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Enzyme III Stimulation of Cyclic AMP Synthesis in an *Escherichia coli crp* Mutant

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Cyclic AMP (cAMP) synthesis in *Escherichia coli* is altered in cAMP receptor protein mutants and in phosphoenolpyruvate:sugar phosphotransferase transport system mutants. The stimulation of cAMP synthesis observed in cAMP receptor protein-deficient mutants is largely dependent upon enzyme III of the phosphoenolpyruvate:sugar phosphotransferase transport system. The phosphoenolpyruvate:sugar phosphotransferase transport system enzyme I is not required for elevated cAMP synthesis. These results suggest that enzyme III plays an important role in regulating adenylate cyclase activity.

Cyclic AMP (cAMP) is a major mediator of gene expression in bacteria (13), yet regulation of its synthesis via adenylate cyclase is poorly understood at the molecular level. To date, most studies have been directed toward elucidation of the molecular mechanism underlying the control of adenylate cyclase activity by the phosphoenolpyruvate:glycose phosphotransferase transport system (PTS) (3, 10, 11). By a genetic approach, it was found that the two common proteins of this phosphorylation cascade, enzyme I (encoded by *ptsI*) and Hpr (encoded by *ptsH*), are involved in regulation of adenylate cyclase activity. Moreover, enzyme III (encoded by *crr*), a component of the main PTS entry system for glucose, appears to be a key intermediate in this control (3, 11).

Another type of regulation of adenylate cyclase activity involves the cAMP receptor protein (CAP). The first evidence for such a control was the observation that bacteria carrying a mutation or a deletion in the gene coding for CAP (*crp* or Δ*crp*) were found to produce 20 to 100 times more cAMP than the corresponding crp^+ strain (6, 14). Conversely, cAMP synthesis is reduced in a crp^* mutant which synthesizes an altered CAP active in the absence of cAMP (5). Since in Δ*crp* strains the seemingly small increase in the adenylate cyclase activity measured in vitro (8) or in permeabilized cells (6) (about threefold) could not account for the large increase in cAMP synthesis in cultures, Joseph et al. (6) concluded that CAP regulates the activity rather than the synthesis of adenylate cyclase. The molecular control exerted by CAP has not yet been elucidated.

We questioned whether the PTS might play a role in the control of adenylate cyclase activity exerted by CAP. We therefore constructed two double mutants, Δ*crp ptsI* and Δ*crp crr* (Table 1). We then compared the rates of cAMP synthesis in these strains to those obtained in the single Δ*crp*, *ptsI*, or *crr* mutants and in the wild type (Table 2). As shown, the Δ*crp* strain synthesized about 25 times more cAMP during exponential growth than the parental crp^+ strain. Similarly, although the *ptsI* strain synthesized low levels of cAMP (3), its Δ*crp* derivative displayed about the same rate of cAMP synthesis as the Δ*crp* $pstI^+$ strain. In contrast, the rate of cAMP synthesis found in cultures of the Δ*crp crr* strain represented only 5 to 10% of that found with the two other Δ*crp* strains.

These results show that activation of adenylate cyclase by lack of CAP requires a functional PTS enzyme III and does not require a functional PTS enzyme I. Consequently, this activation appears to be independent of phosphorylation of enzyme III by enzyme I. The remaining stimulation of cAMP synthesis found in a *crr crp* double mutant could be due to residual enzyme III activity in the *crr* mutant or it could be due to an independent mode of synthesis.

What might be the mechanism of activation of the adenylate cyclase in the absence of CAP? We believe it unlikely that CAP interacts directly with adenylate cyclase. In fact, the study of various partial mutants of the *crp* gene (1) led us to suggest that CAP controls adenylate cyclase activity either via the transcription of a regulatory protein or by modulating the level of a regulatory metabolite (5). (A hypothesis similar to the latter was also proposed by Dietzler et al. [2] for the control of glycogen synthesis by the cAMP-CAP complex). We further show here that the appearance of a large increase in the rate of cAMP synthesis in the absence of CAP is dependent on the presence of the PTS enzyme III, yet we cannot choose at the present time between the two following possibilities: (i) the presumably indirect control of adenylate cyclase activity exerted by CAP might involve enzyme III as a key intermediate; or (ii) the modulation of adenylate cyclase activity in the cell might result from the interplay of two different regulatory mechanisms, one involving enzyme III, the other involving CAP.

When CAP is lacking, enzyme III can activate adenylate cyclase in the absence of a functional enzyme I. This result, together with the fact that even in the presence of glucose as the carbon source large activation of adenylate cyclase occurs in the absence of CAP in a strain wild type for PTS (14; unpublished data), strongly suggests that apparently nonphosphorylated enzyme III can activate adenylate cyclase. These findings appear to contradict the simple model proposed by Postma (11). According to this model, in the absence of glucose permeation into the cells, the phosphorylated form of enzyme III would activate adenylate cyclase. In the presence of glucose, however, enzyme III, being predominantly in its nonphosphorylated form, could not activate the enzyme. We thus propose that some modulators, other than simply the state of phosphorylation of enzyme III through the PTS cascade, should be considered to account for the whole range of regulation of the adenylate cyclase activity. These putative modulators might act on

TABLE 1. *Escherichia coli* K12 strains used[a]

Strain	Genotype	Derivation	Origin
CA8439	Hfr *thi relA rpsL Δcrp-39 Δcya*		(12)
CH11	Hfr (P045) *ptsI211 relA1 thi-1 bglB7? spoT1* λ^-		W. Epstein; (4) through B. Bachmann
LM1	F^-, *thi argG6 metB his-1 galT rpsL crr manA manI nargE*		(7) through P. Postma
CHE11A	Hfr *relA1 thi-1 bglB7? spoT1*	Mannose-utilizing transductant from CHE11 by P1 grown on wild type.	This work
CHE11A1	Hfr *relA1 thi-1 bglB7? spoT1 rpsL Δcrp-39*	Str^r transductant from CHE11A by P1 grown on CA8439; white colonies on EMB maltose in the absence or presence of cAMP.	This work
CHE111	Hfr *relA1 thi-1 bglB7? spoT1 ptsI211 rpsL Δcrp-39*	Str^r transductant from CHE11 by P1 grown on CA8439; white colonies on EMB maltose in the absence or presence of cAMP.	This work
CHE11B	Hfr *relA1 thi-1 bglB7? spoT1 crr*	$(Mannose)^+$ transductant from CHE11 by P1 grown on LM1; succinate nonutilizing.	This work
CHE11B1	Hfr *relA1 thi-1 bglB7? spoT1 crr rpsL Δcrp-39*	Str^r transductant from CHE11B by P1 grown on CA8439; white colonies on EMB maltose in the absence or presence of cAMP; succinate non-utilizing.	This work

[a] Transductions by P1 *vir* were performed as described in (1). LB, EMB, and M63 are described in (9).

adenylate cyclase either directly or through the mediation of enzyme III. In any event, enzyme III appears to play a crucial role in the multiple regulation of adenylate cyclase activity.

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TABLE 2. Differential rates of cAMP synthesis in strains carrying various combinations of *crp*, *ptsI*, and *crr* alleles[a]

Strains and relevant markers	Doubling time (min)	Rate of cAMP synthesis
CHE11A	44	310
CHE11A1 *Δcrp*	69	8,000
CHE11 *ptsI*	39	125
CHE111 *Δcrp ptsI*	203	10,000
CHE11B *crr*	45	60
CHE11B1 *Δcrp crr*	233	530

[a] The bacteria were grown at 37°C in 0.4% gluconate M63 (9) supplemented with 0.1% Casamino Acids. Samples were periodically removed for measurements of cAMP levels in the entire cultures throughout the exponential growth phase. cAMP determinations were made by radioimmunoassay technique (6). The differential rates of cyclic AMP synthesis which were found to be constant under these conditions for each strain (6) are expressed as the increase in picomoles of cyclic AMP per increase in milligrams (dry weight) of bacteria.