An Alternate Pathway for the Processing of the Prolipoprotein Signal Peptide in Escherichia coli*

(Received for publication, March 15, 1985)

John Ghrayeb*, Charles A. Lunn, Sumiko Inouye, and Masayori Inouye

From the Department of Biochemistry, State University of New York at Stony Brook, Stony Brook, New York 11794

Previous studies showed that when the signal sequence plus 9 amino acid residues from the amino terminus of the major lipoprotein of *Escherichia coli* was fused to β-lactamase, the resulting hybrid protein was modified, proteolytically processed, and assembled into the outer membrane as was the wild-type lipoprotein (Ghrayeb, J., and Inouye, M. (1983) J. Biol. Chem. 259, 463-467). We have constructed several hybrid proteins with mutations at the cleavage site of the prolipoprotein signal peptide. These mutations are known to block the lipid modification of the lipoprotein at the cysteine residue, resulting in the accumulation of unprocessed, unmodified prolipoprotein in the outer membrane. The mutations blocked the lipid modification of the hybrid protein. However, in contrast to the mutant lipoproteins, the cleavage of the signal peptides for the mutant hybrid proteins did occur, although less efficiently than the unaltered prolipoprotein. The mutant prolipoprotein proteins were cleaved at a site 5 amino acid residues downstream of the prolipoprotein signal peptide cleavage site. This new cleavage between alanine and lysine residues was resistant to globomycin, a specific inhibitor for signal peptidase II. This indicates that signal peptidase II, the signal peptidase which cleaves the unaltered prolipoprotein, is not responsible for the new cleavage. The results demonstrate that the cleavage of the signal peptide is a flexible process that can occur by an alternative pathway when the normal processing pathway is blocked.

The major lipoprotein of *Escherichia coli* outer membrane undergoes several complex post-translational modification steps before its assembly into the outer membrane (see for review, Ref. 1). The protein is initially synthesized as a higher molecular weight precursor, prolipoprotein, which contains a peptide extension of 20 amino acid residues at the amino terminus (2). The peptide extension (signal peptide) is required for the lipoprotein secretion across the cytoplasmic membrane. Upon translocation of the protein across the membrane, the cysteine residue at the cleavage site of the signal peptide is modified with a glycine group. This modification is essential for the subsequent cleavage of the signal peptide (3, 4). This cleavage was carried out by the prolipoprotein-specific signal peptidase, signal peptidase II. The newly formed free amino group of the lipid-modified cysteine residue is then further acylated with a fatty acid, and the resultant fully modified lipoprotein is assembled into the outer membrane (1).

Various mutations within the prolipoprotein signal peptide have been generated by oligonucleotide-directed site-specific mutagenesis (5-10). Studies of these mutations have detailed residues within lipoprotein's signal peptide that are important for its processing and compartmentation. The lipoprotein signal sequence must contain positively charged residues at its amino terminus (5, 8), a hydrophobic, α-helical core (10) with centrally located glycine residues (9), and glycine and cysteine residues at the signal peptide cleavage site (6, 7) to promote efficient assembly of lipoprotein into the bacterial outer membrane. We have also shown that a hybrid protein composed of the signal peptide plus 9 amino acid residues of the prolipoprotein coupled to β-lactamase is post-translationally modified with lipid, and localized in the outer membrane (11) in a manner similar to lipoprotein. This was somewhat surprising, as lipoprotein and β-lactamase are processed via different signal peptides (1, 12). To further analyze the signal peptidase cleavage site of prolipoprotein-β-lactamase, we constructed two hybrid proteins with mutations at the signal peptide cleavage site, known as C2 (5) and C3 (6). In the C2 mutation, the cysteine residue at position 21 was replaced with a glycine residue, and in the C3 mutation, the glycine residue at position 20 was deleted. These mutations in the lipoprotein signal sequence resulted in complete lack of signal peptide cleavage, resulting in the accumulation of the unmodified prolipoprotein in the outer membrane. However, when these mutations were incorporated into prolipoprotein-β-lactamase, the signal peptide was cleaved, although less efficiently. The cleavage occurred at a new site 5 amino acid residues downstream of the normal cleavage site, between alanine at position 25 and lysine at position 26. The processing was found to be insensitive to globomycin, a specific inhibitor of signal peptidase II (13, 14). These results indicate that the recognition of the signal peptide by the translocation apparatus is sufficiently flexible to allow recognition by an alternative processing pathway when one pathway is blocked.

**MATERIALS AND METHODS**

**Bacterial Strains and Plasmids—** *E. coli* strain SB221 (λ<sup>−</sup> hsdR<sup>+</sup> lac<sup>+</sup> 125 rpsL<sup>−</sup> pro<sup>−</sup> ) was used in all experiments as the host cell. Cells were grown in the following media depending on the experiment: M9 medium (16) supplemented with glucose (4 mg/ml), tryptophan (20 μg/ml), leucine (20 μg/ml), thiamine (2 μg/ml), MgSO<sub>4</sub>-7H<sub>2</sub>O (200 μg/ml), chloramphenicol (10 μg/ml), and casamino acids (0.1%, w/v, Difco Laboratories); L broth was supplemented with ampicillin (50 μg/ml) and/or chloramphenicol (10 μg/ml).

**Plasmids** pJG311<sup>+</sup> (11), pIN-II-<sup>+</sup> (Cys→Gly) (6) and pIN-II-

*This work was supported by Grants GM19043 (to M. I.) and GM09492 (to J. G.) from the National Institute of General Medical Sciences and Grant NP3871 from the American Cancer Society (to M. I.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1794 solely to indicate this fact.

† Present address: Centocor Inc., 244 Great Valley Parkway, Malvern, PA 19355.

<sup>1</sup>pJG311 is identical to pJG310 (11) except that the EcoRI site downstream of the inserted bla gene was removed (J. Ghrayeb and M. Inouye unpublished data).
Alternate Processing of the lpp Signal Peptide

lpp (ΔGly21) (7) were used for the plasmid constructions.

DNA Manipulations—Isolation of plasmid DNA and various manipulations were previously described (11). Restriction enzymes and T4 DNA ligase were obtained from Bethesda Research Laboratories. DNA polymerase, Klenow fragment, was from New England Nuclear. ADP-ribosylated bovine serum albumin (dGGAATTCC) was purchased from New England Biolabs. DNA sequencing was carried out by the method of Maxam and Gilbert (17).

Expression of the Prolipo-β-lactamase Mutants—For growth experiments, 10-ml cultures were grown in M9 medium supplemented with (0.5%) casamino acids at 37 °C to a Klett reading of 50 (blue filter) at which time IPTG (Sigma) was added to a final concentration of 2 mM and Klett readings were taken every 15 min.

For pulse-chase experiments, 10-ml cultures were grown in amino acid supplemented M9 medium (minus casamino acids) to a Klett reading of 50 at which time IPTG was added to 2 mM. After 10 min, [3H]methionine (25 μCi/ml) was added. The chase was begun after 10 min by the addition of nonradioactive methionine to a final concentration of 4 mg/ml. One ml of each sample was removed at various times and added to 1 ml of ice-cold stopping solution (6). Globomycin (obtained from Dr. Arai, Sankyo Pharmaceutical Co., Tokyo, Japan) was used at a final concentration of 100 μg/ml.

Preparation and use of anti-β-lactamase serum were described previously (5). Osmotic shock was performed according to the method of Koshland and Botstein (18), except for the omission of EDTA from the plasmolysis buffers. The separation of the outer and the cytoplasmic membrane was performed by specific solubilization of the cytoplasmic membrane using sodium dodecyl sulfate (19).

Alternate Processing of the lpp Signal Peptide

Fig. 1. Construction of pJG312 from pIN-II lpp C2

(Cys-Gly) (6) and pJG311 (Ref. 11 and Footnote 1). Details of the procedures are described under “Results” and “Materials and Methods.” pJG313 was constructed in the same way but using pIN-II-lpp C3 (ΔGly21) (7). Sequence sources: lpp promoter, lac promoter-operator, mutant lpp signal sequence, lpp signal sequence, lpp gene, TEM 1 β-lactamase gene, lacI gene, etc. Other abbreviations: Cm′, chloramphenicol resistance marker; Amp′, ampicillin resistance marker.

sequences deduced from the DNA sequences of pJG312 (Cys-Gly) and pJG313 (ΔGly21). Except for the mutations in the signal peptide region of the lipoprotein, pJG312 and pJG313 are identical to pJG311.

Expression of the Prolipo-β-lactamase Mutants—For both pJG312 and pJG313, the genes for the hybrid proteins are under control of the lpp promoter and the lac promoter-operator region. Therefore, expression of the genes coding for the hybrid proteins can be induced by a lac inducer such as IPTG. When exponentially growing cells harboring pJG312 or pJG313 were treated with 2 mM IPTG, cell growth ceased within 30 min (Fig. 3). Further incubation in IPTG caused cells harboring pJG313 to lyse. In order to examine whether this cell lysis is related to the amount of the pJG313 gene product, we determined the kinetics of hybrid protein production in cells harboring the plasmids by quantitative rocket immunoelectrophoresis using anti-β-lactamase antiserum. Fig. 3 shows that cells carrying pJG312 produce a maximum of 0.3 μg of hybrid protein/ml of the culture within 30 min of induction. No cell lysis was observed for up to 12 h after induction of the pJG312 products (data not shown). Conversely, cells harboring pJG313 produced 0.8 μg of hybrid protein/ml of the culture. This was followed by cell lysis (Fig. 3), with the loss of the hybrid protein into the culture medium. This suggests that the lysis of cells containing pJG313 is a result of the production of a larger amount of the hybrid protein. At present, it is not known why cells harboring pJG313 produced more protein than cells harboring pJG312.

RESULTS

Construction of Mutant Prolipo-β-lactamases—Fig. 1 presents a summary of the construction of pJG312. Plasmid pIN-II-lpp C2 (Cys21-Gly) (6) was digested with PvuII and the resulting digest was treated with T4 DNA ligase in the presence of 8-mer EcoRI linkers. The ligation mixture was then treated with XbaI and EcoRI and the 100-base pair XbaI-EcoRI fragment, which includes the DNA sequence coding for the 8-mer EcoRI linker oligonucleotide, was purified according to published procedures (20). Preparation and use of anti-β-lactamase serum were described previously.

The amount of lipoprotein in crude cell lysates was determined using quantitative rocket immunoelectrophoresis (21). Purified TEM 1 β-lactamase was used to standardize the electrophoresis system.

Sequential Edman Degradation—Edman degradation was carried out as previously described (15). Proteins were eluted from fixed and stained SDS-polyacrylamide gels by gentle shaking for 24 h at 30 °C, out as previously described (15). Proteins were eluted from fixed and stained SDS-polyacrylamide gel electrophoresis were carried out as previously described (6). Osmotic shock was performed according to the method of Koshland and Botstein (18), except for the omission of EDTA from the plasmolysis buffers. The separation of the outer and the cytoplasmic membrane was performed by specific solubilization of the cytoplasmic membrane using sodium dodecyl sulfate (19).

The abbreviations used are: IPTG, isopropyl-1-thio-β-D-galacto-pyranoside; SDS, sodium dodecyl sulfate.

Footnote 1. lpp (ΔGly21) (7) were used for the plasmid constructions.

Footnote 2. The abbreviations used are: IPTG, isopropyl-1-thio-β-D-galactopyranoside; SDS, sodium dodecyl sulfate.
with 0.2% (w/v) casamino acids (vitamin free, Difco) at 37 °C. At the plotted relative to time after inoculation. The growth of SB221 cultures were removed, the cells were collected by centrifugation, disintegrated by sonication. Each sonicate was then assayed for soluble lipo-β-lactamase by quantitative rocket immunoelectrophoresis. Klett unit of 50, IPTG was added treated with 2 mM IPTG for 10 min. The cells were then analyzed by SDS-polyacrylamide gel electrophoresis. As a control, sonically disrupted extracts from cells carrying pJG311 were labeled for 10 min. The cells were then chased for 1, 5, 10-s pulse of pJG312, lanes 2 and 6, 1-min chase of pJG312, lanes 3 and 7, 5-min chase of pJG312, and lanes 4 and 8, 20-min chase of pJG312. Lane 9, 10-s pulse followed by a 2-min chase of pJG311. a and b designate the position of the precursor and processed forms of the pJG312 hybrid protein, respectively, while c designates the position of the processed form of pJG311 hybrid protein. A portion of the gel is shown.

We next determined whether these hybrid proteins with altered signal peptide cleavage sites were processed in vivo. Exponentially growing cells carrying pJG312 or pJG313 were treated with 2 mM IPTG for 10 min. The cells were then labeled with [35S]methionine for 10 s at 37 °C, followed by a chase for 1, 5, and 20 min with nonradioactive methionine. The soluble and membrane fractions were treated with anti-β-lactamase serum, and the immunoprecipitates were then analyzed by SDS-polyacrylamide gel electrophoresis. As a control, sonically disrupted extracts from cells carrying pJG311 were labeled for 10 s then chased for 2 min. Lane 9 of Fig. 4 shows that the immunoprecipitate prepared from total cell extract of pJG311 contained two species. Previous work (11) showed that these species were a precursor form of the hybrid protein (Fig. 4, band a) and the mature product, lipo-β-lactamase (Fig. 4, band c). Conversion from the precursor to mature form of the hybrid protein was virtually complete within 2 min (11). Immunoprecipitates from pulse-labeled cells containing pJG312 showed a single major species mainly in the soluble fraction (band a, lane 1), running coincident with the precursor of pJG311 (lane 9). Numerous smaller molecular weight species in lane 1 are considered to represent nascent chains of the hybrid protein. Such species were previously observed in pulse-labeled extracts from cells containing pJG311 (11).

After a chase with nonradioactive methionine for 1 min (Fig. 4, lanes 2 and 6), a second species (band b) appeared in both the soluble and membrane-associated fraction. The species migrated at a molecular mass 200-300 daltons smaller than the mature form of the hybrid protein produced by pJG311 (Fig. 4, lane 9). Chasing with nonradioactive methionine for 5 and 20 min showed a decrease in labeling of band a in both the soluble fraction and the membrane fraction, suggesting that band a was a precursor of band b (lanes 3, 4, 7, and 8). The amount of band a material in the membrane fraction decreased little with chasing for from 1 to 20 min (lanes 6–8). This may suggest that a part of the membrane bound precursor cannot be converted to the low molecular
weight form (see "Discussion"). Similar results were obtained with fractions prepared from cells containing pJG313 (data not shown). Finally, both mutant hybrid proteins were associated with the crude membrane fraction. As is shown later, both mutant hybrid proteins were released by osmotic shock, suggesting localization in the periplasmic space.

Characterization of Mutant Protein Processing—The presumed processing of the precursors of the hybrid proteins with cleavage site mutations was surprising, as the prolipoproteins with identical mutations accumulated as unprocessed precursors in the cells (6, 7). We wondered whether the process of converting the band a species to the band b species of pJG312 and pJG313 products utilized the signal peptidase II, which specifically processed prolipoprotein. We therefore tested whether the conversion of species a to species b was sensitive to globomycin, an antibiotic known to block processing by signal peptidase II (13, 14). While the processing of the pJG311 product was blocked by globomycin, the processing of both the pJG312 and pJG313 products were resistant to globomycin (data not shown).

Having shown that both mutant hybrid proteins were not processed by signal peptidase II, and recognizing that the processed forms of both mutant hybrid proteins migrated at an unexpectedly low molecular weight, we next determined the site at which the proteins were cleaved. Exponentially growing cells harboring pJG312 and pJG313 were induced for 10 min with IPTG, then labeled with [3H]isoleucine for 5 min. The soluble fraction prepared from the labeled cells was immunoprecipitated with anti-β-lactamase serum and the immunoprecipitates were submitted to SDS-polyacrylamide gel electrophoresis. The bands corresponding to the processed products produced by pJG312 and pJG313 were excised from the gel and the protein was eluted and subjected to sequential Edman degradation. Fig. 5 shows that isoleucine appeared in the second and sixth degradation cycles for the products of both pJG312 (Fig. 5A) and pJG313 (Fig. 5B). This result demonstrates that the signal peptide cleavage occurred for both pJG312 and pJG313 hybrid proteins between the 5th ( Ala) and 6th (Lys) residues of the mature lipoprotein sequence (see Fig. 2). This is in contrast with the gene product of pJG311, which is cleaved between Gly 20 and Cys 21 (see Fig. 2 and Ref. 11). The fact that the products for both pJG312 and pJG313 migrated faster than the wild-type lipo-β-lactamase in SDS-polyacrylamide gel electrophoresis (Fig. 4) is consistent with the lack of 5 amino acid residues at the amino termini of the mutant products.

Localization of the Products—In order to determine the cellular localization of these two hybrid proteins, cells harboring pJG312 or pJG313 were labeled with [35S]methionine for 2 min, after induction with IPTG for 10 min. The cells were then harvested and divided into the periplasmic fraction, the cytoplasmic fraction, and the membrane fraction. Initial analyses using equal cell equivalents revealed that most all hybrid protein resided in the periplasmic space. Fig. 6 shows an experiment in which equal amounts of labeled protein were analyzed. Greater than 90% of the pJG312 (Fig. 6, lane 1) and pJG313 (Fig. 6, lane 4) products were found in the periplasmic fraction. The majority of this periplasmic pool was processed to the mature lipo-β-lactamase (Fig. 6, large arrow). This suggests that, although the presence of a mutant lipoprotein signal sequence slows processing of the hybrid protein precursor, correct cellular localization within the periplasmic space does occur. The periplasmic fraction also contains a small amount of a single species (band c of Fig. 6, lanes 1 and 4) migrating faster than the mature form of the hybrid protein. This presumably represents an oxidized form of hybrid proteins previously described (20). We also observed that the precursor form of the pJG312 product (Fig. 6, band a) migrated at a slightly slower position than the precursor form of pJG313 (Fig. 6, large arrow).
band b). The difference in molecular weight appears to be greater than the single amino acid difference between the two species. We suppose that this difference corresponds to a conformation difference between the two proteins.

It is important to note that, when cells were broken by sonication without being subjected to osmotic shock, both mutant hybrid proteins were associated with a crude membrane fraction (Fig. 4). Fractionation of this crude membrane fraction, using either isopycnic banding in sucrose gradients or differential solubilization with sodium sarcosinate, showed that both processed and mature forms of the hybrid proteins associated with both membrane fractions (data not shown). This presumably reflects an artificial binding of the proteins to the surface of the membrane vesicles. Such sticking of periplasmic β-lactamase to membrane fractions was also observed when the protein was overproduced by the secretion cloning vector pIN III ompA3 (22).

DISCUSSION

We previously showed that certain mutations at the cleavage site of the prolipoprotein signal peptide resulted in the accumulation of unmodified, uncleaved prolipoprotein in the outer membrane of E. coli (6, 7). To test the complementarity of the two signal peptides, and to determine if those mutants can be affected by changes in the structure of the translocated protein, we introduced the same cleavage site mutations into a hybrid protein composed of an amino-terminal portion of the prolipoprotein (the signal peptide plus 9 amino acid residues) coupled to the amino terminus of mature β-lactamase. Although the unaltered hybrid protein was processed by signal peptidase II (11), we wondered whether mutation of the signal peptidase II cleavage site would promote processing by the signal peptidase which recognizes native β-lactamase in vivo, most likely signal peptidase I (23). These mutant hybrid proteins were constructed in exactly the same manner as prolipo-β-lactamase previously described (11) except that in one mutant (product of pJG312) the amino-terminal cysteine was replaced by glycine (C2 mutation, Ref. 6), while in the second mutant (product of pJG313) the glycine at the carboxyl-terminal residue (GpO) of the prolipoprotein signal peptide was deleted (C3 mutation, Ref. 7).

Our results demonstrate that the precursor forms of both mutant hybrid proteins are processed to mature products, but not by signal peptidase II, as processing was not inhibited by globomycin, a specific inhibitor of signal peptidase II. The signal peptide was cleaved at the new cleavage site between alanine at position 5 and lysine at position 6 of the mature lipoprotein (see Fig. 5). Signal peptidase I is a likely candidate for processing these mutant hybrid proteins, as this protease is involved in processing many secreted proteins, often cleaving after alanine residues (24). The processing of both mutant proteins are slow and incomplete, resulting in the accumulation of precursors. Pulse-chase experiments showed that the mutant hybrid proteins were processed more slowly than the unaltered hybrid proteins (Fig. 4 and Ref. 11). The processed product was first accumulated in the soluble fraction, then chased into the membrane bound form. Since the membrane bound product was found in the periplasmic space, it is likely that the soluble form of the processed product is generated by the fractionation procedure applied in the experiments, and that this product associates with the membrane fraction. As more proteins are synthesized, these mutant hybrid proteins bind to the membrane such that they fracionate with the membrane fraction. At present, we do not know whether the unprocessed hybrid proteins accumulate due to limited amounts of signal peptidase in the cell, or segregation of the hybrid proteins away from this signal peptidase. The IPTG sensitivity of the cells harboring pJG312 and pJG313 is probably due to the accumulation of the precursor forms of the proteins (Fig. 3). We have previously shown that a lipid-modified cysteine residue and 8 additional amino-terminal amino acid residues of the lipoprotein are sufficient to guide a lipoprotein-β-lactamase hybrid to the outer membrane (11). Since the processed products of these mutant hybrid proteins lack the lipid modification and were found in the periplasmic space, it can be concluded that the lipid moiety is essential for the assembly of the hybrid protein into the outer membrane of E. coli.

Our finding that the hybrid proteins of the present study were processed, while the prolipoprotein carrying the same mutations in the signal peptide were not processed, is intriguing. This is in spite of the fact that the unaltered prolipo-β-lactamase is exclusively processed by signal peptidase II, and no cleavage of this hybrid protein is observed even if signal peptidase II is inhibited by globomycin (11). We suggest that lipid modification of the unaltered prolipo-β-lactamase obscures the potential signal peptidase cleavage site, resulting in precursor accumulation in the presence of globomycin. When then is the mutant prolipoprotein not cleaved by signal peptidase? One possibility is that the mutant prolipoprotein has a different conformation at the new cleavage site from the mutant prolipo-β-lactamase. Similar alternative cleavage of Bacillus licheniformis propeptidilace as a result of mutation has been reported (25). Second, the small size of unmodified prolipoprotein may permit translocation to occur before processing. Thus, the prolipoprotein is assembled into the outer membrane, a compartment inaccessible to signal peptidase.

Acknowledgment—We are grateful to Dr. S. Pollitt for his critical reading of this manuscript.

REFERENCES

1. Wu, H. C. (1982) in Membranes and Transport (Martini, A. N., ed) Vol. 1, pp. 299-306, Plenum Press, New York.
2. Inouye, M., and Halleux, S. (1980) CRC Crit. Rev. Biochem. 7, 339-371.
3. Tokunaga, M., Tokumaru, H., and Wu, H. C. (1983). Proc. Natl. Acad. Sci. U.S.A. 79, 2296-2300.
4. Brown, V. (1979) Biochim. Biophys. Acta 515, 335-371.
5. Inouye, S., Soberson, X., Franceschini, T., Nakamura, K., Itakura, K., and Inouye, M. (1982) Proc. Natl. Acad. Sci. U.S.A. 79, 3438-3441.
6. Inouye, S., Franceschini, T., Sato, M., Itakura, K., and Inouye, M. (1983) EMBO J. 2, 87-91.
7. Inouye, S., Sherman-Hsu, C.-P., Itakura, K., and Inouye, M. (1985) Science 225, 69-61.
8. Vlasuk, G. P., Inouye, S., Ito, H., Itakura, K., and Inouye, M. (1983) J. Biol. Chem. 258, 7141-7146.
9. Inouye, S., Vlasuk, G. P., Hao, H., and Inouye, M. (1984) J. Biol. Chem. 259, 3739-3733.
10. Vlasuk, G. P., Inouye, S., and Inouye, M. (1984) J. Biol. Chem. 259, 6165-6169.
11. Ghosh, J., and Inouye, M. (1985) J. Biol. Chem. 259, 463-467.
12. Tokunaga, M., Loranzer, J. M., Wolfe, P. B., and Wu, H. C. (1982) J. Biol. Chem. 257, 9922-9925.
13. Imakai, M., Takeuchi, M., Minishu, K., and Araki, M. (1978) J. Antibiot. (Tokyo) 31, 1203-1205.
14. Hussain, M., Ichihara, H., and Miyazawa, S. (1980) J. Biol. Chem. 255, 5707-5712.
15. Nakamura, K., and Inouye, M. (1979) Cell 18, 1109-1117.
16. Miller, J. H. (1972) in Experiments in Molecular Genetics, p. 431, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY.
17. Maxam, A. M., and Gilbert, W. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 560-564.
18. Kosander, D., and Botstein, D. (1980) Cell 20, 749-760.
19. Filipp, C., Fitcher, G., Wulffe, J. L., and Earhart, C. F. (1973) J. Bacteriol. 115, 717-722.
20. Pollitt, S., and Zalkin, H. (1983) J. Bacteriol. 155, 27-32.
21. Brauch, C. H. (1986) Annu. Rev. Biochem. 55, 45-52.
22. Gstock, J., Kimura, M., Takahara, M., Hsiung, H., Masui, Y., and Inouye, M. (1984) EMBO J. 3, 2437-2444.
23. Zimmermann, R., and Weckern, W. (1983) J. Biol. Chem. 258, 3920-3925.
24. Dufaud, D. G., Lenhardt, S. K., March, P. E., and Inouye, M. (1985) in Curr. Top. Membr. Transp. 24, 65-104.
25. Hoshino, S., Chang, S.-Y., Chang, S., and Wu, H. C. (1984) J. Biol. Chem. 259, 10448-10454.