Evidence for a Small Catalytic Domain in the Adenylate Cyclase from Salmonella typhimurium*

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Deletions of large portions of the carboxyl-terminal end of the adenylate cyclase (ATP pyrophosphate lyase (cyclizing), EC 4.6.1.1) from Salmonella typhimurium do not significantly affect the enzymatic activity exhibited by the shortened polypeptide. The deletion mutations were generated by nuclease Bal31 digestion from the 3'-end of the cya gene fragment cloned by Wang et al. (Wang, J. Y.-J., Clegg, D. O., and Koshland, D. E. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 4684-4688); the shortened cya genes were inserted in pBR322 and used to transform a cya- strain of Escherichia coli. The original gene fragment encodes for an enzymatically active polypeptide having an apparent molecular weight of 77,000. Mutant polypeptides as small as 46,000 Da were found to retain significant enzymatic activity and to confer several cya phenotypes on the E. coli host. More extensive deletions resulting in polypeptides as small as 33,000 Da did not have assayable amounts of adenylate cyclase activity, but the biochemical properties of the transformed cya host implicate the presence of low levels of enzymatic activity. These data suggest that the structure of the intact enzyme is composed of discrete functional domains. Such a structure for this adenylate cyclase would both facilitate investigations of the chemical mechanism of the reaction and allow structure-function relationships in this physiologically important enzyme to be investigated on a molecular level.

Adenylate cyclases (ATP pyrophosphate lyase (cyclizing), EC 4.6.1.1) catalyze the intramolecular transfer of the adenyl group of ATP from pyrophosphate to the 3'-hydroxyl group to form cyclic AMP. Very few investigations of the chemical mechanism of this reaction have been reported due to the extreme difficulty in obtaining large amounts of homogeneous enzyme from any source. This laboratory has reported stereochemical studies of the mechanism of the enzyme isolated from Brevibacterium liquefaciens using phosphorothioate (1) and oxygen chiral phosphate ester (2) methodologies, and in both cases we found that the transfer of the adenyl group occurs with inversion of configuration at phosphorus. These findings are persuasive evidence that the transfer reaction does not involve the formation of an adenylated enzyme intermediate. Eckstein and his coworkers have reached a similar conclusion regarding the mechanism of the enzyme from bovine brain (3). These experiments were possible because stereochemical studies do not require large amounts of pure enzymes but only that the enzyme preparation contain a single enzymatic activity capable of interconverting substrates and products. However, the identification of the general basic catalyst we have proposed to be present in the active site (1) and spectroscopic investigations of bound substrates and products are simply not possible with the currently available sources of enzyme.

Wang et al. recently reported the cloning of the gene for the adenylate cyclase (cyt) produced by Salmonella typhimurium (4). The size of the catalytically active protein encoded by the gene was found to depend reproducibly on the precise vector in which the 7.1-kb EcoRI restriction fragment containing the cya gene was inserted, with the observed polypeptides ranging in size from 78,000 to 81,000 Da. In addition, one deletion mutant that produced measurable adenylate cyclase activity in a cya- strain of Escherichia coli was found to encode for a 67,000-Da polypeptide; this mutant lacked no more than 450 base pairs from one end of the cloned EcoRI fragment. These data were interpreted as evidence for the EcoRI restriction fragment encoding for an active enzyme fragment, i.e. the coding region for the cya gene extended beyond one of the EcoRI sites in the intact chromosome. This conclusion is consistent with an estimated molecular weight of 92,000 for the purified enzyme from E. coli (5).

Although a multiple copy plasmid containing the cya gene fragment might be directly useful in facilitating purification of this biochemically interesting adenylate cyclase, we felt that an investigation of the structure of the cya gene and its relationship to catalytic activity should precede any attempts at purifying the enzyme. The reasons for this decision are as follows.

1) The apparent insensitivity of catalysis to deletion of the carboxy-terminal end of the polypeptide suggested that the active site may be entirely contained in a structural domain considerably smaller than the intact enzyme molecule. If this hypothesis were correct, the ability to obtain only this portion of the molecule would facilitate studies of the reaction mechanism.

2) The presence of a small catalytic domain could suggest that the portion of the protein not required for catalysis has another important function. The enzymes from E. coli and S. typhimurium are involved in catabolite repression (6), and the regulatory properties crucial to this function may reside in a second domain of the intact enzyme molecule.

3) The most effective use of recombinant DNA techniques to obtain large amounts of a protein requires detailed knowledge of the structure of the gene.

We have constructed a family of recombinant plasmids with deletions of increasing length from the 3'-end of the

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1 The abbreviation used is: kb, kilobase.
cloned cya gene fragment from *S. typhimurium*. After transformation of a cya strain of *E. coli* with these plasmids, we found that approximately 900 base pairs could be deleted without a significant change in the adenylate cyclase activity measurable in toluenized cells; the largest such deletion reduced the size of the adenylate cyclase polypeptide from 77,000 to 46,000 Da. Although further deletion of DNA eliminated the ability to detect enzyme activity, at least 500 additional base pairs (for a total of 1400 base pairs) could be deleted without the complete loss of cya phenotypes in the transformed host; the smallest polypeptide having this property had a molecular weight of 33,000. These observations are consistent with this enzyme having its active site located in a structural domain considerably smaller than the intact enzyme molecule.

**EXPERIMENTAL PROCEDURES**

Restriction endonucleases were purchased from New England Biolabs, Bethesda Research Laboratories, and Boehringer Mannheim. T4 DNA ligase was from either New England Biolabs or L. Biochemicals. Nucleases Bal 31 and E. coli DNA polymerase (Klenow fragment) were obtained from New England Biolabs. T4 polynucleotide kinase was the product of L. Biochemicals. All enzymes were used according to the suppliers' instructions.

Octanucleotide EcoRI and HindIII linkers were from New England Biolabs and were phosphorylated with polynucleotide kinase before use. [α-32P]ATP was obtained from New England Nuclear. [32P]Methionine was the product of Amersham Corp. All other reagents were of the highest grade commercially available.

A recombinant plasmid, pCK102, with the 7.1-kb EcoRI restriction fragment of *S. typhimurium* DNA containing the cya gene fragment inserted in the EcoRI site of pBR322, was the generous gift of Professor Daniel E. Koshland, Jr., University of California at Berkeley.

**Bacterial Strains**—Two strains of *E. coli* with deletions in cya and deficient in restriction endonuclease activity were used in these studies. The early part of our work utilized a spontaneous mutant of *E. coli* strain CA8306 (obtained from J. Beckwith via the *E. coli* Genetic Stock Center located at the Yale University School of Medicine, CGSC 6027) selected according to the procedure of Pichaczek et al. (17); the required bacterial strains were generously provided to us by Professor Pichaczek, Laboratoire de Biochimie, Centre Paul Lamarque, Montpellier, France. This strain was assessed to be deficient in both host restriction and modification activity (hadR<sup>−</sup>, hadM<sup>−</sup>) as well as in adenylate cyclase activity (cya<sup>−</sup>).

We later constructed a strain of *E. coli* deficient in adenylate cyclase activity (cya<sup>−</sup>) and in host restriction but not modification activity (hadR<sup>−</sup>, hadM<sup>−</sup>) as a more versatile cloning vector. This strain was obtained as follows. P<sub>1</sub> transducing phage stock was prepared from *E. coli* strain SK2226 (obtained from S. Kushner via the *E. coli* Genetic Stock Center, CGSC 6420); this strain is cya<sup>−</sup> and resistant to tetracycline (*tet<sup>+</sup>*) by virtue of the *rie-290::Tn10* chromosomal marker located at approximately 84 min on the *E. coli* genetic map (8). This phage stock was used to transduce *E. coli* strain LS833 (obtained from L. Soll via the *E. coli* Genetic Stock Center, CGSC 5891); this strain is cya<sup>−</sup> by virtue of a deletion in cya which is located at approximately 84 min on the *E. coli* genetic map. Several cya<sup>−</sup> and *tet<sup>+</sup>* transductants were purified, and P<sub>1</sub> phage stock was prepared from one. The phage stock was used to transduce *E. coli* strain MM294 (obtained from M. Meselson via the *E. coli* Genetic Stock Center, CGSC 6310); this strain is hadR<sup>−</sup>, hadM<sup>−</sup>, and cya<sup>−</sup>. Several cya<sup>−</sup> and *tet<sup>+</sup>* transductants were purified and one was designated strain JG1 and used as the cloning vector in our later experiments; JG1 retains the TnlO chromosomal marker and is *tep*. Also, MM294 is a motile strain, but JG1 is not by virtue of its being cya<sup>−</sup>; however, JG1 is motile in the presence of added cyclic AMP.

*E. coli* strain CSR003 (obtained from A. Sancar via the *E. coli* Genetic Stock Center, CGSC 5830) was used in the maxi-cells procedure.

**Construction of pTL292**—A 2.92-kb piece of the EcoRI insert in pCK102 which contains the cya gene fragment and several hundred additional base pairs upstream from the cya coding region was positioned in pBR322 so that a unique EcoRI site was located at the 3'-end of the cya gene and a unique HindIII site was located at the upstream end; the steps involved in this construction are summarized in Fig. 1.

Briefly, pCK102 contains seven *BglI* restriction sites, and the largest *BglI* fragment includes 3.3 kb of insert DNA and 0.9 kb of pBR322 DNA extending from the 3'-end of the EcoRI construct into the *P-lactamase* gene. The ends of this fragment were made blunt with the *Klenow* fragment of DNA polymerase I, and HindIII linkers were ligated to the ends. Following digestion with EcoRI and HindIII, a 3.3-kb fragment of *S. typhimurium* derived DNA with an EcoRI sticky end at the 3'-end of the cya gene and a HindIII sticky end at the opposite end of the fragment was isolated; this was ligated with the larger EcoRI-HindIII fragment of pBR322 to produce a plasmid designated pTL330 (the number of this and all other plasmids we describe in the text).

![Fig. 1. Steps involved in the construction of pTL292. pTL292 contains a unique EcoRI site at the 3'-end of the cya gene and a unique HindIII site upstream from the promoter for the cya gene.](http://www.jbc.org/)
this paper refers to the number of decibases in the S. typhimurium-derived DNA in the plasmid.

pTL320 was digested with HindIII and subjected to limited digestion by nuclease Bal31 to remove DNA upstream from the promoter region for cya so that coding regions for other proteins known to be present in the original 7.1-kb EcoRI fragment would be deleted. HindIII linkers were directly ligated to the nuclease Bal31-digested DNA, and following digestion with HindIII, ligation, and transformation, the phenotypes on McConkey/lactose plates containing ampicillin were determined. The S. typhimurium insert DNA in cya+ colonies was sized by agarose gel electrophoresis, and a plasmid with approximately 300 base pairs removed from the S. typhimurium DNA and 600 base pairs from pBR322 was chosen and designated pTL292.

Construction of the Family of Plasmids with Deletions at the 3'-end of the cya Gene—Deletions were created at the 3'-end of the cya gene by limited nuclease Bal31 digestion of plasmid linearized with EcoRI; the steps involved in such a construction are summarized in Fig. 2.

In a preliminary investigation of the influence of extent of deletion from the 3'-end of the cya gene on adenylate cyclase activity, pTL292 was linearized with EcoRI, and the purified linear DNA was digested for varying lengths of time with nuclease Bal31 to accomplish deletions as large as 1.3 kb. The mixture of shortened DNA fragments was directly ligated with EcoRI linkers, and following digestion with both EcoRI and HindIII, the pieces of DNA derived from S. typhimurium were isolated by agarose gel electrophoresis. These fragments were ligated with the larger EcoRI-HindIII fragment of pBR322, and the ligation mixture was used for transformation. By virtue of the phenotype on McConkey/lactose plates and determination of the sizes of the associated S. typhimurium DNA, we concluded qualitatively that the conversion of active enzyme to considerably less active forms occurred when 0.6 to 1.0 kb of DNA had been deleted from the 3'-end of the cya gene.

Additional deletions in this critical region were generated by linearizing pTL265 (obtained from the preliminary experiment) with EcoRI and digesting the linearized plasmid with nuclease Bal31 for varying lengths of time. Following ligation with EcoRI linkers, isolation of the EcoRI-HindIII fragments containing S. typhimurium DNA, and ligation with the larger EcoRI-HindIII fragment of pBR322, transformation of JG1 yielded pink, white/pink, and white phenotypes on McConkey/lactose plates and determination of the EcoRI and HindIII sites. The pieces of DNA derived from S. typhimurium were directly ligated with EcoRI linkers, and following digestion with HindIII, ligation, and transformation with pTL265 (obtained from the preliminary experiment) resulted in a family of plasmids with varying extents of deletion from the 3'-end of the cya gene.

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Determination of Extent of Deletion in the Cya Gene—The size of the S. typhimurium DNA in recombinant plasmids was determined by comparison of the electrophoretic mobilities of appropriate restriction fragments with those of standards prepared by restriction endonuclease digestion of pBR322. The size of the entire insert was determined by digestion with EcoRI and HindIII. A more precise estimate of the relative sizes of the cloned fragments was accomplished by digestion with EcoRI and BstEII. The S. typhimurium DNA in plasmids including and derived from pTL292 contains three BstEII sites; pBR322 contains no BstEII sites. The largest EcoRI-BstEII fragment derived from the insert DNA contains the Sau3A site mapped by Wang et al. (4) and possesses the 3'-end of the cya gene.

RESULTS AND DISCUSSION

Phenotype of Transformants on McConkey/Lactose Plates—Using the phenotypes of transformants on McConkey/lactose plates as the

![Fig. 2. Steps involved in deletion of DNA from the 3'-end of the cya gene.](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3875184/)
initial screening criterion, we identified three classes of deletion mutations of the cya gene: Class I, having uniformly pink colonies and *S. typhimurium* DNA sizes ranging from 2.92 kb (no deletion from the 3' end of the cya gene fragment cloned by Wang et al. (4)) to 2.00 kb (0.92 kb deletion); Class II, having predominantly white colonies with pink centers and insert DNA sizes ranging from 1.85 kb (1.07 kb deletion) to 1.54 kb (1.38 kb deletion); and Class III, white colonies with insert DNA sizes less than 1.54 kb. Several Class I and II mutants were selected for further characterization, and the recombinant plasmids chosen are listed in Table I.

**Level of Adenylate Cyclase Activity**—The amount of adenylate cyclase activity found in JG1 transformed with each of the plasmids listed in Table I was measured, and the values obtained from two independent duplicate determinations are included in Table I to provide an indication of the variability we find in assays using toluenized cells as the source of enzyme. Our assay is not sufficiently sensitive to detect enzyme activity in MM294, a cya+ strain, but all the Class I mutants have easily measurable amounts of enzyme activity. We attribute the enzyme activity produced by the Class I mutants to overproduction of catalytically active polypeptides encoded by the cya gene fragments present in the multiple copy plasmids. We do not believe that the high levels of enzyme activity in the Class I mutants can be explained by functional complementation of an inactive polypeptide encoded by the uncharacterized chromosomal deletion mutation in JG1, since we can detect high levels of enzyme activity in the cya+ strain MM294 transformed with the plasmids containing Class I deletion mutations (data not shown). In addition, such an explanation would require that an inactive plasmid-encoded polypeptide would bring about significantly enhanced production of the chromosomal gene product, and we regard this as unlikely.

**Sizes of the Polypeptides Encoded by the Deletion Mutants**—A picture of an autoradiograph showing the sizes of the polypeptides produced by three Class I deletion mutants, pTL292, pTL242, and pTL200, and one Class II deletion mutant, pTL154, is reproduced in Fig. 3. Each plasmid encodes for two polypeptides, the β-lactamase encoded by the bla gene of pBR322 and the cya gene product. The size of β-lactamase, 28,000 Da, is in accord with the established value (12). The size of the cya gene product decreases as the extent of DNA deletion increases, with the decreases in size being in good agreement with those predicted from the amount of DNA deleted. pTL292, which contains the gene fragment cloned by Wang et al. (4), produced a gene product having a molecular weight of 77,000; pTL200, the smallest Class I mutant, produced a gene product having a molecular weight of 46,000; and pTL154, the smallest Class II mutant, produced a gene product having a molecular weight of 33,000.

Reading in the counterclockwise direction from the EcoRI site in the nucleotide sequence for pBR322 (12), termination codons in each of the three reading frames occur at map positions 4317, 4339, and 4358; thus, at most 14 amino acids will be present at the carboxyl termini of the cya gene products by virtue of their termination in the pBR322 sequence.

These measurements confirm the conclusion reached by Wang et al. regarding the cloned EcoRI fragment not having an intact cya gene (4). Together with the adenylate cyclase activities reported in Table I, these data also show that the molecular weight of the cya gene product can be reduced to 46,000 without significantly altering catalytic activity.

**Effect of Deletions on the Rate of β-Galactosidase Synthesis**—The synthesis of β-galactosidase requires the presence of cyclic AMP (6). The data in Table I also reveal that all of the Class I mutants are able to rapidly synthesize β-galactosidase in the presence of the inducer isopropylthiogalactoside; a lower but still significant rate of β-galactosidase synthesis is supported by the cya gene products produced by the Class II mutants. These data are in accord with the phenotypes observed on McConkey/lactose plates.

Wang et al. reported that *E. coli* transformed with pBR322 containing the intact 7.1-kb EcoRI restriction fragment had an intracellular concentration of cyclic AMP nearly double that found in a wild type strain (4). Although we have not measured the intracellular concentrations of cyclic AMP in JG1 transformed with our deletion mutants, we expect the Class I mutants to have similar high levels of cyclic AMP, and this is in agreement with the rapid rates of β-galactosidase synthesis.

**Table I**

| Strain/plasmid | Insert size (kb) | McConkey phenotype | Enzyme activity† | β-Galactosidase* | Generation timeº | Motility* |
|----------------|-----------------|--------------------|------------------|-----------------|-----------------|-----------|
| JG1/pTL292     | 2.92            | Pink               | 2.01, 2.17       | 0.65            | 96              | +++       |
| JG1/pTL385     | 2.65            | Pink               | 0.45, 0.56       | 0.52            | 101             | +++       |
| JG1/pTL242     | 2.42            | Pink               | 0.77, 2.19       | 0.49            | 138             | +++       |
| JG1/pTL228     | 2.28            | Pink               | 0.46, 0.95       | 0.58            | 100             | +++       |
| JG1/pTL221     | 2.21            | Pink               | 1.68, 0.68       | 0.57            | 111             | +++       |
| JG1/pTL217     | 2.17            | Pink               | 0.38, 1.44       | 0.56            | 99              | +++       |
| JG1/pTL212     | 2.12            | Pink               | 0.78, 0.86       | 0.64            | 129             | +++       |
| JG1/pTL203     | 2.03            | Pink               | 2.65, 2.41       | 0.41            | 126             | +++       |
| JG1/pTL200     | 2.00            | Pink               | 0.50, 1.15       | 0.41            | 135             | +++       |
| JG1/pTL185     | 1.85            | White/Pink         | ND*              | 0.10            | 62              | +         |
| JG1/pTL173     | 1.73            | White/Pink         | ND*              | 0.07            | 64              | +         |
| JG1/pTL167     | 1.67            | White/Pink         | ND*              | 0.09            | 72              | +         |
| JG1/pTL154     | 1.54            | White/Pink         | ND*              | 0.09            | 71              | +         |
| JG1/pBR322     |                 | White              | ND*              | 0               | 115             | 0         |
| MM294          |                 | Pink               | ND*              | 0.45            | 60              | +++       |

* Estimated error, ±0.2 nmol of cyclic AMP/30 min/mg of protein.
† Rate of synthesis of β-galactosidase expressed as ΔΔA₄₂₀ nm/min/ΔA₃₉₅ cm. The values are averages of at least two determinations.
º The values are averages of at least three determinations.
‡ ++++, motility unaffected by addition of exogenous cyclic AMP; ++ and +, motility increased by addition of exogenous cyclic AMP; 0, nonmotile in the absence of exogenous cyclic AMP.
* Not detectable.
The mobilities of protein standards (phosphorylase b, bovine serum albumin, pyruvate kinase, ovalbumin, and carbonic anhydrase) are indicated on the left and the sizes of the polypeptides encoded by the blu and cya genes are indicated on the right.

synthesis found for these mutants. Our data demonstrate that Class II mutants are also capable of synthesizing low levels of cyclic AMP, since these can slowly make β-galactosidase. The behavior observed for the Class II mutants cannot be unequivocally explained at this time; either their cya gene products have low levels of catalytic activity or they functionally complement an inactive fragment of adenylate cyclase which is produced by the uncharacterized chromosomal deletion in JG1.

Cell Motility—The synthesis of flagellar proteins requires the presence of cyclic AMP (6). The Class I mutants confer motility on JG1 which is unaffected by the presence of added cyclic AMP; the Class II mutants are motile but the motility is increased by the presence of added cyclic AMP (Table I). The latter observation implies that the Class II mutants are able to synthesize low levels of cyclic AMP.

Generation Times—All Class I mutants grow slowly; Class II mutants grow more rapidly and are similar to MM294 in generation time (Table I). Wang et al. observed that a cya+ strain of E. coli transformed with pBR322 containing the intact 7.1-kb EcoRI fragment grew more slowly than did the untransformed strain (4), and the low growth rates of our Class I mutants may be attributed to the elevated levels of cyclic AMP likely to be present in these cells. That the Class II mutants have generation times similar to that measured for MM294 and considerably shorter than that measured for JG1 implies that these strains are capable of synthesizing low levels of cyclic AMP.

Conclusions and Predictions—We have identified and characterized two classes of deletion mutations of the cya gene from S. typhimurium which permit the synthesis of cyclic AMP despite extensive deletions of the carboxyl-terminal of the intact enzyme molecule. The largest Class I deletion results in the synthesis of an active polypeptide which has a molecular weight of 46,000 or about one-half of that thought to be associated with the intact enzyme (5). Since the catalytic activity of this enzyme species is not significantly different than that measured for the polypeptide with a molecular weight of 77,000, we conclude that the carboxyl-terminal half of the intact enzyme is not directly involved in catalysis. The largest Class II deletion results in the synthesis of polypeptide having a molecular weight of 33,000, or about one-third of the intact enzyme molecule; although this mutant does not have assayable levels of adenylate cyclase activity, the other tests for the presence of cyclic AMP are positive. Irrespective of the reason for the low levels of adenylate cyclase activity implicated for the Class II mutants, it remains clear that at least half of the intact enzyme molecule is not directly involved in catalysis.

The retention of catalytic activity by the NH2-terminal half of the intact enzyme molecule suggests that the intact enzyme is composed of at least two discrete structural and functional domains as outlined in Fig. 4. At present we cannot define the function of the carboxyl-terminal half of the intact enzyme, although it is likely that this portion of the molecule is responsible for the regulation of the level of cyclic activity expressed by the NH2-terminal half. This hypothesis implies that metabolites such as glucose which are known to alter the catalytic activity of the intact enzyme (6) interact (directly or indirectly) with the presumed carboxyl-terminal domain, causing a change in the interdomain interactions and a modulation of the catalytic activity. Such a structure for the cya gene product would predict that mutants in the regulation of catalytic activity should be isolable, and some properties of a mutation of this type were described recently (13). The functional structure of this prokaryotic adenylate cyclase may prove to be conceptually analogous to those of the eucaryotic adenylate cyclases in which separate protein components have been identified and assigned separate catalytic and regulatory functions (14).

Our observations should be relevant to further studies of this adenylate cyclase. Since the intact enzyme is thought to be monomeric (5), the enzyme fragments produced by our deletion mutants should also be monomeric; their reduced molecular weights should permit high resolution: NMR and ESR studies of bound substrates and products in analogy to those which have been performed by Cohn and Reed and their coworkers on various kinases (15) and also simplify chemical investigations of the functions of amino acid residues present in the active site. In addition, in vitro and in vivo experiments comparing the biochemical properties of the separate domains of the adenylate cyclase molecule with those of the intact enzyme should also be possible, thereby providing a method for investigating structure-function relationship in the regulation of activity and the interactions of the enzyme with other biochemical systems in the bacterial cell.

In the absence of purified enzyme, we have been able to use recombinant DNA techniques to infer an interesting and
potentially important feature of the structure of the adenylate cyclase from *S. typhimurium*. We fully expect that further characterization of the cya gene will allow the overproduction of the intact enzyme and its functional domains, and this ability should prove useful in understanding both the reaction mechanism and the *in vivo* function of this very important enzyme.

Subsequent to the completion of the research reported in this article, relevant observations about the structure and function of the *E. coli* cya gene and gene product were published. Roy and Danchin reported that the polypeptide encoded by the cya gene has a molecular weight of 95,000 as determined by the maxicell procedure (16); this value is in excellent agreement with that determined for the purified protein (5). These investigators also reported that carboxyl-terminal deletions of the *E. coli* cya gene produced by restriction endonuclease digestion of the DNA retain the ability to complement a host chromosomal cya deletion; the sizes of these polypeptides were 88,000 and 48,000. In collaboration with Joseph and Ullman, these investigators very recently reported that the deletion mutations are catalytically active but do not retain the ability to be inhibited by glucose (17). Their interpretations are in qualitative agreement with those discussed in this manuscript, and our data provide a precise estimate of the size of the catalytic domain present in the *S. typhimurium* enzyme.

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