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Suppression of Defects in Cyclic Adenosine 3',5'-Monophosphate Metabolism in *Escherichia coli*

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Strain MM6-13 (*ptsI suc lacI sup*) of *Escherichia coli* contains a suppressor of the succinate-negative phenotype. In MM6-13, *sup* caused enhanced growth in glycerol, maltose, melibiose, and succinate media and increased activity of β-galactosidase and tryptophanase relative to an isogenic strain without *sup*. In strain A61 (*cya sup*), *sup* partially suppressed *cya*. Cyclic guanosine monophosphate increased β-galactosidase activity sevenfold in A61 and enabled this strain to grow on maltose, galactose, succinate, and arabinose. Strain A61 responded to much lower concentrations of cyclic adenosine monophosphate than cyclic guanosine monophosphate. It appears that *sup* is located in the *crp* locus. These results suggest that *sup* mutants have an altered cyclic adenosine monophosphate receptor protein which is activated by cyclic guanosine monophosphate and has an increased affinity for cyclic adenosine monophosphate.

Enzyme I mutants (*ptsI*) of the phosphoenolpyruvate:sugar phosphotransferase system (PTS) are unable to phosphorylate and transport a number of carbon sources such as glucose, fructose, mannitol, and mannose (PTS compounds). In addition, enzyme I mutants usually are unable to utilize certain carbon sources (non-PTS compounds) that are neither transported nor phosphorylated by this system (13). Non-PTS compounds such as glycerol (3), lactose (9), and succinate (2) can be utilized by enzyme I mutants if cyclic AMP (cAMP) is added to the medium. However, exogenous cAMP is unable to effect the utilization of PTS compounds by enzyme I mutants (2). In *Escherichia coli*, the lack of utilization of non-PTS compounds can be explained by the low levels of cAMP in *ptsI* mutants (8, 10, 11), although other factors may be involved.

Most *ptsI* mutants are able to regain the ability to utilize both PTS and non-PTS compounds by a single back mutation. In contrast, revertants of *E. coli* strain MM6, a *ptsI* mutant, are able to utilize PTS compounds but retain their succinate negativity (2, 6). The succinate-negative phenotype of this strain was found to be due to another mutation, *suc*, which is very closely linked to *ptsI* (2). The ability of *suc* mutants to utilize succinate in the presence of exogenous cAMP suggests that *suc* mutants have low levels of cAMP.

Two extragenic *suc* suppressor mutations that are not linked to *ptsI* have been found (2). Although the location of one of these is unknown, the other suppressor mutation (*sup*) is linked to *rpsL* (formerly *strA*) and appears to be located in the *crp* gene. In the present report, the effect of *sup* on other cAMP-dependent activities is described.

MATERIALS AND METHODS

Bacterial strains. The strains of *E. coli* are listed in Table 1.

Media and growth conditions. OM media containing 0.4% carbon source were used (2). For growth rate determinations, cells were grown in flasks with sidearms (1). MacConkey agar was obtained from Difco Laboratories.

Transductions. Transductions were carried out as described previously (2).

Enzyme Assays. Tryptophanase was assayed by the procedure described by Bilezikian et al. (4). Tryptophanase was induced by the addition of 2 mM L-tryptophane to the growth media. The assay for β-galactosidase has been described previously (1). Enzyme activities are expressed as micromoles per minute per milligram of protein.

Protein determination. Protein content of whole cells was determined by the procedure of Lowry et al. (7), with the addition of hexadecyltrimethylammonium bromide (0.1 mg per ml).

Chemicals. cAMP, cyclic GMP (cGMP), D-galactose (glucose free), and isopropylthiogalactoside were obtained from Sigma Chemical Co.

RESULTS

Effect of *sup* on the utilization of non-PTS compounds. In Table 2, the growth of MM6-13 on non-PTS compounds, namely arabinose, gluconate, glycerol, maltose, melibiose, and succinate is compared with the growth of isogenic strains. Strain MM6-13 (*ptsI suc lacI sup*), a *sup* mutant, utilized glycerol, maltose, melibiose, and succinate, whereas strain MM6

(*ptsI suc lacI*), which lacks *sup*, was unable to utilize these compounds. Thus, the *sup* mutation enabled strain MM6-13 to utilize these compounds. The growth of MM6-13 on non-PTS compounds was similar to that of 3300, except that a lag period preceded utilization of arabinose by MM6-13. The growth rate of MM6 in gluconate, a non-PTS compound that supports growth of *ptsI* mutants (2) and *sup* mutants (Alexander, unpublished observation), was faster than the growth rate of MM6-13. Comparison of the growth of MM6-1 (*suc*) with MM6-13M (*suc sup*) shows that *sup* suppressed the *suc* mutation.

TABLE 1. *Strains of E. coli*

Strain	Genotype	Source or reference
3300	*lacI*	(2)
MM6	*ptsI suc lacI*	(2)
MM6-1	*suc lacI*	(2)
MM6-13	*ptsI suc lacI sup*	(2)
MM6-13M	*suc lacI sup*	Reversion of MM6-13 on mannitol
A106	*ptsI suc rpsL*	(2)
A107	*ptsI suc rpsL lacI*	(2)
A39	*lacI*	(1)
CA7901	*crp*	B. Tyler
CA8306	*cya*	B. Tyler
A23	*ptsI suc lacI sup rpsL*	Transduction of MM6-13, using A106 as donor
A25	*lacI sup*	Transduction of MM6-13, using 3300 as donor
A50	*ptsI suc lacI rpsL crp*	Transduction of MM6-13, using A70 as donor
A60	*cya rpsL*	Transduction of CA8306, using A23 as donor
A61	*cya rpsL sup*	Transduction of CA8306, using A23 as donor
A62		Transduction of CA8306, using A23 as donor
A70	*crp rpsL*	Transduction of CA7901, using A106 as donor

Effect of *sup* on the levels of β-galactosidase and tryptophanase. The relative activities of isogenic strains with and without *sup* is shown in Table 3. MM6-13 and A39 (a *pts*$^+$ *suc*$^+$ transductant of MM6) had much higher activities of both β-galactosidase and tryptophanase than did MM6. Thus, *sup* had a significant effect on the levels of two enzymes that are under cAMP control (8).

The relatively high level of β-galactosidase in MM6-13M (*suc sup*) compared with MM6-1 (*suc*) (Table 3) is further evidence that *sup* suppressed the *suc* mutation.

Partial suppression of *cya* by *sup*. In the absence of added nucleotides strain A61 (*cya sup*) had much higher levels of β-galactosidase than did A60 (*cya crp*$^+$), although the β-galactosidase level in A61 was only about one-fourth as high as that in A62 (*cya*$^+$ *crp*$^+$) (Fig. 1). These results indicate that *sup* was able to partially suppress *cya*.

Effect of cAMP and cGMP on β-galactosidase activity. β-Galactosidase activity in A61 was increased sevenfold by cGMP (Fig. 1). cGMP was almost as effective as cAMP. By contrast, cGMP only resulted in a twofold increase in β-galactosidase activity in A60, and it caused a slight decrease in A62. The large increase in activity in A61 indicates that *sup* responds to both cAMP and cGMP.

TABLE 3. *Effect of sup on tryptophanase and β-galactosidase activities*[a]

Strain	Genotype	Tryptophanase activity	β-Galactosidase activity
MM6-13	*ptsI suc lacI sup*	0.108	17.2
MM6	*ptsI suc lacI*	0.008	5.2
A39	*lacI*	0.093	14.2
A25	*lacI sup*	0.130	20.1
MM6-13M	*suc lacI sup*		17.5
MM6-1	*suc lacI*	0.007	5.5

[a] Cells were grown in OM medium containing 0.4% glycerol and 0.1% Casamino Acids.

TABLE 2. *Effect of sup on growth on non-PTS compounds*

Strain	Genotype	Growth on carbon source[a]:						
		Arabinose	Gluconate	Glucose	Glycerol	Maltose	Melibiose	Succinate
3300	*lacI*	0.52	0.59	0.59	0.42	0.56	0.52	0.24
MM6	*ptsI suc lacI*	–[b]	0.55	ND[c]	–	–	–	<0.09
MM6-13	*ptsI suc lacI sup*	–	0.42	ND	0.36	0.35	0.52	0.19
MM6-13M	*suc lacI sup*	0.28	0.46	0.52	0.45	0.42	0.42	–
MM6-1	*suc lacI*	–	0.52	0.35	–	–	–	<0.09
A25	*lacI sup*	–	0.59	0.57	–	0.36[d]	0.38[d]	–

[a] Specific growth rates per hour. Inocula were grown overnight in OM medium containing 0.4% gluconate.
[b] –, No growth until after a lag period of more than 3 h.
[c] ND, Not determined.
[d] Initial growth was not exponential.

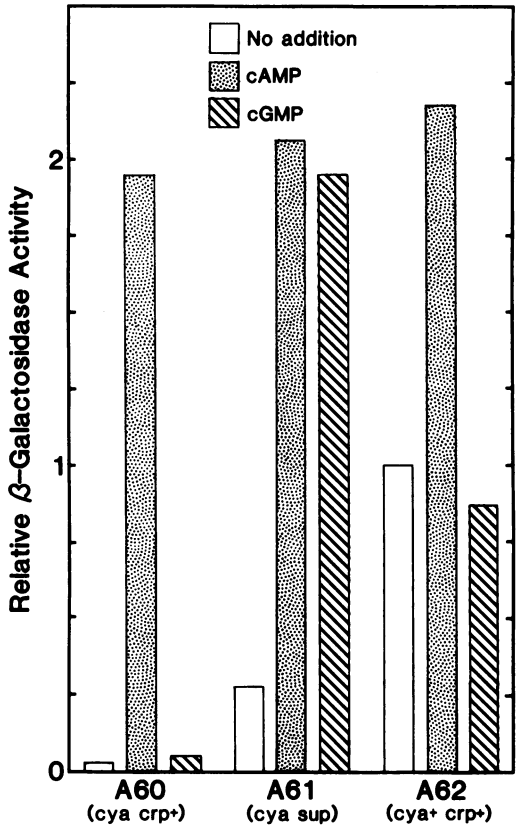

FIG. 1. *Effect of cAMP and cGMP on β-galactosidase activity in strains A60, A61, and A62. Cells were grown in OM medium containing 0.4% glucose. The concentration of cyclic nucleotides was 1 mM. β-Galactosidase was induced with 2 mM isopropyl-thiogalactoside.*

β-Galactosidase activity in A61 increased 1.8- to 4.3-fold by 5×10^{-5} to 1×10^{-3} M cGMP, whereas the activity was increased 1.5- to 3.7-fold by 5×10^{-6} to 1×10^{-4} M cAMP (Fig. 2). These data show that much lower concentrations of cAMP were required.

Effect of cAMP and cGMP on the utilization of carbon sources. With exogenous cGMP, the specific growth rate of A61 on galactose, succinate, and arabinose ranged from 0.35 to 0.59, whereas no significant growth occurred in the absence of cGMP (Table 4). Moreover, the addition of 1 mM cGMP enabled A61 to grow on maltose plates. By contrast, 1 mM cAMP enabled A60 to grow on maltose, galactose, and succinate plates; however, 1 mM cGMP was ineffective with this strain. Strain A62 grew on these compounds both in the presence and absence of cyclic nucleotides.

The effect of the concentration of cyclic nucleotides on maltose utilization in strain A61 is

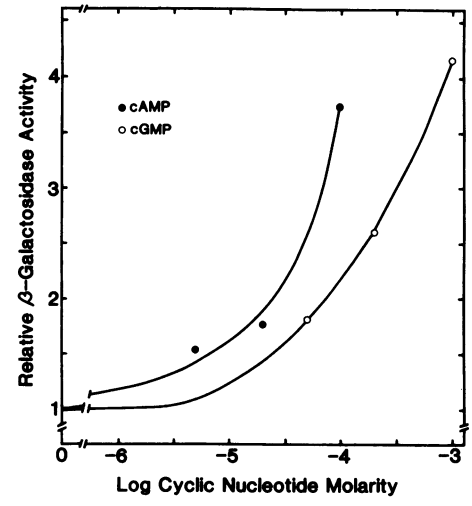

FIG. 2. *Effect of concentrations of cAMP and cGMP on β-galactosidase activity in strain A61 (cya sup). Conditions were the same as those described in the legend to Fig. 1.*

TABLE 4. *Effect of cGMP on the utilization of different carbon sources in strain A61 (cya sup)*

Carbon source[a]	Specific growth rate (h^{-1})
Glucose	0.59
L-(+)-Arabinose	<0.09
L-(+)-Arabinose plus cGMP	0.35
Galactose	<0.12
Galactose plus cGMP	0.46
Succinate	<0.09
Succinate plus cGMP	0.42

[a] The concentration of cGMP was 1 mM.

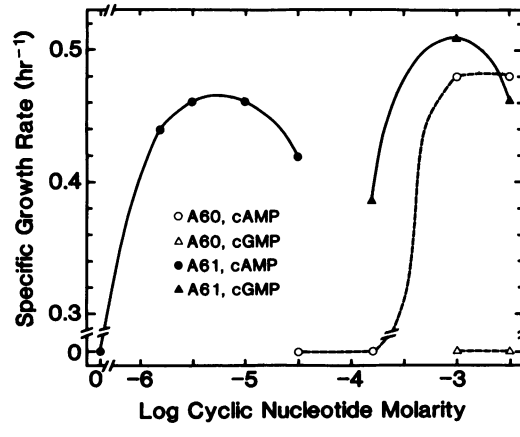

FIG. 3. *Effect of concentrations of cAMP and cGMP on the growth rates of strain A60 (cya crp+) and strain A61 (cya sup) in OM medium containing 0.4% maltose. Inocula were grown overnight in OM medium containing 0.4% glucose.*

shown in Fig. 3. The fastest growth occurred with 5×10^{-6} to 2×10^{-5} M cAMP, whereas 1×10^{-3} M cGMP was required for the highest growth rate. The lowest concentrations that inhibited growth were 5×10^{-5} M cAMP and 5×10^{-3} M cGMP. The lack of growth in maltose with A60 in the presence of 5×10^{-5} or 2×10^{-4} M cAMP shows that higher concentrations of cAMP are required in *crp*$^+$ strains than in *sup* strains. The effect of concentrations of cyclic nucleotides on maltose utilization was similar to the effect on β-galactosidase synthesis.

Mapping of *sup*. Transductions with P1 phage were carried out to determine the linkage of *sup* and *crp*. In a transduction with A107 (*crp*$^+$ *rpsL*) as donor, MM6-13 (*sup rpsL*$^+$) as recipient, and selection on streptomycin, 58% of the transductants were *crp*$^+$ (Table 5, line 1). In another transduction with MM6-13 as donor, A50 (*crp rpsL*) as recipient, and selection on succinate, 68% of the transductants were *rpsL*$^+$ (Table 5, line 2). These data confirm previous results (2) which indicated that *sup* is linked to *rpsL*. A transduction was carried out with A70 (*crp rpsL*) as donor, MM6-13 as recipient, and selection on streptomycin (Table 5, line 3). If *sup* and *crp* were located on opposite sides of *rpsL*, approximately equal numbers of *crp* and *crp*$^+$ transductants would be expected. Since less than 2% of the transductants were *crp*$^+$, it is concluded that *sup* and *crp* are closely linked; however, it is not possible to determine from these results whether *sup* is located in the *crp* locus. If *sup* and *crp* are not located in the same locus, then the transduction in Table 5, line 2 would be expected to give two classes of transductants with readily discernible phenotypes, namely, *crp*$^+$ *sup* and *crp sup*. Failure to detect these two classes of transductants argues strongly against the possibility that *sup* and *crp* are separate genes.

The apparent difference in linkage between *crp* and *rpsL* in Table 5, lines 1 and 3 presumably is due to the selective disadvantage of *crp* transductants which form smaller colonies and have slower growth rates than do *crp*$^+$ transductants. This is supported by the results in Table 5, line 4 in that only 15% of the transductants were *crp* as opposed to nearly 60% of the transductants were *crp*$^+$ in Table 5, line 1.

DISCUSSION

The suppressor mutation *sup* was acquired by MM6-13 in a selection on succinate medium. In addition to suppression of succinate negativity, *sup* enhanced growth on glycerol, maltose, and melibiose as the sole carbon source (Table 2). In agreement with the previous finding that *suc* prevents the utilization of the non-PTS compound succinate (2), the inability of MM6-1 (*suc lacI*) to utilize arabinose, glycerol, maltose, and melibiose indicates that *suc* prevents the utilization of other non-PTS compounds (compare MM6-1 and 3300, Table 2). In contrast to the results obtained with MM6-1, the ability of MM6-13M (*suc lacI sup*) to utilize arabinose, glycerol, maltose, and melibiose confirms our previous conclusion that *sup* supresses *suc* (2). The inability of A25 (*lacI sup*) to utilize non-PTS compounds suggests that *sup* interferes with the regulation of metabolism of these compounds in *ptsI*$^+$ *suc*$^+$ strains.

In addition to the profound effect of *sup* on the utilization of non-PTS compounds, *sup* alters the regulation of synthesis of catabolite-sensitive enzymes. As shown in Table 3, *sup* greatly increased the levels of tryptophanase and β-galactosidase. Moreover, *sup* partially suppressed *cya* (Fig. 1). It appears that all the known effects of *sup* involve activities that are cAMP dependent.

By mapping, it has been established that *sup* is closely linked to *crp*. Since there is no evidence for a novel gene located in this region which is involved in cAMP metabolism, it seems likely that the *sup* mutation lies within the *crp* locus. Both the results of mapping and the effects of *sup* are consistent with the idea that the *sup*

TABLE 5. *Cotransduction of sup with rpsL*

Donor		Recipient		Selected marker of donor	No. of colonies examined	No. of colonies with unselected character of donor[a]
Strain	Relevant genotype	Strain	Relevant genotype			
A107	*crp*$^+$ *rpsL*	MM6-13	*sup rpsL*$^+$	*rpsL*	143	83 (58)
MM6-13	*sup rpsL*$^+$	A50	*crp rpsL*	*sup*	354	240 (68)
A70	*crp rpsL*	MM6-13	*sup rpsL*$^+$	*rpsL*	223[b]	79 (35)
A70	*crp rpsL*	MM6	*crp*$^+$ *rpsL*$^+$	*rpsL*	69	10 (15)

[a] Number within parentheses denotes the percentage of colonies with unselected marker.

[b] Three colonies had the crp$^+$ phenotype as determined on MacConkey-lactose agar. *sup* transductants were inhibited by lactose in the medium (1).

mutation results in an altered cAMP receptor protein.

Although it has been reported that *crp* mutants produce abnormally high amounts of cAMP (5, 12), it does not seem likely that the effects of *sup* are due to increased cAMP because of the ability of *sup* to suppress a *cya* deletion in strain A61 (Fig. 1).

In strain A61 (*cya sup*), cGMP caused increased β-galactosidase activity and enabled this strain to grow on maltose, glycerol, succinate, and arabinose. These results suggest that *sup* mutants have a cAMP receptor protein which can be activated by cGMP. In view of the effects of cGMP and the mapping data, it seems likely that *sup* is similar to the mutation described by Sanders and McGeach (14). However, *sup* is clearly distinct from the *crp* mutation (*crp*-1) selected by Botsford and Drexler (5). With *crp*-1, utilization of maltose, succinate, and arabinose was reported by a strain with a *cya* deletion, whereas these compounds were not utilized with *sup* (Fig. 3 and Table 4). Since *crp*-1 and *sup* were present in the same genetic background, namely that of strain CA8306, it is unlikely that these differences are due to other variations in the strains. Another striking difference is that *sup* responds to cGMP, whereas *crp*-1 does not.

Relatively low concentrations of cAMP were required by A61 to stimulate β-galactosidase levels and to enhance growth in maltose media. Although these results do not necessarily reflect intracellular levels of cAMP and cGMP, they suggest that *sup* mutants contain an altered cAMP receptor protein with increased affinity for cAMP. Accordingly, suppression of *suc* can be explained by increased affinity of the *sup* protein for cAMP. On the other hand, suppression of *cya* may be mediated by cGMP (14), although the possibility that this suppression is due to the ability of the *sup* protein to act independently of cAMP has not been excluded in the present work.

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