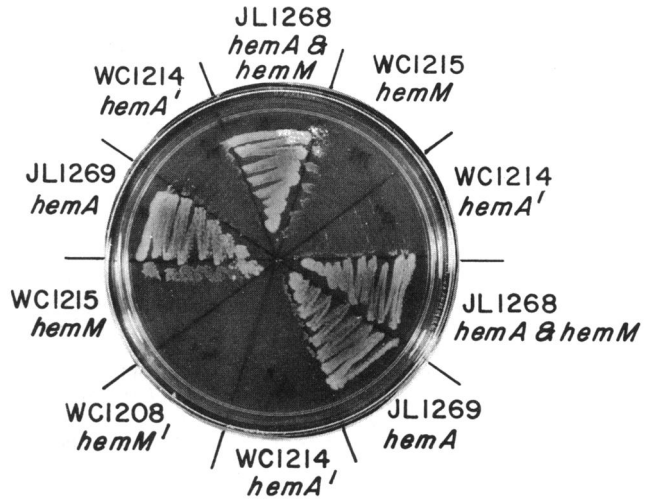

FIG. 4. Growth of strains and their abilities to feed ALA-requiring strains on LB agar medium.

harboring *hemM*, the resultant strain, WC1215, required hemin or ALA for growth. WC1214 and WC1208, strains derived from HU227 and transformed with plasmids containing partial *hemA* and partial *hemM*, respectively, also required ALA for growth. The growth rate dependencies of these three strains for different concentrations of ALA were almost superimposable (data not shown), and while 25 μg/ml was adequate for optimum growth, 100 μg/ml did not appear to be toxic.

JL1269, the strain with *hemA* alone, accumulated ALA because (i) colonies fluoresced after 2 days, and it is known that ALA supplementation causes accumulation of porphyrins in wild-type strains (19), and (ii) it could feed ALA-deficient strains derived from HU227 which contain *hemM* (WC1215), partial *hemA* (WC1214), or partial *hemM* (WC1208) on a plasmid. WC1215, the strain with *hemM* on a plasmid, did not fluoresce or feed our ALA-deficient mutants; therefore, we concluded that it cannot make ALA (Fig. 4). It is significant that the strain containing a plasmid harboring both *hemA* and *hemM*, JL1268, accumulated more ALA and fluoresced much sooner, after 1 day, and more strongly than JL1269, which has a plasmid harboring only *hemA*. Figure 5 shows the accumulation of ALA as a function of time for four host strains, HU227 (*hemA*; hemin permeable), Hfr Cavalli (wild-type parent), SASX41B (*hemA*; not hemin permeable), and I14 (*hemM*; not hemin permeable), each containing, variously, a plasmid with *hemA* and *hemM* together or *hemA* or *hemM* separately. In every case, the two genes together caused the greatest accumulation of ALA. *hemA* caused some accumulation, and *hemM* caused none or a little in strain I14. Our *hemM* construct did not support the growth of I14. Thus, for maximum ALA accumulation, a larger dosage of both genes is required.

The apparent contradiction between our results and those of Murooka and coworkers (10) may reflect the need for both *hemM* and *hemA* gene products, although the *hemA* gene product appears to be an enzyme with the size of GTR45. It is probable that ALA-requiring mutant strain HU227 has a mutation in *hemA* and has a normal *hemM* gene, since *hemA* is sufficient to complement the mutation. The mutation in *hemA* is most likely in the structural gene because if the promoter region did not function, the mutation would be lethal since release factor 1 depends on the same promoter region (6). The mutant studied by Murooka and coworkers may be a *hemM* mutant with a normal *hemA* gene. However, our *hemM*-containing plasmid did not complement this strain. It is possible that the mutant studied by Murooka and coworkers (10) has a mutation in the upstream region of *hemA*, and their plasmids containing *hemM* may correct the mutation by recombination. The accumulation of ALA in these studies indicates that while a normal *hemM* gene and many copies of *hemA* are sufficient for accumulation, the strain with many copies of both genes accumulated more ALA. It is possible that the *hemM* gene product is a regulatory protein or a regulatory subunit of

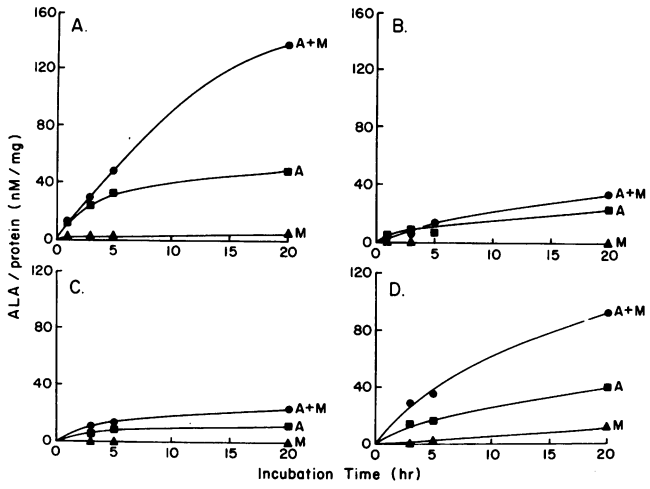

FIG. 5. Accumulation of ALA by various strains containing multicopy plasmids harboring *hemA*, *hemM*, and *hemA* plus *hemM*. Host strains: panel A, HU227; panel B, Hfr Cavalli; panel C, SASX41B; panel D, I14.

GTR reductase. It is also possible that GTR85 (11, 25) is some combination of the *hemA* and *hemM* gene products which survives sodium dodecyl sulfate treatment.

This work was supported by grants NSF DCB89-14913, NIH-RCMI, RR03060, NIH-MBRS RR08168, and PSC-CUNY 661150 and 662151.

We thank Lavern Wright for technical assistance.

REFERENCES

1. **Avissar, Y. J., and S. I. Beale.** 1988. Biosynthesis of tetrapyrrole pigment precursors. Formation and utilization of glutamyl-tRNA for δ-aminolevulinic acid synthesis by isolated enzyme fractions from *Chlorella vulgaris*. Plant Physiol. **88:**879–886.
2. **Avissar, Y. J., and S. I. Beale.** 1989. Identification of the enzymatic basis for δ-aminolevulinic acid auxotrophy in a *hemA* mutant of *Escherichia coli*. J. Bacteriol. **171:**2919–2924.
3. **Beale, S. I., and P. Castelfranco.** 1974. The biosynthesis of δ-aminolevulinic acid in higher plants. Plant Physiol. **53:**291–296.
4. **Chen, M.-W., D. Jahn, G. P. O'Neill, and D. Söll.** 1990. Purification of the glutamyl-tRNA reductase from *Chlamydomonas reinhardtii* involved in δ-aminolevulinic acid formation during chlorophyll biosynthesis. J. Biol. Chem. **265:**4058–4063.
5. **Drolet, M., L. Peloquin, Y. Echelard, L. Cousineau, and A. Sasarman.** 1989. Isolation and nucleotide sequence of the *hemA* gene of *Escherichia coli* K12. Mol. Gen. Genet. **216:**347–352.
6. **Elliott, T.** 1989. Cloning, genetic characterization, and nucleotide sequence of the *hemA-prfA* operon of *Salmonella typhimurium*. J. Bacteriol. **171:**3948–3960.
7. **Friedmann, H. C., R. F. Thauer, S. P. Gough, and C. G. Kannangara.** 1987. Δ-Aminolevulinic acid formation in the archaebacterium, *Methanobacterium thermoautotrophicum* requires $tRNA^{glu}$. Carlsberg Res. Commun. **52:**363–371.
8. **Hoober, J. K., A. Kahn, D. E. Ash, S. Gough, and C. G. Kannangara.** 1988. Biosynthesis of Δ-aminolevulinate in greening barley leaves. IX. Structure of the substrate, mode of gabaculine inhibition, and the catalytic mechanism of glutamate 1-semialdehyde aminotransferase. Carlsberg Res. Commun. **53:**11–25.
9. **Huang, D.-D., and W.-Y. Wang.** 1986. Chlorophyll biosynthesis in *Chlamydomonas* starts with the formation of glutamyl-tRNA. J. Biol. Chem. **261:**13451–13455.
10. **Ikemi, M., K. Murakami, M. Hashimoto, and Y. Murooka.** 1992. Cloning and characterization of genes involved in the biosynthesis of δ-aminolevulinic acid in *Escherichia coli*. Gene **121:**127–132.
11. **Jahn, D., U. Michelsen, and D. Söll.** 1991. Two glutamyl-tRNA reductase activities in *Escherichia coli*. J. Biol. Chem. **266:**2542–2548.
12. **Kannangara, C. G., and S. P. Gough.** 1978. Biosynthesis of Δ-aminolevulinate in greening barley leaves: glutamate-1-semialdehyde aminotransferase. Carlsberg Res. Commun. **43:**185–194.
13. **Li, J.-M., O. Brathwaite, S. D. Cosloy, and C. S. Russell.** 1989. 5-Aminolevulinic acid synthesis in *Escherichia coli*. J. Bacteriol. **171:**2547–2552.
14. **Li, J.-M., S. D. Cosloy, and C. S. Russell.** 1988. 5-Aminolevulinic acid synthesis in *Escherichia coli*. J. Cell Biol. **107:**617a.
15. **Li, J.-M., C. S. Russell, and S. D. Cosloy.** 1989. Cloning and structure of the *hemA* gene of *Escherichia coli* K-12. Gene **82:**209–217.
16. **O'Neill, G. P., M.-W. Chen, and D. Söll.** 1989. δ-Aminolevulinic acid biosynthesis in *Escherichia coli* and *Bacillus subtilis* involves formation of glutamyl-tRNA. FEMS Microbiol. Lett. **60:**255–260.
17. **O'Neill, G. P., D. M. Peterson, A. Schön, M.-W. Chen, and D. Söll.** 1988. Formation of the chlorophyll precursor δ-aminolevulinic acid in cyanobacteria requires aminoacylation of a $tRNA^{Glu}$ species. J. Bacteriol. **170:**3810–3816.
18. **Peterson, D. M., A. Schon, and D. Söll.** 1988. The nucleotide sequences of barley cytoplasmic glutamate transfer RNA's and structural features essential for formation of δ-aminolevulinic acid. Plant Mol. Biol. **11:**293–299.
19. **Philipp-Dormston, W. K., and M. Doss.** 1975. Overproduction of porphyrins and heme in heterotrophic bacteria. Z. Naturforsch. Teil C **30:**425–426.
20. **Sancar, A., and W. D. Rupp.** 1978. Correction of the map location for the *phr* gene in *Escherichia coli* K-12. Mutat. Res. **51:**139–143.
21. **Sasarman, A., M. Suredeanu, and T. Horodniceanu.** 1968. Locus determining the synthesis of δ-aminolevulinic acid in *Escherichia coli* K-12. J. Bacteriol. **96:**1882–1884.
22. **Schneegurt, M. A., and S. I. Beale.** 1988. Characterization of the RNA required for biosynthesis of δ-aminolevulinic acid from glutamate. Plant Physiol. **86:**497–504.
23. **Schon, A., G. Krupp, S. P. Gough, S. Berry-Lowe, C. G. Kannangara, and D. Söll.** 1986. The RNA required in the first step of chlorophyll synthesis is a chloroplast glutamate tRNA. Nature (London) **322:**281–284.
24. **Verkamp, E., and B. K. Chelm.** 1989. Isolation, nucleotide sequence, and preliminary characterization of the *Escherichia coli* K-12 *hemA* gene. J. Bacteriol. **171:**4728–4735.
25. **Verkamp, E., M. Jahn, D. Jahn, A. M. Kumar, and D. Söll.** 1992. Glutamyl-tRNA reductase from *Escherichia coli* and *Synechocystis* 6803. J. Biol. Chem. **267:**8275–8280.
26. **Wang, W.-Y., S. P. Gough, and C. G. Kannangara.** 1981. Biosynthesis of Δ-aminolevulinate in greening barley leaves. IV. Isolation of three soluble enzymes required for the conversion of glutamate to Δ-aminolevulinate. Carlsberg Res. Commun. **46:**243–257.
27. **Wang, W.-Y., D. D. Huang, D. Stachon, S. P. Gough, and C. G. Kannangara.** 1984. Purification, characterization and fractionation of the δ-aminolevulinic acid-synthesizing enzymes from light-grown *Chlamydomonas reinhardtii* cells. Plant Physiol. **74:**569–575.