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Effect of Carbon Source and the Role of Cyclic Adenosine 3',5'-Monophosphate on the *Caulobacter* Cell Cycle

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The expression of cell cycle events in *Caulobacter crescentus* CB13 has been shown to be associated with regulation of carbohydrate utilization. Growth on lactose and galactose depends on induction of specific enzymes. Prior growth on glucose results in a delay in enzyme expression and cell cycle arrest at the nonmotile, predivisional stage. Dibutyryl cyclic adenosine 3',5'-monophosphate (AMP) was shown to stimulate expression of the inducible enzymes and, thus, the initiation of the cell cycle. β-Galactosidase-constitutive mutants did not exhibit a cell cycle arrest upon transfer of cultures from glucose to lactose. Furthermore, carbon source starvation results in accumulation of the cells at the predivisional stage. The cell cycle arrest therefore results from nutritional deprivation and is analogous to the general control system exhibited by yeast (Hartwell, Bacteriol. Rev. 38:164-198, 1974; Wolfner et al., J. Mol. Biol. 96:273-290, 1975), which coordinates cell cycle initiation with metabolic state. Transfer of *C. crescentus* CB13 from glucose to mannose did not result in a cell cycle arrest, and it was demonstrated that this carbon source is metabolized by constitutive enzymes. Growth on mannose, however, is stimulated by exogenous dibutyryl cyclic AMP without a concomitant increase in the specific activity of the mannose catabolic enzymes. The effect of cyclic AMP on growth on sugars metabolized by inducible enzymes, as well as on sugars metabolized by constitutive enzymes, may represent a regulatory system common to both types of sugar utilization, since they share features that differ from glucose utilization, namely, temperature-sensitive growth and low intracellular concentrations of cyclic guanosine 3',5'-monophosphate.

The cell cycle of *Caulobacter crescentus* is characterized by a set of spatially and temporally regulated morphological changes. The sequence of these events (stalk formation and the initiation of deoxyribonucleic acid synthesis, followed by the simultaneous appearance of flagella, pili and phage receptor sites, and asymmetric cell division) can be represented by fractions of a unit cell cycle, since their order is independent of the generation time (Fig. 1). This gram-negative bacterium has proved useful for studies on the regulation of differentiation in procaryotes with respect to the timing of gene expression and the localization of gene products within the cell. The precise coordination of growth, cell division, and cell differentiation within each *Caulobacter* cell cycle suggests that a regulatory mechanism functions to maintain the sequential expression of these various functions.

In this report we present experiments that demonstrate that the progress of cell cycle events in *C. crescentus* is dependent on the metabolic state. Evidence for a specific "start" period in the *Caulobacter* cell cycle was obtained when glucose-grown cultures were shifted to media containing lactose or galactose as the sole carbon source. Delayed expression of inducible enzymes results in cell cycle arrest at a specific cell stage before initiation of a new cell cycle. This cell cycle arrest can be overcome by exogenous dibutyryl cyclic adenosine 3'5'-monophosphate (AMP), leading to stimulation of expression of the inducible catabolic enzymes. The induction of catabolic enzymes, and thus carbohydrate utilization, permits resumption of the cell cycle.

Although the effect of dibutyryl cyclic AMP appears to be similar in nature to the effect observed in the enteric bacteria, evidence reported here demonstrates that dibutyryl cyclic AMP stimulates growth on sugars catabolized by constitutive enzymes, in addition to its role in the expression of inducible catabolic enzymes. In general, stimulation of growth rate was observed in cultures grown on sugars

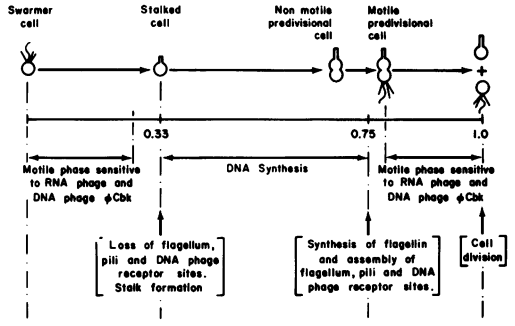

FIG. 1. *Schematic representation of C. crescentus cell cycle events. Data have been compiled from Shapiro and Agabian-Keshishian (12), Newton (4), and Stove and Stanier (17).*

that serve as poor carbon sources. Features shared by inducible and constitutive catabolic enzymes of *C. crescentus* include temperature-sensitive growth and low intracellular concentrations of cyclic guanosine 3',5'-monophosphate (GMP) compared with those of glucose-grown cultures, suggesting that the stimulation of growth rate by dibutyryl cyclic AMP may occur via a shared regulatory pathway. This regulatory system can be dissociated by a mutation affecting the response of inducible catabolic enzymes to dibutyryl cyclic AMP with no effect on the stimulation of growth by this cyclic nucleotide in the presence of constitutively metabolized sugars.

MATERIALS AND METHODS

Materials. [^{14}C]lactose D-[1-^{14}C]glucose, 20 mCi/mmol, and [*U*-^{14}C]mannose, 5 mCi/mmol, were purchased from the Amersham/Searle Corp. ^{14}C-reconstituted protein hydrolysate yeast profile (1 mCi/ml) was purchased from Schwarz/Mann. The radioimmune assay kit for cyclic AMP and cyclic GMP was obtained from Collaborative Research. Cyclic AMP-specific phosphodiesterase from *Blastocladiella emersonii* was a gift of P. Silverman. N^6,O^2-dibutyryl cyclic 3',5'-AMP and *N*-methyl-*N'*-nitrosoguanidine were purchased from the Sigma Chemical Co. Rifampin was obtained from Calbiochem.

Growth conditions and mutant strains. *C. crescentus* strain CB13 was grown at 30°C in minimal medium (12) in the presence of a variety of carbon sources (0.2%, wt/vol) or in complex medium PYE (12). Mutant strains AE7 and AE9 were isolated following NTG mutagenesis of *C. crescentus* CB13 (1). Mutant AE7 (*lac^c-505*) is a β-galactosidase-constitutive strain isolated on x-gal media. Mutant AE9 (*lac$^-$-507*) is unable to grow on either lactose or galactose, even in the presence of dibutyryl cyclic AMP.

Carbohydrate uptake assays. Cultures grown at 30°C were harvested by centrifugation (10,000 rpm in a Sorvall SS-34 rotor for 10 min at room temperature) and washed once with minimal salt medium, and the pellet was suspended in minimal salt medium at room temperature. The cell suspension was adjusted to an optical density (OD) at 660 nm of 0.155, and unlabeled sugar was added and then incubated for 5 min at 30°C with shaking. The experiment was initiated by addition of the corresponding ^{14}C-sugar to yield 1×10^5 to 4×10^5 cpm per ml. At specified times, 2 portions of 1 ml were withdrawn and filtered on membrane filters (0.45 μm; Millipore Corp.). The filters were washed twice with 5 ml of minimal salt medium, dried, and then counted in Omnifluor-toluene (New England Nuclear) liquid scintillation fluid. The initial rate of sugar uptake by the cells was determined at various substrate concentrations, and the kinetic constants for uptake were calculated from Lineweaver-Burk double reciprocal plots. Mannose uptake was measured at an initial concentration of 0.5 to 20 mM. Lactose uptake by glucose-grown cells was measured at an initial concentration of 0.3 to 10 mM and in lactose-grown cells at an initial concentration of 0.014 to 1 mM.

Determination of intracellular concentration of cyclic AMP and cyclic GMP. Cultures were grown in minimal medium with the appropriate carbon source to either mid-log phase or late log phase. Cells were collected by rapid filtration of 25-ml portions on membrane filters (0.65 μm, 47 mm; Millipore Corp.). The filters were suspended in cold water, and concentrated formic acid was added to a concentration of 1 M. The suspensions were frozen and thawed three times before removal of the filters and centrifugation at 15,000 rpm in a Sorvall SS-34 rotor for 20 min at 4°C. The supernatants were decanted and lyophilized. The dry residues were suspended in 0.05 M sodium acetate buffer (pH 6.2), and the cyclic AMP content was measured by radioimmune assay without further purification. Cyclic AMP was not detected in samples pretreated with cyclic AMP-specific phosphodiesterase from *B. emersonii* (16). The cyclic GMP concentration was determined in samples pretreated with cyclic AMP-specific phosphodiesterase, since cyclic AMP interfered with the cyclic GMP determination. The enzymatic hydrolysis of cyclic AMP was carried out as described previously (13). All measurements were carried out in triplicate with reference standards of 0.05 to 10 pmol. The intracellular concentration, based on cell volume, was determined as described previously (13).

Electron microscopy. Bacterial preparations were negatively stained with 1% phosphotungstic acid by the procedure described previously (12). Specimens were examined in a Siemens Elmskop 1A electron microscope at a voltage of 80 kV.

Resolution of labeled proteins by gel electrophoresis. Low levels of protein synthesis were maintained by cell cultures during the course of the cell cycle arrest. Cultures (3 ml) in arrest, rescued by dibutyryl cyclic AMP, or self-induced by lactose, were pulse-labeled with 50 μCi of ^{14}C-reconstituted protein hydrolysate (1 mCi/ml) for 15 min at 30°C with shaking. The pulse was terminated by addition of trichloroacetic acid to a concentration of 5%, and the sample was cooled in an ice bath for 20 min. The

samples were then frozen and thawed, and the precipitate was collected by centrifugation at 15,000 rpm in a Sorvall SS-34 rotor for 20 min at 4°C. The pellet was washed once with acetone and dried at room temperature. The pellet was then dissolved in buffer containing 0.5 M tris(hydroxymethyl)-aminomethane (Tris)-hydrochloride (pH 7.0), 1% sodium dodecyl sulfate (SDS), and 0.1% 2-mercaptoethanol. Samples (40 μl) containing about 200 μg of protein were mixed with 10 μl of sample buffer (62 mM Tris-hydrochloride [pH 6.8]-2% SDS-7.5% glycerol-5% 2-mercaptoethanol) and 3 μl of 0.02% phenol red. The samples were boiled for 1 min and applied to acrylamide gradient slab gels (15 by 10 cm) containing 0.1% SDS, 0.35 M Tris-hydrochloride (pH 8.8), and a gradient of 7.5% acrylamide, 0.2% methylenebisacrylamide to 15% acrylamide, 0.4% methylenebisacrylamide. The gels were polymerized with 5 μl of tetramethylene diamine per 15 ml and 0.1 ml of 5% ammonium persulfate per 15 ml. The stacking gel contained 3% acrylamide, 0.08% methylenebisacrylamide, 0.062 M Tris-hydrochloride (pH 7.0), and 0.1% SDS and was polymerized with 5 μl of tetramethylene diamine per 10 ml and 0.25% ammonium persulfate. Electrophoresis was performed at a constant 33 mA. Gels were stained overnight at room temperature with 0.4 mg of Coomassie brilliant blue in 50% methanol per ml-7.5% acetic acid and destained in 7.5% acetic acid-5% methanol. For autoradiography, gels were dried under vacuum at 100°C and exposed to Kodak No-Screen X-ray film. Autoradiograms were developed with Kodak liquid X-ray developer and Kodak rapid fixer.

Enzyme assays. All enzyme activities were measured in crude cell extracts and found to be linearly dependent on protein concentration and time of incubation. β-Galactosidase activity was measured by the method of Pardee et al. (6). Mannokinase activity was measured in sonically treated cell extracts by a modification of the methods of Sebastian and Asensio (11) and Wilson and Hogness (19). The reactions were carried out in 0.1 ml containing 10 mM phosphate buffer (pH 7.0), 50 mM adenosine 5'-triphosphate, 40 mM $MgCl_2$, and 2 μCi of [1-^{14}C]-mannose (59 mCi/mmol). Phosphomannose isomerase activity was measured in sonically treated cell extracts by the method of Rosen et al. (9).

RESULTS

Growth of *C. crescentus* CB13 on a variety of sugars as sole carbon source. The ability of *C. crescentus* CB13 to grow in minimal medium supplemented with different carbohydrates as sole carbon source is shown in Table 1. The carbohydrates tested were glucose, maltose, sucrose, lactose, galactose, mannose, ribose, and fructose. *C. crescentus* CB13 was able to grow on each of these sugars as sole carbon source at the optimal growth temperature, 30°C. Growth on galactose, however, was possible only in the presence of 3 mM dibutyryl cyclic AMP. The growth rates varied considerably, with slowest rates observed on mannose, ribose, and fructose.

TABLE 1. *Growth rate of C. crescentus CB13 on various carbon sources and the effect of dibutyryl cyclic AMP*

Carbon source	Doubling time (h)			
	30°C		37°C	
	− Dibutyryl cyclic AMP	+ Dibutyryl cyclic AMP	− Dibutyryl cyclic AMP	+ Dibutyryl cyclic AMP[b]
Glucose	4	4	4	4
Maltose	4	4	4	4
Sucrose	4	4	4	4
Lactose	10	10	NG	16
Galactose	NG[c]	26	NG	
Mannose	32	18	42	12
Ribose	28	18	35	12
Fructose	42	24	58	18

[a] Cultures were grown in minimal salt media containing 0.2% of either glucose, maltose, or sucrose and 0.5% of the other sugars listed.
[b] Addition of 3×10^{-3} M dibutyryl cyclic AMP.
[c] NG, No growth observed.

The growth rate on glucose, maltose, or sucrose was the same at 37 and 30°C. The growth rate on mannose, ribose, or fructose, however, was reduced at the elevated temperature, and growth on lactose was possible at the elevated temperature only in the presence of 3 mM dibutyryl cyclic AMP. Other cyclic nucleotides or cyclic nucleotide derivatives such as cyclic AMP, cyclic GMP, dibutyryl cyclic GMP, 8-bromo cyclic GMP, or 8-bromo cyclic AMP did not stimulate growth on lactose at 37°C. Growth on glucose, maltose, and sucrose was not affected by exogenous dibutyryl cyclic AMP, whereas growth on mannose, ribose, and fructose at both temperatures was stimulated by this nucleotide.

There appears, therefore, to be a differential effect of temperature on carbon source utilization. The cyclic nucleotide dibutyryl cyclic AMP functions to stimulate growth at the restrictive temperature in the case of lactose utilization and at both 30 and 37°C in the case of mannose, ribose, and fructose utilization.

Effect of carbon source on catabolic enzyme expression. Cell extracts of cultures grown on glucose, lactose, and mannose in the presence and absence of exogenous cyclic AMP were assayed for the enzyme activities shown in Table 2. β-Galactosidase and the lactose uptake system were induced by growth on lactose, and the induction of β-galactosidase was stimulated by exogenous dibutyryl cyclic AMP (Table 3) (13). As in other bacteria, mannokinase

TABLE 2. *Enzyme activities and transport constants during growth on various carbon sources*[a]

Strain	Carbon source	β-Galactosidase (nm/mg per h)	Lactose uptake[b]	Galactokinase (nm/mg per h)	Phosphomannose isomerase (μm/mg per h)	Mannokinase (nm/mg per h)	Mannose uptake[b]
Wild type	Glucose	1.5	0.30	2.15	0.42	65.0	507
AE7 *lac*[c]	Glucose	28.8	0.22				
Wild type	Lactose	33.0	3.08	125.0			
Wild type	Mannose				0.45	93.0	475
Wild type	Mannose + dibcAMP[c]				0.47		476
Wild type	Galactose + dibcAMP[c]			45.0			

[a] Enzyme activities and transport constants were assayed as described in the text.
[b] First-order kinetic constant (C) = V_{max}/K_m (per minute).
[c] 3 mM dibutyryl cyclic AMP (dibcAMP).

TABLE 3. *β-Galactosidase activity during growth on glucose and lactose and the effect of dibutyryl cyclic AMP*

Carbon source	Stage of growth (h)	β-Galactosidase activity[a]
Glucose[b]	Mid-log	1.5
Lactose[c]	Mid-log	33.0
Glucose → lactose	1	2.5
	5	2.7
	12	3.2
	26[d]	13.0
Glucose → lactose + 3 mM dibutyryl cyclic AMP	3	5.4
	6	8.1
	12	13.5
	26	45.0

[a] β-Galactosidase activity was assayed as described in the text.
[b] 0.2% (wt/vol) glucose in minimal salt medium.
[c] 0.5% (wt/vol) lactose in minimal salt medium.
[d] Self-induced growth on lactose.

(11) and phosphomannose isomerase (9) activities were found to be constitutively expressed. Since dibutyryl cyclic AMP was found to stimulate growth when mannose was used as the sole carbon source, mannokinase, phosphomannose isomerase, and mannose uptakes were measured in cultures grown on mannose in the presence and absence of 3 mM dibutyryl cyclic AMP. Dibutyryl cyclic AMP had no effect on these activities, suggesting that the stimulatory effect of this cyclic AMP derivative on growth was not at the level of either transport or metabolism of mannose.

Effect of carbon source limitation on cell cycle events. Upon shift of cultures from glucose to either lactose or galactose, cells accumulated at the nonmotile predivisional cell stage without loss of viability. Electron microscopic examination of the culture at this stage showed homogeneous populations of the nonmotile predivisional cells. These cells lacked flagella and pili, but septum formation had occurred. This arrest in the cell cycle was overcome spontaneously after a growth lag of about 24 h in lactose (Fig. 2). The resumption of growth, the appearance of motile cells, and susceptibility to phage infection were parallel to the induction of β-galactosidase (13). Cultures arrested at the predivisional cell stage on galactose-minimal media were unable to overcome the cell cycle block unless dibutyryl cyclic AMP was added (Fig. 2). Addition of 3 mM dibutyryl cyclic AMP at any time during the block period in the presence of lactose resulted in stimulation of growth, cellular development, and induction of β-galactosidase. The addition of rifampin (50 μg/ml) prevented the appearance of β-galactosidase activity in the presence of dibutyryl cyclic AMP.

To determine the pattern of protein synthesis during and after cell cycle arrest, cultures were pulse-labeled with ^{14}C-amino acids. Autoradiograms of proteins resolved by SDS-polyacrylamide gel electrophoresis are shown in Fig. 3. The profile of newly synthesized proteins was similar in cultures induced for growth on lactose either spontaneously or after stimulation by dibutyryl cyclic AMP. The rate of protein synthesis was 50% higher in cultures induced in the presence of the cyclic nucleotide than in the self-induced cultures. Comparison of arrested and induced cultures, however, revealed major differences in protein profiles. Two major differences in the band patterns can be attributed to outer membrane proteins. Protein bands designated O.M., as shown in Fig. 3, have been identified as major components of the *C. crescentus* CB13 outer membrane during growth on lactose (Agabian, manuscript in preparation). Flagella were not observed on arrested cell populations, and the synthesis of flagellin, the flagellar subunit (14), was repressed in the arrested culture. Although a residual level of flagellin synthesis can be observed in Fig. 3, the flagellin band was rou-

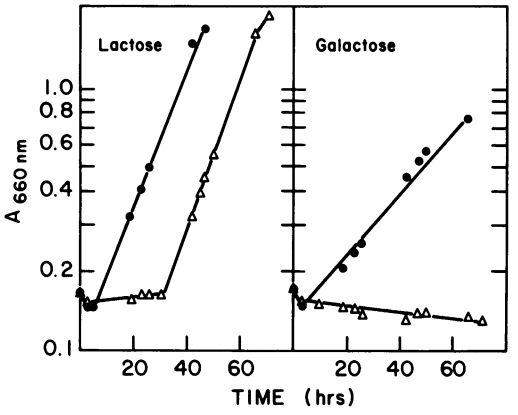

FIG. 2. *Growth of C. crescentus CB13 on lactose (0.5%) and galactose (0.5%) after a shift from glucose (0.2%)* (△) *and stimulation of growth by exogenous dibutyryl cyclic AMP (3 × 10^{-3} M)* (●).

tinely decreased in pulse-labeled, blocked cultures and, in some experiments, was totally absent.

When cultures grown for several generations on glucose were shifted to mannose, which is utilized constitutively, growth resumed after a lag period of about 8 to 10 h (Fig. 4). Unlike the shift from glucose to either galactose or lactose, the cell cycle was not arrested; microscopic examination of the cultures showed the existence of all cell types. Furthermore, a β-galactosidase-constitutive mutant (AE7; Table 2) did not exhibit a cell cycle arrest upon shift from glucose to lactose, although a lag occurred before onset of growth (Fig. 4). Thus, a mutation, which allows immediate utilization of lactose, eliminated the cell cycle arrest that normally follows the shift from glucose to lactose. Similarly, a cell cycle arrest did not occur upon shift of wild-type cells from glucose to either ribose or fructose.

Effect of dibutyryl cyclic AMP on constitutive carbon source utilization. Exogenous dibutyryl cyclic AMP (3 mM) was found to stimulate growth on mannose, ribose, and fructose (Fig. 4; Table 1) and to shorten the lag period, but not the generation time, of the β-galactosidase-constitutive mutant growing on lactose.

The role of cyclic AMP in stimulating growth on a constitutive carbon source was further studied by using a mutant strain of *C. crescentus*, AE9. This strain cannot grow on either lactose or galactose, even in the presence of dibutyryl cyclic AMP. Strain AE9 grew, however, on mannose, and growth was stimulated by exogenous dibutyryl cyclic AMP (Fig. 5). Neither phosphomannose isomerase nor mannokinase activity is stimulated by dibutyryl cyclic AMP under conditions where the growth rate on mannose is increased in the presence of this cyclic nucleotide. It is of interest that AE9 was capable of growth on both ribose and fructose and that growth was stimulated by dibutyryl cyclic AMP in a manner similar to that observed for mannose.

Effect of carbon source on intracellular concentrations of cyclic AMP and cyclic GMP. The intracellular concentrations of both cyclic AMP and cyclic GMP were measured during growth on minimal media containing glucose, mannose, or lactose and during the period of cell cycle block after a shift from glucose to lactose (Table 4). The intracellular concentration of cyclic AMP did not vary significantly with the carbon source. Similar results have been reported previously (13) for cultures grown in enriched medium supplemented with either glucose or lactose.

The intracellular concentration of cyclic GMP, however, did depend upon carbon source and stage of growth. Mid-log-phase cultures of both lactose- and mannose-grown cells had three- to fivefold-lower intracellular concentrations of cyclic GMP than that observed in glucose-grown, log-phase cultures. Cyclic GMP levels were lower in late-log-phase cultures grown on glucose. After the shift of a late-log-phase culture from glucose to lactose, the levels of cyclic GMP increased to that of mid-log-phase cultures and then dropped to the level of lactose-grown cultures as the culture recovered from the cell cycle arrest.

DISCUSSION

Carbon source availability and utilization have been shown to affect the cell cycle. Cultures grown on glucose and transferred to lactose utilized by inducible enzymes arrest at a specific cell stage. The cells accumulate at the nonmotile predivisional stage. This stage occurs before the expression of polar differentiation events, i.e., the formation of flagellum, pili, and the transient receptor sites for DNA phage φCbK at the incipient swarmer cell pole (13). It was previously shown that flagellin is synthesized at a defined time in the cell cycle, just prior to the coordinate assembly of the surface polar structures (14). Pulse-labeling of the arrested cultures with ^{14}C-amino acids showed reduction in the synthesis of specific proteins used for the assembly of surface structures: flagellin, the subunit for flagellum assembly, and major components of the outer membrane, which are characteristic of lactose-grown cultures. The nonmotile predivisional cell stage is further characterized by initiation

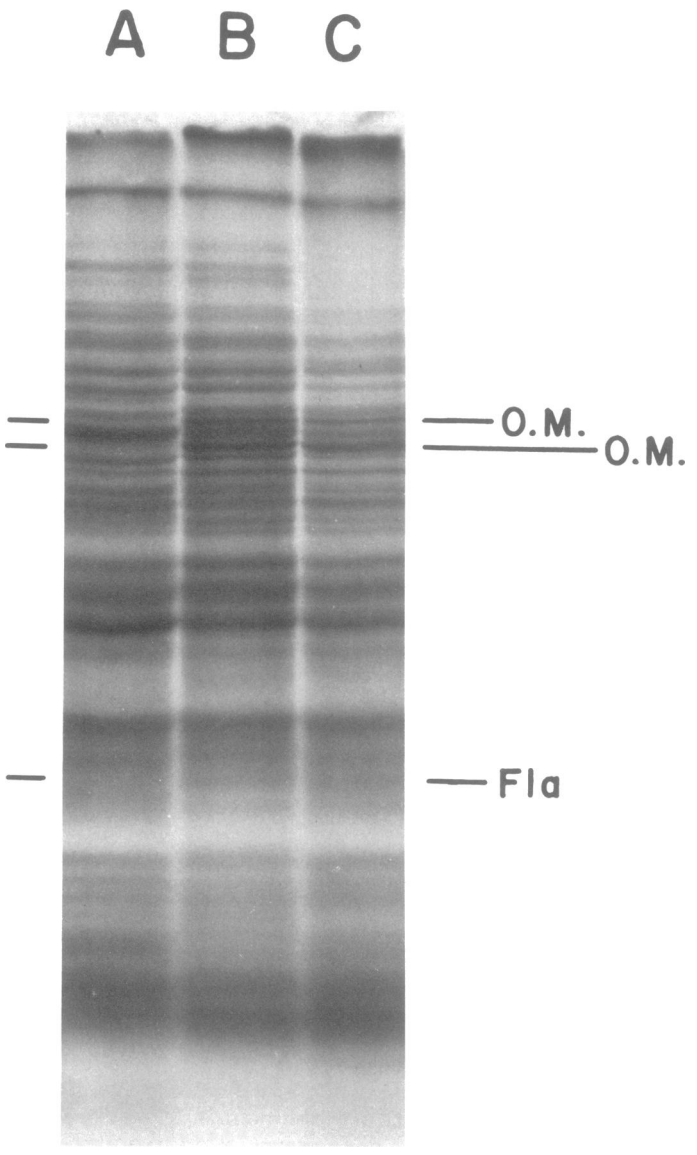

FIG. 3. *Autoradiogram of pulse-labeled C. crescentus CB13 proteins separated by polyacrylamide gel electrophoresis. Labeling of cellular proteins with ^{14}C-amino acids and electrophoresis and autoradiography were carried out as described in the text. Marker gels (not shown) contained chymotrypsinogen, bovine serum albumin, C. crescentus flagellin, and C. crescentus ribonucleic acid polymerase. (A) C. crescentus culture (OD = 0.295) pulse-labeled during a glucose-to-lactose arrest period. Total protein applied to the gel was 115 μg containing 1.08 × 10^6 cpm. (B) Pulse-labeled culture (OD = 0.325) induced for growth on lactose by exogenous dibutyryl cyclic AMP (3 × 10^{-3} M). Total protein applied to gel was 63.4 μg containing 0.99 × 10^6 cpm. (C) Pulse-labeled culture (OD = 0.330) self-induced for growth on lactose after prior growth on glucose. Total protein applied to gel was 81.2 μg containing 1.12 × 10^6 cpm. O.M. indicates outer membrane protein, and Fla indicates flagellin.*

of septum formation, and the completion of chromosomal replication occurs at approximately this stage in the cell cycle (2). The progression of the cell cycle is dependent on active metabolism, since the period of cell cycle arrest was shown to coincide with the period of delay in the induction of catabolic enzymes. Exogenous dibutyryl cyclic AMP stimulated induction of these catabolic enzymes, and, consequently, the cell cycle could be resumed. This

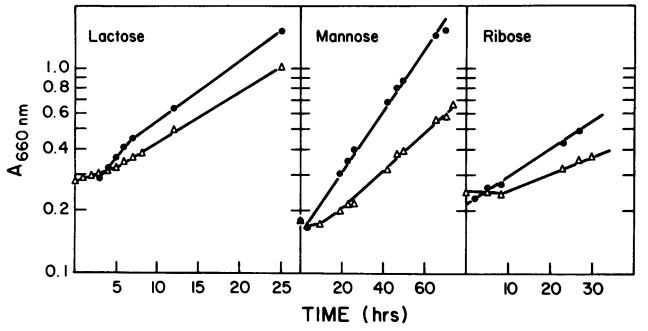

FIG. 4. *Growth of the β-galactosidase-constitutive strain C. crescentus AE7 on lactose (0.5%) and CB13 on mannose (0.2%) and ribose (0.2%) after a shift from glucose (0.2%) (△) and stimulation of growth by exogenous dibutyryl cyclic AMP (3 × 10⁻³ M) (●). The generation time of AE7 on lactose was 12 h, in both the absence and presence of dibutyryl cyclic AMP.*

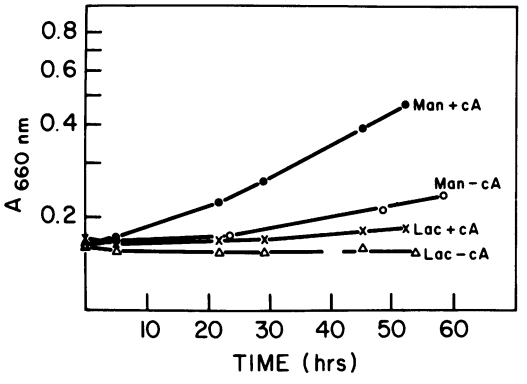

FIG. 5. *Differential effect of exogenous dibutyryl cyclic AMP (3 × 10⁻³ M) on growth on lactose and mannose of C. crescentus AE9.*

TABLE 4. *Intracellular concentrations of cyclic AMP and cyclic GMP*[a]

Carbon source (%)	Growth stage	Cyclic GMP (×10⁻⁸ M)	Cyclic AMP (×10⁻⁷ M)
Glucose (0.2)	Stationary phase	0.23	
		0.27	
		0.19	
Glucose (0.2)	Mid-log phase	0.98	1.70
Mannose (0.2)	Mid-log phase	0.23	
Lactose (0.5)	Mid-log phase	0.33	1.90
Glucose → lactose[b]	6 h (cell cycle arrest)	0.46	
		0.41	
	14 h (cell cycle arrest)	0.58	2.30
	18 h (cell cycle arrest)		1.87
	22 h induced[c]	0.26	2.00
		0.29	

[a] Intracellular concentrations of cyclic nucleotides were determined by radioimmune assay as described in the text.

[b] A culture was grown to late log phase in minimal medium containing 0.2% glucose. Cells were collected by centrifugation, washed with minimal salt media, and suspended in lactose (0.5%) minimal medium at an OD of 0.340.

[c] Self-induced growth on lactose.

cell cycle arrest can be explained by assuming that the nonmotile predivisional cell represents a stable hypometabolic stage and that the cell must sense the metabolic state before initiating a new cell cycle. This assumption is further supported by the following. (i) A cell cycle arrest did not occur after transfer from glucose to lactose of a β-galactosidase-constitutive mutant (ii) Similarly, transfer to mannose, which is metabolized by constitutively expressed enzymes, did not result in a cell cycle arrest. (iii) A cell cycle arrest did occur when cultures were transferred from glucose to galactose, a carbon source that cannot be utilized in the absence of exogenous dibutyryl cyclic AMP. (iv) Transfer of glucose-grown cultures to minimal medium without any carbon source resulted in accumulation of cells at the nonmotile predivisional stage.

The control of cell cycle initiation has been shown to be dependent on the metabolic state of eucaryotic cells. Hartwell has observed that the cell cycle in wild-type yeast is arrested early in G1 by starvation for essential nutrients (3). The functional state of amino acid biosynthesis in yeast, furthermore, can apparently be read by the cell as part of a "start" signal for the cell cycle (20). Yeast mutants aberrant in the general control of amino acid biosynthesis are temperature sensitive for cell cycle initiation and arrest early in G1 (20), indicating that there exists a regulatory system which coordinates the progress of the cell cycle and the metabolic state of cells.

To establish a correlation between a cell cycle start period and carbon source utilization, we studied the growth of *C. crescentus* on a number of carbohydrate carbon sources and determined the inducible or constitutive nature of

the enzymes for the initial catabolic steps. The common pathway of hexose utilization in *Caulobacter* proceeds via the Entner-Doudoroff pathway (7) analogous to carbohydrate metabolism in *Pseudomonas* (5). 2-Keto-3-deoxy-6-phosphogluconate aldolase has been detected in extracts of *C. crescentus* (7). We have determined that both transaldolase and transketolase activities are present in *C. crescentus* CB13 cell extracts (assayed by the method of Tsolas and Horecker [18] and Rutter et al. [10]), but not fructose 1,6-diphosphate aldolase. High levels of glucose-6-phosphate dehydrogenase were reported in extracts of *C. crescentus*, and the enzyme activity was shown to be induced by growth on glucose (7, 15). A pathway for galactose catabolism similar to that of *Pseudomonas* has been demonstrated in *C. crescentus* (in preparation) and was shown to be induced by growth on galactose. The components of the phosphotransferase system responsible for sugar transport in many bacteria (8) could not be detected in *C. crescentus* (M. Saier, personal communication).

In the present study we have determined the inducible and constitutive nature of the initial steps in the catabolism of lactose and mannose. Both β-galactosidase and lactose uptake were shown to be induced by growth on lactose. The enzymes for mannose catabolism were shown to be constitutively expressed. Dibutyryl cyclic AMP was shown to stimulate induction of lactose catabolism and, consequently, growth on lactose. This cyclic nucleotide was also shown to stimulate growth on sugars utilized by constitutive enzymes. The short growth lag after the transfer from glucose to mannose, ribose, or fructose was eliminated by exogenous dibutyryl cyclic AMP, and the growth on these sugars was faster in the presence of this cyclic nucleotide. The stimulation of growth rate on mannose by dibutyryl cyclic AMP did not result from increased levels of the corresponding catabolic enzymes. Since growth on mannose, ribose, and fructose was very slow, we conclude that these sugars are poor carbon sources. It is thus possible that the stimulation of the rate of growth on these sugars by dibutyryl cyclic AMP resulted from interference with a growth inhibitor or the effect of this cyclic nucleotide on the general regulation of balanced growth, i.e., by affecting the relative rate of carbon and nitrogen metabolism.

Comparison of growth on mannose or lactose to growth on glucose suggests that utilization of lactose and mannose may be regulated via a mechanism that is distinct from that of glucose utilization. Characteristics shared by lactose and mannose utilization are as follows. (i) Growth on glucose is not affected by dibutyryl cyclic AMP, whereas growth on lactose and mannose is stimulated by this nucleotide. (ii) Growth on lactose and mannose is temperature sensitive. Growth on lactose at 37°C is possible only in the presence of dibutyryl cyclic AMP. Growth on mannose, ribose, and fructose is slower at the restrictive temperature and can be stimulated by dibutyryl cyclic AMP at this temperature. (iii) Intracellular concentrations of cyclic GMP in cultures grown on lactose and mannose are lower than the concentrations found in cultures grown on glucose. In the gram-negative bacterium *Caulobacter*, glucose repression of catabolic enzymes was previously reported, and dibutyryl cyclic AMP was shown to stimulate the expression of inducible enzymes, although the intracellular concentration of cyclic AMP does not vary with carbon source (13). The target for dibutyryl cyclic AMP and the role of cyclic GMP in growth regulation, however, are not clear at present.

Dibutyryl cyclic AMP stimulation of growth on sugars metabolized by constitutive enzymes, on the one hand, and stimulation of the formation of inducible enzymes, on the other hand, may reflect interaction of this cyclic nucleotide with different components of a general regulatory mechanism. The differential effect of dibutyryl cyclic AMP on mutant strain AE9, grown on inducibly and constitutively utilized carbon sources, may indicate that two aspects of this general control mechanism can be dissociated. The mutant was incapable of growth on either lactose or galactose, even in the presence of dibutyryl cyclic AMP. This mutation, however, did not affect the control mechanism that operates in the case of growth on the constitutively utilized sugars that remained sensitive to exogenous dibutyryl cyclic AMP. No revertants that would grow on either lactose or galactose in the absence or presence of dibutyryl cyclic AMP could be recovered (frequencies less than 10^{-10}), although spontaneous drug-resistant mutants of this strain were obtained at frequencies similar to that of the wild-type strain. It thus appears unlikely that the phenotype results from a double mutation in lactose and galactose metabolism and that the mutation may, in fact, be a deletion of, or a multiple mutation in, a function necessary for the induction of the catabolic enzymes for lactose and galactose utilization. An alternative to saying that the mutation has an altered response to cyclic nucleotides is that, if there were a mutation in a common catabolic step in lactose and galactose utilization, it might render the mutant strain incapable of

growth on these two sugars and that the phenotype is independent of cyclic nucleotide control. The observation that growth of AE9 on mannose as well as ribose and fructose is considerably slower than the growth of the wild-type strain suggests that the mutation is not in a structural gene for lactose and galactose utilization, but in a general regulatory function.

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