Cyclic Nucleotides and the Regulation of Cell Growth

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Preface

The understanding of the mechanism(s) which are involved in the control of cell growth has for a long time been a black box, and still remains obscure at the present time. However, recent advances in cyclic nucleotide research have introduced a considerable body of information implicating cyclic AMP and cyclic GMP in the regulation of cell growth. The actual mechanism(s) through which these cyclic nucleotides take part in the control of cell growth remains largely unknown at the present time.

Due to the widening scope of these investigations, both at the metabolic and mechanistic levels, a specialized conference, representing a variety of views and dealing with different cell systems, both eukaryotic and prokaryotic, was held for a discussion of the recent advances in this area of cyclic nucleotide research.

The proceedings of this conference, published herein, present the current view of cyclic nucleotide involvement in the regulation of cell growth, as well as an up-to-date review of the field in a number of principal areas of cell growth.

For an appropriate sequence of information, this book is divided into five sections, three of which deal with the role of cyclic nucleotides in the regulation of bacterial growth, cell cycle, and cell proliferation; and two dealing with cyclic nucleotide metabolism and the regulation of cell growth, and mechanisms of growth regulation.

It is hoped that this book will provide for the student and the research scientist an updated view of the field with a detailed account of original discoveries in the area of cyclic nucleotides and the regulation of cell growth.

I would like to take this opportunity to acknowledge Rutgers University Research Council, Charles and Johanna Busch Memorial Fund to the Bureau of Biological Research, Roche Institute of Molecular Biology, Johnson & Johnson, E. R. Squibb, Merck and Company, Ortho Pharmaceutical, Ethicon Inc., and Pharmacia Laboratory for their support which made this conference possible.

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Contents

Part I

CYCLIC NUCLEOTIDES AND THE REGULATION OF BACTERIAL GROWTH

cAMP and the Regulation of Bacterial Growth

Morad Abou-Sabé

Bacterial growth may be defined in terms of either cellular mass or number of cells. The two remain proportional under conditions of steady growth, but this ratio can vary with growth conditions. As defined, regulation of bacterial growth thus becomes a reflection of the effect of growth conditions, presence or absence of growth factors in the growth medium and/or the culture response to molecules which do not, in themselves, serve as growth factors. Regulation of bacterial growth by the latter category thus implies a direct or indirect effect on one or more aspects of the cellular growth machinery, i.e., its metabolic proficiency (anabolic and amphibolic). Of such factors are metabolic inhibitors, metabolic stimulants, and factors that affect the cell in both directions inhibiting some reactions while stimulating others, such as cyclic 3',5'-adenosine-monophosphate (cAMP). Studies of the role of cAMP in many biological functions in a variety of prokaryotic systems have shown the involvement of cAMP in the transcription of inducible enzymes, the development of competence in bacteria, the cell cycle and development of *Caulobacter* (see Kurn and Shapiro, this volume), the formation of flagella and the sporulation process as well as many other specific functions (for recent reviews, see 40,41). The role played by cAMP in these diverse functions does not follow a uniform pattern, e.g., while cAMP stimulates transcription it inhibits uracil uptake in *Escherichia coli* (16,28).

If, at the risk of generalization, one considers

growth a final expression of all reactions and interactions that occur in the bacterial cell, cAMP effect on cell growth would become an expected result of its diverse effects on the cell. The difficulty in this conception is the fact that no primary biochemical reaction directly influencing or responsible for growth determination has been identified in any living system. Thus, growth remains a final expression of many biological reactions. Consideration of DNA synthesis and replication as the ultimate precursor of cell growth (reproduction) still does not satisfy the criteria of a primary biochemical reaction directly responsible for growth since DNA synthesis and replication are dependent upon many other prerequisite reactions in the cell. In order to define a primary step for cell growth, therefore, one would have to consider the cell as a whole, in its environment, define its interactions with the growth medium (under normal growth conditions), and characterize the first result of such interactions upon which the final cell growth characteristics are determined. Once studies direct themselves at the internal interactions, they have already passed the primary step. Interactions at the membrane level thus become of crucial importance in defining what (if anything) regulates cell growth.

Of the many biological effects of cAMP in bacteria, two have received most detailed analysis. The role of cAMP in catabolite repression has received extensive attention, resulting in the identification of the requirement of cAMP for the transcription of mRNA in inducible operons (16,46). On a different level, the role of glucose in the regulation of the cAMP level in bacteria, has been studied at great length (see below). These two specific levels of investigation were directed at two sides of the same coin. On the one hand, are the variations in the cAMP level directly related to the phenomenon of catabolite repression? On the other hand, how is the cAMP level itself regulated? These two questions are thus directly related since whatever the mechanism of cAMP regulation, its ultimate level itself will regulate transcription (as well as all other functions sensitive to cAMP) influencing, in the final analysis, cell growth. As pointed out above, in relation to cell growth, the regulation of the cAMP level thus becomes an essential step which ultimately affects cell growth. With this concept in mind, we have directed our attention in the past few years toward the question of the regulation of cAMP in bacteria.

REGULATION OF cAMP LEVEL IN BACTERIA

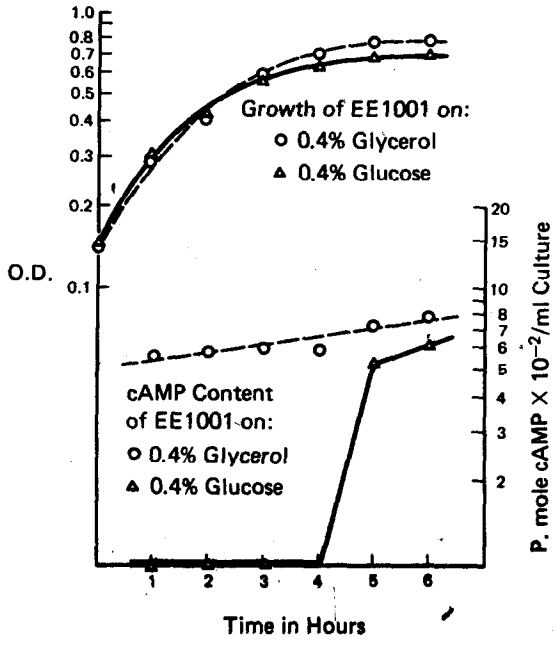
Metabolism of cAMP in bacteria involves two basic reactions, one in which cAMP is synthesized from ATP by the enzyme adenylate cyclase and a second reaction in which cAMP is hydrolyzed to 5'-AMP by the enzyme cAMP phosphodiesterase (35,44,45). Localization of these enzymes in *E. coli* showed the adenylate cyclase to be membrane bound and the cAMP phosphodiesterase to be soluble,

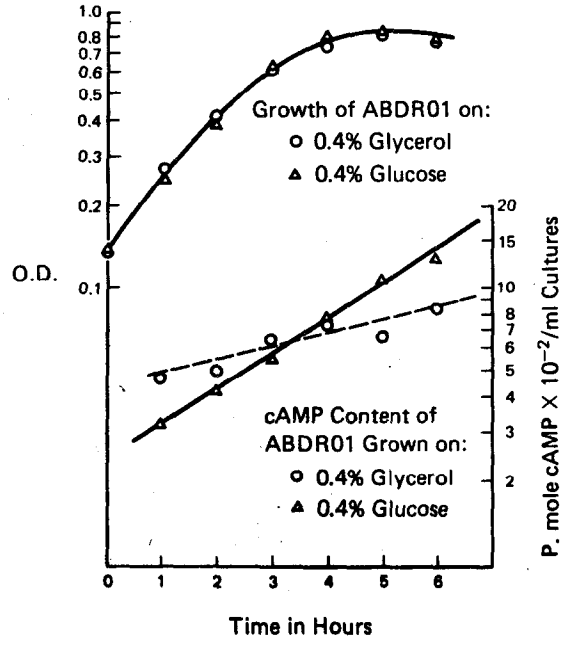
i.e., cytoplasmic (27,35,44,56).

Regulation of cAMP level in bacteria was first shown by Makman and Sutherland (33), they observed a glucose effect on the intracellular level of cAMP,where cells grown on glucose as a sole carbon and energy source had a lower level of cAMP than cultures grown in glycerol media. The level of cAMP also rose upon the depletion of glucose from the culture medium. This observation was explained to mean that in the presence of glucose cAMP was excreted from the cells at a higher rate, resulting in a lower level of intracellular cAMP. Glucose sensitive cells thus retain a low level of cAMP, and inducible enzyme synthesis, e.g., β-galactosidase, appears inhibited by glucose. Addition of exogenous cAMP to cultures grown in glucose was found to reverse the glucose effect on β-galactosidase synthesis (36). The question of glucose reduction of the level of cAMP in bacteria thus became a focal point in the understanding of the glucose affect (catabolite repression). Attempts by Ide (27) to show whether glucose or glucose metabolites had an affect on the adenylate cyclase in *E. coli* were unsuccessful. In 1970, we isolated a number of mutants of *E. coli* B/r (*rbs*$^+$) which were insensitive to glucose catabolite repression (1). Studies on one of these mutants (*E. coli* B/r ABDR01), showed that it was not only insensitive to catabolite repression, but also stimulated by glucose in its capacity to synthesize the inducible enzymes, β-galactosidase (β-gal) and tryptophanase (TPase) (2). Determination of the rate of cAMP synthesis in this mutant and its catabolite sensitive parent strain (*E. coli* B/r EE1001), showed that while cAMP synthesis was inhibited in EE1001 by glucose, it was synthesized throughout the culture's growth in glucose media at a rate one and one half times its rate of synthesis in glycerol grown cultures (Figures 1 and 2) (2). The availability of this mutant, ABDR01, facilitated our analysis of how the cAMP level is regulated in *E. coli*. Studies by Peterkofsky and Gazdar (37) on the effect of glucose on cAMP metabolism in *E. coli* showed that while cAMP formation depends on the absence of glucose, its excretion from the cells must be dependent upon some factor other than glucose. Determination of whether the adenylate cyclase and/or the cAMP phosphodiesterase activities or synthesis were subject to glucose presence (at different concentrations) in the culture media showed no significant alteration of either enzyme activity and the authors concluded that an explanation of the glucose effect on cAMP accumulation could not be based on changes in the activities of the synthetic or degradative enzymes of cAMP (37). Similar studies in Rickenberg's laboratory (9), however, supported Makman and Sutherland's idea of cAMP excretion in the presence of glucose.

REGULATION OF cAMP SYNTHESIS IN ESCHERICHIA COLI

It, therefore, appeared to us that a most direct way to elucidate whether the mechanism of glucose regulation





FIGURES 1 and 2. *cAMP synthesis in Escherichia coli B/r EE1001 and ABDR01 grown on minimal glycerol and glucose media (0.4%). Two ml samples were collected at 1 h intervals for cAMP assay, and 3 ml collected for turbidity measurements. cAMP assay samples were incubated in a boiling water bath for 3 min, and centrifuged at 1,000 rpm to remove cell debris. cAMP determination was performed by the Gilman binding assay (23). Figure 1 shows cAMP synthesis and culture growth in glycerol and glucose for EE1001, and Figure 2 shows cAMP synthesis and growth of ABDR01 in the same media. From Abou-Sabé (2).*