Cyclic AMP and Cell Division in Escherichia coli

RICHARD D'ARI,* ALINE JAFFÉ, PHILIPPE BOULOC, AND ALINE ROBIN

Institut Jacques Monod,† 75251 Paris Cedex 05, France

Received 22 June 1987/Accepted 5 October 1987

We examined several aspects of cell division regulation in Escherichia coli which have been thought to be controlled by cyclic AMP (cAMP) and its receptor protein (CAP). Mutants lacking adenyl cyclase (cya) or CAP (crp) were rod shaped, not spherical, during exponential growth in LB broth or glucose-Casamino Acids medium, and lateral wall elongation was normal; in broth, stationary-phase cells became ovoid. Cell mass was smaller for the mutants than for the wild type, but it remained appropriate for their slower growth rate and thus probably does not reflect early (uncontrolled) septation. The slow growth did not seem to reflect a gross metabolic disorder, since the mutants gave a normal yield on limiting glucose; surprisingly, however, the cya mutant (unlike crp) was unable to grow anaerobically on glucose, suggesting a role for cAMP (but not for CAP) in the expression of some fermentation enzymes. Both cya and crp mutants are known to be resistant to mecillinam, an antibiotic which inhibits penicillin-binding protein 2 (involved in lateral wall elongation) and also affects septation. This resistance does not reflect a lack of PBP2. Furthermore, it was not simply the result of slow growth and small cell mass, since small wild-type cells growing in acetate remained sensitive. The cAMP-CAP complex may regulate the synthesis of some link between PBP2 and the septation apparatus. The ftsZ gene, coding for a cell division protein, was expressed at a higher level in the absence of cAMP, as measured with an ftsZ::lacZ fusion, but the amount of protein per cell, shown by others to be variable over a 10-fold range of cAMP, was independent of cAMP, suggesting that ftsZ expression is not regulated by the cAMP-CAP complex.

Cell division in gram-negative bacilli such as Escherichia coli involves invagination of the three envelope layers—cytoplasmic membrane, peptidoglycan, and outer membrane—with concomitant formation at the cell center of a septum, which then provides the new hemispherical poles for the two daughter cells. This complex biochemical process is carried out by a number of enzymes. The rigid peptidoglycan layer responsible for maintaining the cylindrical shape of the cell is synthesized and cross-linked at least in part by penicillin-binding proteins (PBPs), some of which seem to be specifically involved in septation. Other septation functions have been identified genetically in E. coli, generally by fts (filamentation thermosensitive) mutations, although the molecular activities of these gene products remain to be elucidated.

During steady-state growth, E. coli cell division appears to be strictly regulated; septation takes place precisely at the center of the cell and at a precise cell mass whose value depends on the growth rate (9, 21). Virtually nothing is known about the molecular bases of this spatial and temporal regulation, and no specific regulatory functions have been clearly identified. Several observations, however, summarized below, have led to the tentative suggestion that cyclic AMP (cAMP) and its receptor protein (CAP) may regulate certain aspects of cell division in E. coli.

The cAMP-CAP complex is a positive transcriptional regulator of a number of catabolite operons in E. coli and as such plays a role in catabolite repression, whereby secondary carbon sources are not catabolized in the presence of glucose (4, 23, 32). The cAMP-CAP complex regulates other operons as well, both positively and negatively, and mutants lacking either adenylate cyclase (cya) or CAP (crp) have a highly pleiotropic phenotype in addition to their inability to utilize a broad range of normally well catabolized carbon sources. Several aspects of their phenotype seem to involve the regulation of cell division, suggesting that the cAMP-CAP complex regulates the transcription of one or more division genes. First, cya and crp strains have abnormal cell morphology; some authors have reported spherical cells rather than rods (15, 16), suggesting an excess of septation over lateral wall elongation, and cell mass is small. Second, cya and crp mutants are resistant to the β-lactam antibiotic mecillinam (1, 13). This antibiotic binds preferentially to PBP2 and prevents lateral wall elongation, leading to the formation of spherical cells (22, 30). Third, transcription of the cell division gene ftsZ has been reported to be higher in a cya mutant (9), possibly resulting in an excess of septation, since the FtsZ protein is thought to be limiting in cell division (37). Fourth, we have described a type of cell division that seems to require a functional cAMP-CAP complex: anucleate cells are formed whenever DNA synthesis is blocked in the absence of SOS-associated division inhibition, and the aberrant divisions giving rise to these cells are more frequent when the amount of cAMP-CAP complex is increased and abolished in cya and crp mutants (14). Finally, there is a mutant (fic) which stops dividing in the presence of exogenous cAMP at 43°C (35).

In the present study we reexamine these questions in an attempt to determine whether the cAMP-CAP complex is a direct regulator of some cell division function in E. coli.

MATERIALS AND METHODS

Bacterial strains. For the present work, we chose the wild-type E. coli K-12 F- λ- strain GC2700, also known as F88 and UTH1038, because it has not been subjected to the numerous cycles of mutagenesis typical of many laboratory strains. Isogenic cya and crp derivatives were constructed by P1 vir-mediated transduction. GC2700 was first transduced to ilv::Tn5 or cysG::Tn5 and selected on LB plates containing kanamycin (40 μg/ml). The former was then

* Corresponding author.
† A joint institute of the Centre National de la Recherche Scientifique and the Université Paris 7.
transduced to Ilv \(^*\) cya-854 (strain GC2793) and the latter to Cys \(^*\) crp-96 (strain GC2846); these transductants were Mal \(^*\) Lac \(^{-}\) Km \(^*\). A. Ullmann kindly provided the donor strains as well as GC2700; the cya-854 allele is a deletion (6), and the crp-96 allele is nonreverting (26). Phase \(\lambda\) JFLL100, which carries lacZ fused to the ftsZ promoters (9), was used to lysogenize strain GC2793 on a plate containing cAMP, to permit expression of the receptor. The lysogen (strain GC2961) was transduced to cya \(^*\) (selection for Mal \(^*\)) to have an isogenic control (strain GC2962).

**Media and growth conditions.** M63 minimal salts medium (20) was used, containing 0.4% glucose or 0.2% acetate for aerobic growth and 1% glucose or 1% glycerol for anaerobic growth, with further supplements as indicated in the text; Casamino Acids, when used, was added at 0.05%. Complete medium was LB broth (20). Solid media contained 1.5% agar.

Cultures were grown aerobically in Erlenmeyer flasks with vigorous agitation. For anaerobic growth, plates were incubated in a Forma Scientific anaerobic glove box (model 1024) with a carbon dioxide-hydrogen-nitrogen (1:1:18) atmosphere.

All experiments were carried out at 37°C.

**Evaluation of cell number, volume, and morphology.** Bacteria were counted in a model ZI Coulter counter with a 30-\(\mu\)m orifice. Volume distributions were determined with a C1000 channelizer. Photomicrographs were taken through a Zeiss Jenaamed phase contrast microscope; samples were fixed in 3.7% formaldehyde.

**Miscellaneous methods.** P1 transductions and \(\beta\)-galactosidase assays were performed as described by Miller (20).

### RESULTS

**Cell morphology.** Wild-type, cya, and crp bacteria were uniformly rod shaped during exponential growth in LB broth (Fig. 1) and in glucose-Casamino Acids medium (not shown). In stationary phase, when cell volume was smaller (Table 1), all cells remained rod shaped in glucose-Casamino Acids medium (not shown); in LB broth, the mutant cells were ovoid (Fig. 1). These were the only culture conditions in which we observed nearly spherical cells. As expected, addition of 1 mM cAMP to the medium partially corrected the phenotype of the cya mutant but had little effect on the crp and wild-type strains (Fig. 1, Table 1).

**Cell mass and growth.** During steady-state growth, cell division occurs at a precise cell mass, the absolute value of which increases with growth rate (9, 21). Mutants with smaller than normal cell mass could be affected in the temporal regulation of division, triggering septation too early in the cell cycle. To compare the cell mass of the wild-type, cya, and crp strains under steady-state conditions, we used very dilute exponential-phase cultures in which the cell number and the cell volume distribution could be measured with a Coulter counter. Overnight cultures were diluted 10\(^4\)-fold in fresh medium. After 2.5 h, samples were withdrawn periodically for analysis. Cell number increased exponentially in all cultures for over 3 h, while the cell volume distribution remained essentially constant, indicating that the bacteria were in steady state. Under these conditions the cya and crp mutants had a smaller cell volume, both in glucose-Casamino Acids medium and in LB broth (Table 1). Addition of 1 mM cAMP partially corrected the volume of the cya mutant but did not affect that of the wild-type or crp strain (Table 1). During steady-state growth in glucose-Casamino Acids medium, the wild-type strain had a doubling time of 27 min, whereas the cya and crp mutants doubled in 45 and 44 min, respectively (Fig. 2).

The morphology of cya and crp mutant cells during steady-state growth was appropriate for their growth rate; wild-type cells growing in minimal glucose medium with a 51-min doubling time formed rods of similar volumes (Table 1). The smaller cell mass of the mutants is thus more likely to be a secondary result of their slower growth rate than a primary defect in the temporal regulation of division.

It is unclear why the cya and crp mutants grew more slowly than the wild-type strain. The cya mutant has been reported to synthesize low levels of 2-oxoglutarate dehydrogenase, a key enzyme of the tricarboxylic acid cycle (27).

**TABLE 1.** Modal volume of wild-type, cya, and crp strains

<table>
<thead>
<tr>
<th>Phase and medium</th>
<th>1 mM cAMP added</th>
<th>Modal cell volume ((\mu)m(^3))</th>
<th>Wild type</th>
<th>cya</th>
<th>crp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exponential phase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LB broth</td>
<td></td>
<td>2.52</td>
<td>1.36</td>
<td>1.36</td>
<td></td>
</tr>
<tr>
<td>Glucose-Casamino Acids</td>
<td></td>
<td>2.48</td>
<td>2.09</td>
<td>1.32</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td>1.37</td>
<td>1.04</td>
<td>1.06</td>
<td></td>
</tr>
<tr>
<td>Stationary phase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LB broth</td>
<td></td>
<td>0.78</td>
<td>0.50</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>Glucose-Casamino Acids</td>
<td></td>
<td>0.81</td>
<td>0.85</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.85</td>
<td>0.70</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.81</td>
<td>0.74</td>
<td>0.66</td>
<td></td>
</tr>
</tbody>
</table>

* The strains used were GC2700 (wild type), GC2793 (cya), and GC2846 (crp); data for LB broth are from the experiments described in the legend to Fig. 2.
FIG. 1. Morphology of cya and crp mutants. Cultures of strains GC2700 (WT), GC2793 (cya), and GC2846 (crp) were grown to saturation in LB broth, and samples were fixed and photographed. These cultures were diluted 10^4-fold in fresh LB broth with or without 5 mM cAMP and grown to exponential phase; samples were concentrated by filtration, fixed, and photographed. The only cAMP-containing culture shown is in the "+" panel of the cya mutant in exponential phase.

observation that glycerol kinase requires an active cAMP-CAP complex for expression, both aerobically and anaerobically (10).

Mecillinam resistance. Although cya and crp mutants are resistant to mecillinam (25 μg/ml), several observations indicate that PBP2, the principal target of this antibiotic, is still functional in the mutants. First, it is able to bind penicillin (13); second, the addition of mecillinam (1 μg/ml) to cultures of the cya and crp mutants induced spherical morphology, indicating that mecillinam is still able to inhibit PBP2 in these strains; third, exponentially growing mutant cells were rod shaped (Fig. 1), whereas pbpA mutants lacking PBP2 are spherical (28), and when cell division is blocked (for example, by thymine starvation) cya and crp mutants are able to elongate into filaments (not shown) (14).

As shown above, cya and crp mutants exhibit slow growth and small cell mass. Other mutations conferring mecillinam resistance are also associated with slow growth and small mass or spherical morphology (19, 38). It seemed possible that slowly growing cells, which are shorter than fast-growing cells, carry out less cell wall elongation and are therefore resistant to mecillinam (28a). To test this idea, we grew wild-type cells in acetate medium; the generation time was 100 min and the average cell volume was 0.7 μm³. The
This ratio, rather than the amount of FtsZ per mass, is the physiologically relevant parameter and suggests that the cAMP-CAP complex does not regulate ftsZ transcription.

**DISCUSSION**

The first role attributed to the cAMP-CAP complex in *E. coli* was as a mediator of catabolite repression, acting as a positive transcriptional regulator of a number of catabolic operons (4, 23, 32). Subsequently, it has been found to be involved in other major regulatory networks. The phosphoenolpyruvate-dependent phosphotransferase system, responsible for the transport of numerous hexoses, is intimately related to the level of cAMP-CAP activity in the cell (25), and there are less well defined connections between cAMP-CAP and the stringent response (5, 11). In the present study we have examined the various lines of evidence that the cAMP-CAP complex may play a role in cell division regulation.

Cell mass at the moment of division is a function of growth rate and presumably reflects the temporal control of division. We show here that the smaller cell mass of cya and crp mutants is appropriate to their growth rate, being essentially
the same as that of wild-type cells growing in a poorer medium at the same growth rate. This suggests that the mechanisms coupling cell division to growth rate function correctly during steady-state growth in the absence of a functional cAMP-CAP complex.

The slow growth of cya and crp mutants remains unexplained. Certain enzymes that are involved in the central metabolic pathways and known to have low levels of expression in the absence of CAMP (8, 27) may become growth rate limiting. In the course of our metabolic studies, we found that our cya mutant was unable to ferment glucose, although the crp mutant grew well anaerobically. This is presumably a direct result of the loss of adenyl cyclase, since the addition of exogenous cAMP restored growth. The cya defect seems to be specific to fermentation, without affecting anaerobic respiration. This suggests that cAMP is required for the expression of some fermentation enzyme but that its mediator is not CAMP. It is interesting, however, that a crp* allele, which permits CAMP-promoted expression of certain catabolic operons in the absence of CAMP (26), restored fermentation ability to the cya mutant. It has been reported that CAMP, but not CAMP, is required for the expression of fumarate reductase (34), but it was not clear that there was growth without CAMP or Casamino Acids (33). Fermentation ability is one of the few instances in which the cya and crp mutants exhibit distinct phenotypes. Other examples, observed in aerobic conditions, may be related to this. Whereas both mutants make small colonies on plates, as expected from their slower growth rate and lack of motility (39), the size of the crp mutant is distinctly smaller than those of the crp strain; this may reflect oxygen limitation at some stage of colony formation. Similarly, although both mutants have the same growth yield as the wild type in limiting glucose, the maximum yield in excess glucose is slightly higher for the cya mutant than for the crp and wild-type strains; oxygen limitation may result in carbon wasting in the latter strains via less efficient fermentative pathways.

The β-lactam mecillinam specifically inhibits PBPs and induces spherical cell shape (22, 30). Certain resistant mutants have lost the ability to carry out cell elongation. These include mutations in pbpA, the PBP2 structural gene (28), and rodA, an adjacent gene of unknown function (29). Such mutants are spherical even in the absence of mecillinam and apparently grow by constant septation. The cya and crp mutants, on the other hand, have retained the ability to elongate: they are rod shaped during growth in the absence of mecillinam (Fig. 1) and form filaments when DNA synthesis is interrupted. Their mecillinam resistance is not solely due to their slow growth or small size, since the wild-type strain growing in poor medium remains sensitive.

It has been suggested that the lethality of mecillinam is due to an effect on septation (9). This could be brought about by protein-protein interactions. Evidence has recently been presented for direct interactions between the RodA protein and PBP1 (2) and between PBP3 and FtsA (31), both involved in septation, so the mecillinam-PBP interaction could conceivably affect the septation machinery. If so, this would imply that in the cya and crp mutants some component of the septation apparatus is qualitatively or quantitatively altered, at least in the presence of mecillinam. Further evidence for this comes from the observation that mecillinam stimulates septation at the nonpermissive temperature in the cell division mutant BUG-6 (7), which has been shown to carry a crp(Ts) mutation in addition to the fis allele (3).

The observation (9) that transcription of the fisZ gene is increased in the absence of CAMP suggested a possible target for CAMP-CAP regulation of septation. The FtsZ protein, of unknown molecular activity, seems to be required early in the septation process (36). Its activity is limiting (37), and it has been suggested that FtsZ may be a septation regulator (12). The fisZ gene itself is apparently subject to an unusual type of regulation: the amount of protein per cell (evaluated by means of an fisZ::lacZ fusion) has been reported to be constant over a 10-fold range of cell mass (9). We show here that this regulation does not require CAMP; the higher specific activity in the cya mutant is exactly compensated for by its smaller cell size (Fig. 3). By this criterion, then, fisZ expression is not regulated by the CAMP-CAP complex.

The CAMP-CAP complex has been shown to play a division-promoting role, probably involving the FtsZ protein, in the aberrant divisions that produce anucleate cells when DNA synthesis is arrested (14). The direct target of CAMP-CAP regulation has not yet been identified. We have recently isolated a mutant with a phenotype similar to that of the cya and crp strains (slow growth, small cell mass, and mecillinam resistance), although its CAMP-CAP complex remains functional (manuscript in preparation). We are currently studying the regulation of this gene and the relation of its product to PBPs and the septation apparatus.

ACKNOWLEDGMENTS

We thank Willie Donachie for stimulating discussions and for providing the fisZ::lacZ fusion and Emmanuelle Maguin for her constant encouragement. We are grateful to Agnès Ullmann for the gift of bacterial strains, to Danièle Touati for the use of the anaerobic chamber, to François Képès for help with the Coulter counter, and to Richard Schwartzmann for developing the photographs.

P.B. is the recipient of a postdoctoral fellowship from the Ministère de la Recherche et de l’Enseignement Supérieur. This work was supported in part by grant 6696 from the Association pour la Recherche sur le Cancer.

LITERATURE CITED


