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Catabolite Repression of *Escherichia coli* Biofilm Formation

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Biofilm formation was repressed by glucose in several species of *Enterobacteriaceae*. In *Escherichia coli*, this effect was mediated at least in part by cyclic AMP (cAMP)-cAMP receptor protein. A temporal role for cAMP in biofilm development was indicated by the finding that glucose addition after ~24 h failed to repress and generally activated biofilm formation.

In the natural environment, bacteria predominantly exist in matrix-enclosed, sessile communities referred to as biofilms (4). Biofilms protect cells from deleterious conditions, such as attack by the mammalian immune system (5). Biofilms are complex assemblages of cells which exhibit channels and pillars that are thought to permit the exchange of nutrients and wastes. A recent model for biofilm development proposes that it is initiated by the attachment of individual cells to a surface, followed by their migration and replication to form microcolonies that eventually produce the mature biofilm (20, 22). A variety of extracellular molecules and surface organelles participate in *E. coli* biofilm formation (6, 7, 23, 33).

Central carbon flux and its regulation may represent key features of bacterial biofilm development. We recently reported that the RNA binding protein CsrA of *Escherichia coli* represses biofilm formation and activates biofilm dispersal (13). The effect of CsrA on biofilm formation is mediated largely through its regulatory role in central carbon flux and intracellular glycogen synthesis and catabolism (17, 18, 24, 25, 28, 34). The influence of CsrA is substantially greater than that of other regulators of *E. coli* biofilm formation, OmpR, RpoS, or the Cpx two-component system (1, 8, 33). Studies with other species have revealed that the global regulator Crc (catabolite repression control) of *Pseudomonas aeruginosa* activates biofilm formation (21), and the expression of the staphylococcal biofilm polysaccharide PIA (polysaccharide intracellular adhesin) requires a functional glucose phosphoenolpyruvate:sugar phosphotransferase system (15).

During studies of biofilm formation, we noted that the addition of glucose to media was inhibitory. To substantiate this observation, *E. coli* K-12 parental strains MG1655, MC4100, and W3110 and their isogenic *csrA* mutants (Table 1) were grown in microtiter wells in colony-forming antigen (CFA) medium (9) with or without glucose (0.2% wt/vol), and biofilm was quantitated after 24 h of growth using crystal violet staining (A_{630}), as described previously (13) (Fig. 1A). Essentially identical results were observed in Luria-Bertani (LB) medium (19) (data not shown). These and other biofilm experiments described in this article were performed at least in triplicate with three samples per experiment, and data were analyzed by Tukey multigroup analysis (Stat View; SAS Institute Inc., Cary, N.C.). Glucose caused a statistically significant decrease in biofilm formation in every case, which varied from ~30 to 95% reduction, depending primarily on the strain background but also on the medium. Biofilm formation by related clinical isolates, including urinary catheter isolates of *E. coli*, *Citrobacter freundii*, and *Klebsiella pneumoniae* and the intestinal pathogens *Salmonella enterica* Typhimurium and *E. coli* O157:H7, was also repressed by glucose in CFA medium (Fig. 1B) or LB medium (data not shown). These effects generally varied from ~50 to 75%. The three urinary catheter isolates exhibited similar repression by glucose in artificial urine medium (13), which mimics the urinary tract environment (data not shown).

TABLE 1. Bacterial strains or phage used in this study

Strain or phage	Relevant genotype or description	Reference or source
E. coli K-12 strains		
MG1655	F^- λ^-	Michael Cashel
CAG18642	*zhf-331*::Tn*10*; 57.5 min	30
MC4100	F Δ(*argF-lac*)*U169 rpsL relA flhD deoC ptsF rbsR*	11
ML2[a]	*met gal hsdK*$_R$ *supE supF* Δ*cya*::*Kan*^{r}	10
SA2777[a]	F *rpsL relA* Δ*crp*::*cam*	S. Garges and S. Adhya
W3110	F^- λ^- *mcrA mcrB* IN(*rrnD-rrnE*)*1*	Richard E. Wolf, Jr.
TR1-5BW3414[a]	*csrA*::Kan^r	25
Clinical strains		
P5	*C. freundii*	14
P18	*E. coli*	14
P30	*K. pneumoniae*	14
EF302	*E. coli* O157:H7	16
ATCC 14028	*S. enterica* serovar Typhimurium	2
Bacteriophage P1*vir*	Strictly lytic P1; forms clear plaques	30

[a] Mutant alleles Δ*cya*::*kanR*, Δ*crp*::*cam*, and *csrA*::*kanR* were moved among strains by P1*vir* transduction or cotransduction. The strain prefix TR indicates the presence of the *csrA*::Kan^r allele.

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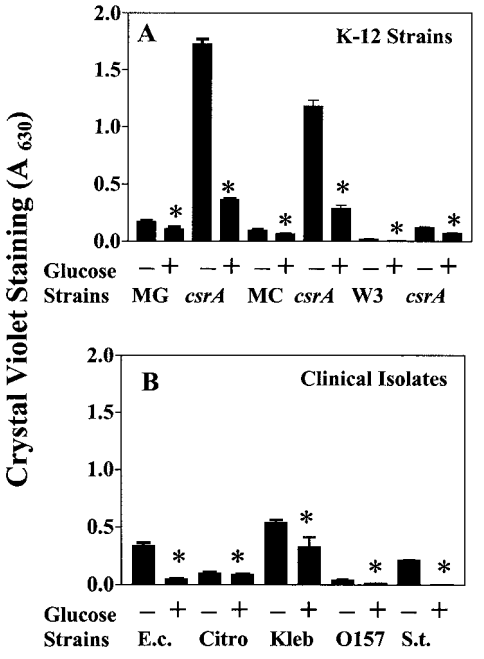

FIG. 1. Effects of glucose on biofilm formation by *E. coli* K-12 strains and their *csrA* mutants (A) and by enteric pathogens (B) in CFA medium. The K-12 parent strains were MG1655 (MG), MC4100 (MC), and W3110 (W). Clinical strains were *E. coli* P18 (E.c.), *C. freundii* P5 (Citro), *K. pneumoniae* P30 (Kleb), *E. coli* O157:H7 EF302 (O157), and *S. enterica* Typhimurium ATCC 14028 (S.t.). Biofilm was determined after 24 h of growth at 26°C in the presence or absence of 0.2% glucose. Each bar shows the average and standard error of three separate experiments ($P < 0.0001$). *, significant difference with respect to cultures lacking glucose. Essentially identical effects of glucose were observed in LB medium (not shown).

The glucose effect, or catabolite repression, is mediated in part by cyclic AMP (cAMP) and cAMP receptor protein (CRP) in *E. coli* (reviewed in references 3 and 29). In classical catabolite repression, transport of glucose leads to dephosphorylation of IIA^{Glc} of the phosphoenolpyruvate:sugar phosphotransferase system, which prevents this protein from activating membrane-bound adenylate cyclase (Cya). The binding of cAMP to CRP forms a complex that interacts specifically and with high affinity to its *cis* elements in the promoter regions of cAMP-regulated genes and thereby regulates transcription. CRP levels also decline during catabolite repression (12, 32). Through these mechanisms, glucose affects the expression of genes located throughout the genome.

To determine whether biofilm formation was subject to classical catabolite repression by cAMP and CRP, the effects of *crp* and *cya* deletions and exogenous cAMP on biofilm formation by MG1655 and its *csrA* mutant were examined. Because cAMP and CRP may have pleiotropic effects on growth, the growth curves of these strains were compared in LB medium (with 0.2% glucose) or CFA medium (lacking glucose) at 26°C with shaking at 280 rpm. Growth rates in LB medium containing 0.2% glucose were unaffected by *cya* or *crp* mutations in MG1655 and very slightly decreased in the *csrA* mutant background (Fig. 2A). However, all of the *cya* and *crp* mutants exhibited substantial growth defects in CFA medium (Fig. 2B). Because of these effects, biofilm formation was corrected for total cell protein to yield specific biofilm values (A_{630} per milligram of protein) in experiments with *cya* and *crp* mutants. Protein assays on cultures containing planktonic and sessile cells were conducted as described previously (13).

The disruption of *crp* in MG1655 or its *csrA* mutant significantly decreased specific biofilm formation (Fig. 3). The effect of *crp* was ~30% in MG1655 and ~75% in the *csrA* mutant both in CFA medium (Fig. 3) and in LB medium containing 0.2% glucose (data not shown). The magnitudes of these effects were comparable to those of the glucose effects on these strains (Fig. 1). Disruption of *cya* also decreased biofilm formation in these strains (Fig. 4). The addition of cAMP (2 or 5 mM) to the growth medium of *cya* mutants significantly increased specific biofilm formation (about two- to fivefold) in all experiments, and in most cases it increased biofilm formation by *cya* wild-type strains. Taken together, these results reveal that glucose effects on biofilm formation in *E. coli* are mediated at least in part by the classical catabolite repression system, i.e., cAMP and CRP.

We previously evaluated the time courses of biofilm formation by MG1655 and its *csrA* mutant, TRMG1655, and observed that biofilm accumulated considerably beyond 24 h in both strains (13). Therefore, a final study was conducted to assess the temporal effects of catabolite repression on biofilm formed by MG1655 and its *csrA* mutant, TRMG1655 (Fig. 5). In this experiment, 0.2% glucose (wt/vol, final concentration) was added to cultures at various times during growth, and biofilm was assayed at 24 or 48 h. The presence of glucose at

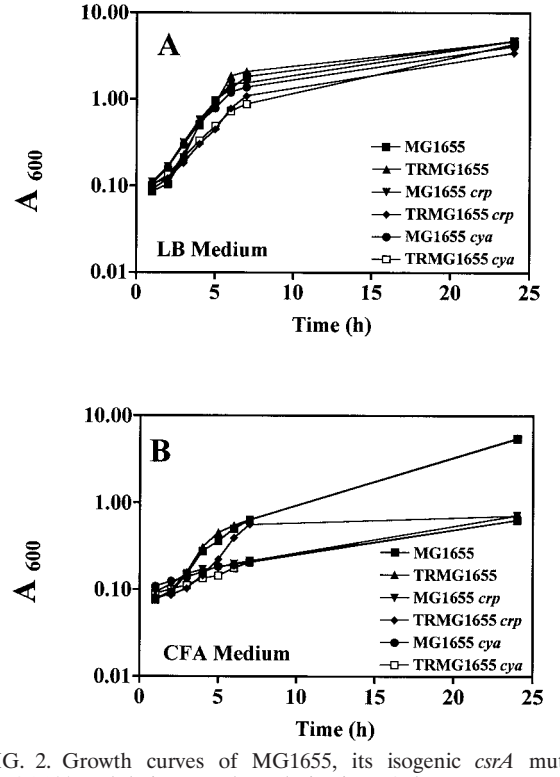

FIG. 2. Growth curves of MG1655, its isogenic *csrA* mutant, TRMG1655, and their *crp* and *cya* derivatives. Cultures were grown at 26°C in LB medium containing 0.2% glucose (A) or CFA medium (B) and sampled at the indicated times, and growth was determined (A_{600}).

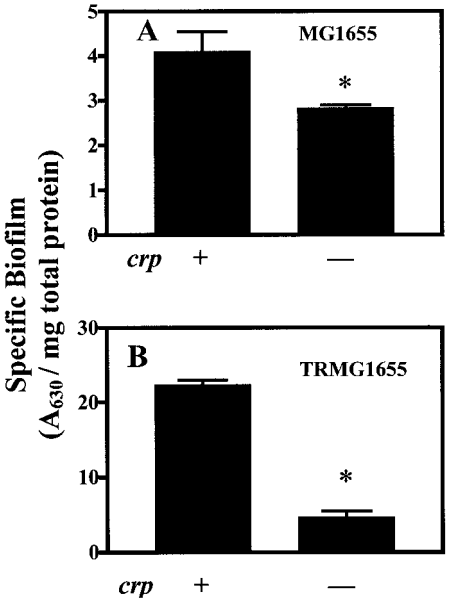

FIG. 3. Effects of *crp* on specific biofilm formation by MG1655 and its isogenic *csrA* mutant TRMG1655. Cultures of *crp* wild type or isogenic mutant strains were grown for 24 h in CFA medium, and biofilm was determined after 24 h at 26°C. Each bar shows the average and standard error of three experiments ($P < 0.0001$). *, significant difference between the *crp* mutant and its parent strain. Results were essentially identical in LB medium containing 0.2% glucose (not shown)

the time of inoculation led to statistically significant inhibition in every case (Fig. 5A, C, E, and G). Thereafter, glucose effects became progressively weaker. One of the 24-h biofilms, that of TRMG1655 in CFA medium, no longer was inhibited but exhibited a modest yet statistically significant increase when glucose was added at 12 h (Fig. 5C). The addition of glucose after 24 h invariably failed to inhibit biofilm formation at 48 h. In fact, glucose addition after 24 h tended to increase biofilm formation, with the exception of TRMG1655 growing in LB medium. These observations suggest that the cAMP-dependent steps of biofilm development are completed by 24 h of growth under these conditions.

Conclusions. Several global regulatory factors influence biofilm development in *E. coli*. The most striking effects to have been recognized thus far are those of CsrA (13), while those of cAMP-CRP and the other regulators (1, 8, 33) are more modest. Previous studies demonstrated that cAMP-CRP activates the expression of certain operons involved in biofilm formation, *flhDC*, which encodes the activator protein for the flagellar cascade of gene expression (23, 31), and *glgCAP*, which encodes glycogen biosynthetic and degradative enzymes (13, 26, 27). However, the relative contributions of these or other genes to the observed catabolite repression remain to be determined. Six *E. coli* K-12 strains and five pathogens, representing four genera of *Enterobacteriaceae*, exhibited glucose inhibition of biofilm formation, suggesting that catabolite repression of biofilm development is a common theme in this family of bacteria. While cAMP exhibits a relatively broad phylogenetic distribution (3), its role in biofilm formation is unknown outside of the *Enterobacteriaceae*. Interestingly, the cAMP-independent catabolite repression control protein Crc of *P. aeruginosa* is essential for biofilm formation (21). Perhaps Crc and cAMP-CRP represent evolutionarily convergent regulatory features of biofilm development in the *Pseudomonadaceae* and the *Enterobacteriaceae*, respectively.

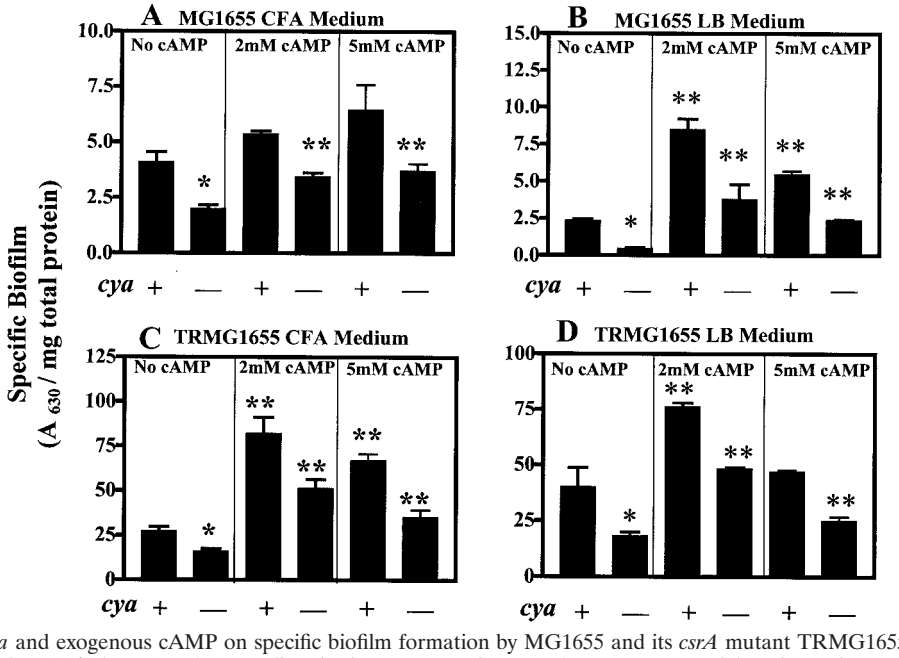

FIG. 4. Effects of *cya* and exogenous cAMP on specific biofilm formation by MG1655 and its *csrA* mutant TRMG1655. Cultures were grown for 24 h in LB medium plus 0.2% glucose or CFA medium in the presence of 0, 2, or 5 mM cAMP. Each bar shows the average and standard error of three experiments. *, that *cya* disruption significantly decreased biofilm formation ($P < 0.0001$); **, addition of cAMP to the culture resulted in a significant increase in biofilm ($P < 0.0001$).

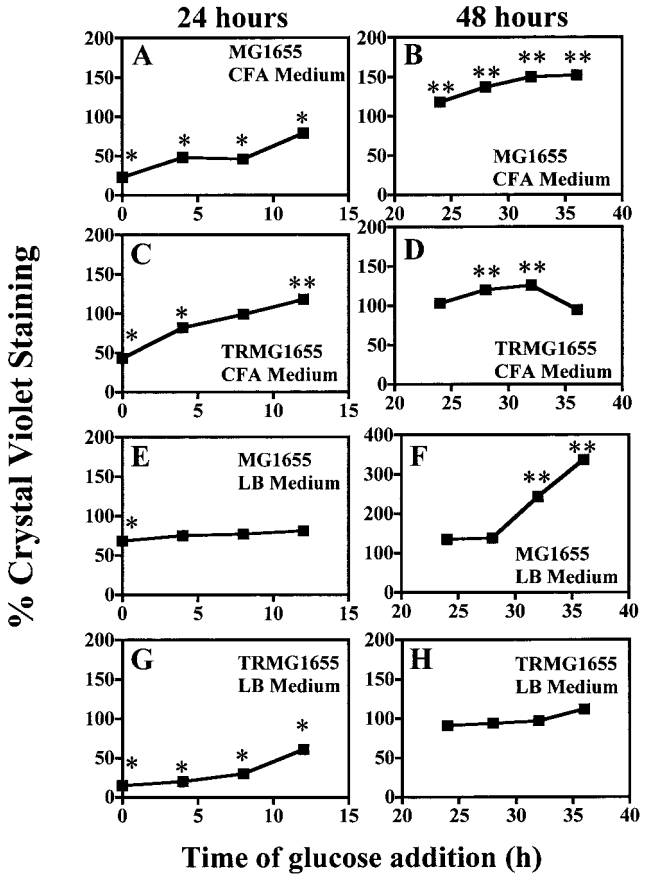

FIG. 5. Temporal effects of glucose on biofilm formation at 24 or 48 h of growth. Glucose (0.2% [wt/vol], final concentration) was added at the indicated times after inoculation of MG1655 or its *csrA* mutant, TRMG1655, into CFA or LB medium. Crystal violet staining was measured at A_{630} at either 24 or 48 h of growth, and results were calculated as percentages of values for the control cultures that lacked glucose. Each point is the average and standard error of three separate experiments. * and **, significant decrease or increase ($P < 0.0001$), respectively, in biofilm formation relative to controls lacking glucose.

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