of Panel Reports (Washington, D.C., 1969).

8. The unrestricted use of outer space for peaceful purposes is assured by the U.N. Treaty on the Peaceful Uses of Outer Space; both the United States and the Soviet Union are officially committed to space-based earth resources programs; some 60 nations participated in the Conference on the Peaceful Uses of Outer Space in Vienna in August 1968 and expressed no reservations in extensive discussion of earth resource surveys; and the United Nations has unanimously affirmed several measures to facilitate information and participation in this field by member states.
9. A. W. Frutkin, *International Cooperation in Space* (Prentice-Hall, Englewood Cliffs, N.J., 1965), chap. 3, pp. 85–131.
10. See *Congr. Rec.*, pp. S14028–29. The full text of the material cited by Senator Pastore on 10 November 1969 appears in a staff paper, "U.S./U.S.S.R. Cooperation in Space Research," prepared by the Office of International Affairs, NASA. The paper is periodically updated to record continuing U.S. initiatives. The latest amendment is dated 6 March 1970.
11. For an elaboration of these current efforts to increase foreign participation and a more detailed report on many elements of this article, see testimony by the administrator of NASA, Thomas O. Paine, in *Hearings before the Committee on Aeronautical and Space Sciences, United States Senate, Ninety-first Congress, Second Session, on S. 3374* (11 March 1970), *Part 3: International Space Cooperation.*

Cyclic Adenosine Monophosphate in Bacteria

In many bacteria the synthesis of inducible enzymes requires this cyclic nucleotide.

Ira Pastan and Robert Perlman

Escherichia coli contains the genetic information for the synthesis of enzymes needed to utilize many substances as sources of carbon and energy (*1*). Ordinarily, however, this genetic information is not expressed; the organisms only make the enzymes required for the utilization of a particular compound when that compound (or an analog) is present in the medium (*2*). For example, two proteins are required for the utilization of lactose: a galactoside permease, which permits the entry of lactose into the cell, and β-galactosidase, which catalyzes the hydrolysis of lactose to glucose and galactose. (A third protein, thiogalactoside transacetylase, is synthesized coordinately with the other two; its role in lactose metabolism is unknown.) The addition of lactose, or a nonmetabolizable lactose analog such as isopropylthio-β-D-galactoside (IPTG), to a culture of *E. coli* induces the synthesis of large amounts of these three proteins; in the absence of an inducer they are present in only very small amounts (*2*). Similarly, other potential carbon sources induce the enzymes required for their metabolism. The mechanism by which β-galactosides induce β-galactosidase synthesis has recently been reviewed (*3*).

The presence or absence of inducer is not the only factor which regulates the synthesis of inducible enzymes. Even in the presence of lactose or IPTG, the differential rate of β-galactosidase synthesis (the rate of enzyme synthesis divided by the rate of total protein synthesis) can vary greatly, depending on the medium in which the cells are growing. The differential rate is high in cultures where succinate is a carbon source, in which growth is slow, and low in cultures with carbon sources such as glucose, which permits rapid growth. The repression of inducible enzyme synthesis by glucose has been known for many years and was originally called the "glucose effect" (*4*). More recently, other carbon sources have been found to cause a similar repression, and so new names, such as "metabolic repression" (*5*) or "catabolite repression" (*6*), have been used to describe the phenomenon.

In 1965, Makman and Sutherland reported that *E. coli* contained the cyclic nucleotide adenosine 3′,5′-monophosphate (cyclic AMP) and that glucose lowered the concentration of cyclic AMP in these organisms (*7*). Cyclic AMP has an important regulatory role in animal cells (*8*), and it seemed possible that it had an equally important regulatory role in bacteria. We postulated that the repression of enzyme synthesis by glucose and other carbon sources might be due to the lowering of the concentration of cyclic AMP by these compounds. The addition of cyclic AMP to cultures in which β-galactosidase synthesis was repressed by glucose or other carbohydrates largely overcame this repression (*9–11*) and increased enzyme synthesis toward the level found in cells grown with succinate (*12, 13*) (Table 1). The effect was specific for cyclic AMP. Other adenine nucleotides, such as adenosine triphosphate (ATP), adenosine diphosphate (ADP), 5′-AMP, and 3′-AMP, and analogs of cyclic AMP, including 2′-deoxy cyclic AMP, N^6,$O^{2'}$-dibutyryl cyclic AMP, N^6-monobutyryl cyclic AMP, and cyclic guanosine 3′,5′-monophosphate were ineffective.

A second effect of glucose is observed when glucose is added to cultures grown on a poorer energy source, such as succinate or glycerol, and induced to make β-galactosidase. After the addition of glucose, there is a transient period of complete or almost complete repression of β-galactosidase synthesis. The duration of this "transient repression" is variable, but is often on the order of 20 to 30 minutes (*14–16*). After the period of transient repression, β-galactosidase synthesis resumes but now at the lower differential rate characteristic of cultures grown on glucose. Cyclic AMP prevents both types of repression (Fig. 1). Therefore, both forms of repression appear to be due to lowered cyclic AMP concentrations; apparently, the cyclic AMP concentration is lower during transient repression.

Glucose represses the synthesis of a number of inducible enzymes and trans-

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port proteins, in addition to β-galactosidase. We have studied the effect of cyclic AMP on the synthesis of many of these (*9*). In every case, cyclic AMP stimulated enzyme synthesis in cultures repressed by glucose. Although cyclic AMP stimulated the synthesis of a diverse group of proteins, including those involved in carbohydrate transport and metabolism, amino acid metabolism, and pyrimidine metabolism, its action was still specific; the nucleotide did not stimulate total RNA or protein synthesis and had no effect on the synthesis of enzymes not subject to glucose repression, such as tryptophan synthetase and alkaline phosphatase (*9*).

Cyclic AMP controls the synthesis of inducible enzymes in a number of other Gram-negative organisms in addition to *E. coli*. In every organism studied, cyclic AMP prevents the glucose repression of the synthesis of an inducible enzyme (*17*). We have not yet tested the effect of cyclic AMP in any Gram-positive bacteria.

Cyclic AMP Concentrations

Exogenous cyclic AMP prevents the repression of enzyme synthesis by glucose. To show that glucose repression is due to lowered cyclic AMP concentrations, we measured these concentrations in cultures repressed by glucose and other substances under conditions identical to those used for the study of enzyme synthesis. The concentration of cyclic AMP in cultures growing exponentially on glycerol was about 5 nanomoles per gram (wet weight) of cells. Five minutes after the addition of glucose, the concentration fell to 0.5 nanomoles per gram; after the addition either of α-methyl glucoside (a glucose analog) or of pyruvate, the concentration fell to 1.3 nanomoles per gram. All of these substances repress β-galactosidase synthesis, but the repression produced by glucose is the most severe. There is at least a qualitative correlation between the ability of a compound to repress β-galactosidase synthesis and its ability to lower cyclic AMP concentrations (*18*).

Table 1. Effect of cyclic AMP on the differential rate of enzyme synthesis in cells growing on various carbon sources [from table 1 (*2*)].

Carbon source (percent)	Enzyme synthesis (unit/mg)	
	No cyclic AMP	$5 \times 10^{-3} M$ cyclic AMP
Succinate (0.5)	350	368
Glycerol (0.5)	313	378
Glucose (0.5)	203	378
Glucose-6-phosphate (0.2)	156	323
Glucose (0.2) + gluconate (0.2)	87	271

Cyclic AMP–Deficient Mutant

The physiological functions of cyclic AMP can best be determined by studying the properties of a mutant unable to synthesize the nucleotide. In *E. coli*, as in animal cells, cyclic AMP is synthesized from ATP by the enzyme adenyl cyclase, according to the reaction

$$\text{ATP} \rightarrow \text{cyclic AMP} + \text{P-P}_i$$

Adenyl cyclase from *E. coli* has been detected and characterized by Ide (*19*) and by Tao and Lipmann (*20*). Since cyclic AMP has no important effect on total RNA or protein synthesis or on the synthesis of biosynthetic enzymes, it seemed possible that an adenyl cyclase deficiency would not be lethal. Rather, if cyclic AMP were required only for the synthesis of inducible enzymes such as β-galactosidase and galactokinase, an adenyl cyclase–deficient mutant would be unable to utilize normally a number of carbon sources, including lactose and galactose. Beckwith suggested that, by selecting on a single plate the mutants that could not ferment lactose and galactose, we might find a mutant deficient in cyclic AMP. By such a procedure, a mutant (strain 5336) was isolated which was markedly deficient in adenyl cyclase activity and which contained undetectable concentrations of cyclic AMP (*18*, *21*) (Table 2). This mutant was unable to grow on lactose, maltose, arabinose, mannitol, or glycerol, and grew slowly on glucose, fructose, and galactose (*21*). The addition of cyclic AMP to the medium permitted the normal utilization of all of these carbon sources (Table 3). When induced with IPTG, the adenyl cyclase–deficient mutant made only 5 percent as much β-galactosidase as did its parent strain; the addition of cyclic AMP increased β-galactosidase synthesis toward wild-type concentrations. As expected, the physiological defects in this mutant were not restricted to carbohydrate metabolism. Indole production is a measure of tryptophanase activity. The adenyl cyclase–deficient mutant was unable to produce indole when grown in the absence of cyclic AMP, but was able to produce indole when grown with cyclic AMP. Further, the mutant made D-serine deaminase at a decreased rate in the absence of the nucleotide and at a normal rate in its presence (*22*). The fact that cyclic AMP corrected all of the metabolic defects in this strain strongly suggests that these other abnormalities are all secondary to the adenyl cyclase deficiency. Further evidence comes from an analysis of revertants of this mutant. Ten revertants were isolated independently on the basis of their ability to grow on lactose. All ten had regained adenyl cyclase activity (*23*).

Control of Cyclic AMP Concentrations

The regulation of inducible enzyme synthesis by cyclic AMP involves two general questions. First, what are the mechanisms which regulate the concentration of cyclic AMP; and, second,

Cyclic AMP stimulates the synthesis of

- β-Galactosidase (E.C. 3.2.1.23)
- *lac* Permease
- Galactokinase (E.C. 2.7.1.6)
- Glycerol kinase (E.C. 2.7.1.30)
- α-Glycerol phosphate permease
- L-Arabinose permease
- Fructose enzyme II (phosphotransferase)
- Tryptophanase
- D-Serine deaminase
- Thymidine phosphorylase (E.C. 2.4.2.4)

Bacteria affected by cyclic AMP

Organism	Enzyme
Escherichia coli	Many
Salmonella typhimurium	Galactokinase
Salmonella typhimurium F′-lac$^+$	β-Galactosidase
Aerobacter aerogenes	β-Galactosidase
Serratia marcescens F′-lac$^+$	β-Galactosidase
Proteus inconstans P-lac$^+$	β-Galactosidase

Table 2. Adenyl cyclase activity of strains of *E. coli* [adapted from (*21*)]. The activity is expressed as the number of picomoles of cyclic AMP formed per 30 minutes per milliliter.

Strain	Adenyl cyclase (pmole 30 min^{-1} ml^{-1})	Specific activity (pmole 30 min^{-1} mg^{-1})
1100	5720	311
5336	200	19

what are the mechanisms by which cyclic AMP stimulates enzyme synthesis? Three processes appear to participate in the regulation of cyclic AMP concentrations in *E. coli.*

1) As has been mentioned above, cyclic AMP is synthesized from ATP by adenyl cyclase. Ide screened a large number of compounds for their effect on *E. coli* adenyl cyclase activity (*19*). Although the enzyme was inhibited by high concentrations of pyridoxal phosphate, oxaloacetate, and malate, and was stimulated by high concentrations of guanosine 3′,5′-monophosphate and phosphoenolpyruvate (PEP), there is no evidence that these or any other compounds tested participate in the physiological regulation of adenyl cyclase activity in *E. coli.* Catecholamines, which stimulate adenyl cyclase activity in a number of animal tissues, had no effect on the *E. coli* enzyme (*19*).

2) In animal cells, cyclic AMP is hydrolyzed to 5′-AMP by a cyclic AMP phosphodiesterase. Methylxanthines elevate tissue concentrations of cyclic AMP by inhibiting this phosphodiesterase. Some strains of *E. coli,* including *E. coli* K12, contain a cyclic AMP phosphodiesterase (*24*, *25*), but the activity of this enzyme is very low or absent in other strains, including *E. coli* B and Crooke's strain (*23*). Cyclic AMP phosphodiesterase activity in *E. coli* extracts is critically dependent on the presence of a reducing agent; the function of the reducing agent may be to keep ferrous ion reduced, since the phosphodiesterase requires ferrous ion for activity (*23*). In contrast to the mammalian enzyme, the *E. coli* phosphodiesterase is not inhibited by methylxanthines. Conceivably, cyclic AMP phosphodiesterase activity in vivo is regulated by some oxidation-reduction system. However, because of the very high activation constant (K_m) of this enzyme for cyclic AMP (5×10^{-4} to $10^{-3}M$) (*23*), and because the glucose repression of β-galactosidase synthesis is similar in K12 3.000 and in Crooke's strain, strains with and without the phosphodiesterase, the role of the cyclic AMP phosphodiesterase in the regulation of cyclic AMP concentrations in *E. coli* is uncertain.

3) A third mechanism that controls cyclic AMP concentrations is its release from the cells into the medium. Makman and Sutherland showed that the lowering of cyclic AMP concentrations by glucose was accompanied by the appearance of cyclic AMP in the medium, and suggested that a major action of glucose was to facilitate the exit of the nucleotide from the cell (*7*). Cyclic AMP has also been identified in the culture media of *E. coli* by Konijn *et al.* (*26*), who showed that the nucleotide is the acrasin which attracts the slime mold *Dictyostelium discoideum* to *E. coli* (*26*). The lowering of cyclic AMP concentrations by glucose, particularly the acute fall in cyclic AMP during the period of transient repression, may be due to the excretion of cyclic AMP from the cell.

Whatever the mechanisms by which glucose regulates cyclic AMP concentrations, it is clear that glucose can lower cyclic AMP under conditions in which the sugar is not extensively metabolized. α-Methylglucoside and 2-deoxyglucose, analogs of glucose that are phosphorylated but not further metabolized, cause transient repression of β-galactosidase synthesis (*16*, *27*), and α-methylglucoside does lower cyclic AMP concentrations.

The regulation of β-galactosidase synthesis by glucose in mutants of the PEP-phosphotransferase system has also been studied. This system, described by Roseman and co-workers (*28*), can be diagrammed as follows

$$\text{PEP} + \text{HPr} \xrightarrow{\text{Enz I}} \text{P}\sim\text{HPr} + \text{Pyruvate}$$

$$\text{P}\sim\text{HPr} + \text{Sugar} \xrightarrow{\text{Enz II}} \text{Sugar-P} + \text{HPr}$$

Enzyme I catalyzes the transfer of phosphate from PEP to a histidine residue on a small, heat-stable protein, HPr. Enzyme II catalyzes the subsequent transfer of phosphate from P ~ HPr to a sugar acceptor. There appears to be a family of enzyme II molecules with specificity for different sugars or groups of sugars (*28*). Mutants deficient in enzyme I or in HPr are unable to accumulate or phosphorylate a large number of carbohydrates, including glucose, α-methylglucoside, and mannitol (*28*). However, β-galactosidase synthesis in some of these mutants was very sensitive (even more sensitive than in wild type strains) to transient repression by all three (*29*). These sugars did not produce permanent repression in the mutants, because the sugars are not metabolized. As in wild type cells, repression in the mutants was accompanied by a fall in cyclic AMP concentration (*18*) and was prevented by addition of cyclic AMP (*29*). Since glucose and α-methylglucoside had no effect on the activity of adenyl cyclase or cyclic AMP phosphodiesterase in *E. coli* extracts, they probably lowered cyclic AMP concentrations in these mutants by promoting the release of the nucleotide from the cells.

In contrast, β-galactosidase synthesis in a mutant deficient in enzyme II activity for glucose was resistant to repression by glucose (*29*). It appears that repression of β-galactosidase synthesis (and, therefore, lowering of cyclic AMP concentrations) requires only the interaction of a sugar with its enzyme II,

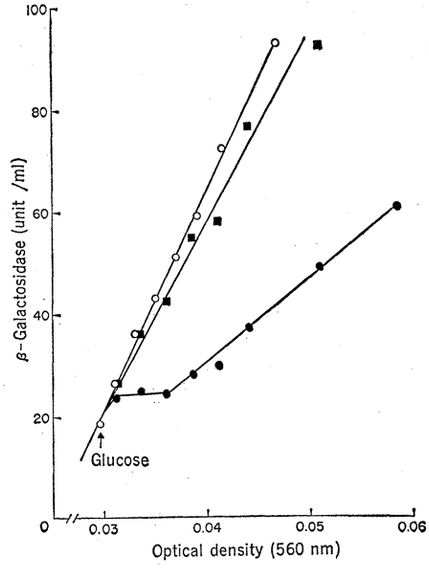

Fig. 1. Effect of glucose and cyclic AMP on the rate of synthesis of β-galactosidase. All samples contain IPTG. Glucose (●——●) or glucose and cyclic AMP (5 m*M*) (■——■) were added at the arrow.

Table 3. Ability of the mutant lacking adenyl cyclase to ferment and grow on various sugars [adapted from (*21*)].

Carbon source	Fermentation		Doubling time (min)	
	Without cyclic AMP	With cyclic AMP	No cyclic AMP	0.002*M* cyclic AMP
Lactose	−	+	>720	85
Maltose	−	+	>720	90
Arabinose	−	+	>720	70
Glycerol	−	+	>720	90
Mannitol	−	+	>720	65
Glucose	−	+	110	65
Fructose	−	+	110	70
Galactose	−	+	110	65

Table 4. The effect of IPTG, cyclic AMP, and glucose on the relative rates of *lac* mRNA synthesis, β-galactosidase synthesis, and the concentration of *lac* mRNA.

Additions	Relative *lac* mRNA synthesis rate	Relative *lac* mRNA concentration	Relative β-galactosidase synthesis rate
None	7	4	1
IPTG	100	100	100
IPTG + glucose	12	10	15
IPTG + glucose + cyclic AMP	119	82	130

and does not require its phosphorylation or subsequent metabolism. Enzyme II is located in the plasma membrane and probably mediates the entry of glucose by facilitated diffusion as well as its phosphorylation; the exit of cyclic AMP may be coupled to the entry of glucose and other sugars mediated by enzyme II. This process has not been studied further.

In contrast to transient repression, permanent repression does require glucose metabolism. Whether glucose metabolites influence the activity of adenyl cyclase, cyclic AMP phosphodiesterase, or the cyclic AMP exit system, or more than one of these processes, is not known.

Mechanism of Cyclic AMP Action: β-Galactosidase

We next turn to the mechanisms by which cyclic AMP stimulates the synthesis of inducible enzymes. The effect of the nucleotide on the synthesis of two enzymes, β-galactosidase and tryptophanase, has been studied. With respect to the stimulation of β-galactosidase synthesis by cyclic AMP, we asked two questions. (i) Does cyclic AMP stimulate the synthesis of β-galactosidase messenger RNA, or does it increase the translation of this RNA into enzyme protein? (ii) Is any chromosomal site in the *lac* operon required for cyclic AMP action? The first question had previously been answered indirectly, first by Nakada and Magasanik (*30*), and then by ourselves (*10*). Nakada and Magasanik estimated the concentration of *lac* messenger RNA (mRNA) by the capacity of cells to synthesize β-galactosidase after removal of the inducer, and showed that, under certain conditions, glucose lowered the concentration of this mRNA. In similar experiments, cyclic AMP increased the amount of *lac* mRNA in glucose-repressed cultures (*10*). Recently, this question was studied more directly with a DNA-RNA hybridization assay performed on nitrocellulose filters as described by Gillespie and Spiegelman (*31*) to measure *lac* mRNA. Briefly, this assay involves pulse labeling RNA with [^{3}H]uridine, and comparing the ability of this RNA to hybridize with DNA from the hybrid transducing phage λh80dlac, in which some of the phage DNA is replaced by *lac* operon DNA, and with DNA from the parent phage, λh80. The difference in hybridization of RNA with these two DNA species is a measure of the amount of *lac* mRNA synthesized by the culture.

With this assay, uninduced cultures made 5 percent as much *lac* mRNA as did cultures induced with IPTG (*32*). The addition of glucose to induced cultures caused a marked decrease in the rate of *lac* mRNA synthesis; the addition of cyclic AMP to glucose-repressed cultures restored *lac* mRNA synthesis to its unrepressed rate (Table 4). Glucose did not affect the half-life of *lac* mRNA. A modification of the hybridization assay (*33*) permits one to measure the amount of *lac* mRNA in the culture (Table 4). Under all conditions there was a good correlation between the rate of *lac* mRNA synthesis, the amount of *lac* mRNA in the cell, and the rate of β-galactosidase synthesis (*34*). With the techniques currently available, it is difficult to distinguish between two possible mechanisms of cyclic AMP action; the nucleotide might increase the frequency of initiation of *lac* mRNA chains, or it might increase the rate of polymerization of these chains. An indirect answer to this question, in favor of the first alternative, has recently been provided by the work of Jacquet and Kepes (*35*), who measured the time required for the action of cyclic AMP to become resistant to actinomycin D and rifampicin. Their results suggest that cyclic AMP stimulates an early step in transcription—a step experimentally indistinguishable from that inhibited by rifampicin. Rifampicin specifically inhibits the initiation, and not the polymerization, of RNA chains (*36*).

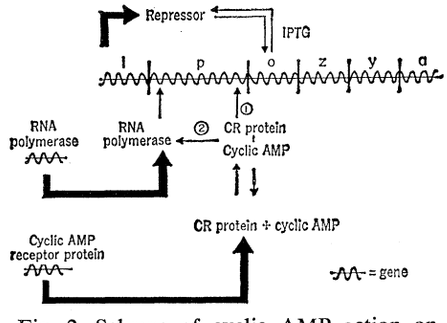

Fig. 2. Scheme of cyclic AMP action on the *lac* operon. The heavy arrows indicate gene products and the light arrows indicate reactions that these proteins undergo (see text). Cyclic AMP receptor protein, CR protein.

The conclusion that cyclic AMP stimulates the initiation of *lac* mRNA synthesis agrees well with the answer to the second question, the chromosomal site through which the effect of cyclic AMP is exerted. Figure 2 shows the lac operon: *z*, *y*, and *a* are the three structural genes of the operon, which code for the synthesis of β-galactosidase, galactoside permease, and thiogalactoside transacetylase, respectively; *i*, *p*, and *o*, are the regulatory genes of the operon. The *i* gene codes for a repressor protein which binds to the DNA of the operator (*o*) region, thereby preventing transcription of *lac* mRNA (*37*). Inducers, such as IPTG, stimulate *lac* mRNA synthesis by binding to the repressor and reducing its affinity for the operator (*38*). Most *i* and *o* gene mutants synthesize the *lac* proteins constitutively (in the absence of inducers) because the mutations decrease the tightness of the repressor-operator binding. [A few *i* gene mutants, called superrepressed mutants, are uninducible (*39*). These mutants make a repressor which binds to the operator but which has lost affinity for the inducer. We will not be concerned with this class of mutants.] The promoter, or *p* gene, controls the maximum rate of *lac* operon expression. Mutants of the promoter respond to inducer, but when fully induced make only a few percent as much of the *lac* proteins as do wild type strains. Because of its location in the operon, it has been proposed that the promoter is the site at which RNA polymerase binds to the *lac* DNA and the site at which *lac* mRNA synthesis is initiated (*40*). We have studied the effect of cyclic AMP in mutants of all three regulatory genes (*10*, *11*, *41*). β-Galactosidase synthesis in mutants of the *i* and *o* genes was repressed normally by glucose (*6*) and responded normally to cyclic AMP (*10*, *13*). In contrast, some promoter mutants were altered in their response to cyclic AMP. One mutant, which carries a deletion of most of the *p* and part of *i* gene (L1) was completely unresponsive to cyclic AMP. Another strain, which contains a point mutation in the *lac*

Table 5. The effect of cyclic AMP on the rate of β-galactosidase synthesis and *lac* mRNA synthesis in cell-free extracts of *E. coli*. A unit (U) of β-galactosidase is defined as the amount of enzyme that catalyzes the hydrolysis of 1 nanomole of *o*-nitrophenyl-β-D-galactoside per minute.

DNA	Cyclic AMP ($10^{-3}M$)	β-Galactosidase (U × 10^{-2})	*lac* mRNA (count/min)
None	−	0	0
λh80	−		46
λh80dlac	−	0.3	129
λh80dlac	+	28.0	209

promoter (L8), manifested a decreased sensitivity to cyclic AMP (*11*, *41*). The finding that cyclic AMP acts at the promoter site agrees with the observation of Silverstone *et al.* (42) that *trp-lac* fusion strains in which the promoter is deleted were resistant to glucose repression. The conclusion that cyclic AMP action requires an intact promoter site is also consistent with its suggested role in facilitating the initiation of *lac* mRNA synthesis.

Mechanism of Cyclic AMP Action: Tryptophanase

The action of cyclic AMP on tryptophanase synthesis differs from its action on β-galactosidase. The concentration of cyclic AMP required to restore enzyme synthesis in cultures repressed by glucose to 50 percent of the unrepressed rate was about 0.8 mmole/liter for β-galactosidase and galactokinase, but was 2 mmole/liter for tryptophanase (*12*, *17*). Further, studies on the regulation of tryptophanase synthesis revealed that cyclic AMP stimulated the amount of tryptophanase made when the nucleotide was added after mRNA synthesis was arrested by treatment of cells with proflavine or actinomycin D or by removal of the inducer (*43*). These results indicated that cyclic AMP was acting at a translational level. Cyclic AMP had no effect on the rate of breakdown of the tryptophanase mRNA, as assessed by the rate of fall of tryptophanase synthesis after the addition of proflavine, nor on the rate of conversion of tryptophanase precursor to active enzyme (*44*). Thus cyclic AMP appeared to increase the rate of polypeptide chain elongation. This action may be related to the observation of Kurvano and Schlessinger (*45*) that ribosomal G factor, the protein which participates in the translocation of the growing peptide chain along the ribosome, binds cyclic AMP in the presence of guanosine triphosphate.

Cyclic AMP Action in Cell-Free Extracts

A detailed investigation of the mechanism of action of cyclic AMP requires studies in a cell-free system. Zubay and associates have described a cell-free system in which β-galactosidase is synthesized in vitro by an extract from *E. coli* upon the addition of DNA from a phage carrying the *lac* operon (*46*). The synthesis of β-galactosidase in this system is highly dependent on the addition of cyclic AMP (*46*). We employed DNA-RNA hybridization to determine whether cyclic AMP affected the rate of *lac* mRNA synthesis in these extracts. *Lac* operon DNA comprises only about 5 percent of the added phage (λh80dlac) DNA. We detected *lac* mRNA by hybridization to a DNA which shares *lac* genes but no other genes with λh80dlac DNA. For this purpose we used DNA from *Proteus mirabilis* F'lac which carries the *lac* genes on the F'lac episome. As shown in Table 5, cyclic AMP increased the rate of synthesis of *lac* mRNA in these extracts (*47*). The finding that considerable *lac* mRNA, but very little β-galactosidase, is made in extracts without cyclic AMP suggests that much of the RNA made in the absence of cyclic AMP cannot be translated into active enzyme. Presumably, cyclic AMP directs RNA polymerase to initiate transcription at the *lac* promoter, and thereby decreases the frequency of random transcription.

Although cyclic AMP stimulated *lac* mRNA synthesis in unfractionated extracts of *E. coli*, it did not stimulate transcription when purified RNA polymerase was incubated with λh80dlac DNA. Thus one or more additional components must be involved in cyclic AMP action.

Cyclic AMP Receptor Protein

As mentioned earlier, mutants deficient in adenyl cyclase are unable to use a variety of carbon sources for growth. There exists a second class of mutants which have a similar phenotype, but which contain normal or increased concentrations of adenyl cyclase and cyclic AMP. It seemed likely that such mutants were defective in a protein (or proteins) with which cyclic AMP might interact to produce its effect. In an attempt to isolate this hypothetical cyclic AMP receptor, we searched in normal cells for a protein with a high affinity for cyclic AMP. We found and purified

Table 6. Stimulation of β-galactosidase synthesis by cyclic AMP receptor protein in cell-free extracts of a mutant containing a defective cyclic AMP receptor protein. β-Galactosidase activity is defined in Table 5.

Cyclic AMP ($10^{-3}M$)	Cyclic AMP receptor protein (μg)	β-Galactosidase (U × 10^{-2})	
		Mutant	Wild type
0	0	2	4
+	0	14	109
+	5	30	
+	25	40	86
0	5	2	4

such a protein ($K_a = 1 \times 10^6$ liters per mole). Two pleiotropic mutants have proteins with a decreased affinity for cyclic AMP, suggesting that the altered protein which binds cyclic AMP causes the pleiotropic phenotype (*48*). Cell-free extracts of these pleiotropic mutants are deficient in their ability to support β-galactosidase synthesis in vitro (Table 6). Enzyme synthesis in these extracts is stimulated by the addition of increasing amounts of purified cyclic AMP binding protein. The protein is inactive in the absence of cyclic AMP and does not stimulate enzyme synthesis in extracts from normal cells. We have named this protein the cyclic AMP receptor protein until its enzymatic function is established. Zubay, Schwartz, and Beckwith (*49*) have also purified a protein required for β-galactosidase synthesis in vitro, and have isolated mutants deficient in this protein. The "catabolite–gene activator protein" purified by these workers may be identical to the cyclic AMP receptor protein.

We have summarized the proposed site and mechanism of cyclic AMP action in the *lac* operon (Fig. 2). The nucleotide stimulates the synthesis of *lac* mRNA by increasing the frequency of initiation of *lac* mRNA chains. Cyclic AMP appears to act at the promoter site, since *p* mutants do not respond to the nucleotide; *i* and *o* mutants respond in a normal manner. Cyclic AMP itself does not act directly on DNA or RNA polymerase but acts by way of one (or possibly more) protein intermediates. One intermediate is the cyclic AMP receptor protein. This protein probably facilitates the initiation of transcription, but it is unclear whether it produces some change in the *lac* DNA (Fig. 2, arrow 1), or RNA polymerase (Fig. 2, arrow 2). Two factors stimulating the initiation of transcription of DNA from bacteriophage T4 and T7 have recently been described (*50*). These proteins, designated sigma factors, combine with RNA polymerase.

It is possible that the cyclic AMP receptor protein acts in a similar manner. In animal cells cyclic AMP increases the activity of various protein kinases which subsequently leads to the activation of phosphorylase or to the phosphorylation of histones (*8*). However, we have as yet been unable to detect any protein kinase activity in our preparation of cyclic AMP receptor protein. The finding that cyclic AMP stimulates gene transcription and enzyme synthesis in cell-free extracts promises to lead to the elucidation of the precise mechanism of cyclic AMP action.

Summary

Both cyclic AMP and a specific inducer acting in concert are required for the synthesis of many inducible enzymes in *E. coli*. Little enzyme is made in the absence of either. In contrast to the specific inducers which stimulate the synthesis only of the proteins required for their metabolism, cyclic AMP controls the synthesis of many proteins. Glucose and certain other carbohydrates decrease the differential rate of synthesis of inducible enzymes by lowering cyclic AMP concentrations. In the *lac* operon, cyclic AMP acts at the promoter site to facilitate initiation of transcription. This action requires another protein, the cyclic AMP receptor protein. The nucleotide stimulates tryptophanase synthesis at a translational level. The action of cyclic AMP in *E. coli* may serve as a model to understand its action on transcriptional and translational processes in eukaryotes.

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Notes on Science in Cuba

Ample moral and financial support may soon overcome the immaturity of Cuban science and technology.

Marcel Roche

In the Ilyushin turboprop from the Compañía Cubana de Aviación which flew us from Mexico to Havana, every seat was filled, mostly with long-haired students from the University of California and other schools, bound to Cuba to cut sugar cane for the Revolution. Enthusiasm was at a high pitch, and applause and cries of "Viva la Revolución" in strong North American accents broke out when the plane took off.

Fervor was indeed the keynote in Cuba among the many scientists and students I was able to interview, and I have allowed for this factor in the following notes. I was able, however, to visit all institutions that I requested to see after discussion with members of the Unesco staff. I was also able to roam about the city as I wished, and my wife often took the bus by herself and saw all of Havana that she wished to see (*1*).

Discussions with the Cubans were very free, and it was easy to obtain information. It was, however, almost impossible to obtain accurate data on budgets and expenses, except for the few examples that I give in the text. I was told that money means nothing in Cuba, that institutions do not have