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Control Mechanism of the *Escherichia coli* K-12 Cell Cycle Is Triggered by the Cyclic AMP-Cyclic AMP Receptor Protein Complex

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The role of cyclic AMP (cAMP) in the cell cycle of *Escherichia coli* K-12 was studied in three mutant strains. One was KI1812, in which the *cya* promoter is replaced by the *lacUV5* promoter. In KI1812, isopropyl-β-D-thiogalactopyranoside induced the synthesis of *cya* mRNA, and at the same time cell division was inhibited and short filaments containing multiple nuclei were formed. The other strains were constructed as double mutants {NC6707 *cya sulB* [*ftsZ*(Ts)] and TR3318 *crp sulB* [*ftsZ*(Ts)]}. In both double mutants, filamentation was repressed at 42°C, but it was induced again by addition of cAMP in strain NC6707 and introduction of pHA7 containing wild-type *crp* in TR3318. These results indicate that lateral wall synthesis in the *E. coli* cell cycle is triggered by the cAMP-cAMP receptor protein complex.

We have been studying how cyclic AMP (cAMP) controls cell division and growth in *Escherichia coli* by using the *fic-1* (filamentation induced by cAMP) mutant (7, 11, 12, 27, 28, 31). Formation of rod-shaped cells (6, 13) and production of non-nucleus-containing cells (9, 15) were shown to require the cAMP-cAMP receptor protein (CRP) complex. These results indicate that in *E. coli* a cascade reaction of the cell growth cycle controlled by cAMP should exist. However, exogenous cAMP did not induce cell filamentation in wild-type cells (11, 12, 31), because synthesis of cAMP is controlled negatively by the cAMP-CRP complex at the transcriptional level (2, 10, 26).

In this study, three types of mutants were constructed: a mutant in which the *cya* promoter was replaced by *lacUV5* (KI1812) and two double mutants {NC6707 *cya sulB* [*ftsZ*(Ts)] and TR3318 *crp sulB* [*ftsZ*(Ts)]}. The effects of the cAMP-CRP complex on cell division control in these strains were studied. The results obtained clearly demonstrated that *E. coli* has á cell cycle control mechanism triggered by the cAMP-CRP complex.

We reported that cell filamentation was induced by isopropyl-β-D-thiogalactopyranoside (IPTG) in the *E. coli fic-1* (formerly *fic* [11])-carrying strain harboring pLCR1 in which the *lacUV5* promoter was fused to the *cya* structural gene (26). This result suggested that expression of *cya* could be controlled coordinately with cell growth, because a high level of intracellular cAMP induced abnormal cell length. To confirm this, the KI1812 strain was constructed in which *cya* expression on chromosomes could be induced by addition of IPTG. Strain HY1025 [*polA*(Ts) Δ*cya*::Km^r] (Table 1; 29) harboring pLCR1 was incubated in L broth containing 50 μg of ampicillin per ml and 50 μg of kanamycin per ml at 30°C. The stationary culture was diluted 10-fold in fresh L broth containing 0.1 mg of cAMP per ml and 50 μg of ampicillin per ml and incubated for 4 h at 42°C. The appropriately diluted culture was then spread on MacConkey agar plates containing 1% maltose and 50 μg of ampicillin per ml and incubated at 42°C overnight. One of the white colonies that appeared, the mutant in which the *lac* promoter is fused to *cya* on the chromosome, was isolated as red colonies on a MacConkey plate containing 1% maltose and 0.5 mg of IPTG per ml and named KI1812. We tested whether *cya* mRNA was induced by IPTG or not by using the dot blot method (Fig. 1), which clearly showed that *cya* mRNA was induced by IPTG in KI1812 but not in HY1025.

The effect of *cya* expression on the *E. coli* cell growth cycle was investigated with KI1812. Addition of IPTG to the culture of KI1812 induced short filaments containing multiple nuclei (Fig. 2A) which were not apparent in the absence of IPTG (Fig. 2B). These results show that inhibition of cell division by cAMP can be induced in the wild type but not in the *fic-1* mutant by removing the negative control of *cya* by promoter replacement (2, 10, 26). This led us to conclude

TABLE 1. *E. coli* strains used

Strain	Genotype	Source or reference
MM386	*polA*(Ts) *rha lac* Str^r	T. Horiuchi
HY1025	MM386 Δ*cya*::Km$^{r a}$	R. Utsumi; 26
KI1812	MM386 $P_{lacUV5\text{-}cya}$[b] Ap^r Km^r	This study
5333	HfrH *thi rel crp*	R. Perlman
KS1026	5333 *crp zhd*::Tn*10*	R. Utsumi; 27
MC4100	*araD139* Δ*lacIPOZYA rpsL thi*	M. J. Casadaban
K170	MC4100 Spc^r	K. Ito
OK6201	KI70 *crp zhd*::Tn*10*	P1 (KS1026) × KI70
PAT84	*ftsZ thr leu thi argH thyA his trp lacY1 xyl malA* λ^r *mtl tonA supE mel* Str^r	Y. Hirota; 16
NC6707	PAT84 Δ*cya*::Km^r	P1 (HY1025) × PAT84
TR3318	PAT84 *crp zhd*::Tn*10*	P1 (OK6201) × PAT84

[a] In HY1025, the kanamycin resistance gene derived from pUC4k (purchased from Pharmacia) and inserted in the *cya* structural gene prevented *cya* mRNA from being transcribed.

[b] The *cya* promoter was replaced by the *lacUV5* promoter on the chromosome.

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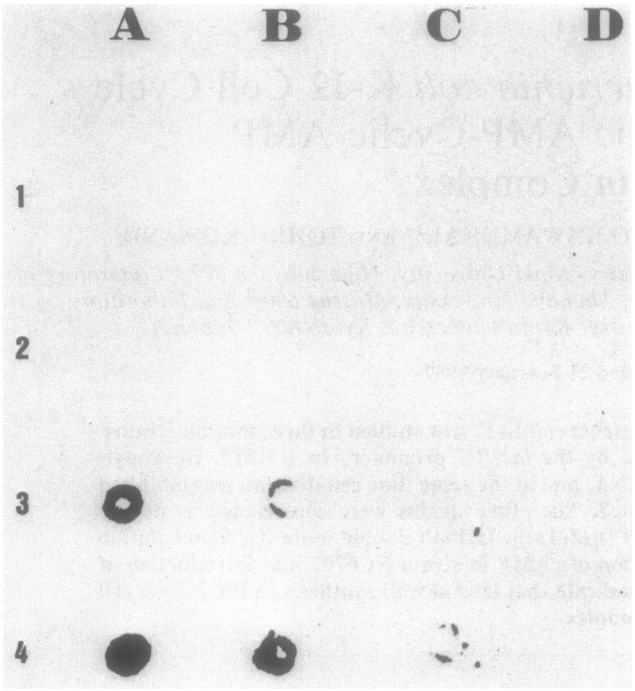

FIG. 1. Induction of *cya* mRNA by IPTG. Overnight cultures of *E. coli* cells (HY1025 in lanes 1 and 2 and KI1812 in lanes 3 and 4) were diluted 10-fold with L broth in the presence (lanes 2 and 4) or absence (lanes 1 and 3) of IPTG (0.25 mg/ml) and incubated for 2 h at 37°C. Total RNAs of HY1025 and KI1812 were prepared by the procedure of Aiba et al. (3). A 2,000-base-pair *Bam*HI-*Hin*dIII fragment of the *cya* structural gene (25) was used as the probe. A 700-ng sample of this fragment was labeled with peroxidase with a Labezyme-POD kit purchased from Wako Chemical Inc., Osaka, Japan. The RNA dot blot method was used as reported previously (19). Denatured cellular RNAs (0.5, 0.25, 0.1, or 0 μg) were spotted on nitrocellulose paper (lanes A, B, C, and D, respectively).

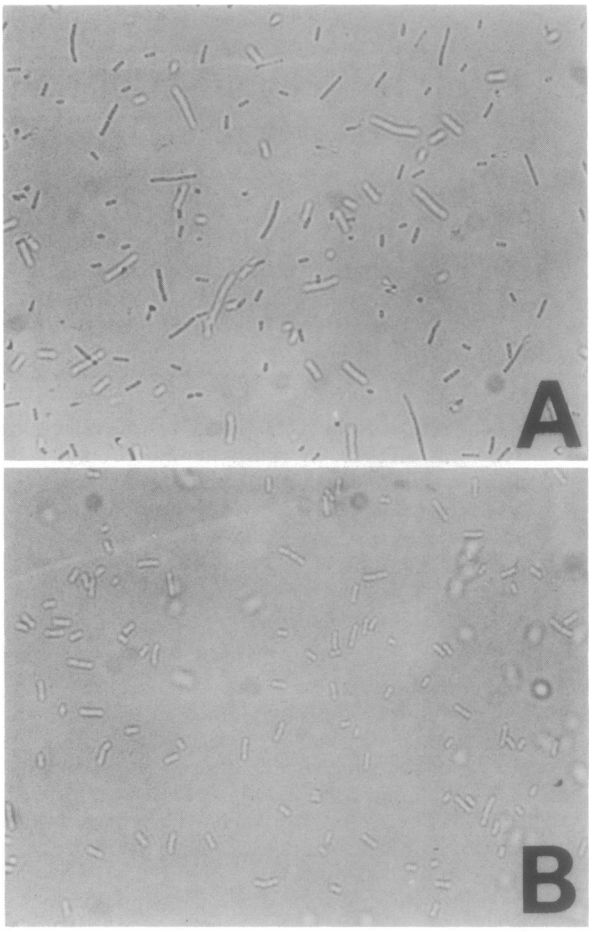

FIG. 2. Induction of cell elongation by constitutive expression of *cya*. A culture of KI1812 was diluted 10-fold in L broth containing IPTG (0.25 mg/ml) and incubated for 2 h at 37°C. The cells were then stained with 4,6-diamidino-2-phenylindole (DAPI) and observed.

that cAMP induces lateral wall synthesis in the *E. coli* cell cycle. To prove this, we constructed double mutants {NC6707-PAT84 *sulB* [*ftsZ*(Ts)] *cya* and TR3318-PAT84 *sulB* [*ftsZ*(Ts)] *crp*} by using P1 transduction from HY1025 (Δ*cya*::Km^r) or OK6201 (KI70 *crp zhd*::Tn*10*) to PAT84 {*sulB* [*ftsZ*(Ts)] mutant} provided by Y. Hirota and investigated the effect of the cAMP-CRP complex on cell division control. The defective properties of these mutants were confirmed. (i) Growth of both strains was temperature sensitive. (ii) The *cya* mutation of NC6707 was complemented by pLCR1 (26) or addition of cAMP. The *crp* mutation of TR3318 was complemented by pHA7 (4) containing the wild-type *crp* gene. In these double mutants, filamentation at 42°C, which is defective because of *sulB* [*ftsZ*(Ts)], was repressed (Fig. 3C and E) but the temperature sensitivity was not suppressed. On the other hand, in NC6707 harboring pLCR1 (data not shown) or cultured in the presence of cAMP, filaments were induced (Fig. 3D), as in the original *sulB* [*ftsZ*(Ts)] phenotype (Fig. 3A). Furthermore, in TR3318 harboring pHA7, filamentation occurred (Fig. 3F). These results clearly indicate that the cAMP-CRP complex triggered the lateral wall in *E. coli*. The phenotypes of NC6707 and TR3318 at 30°C were the same as that of PAT84 (Fig. 3B). It is known that the SulB protein occurs at an early stage of cell division (5) and its overproduction induces minicells (32). The results obtained here indicate that at the restrictive temperature, filamentation in an *sulB* [*ftsZ*(Ts)] mutant is defective because of switching from lateral wall synthesis induced by the cAMP-CRP complex to septum formation. Furthermore, we can understand that during the normal cell cycle, synthesis of the lateral wall induced by the cAMP-CRP complex could occur before the SulB protein works at cell division.

Bacterial rod shape is assumed to depend on the balance of two specific cell wall growth systems; one is responsible for cell elongation, and one is for septum formation (8, 21, 22). Kumar et al. reported that the morphology of a *cya* or *crp* mutant could be defective in lateral wall synthesis (14). We believe that *E. coli* has two peptidoglycan synthesis pathways which are dependent on or independent of the cAMP-CRP complex. In fact, we have succeeded in isolating a cAMP-requiring mutant which seems to lack both the cAMP-dependent and cAMP-independent pathways of peptidoglycan synthesis (30). Short filaments (Fig. 3C and E) were caused by cAMP-independent peptidoglycan synthesis with inhibition of cell division by inactive SulB protein.

The cAMP-CRP complex regulates some kinds of genes positively (24) or negatively (1, 2, 10, 19) at the transcriptional level. To trigger lateral wall synthesis, which genes were controlled by the cAMP-CRP complex? Previously, we succeeded in isolating the cAMP-requiring mutant and

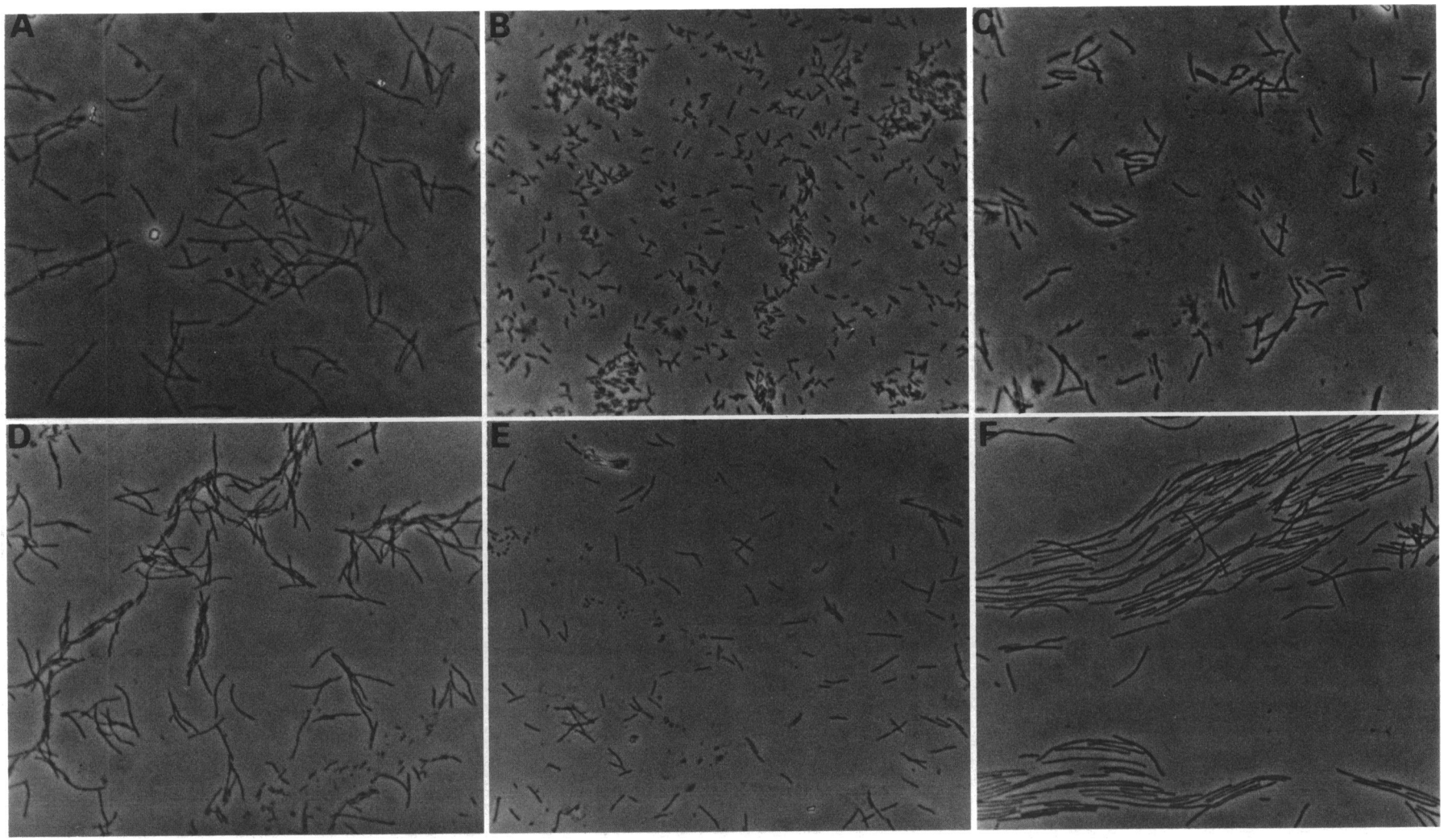

FIG. 3. Lateral wall synthesis triggered by the cAMP-CRP complex. PAT84 (A), TR3318 (E), or TR3318 containing pHA7 (F) was diluted 10-fold in L broth without NaCl and incubated for 2 h at 30°C. NC6707 was cultured in the presence (D) or absence (C) of cAMP (0.2 mg/ml). The culture temperature was then shifted up to 42°C. After 2 h, the cells were observed under a microscope. Panel B shows PAT84 cultured at 30°C. Magnification, ×400.

showed that the mutant needed *N*-acetylglucosamine instead of cAMP to grow (30). From these results, we obtained evidence of growth regulation via *N*-acetylglucosamine metabolism controlled by cAMP. Elongation and septation during cell cycle growth could be switched on and off via amino sugar metabolism controlled by cAMP. In fact, the chemical composition of bacterial cell walls is modulated as a function of the composition of the growth medium and the phase of growth of the organism (18, 20). Furthermore, Toumanen et al. proposed that the chemical composition of the *E. coli* cell wall changes with the growth rate in a manner consistent with alterations in the activity of penicillin-binding proteins and cell shape (23). Such a change could be associated with the control of amino sugar metabolism.

Recently, it has been shown that cAMP has a control role in cell cycle progression in *Saccharomyces cerevisiae* (17). What cAMP controls during such a process remains obscure. We think that the *E. coli* model of growth control by cAMP could apply to *S. cerevisiae*.

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