Cyclic AMP and the Induction of Eukaryotic Gene Transcription*

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There's no more important or ubiquitous a regulatory molecule than cAMP. It functions to coordinate diverse metabolic processes ranging from the breakdown of glycogen in mammalian liver to the synthesis of a number of specific enzymes involved in energy metabolism in Escherichia coli. The mechanisms by which cAMP exerts these diverse effects are pleiotropic in nature, altering numerous steps in a metabolic pathway simultaneously. Impressive progress has been made over the last 20 years in delineating the site of action of cAMP and in isolating and characterizing components involved in the cAMP-induced regulatory cascade. Equally impressive is the lack of progress in understanding the mechanism responsible for the very marked induction of gene expression by cAMP in eukaryotes, in comparison to the knowledge about the induction of gene transcription by cAMP in E. coli. However, this situation is changing rapidly as the promoter-regulatory regions of an increasing number of cAMP-regulated genes are isolated and characterized and specific regulatory proteins are identified. In this review we will focus on the regulation by cAMP of gene transcription in mammalian cells, using as models several of the better characterized gene systems.

All of the cAMP-regulated genes which have been isolated and characterized to date share several common characteristics. They are expressed in tissues which are responsive to hormones or regulatory factors, and their rate of transcription is rapidly altered by cAMP. Usually the mRNA produced by transcription of these genes has a relatively short half-life (1, 2), so that a change in gene transcription is accompanied by a rapid alteration in the level of synthesis of the protein coded for by the gene (3, 4). The promoter-regulatory regions of several of these genes have been isolated and cAMP regulatory elements (CREs) characterized by functional analysis of detailed deletions through the region (5-9) and by mutations in specific bases within the regulatory element(s). In general, CREs have the properties of enhancers in that they regulate transcription from promoters in a manner independent of position or orientation. When these regulatory elements were first described, there was some speculation that cAMP regulation of gene expression in eukaryotes occurred in a manner analogous to that in E. coli (10). This was based primarily on the occurrence of a sequence in the promoter-regulatory region of several genes transcriptionally regulated by cAMP which is similar to the consensus catabolite repressor protein binding site in the lac operon of E. coli (11). This suggestion was strengthened by the similarity between the regulatory subunit of cAMP-dependent protein kinase type II (RII) and E. coli catabolite repressor protein (12, 13). Despite the attractiveness of this hypothesis and the effort expended in establishing a functional link between the two proteins (13, 14), there is no evidence to date that the RII subunit of protein kinase is a specific DNA binding protein or that catabolite repressor protein-like sequences function as CREs in cAMP-regulated genes.

For the purpose of this review, we have placed genes which are transcriptionally induced by cAMP into two general categories. Group 1 genes are those which are rapidly regulated by cAMP, usually within minutes, while the transcription of genes in Group 2 is increased only after several hours of cAMP treatment (see Table 1). Also, the cAMP inducibility of genes in Group 1, although not demonstrated for all genes in this class, is cycloheximide-insensitive (15). We thus propose the rapidity of response to cAMP as a basis for classifying the cAMP-responsive genes, which may indicate that two different mechanisms are responsible for cAMP regulation of gene transcription. Since transcription of the genes in Group 1 seems to be insensitive to cycloheximide, their responsiveness to cAMP most likely involves the rapid modification of a pre-existing protein to a more active form. In contrast, the relatively slow rate of induction of transcription by cAMP of the genes in Group 2 suggests that ongoing protein synthesis is required for the effect of the cyclic nucleotide. To date, the genes in Group 1 are the most extensively characterized and will be the focus of the remainder of this review.

Anatomy of the cAMP Regulatory Domain in Eukaryotic Genes

There are two classes of cis-acting elements which have been identified in the cAMP-regulated genes in Group 1. The first functionally defined element was the 8-bp palindromic sequence T(G/T)ACGTCA, originally termed a CRE, which is highly conserved in the promoter-regulatory regions of the genes shown in Group 1A of Table I. The first evidence that this sequence was involved in the cAMP regulation of gene transcription came from studies of the promoter-regulatory region of the gene for the tetrodotoxin-sensitive sodium channel, and was labeled as the somatostatin CRE (7). In these studies of the promoter-regulatory region of the tetrodotoxin-sensitive sodium channel, the 8-bp palindromic sequence was found to confer cAMP inducibility to a heterologous promoter (16). Since transcriptionally induced genes are isolated and characterized and specific regulatory proteins are identified, it is reasonable to ask whether these CREs function as CREs in cAMP-regulated genes.

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Minireview: Cyclic AMP and Induction of Gene Transcription

TABLE I

| Consensus | CCCAGGCG | Reference |
|-----------|----------|-----------|
| Metallothionein IIa (human) | −178 CCCCAGGC −171 | 22 |
| Growth hormone (human) | −277 CCCAGGCG −284 | 42 |
| Prolactin (rat) | −76 CCCCTGGC −84 | 43 |
| Plasminogen activator (pig) | −642 CCCCAACC −635 | 44 |
| Tyrosine aminotransferase (rat) | −119 CCCCACCC −126 | 23 |
| Proenkephalin (human) | −77 CCGCCGGC −70 | 6 |

2. Slow responding genes

| Consensus | Reference |
|-----------|-----------|
| Lactate dehydrogenase (mouse) | 45 |
| Chorionic gonadotropin, β-subunit (human) | 46 |

PEPCK gene, can confer cAMP sensitivity to a neutral promoter when transfected into human choriocarcinoma cells, a cell line which does not normally express the PEPCK gene. This observation that a CRE from a gene expressed in rat liver can substitute for a CRE from a gene expressed in human placenta emphasizes the evolutionary conservation of the cis-acting element as well as the factor(s) which bind to this sequence.

There is clearly a limit in the degree to which isolated regulatory elements from a gene can be used to delineate the full extent of cAMP regulation of transcription. For example, we have observed that cAMP induction of transcription of a chimeric gene, containing a 47-bp segment of the P-enolpyruvate carboxykinase promoter-regulatory region linked to a neural promoter, was only a fraction of the level noted when native segment of DNA from the PEPCK gene (~416 to −61) was used (5). This region of DNA contains, in addition to a CRE, several other protein binding domains, as noted by DNase I footprinting. In the P-enolpyruvate carboxykinase promoter-regulatory region, a segment of DNA extending from −123 to −74 is protected from DNase I digestion by nuclear proteins. This region of the promoter contains a CRE at −94 to −75 and a putative CAAT box homology from −121 to −99 (19). Functional analysis of deletions through this region of DNA has shown that removal of the CAAT box results in a marked reduction in the rate of basal transcription of a linked structural gene in stably transfected hepatoma cells, as well as an attenuation in the level of induction by cAMP (5).

The proximity of two regulatory domains within the P-enolpyruvate carboxykinase promoter may facilitate protein-protein interactions, leading to a coordinated control of gene transcription. Thus, individual promoter elements, when tested in isolation, may not provide the same degree of transcriptional activity as observed with the intact promoter.

All of the CREs listed in Group 1A (Table I) lie within the first 150 bp of the 5’-flanking region of their respective genes. Therefore, these elements could be regarded as basal enhancers, in addition to functioning as inducible enhancers (20). A role for the CRE as a basal transcription element was suggested in deletion analysis of the P-enolpyruvate carboxykinase promoter-regulatory region (5). When the CRE was deleted from the promoter, the basal level of gene transcription was reduced and the responsiveness of the promoter to cAMP in hepatoma cells was abolished. Deletion of the region of the α-hCG gene promoter which contained the CRE enhanced the basal rate of transcription of its own promoter in a tissue-specific manner. However, its inducibility by cAMP was not tissue-specific. Also of interest is the recent work on cis-acting promoter elements of several adenovirus early region genes (40, 41). Elements within these promoters specifically bind cellular factors, and this binding is necessary for in vitro transcription. A subset of these elements bind to common factor and consensus-like binding sites. Available evidence thus supports a dual role for the CRE as both a basal and an inducible transcriptional enhancer element.

More recently, a different cAMP-responsive element was identified. This sequence, termed Activator Protein 2 (AP-2) binding site, was originally shown to be a basal transcription enhancer present in the SV40 and human metallothionine promoters (21). Its role as an inducible enhancer was shown when five copies of the AP-2 sequence were linked’ to the β-globin promoter and transfected into HeLa cells (22). Transcription from this promoter was induced by treatment of the cells with either phorbol esters, which activate protein kinase C, or by forskolin, which raises the concentration of cAMP. Addition of both provided an additive effect. These agents did not alter gene transcription when this chimeric-gene was transfected into HepG2 cells, suggesting that AP-2 is a cell-specific factor.

The identification of the AP-2 element provided an additional classification for genes which are acutely regulated by cAMP but which lack a CRE. We have listed these under the category of Group 1B genes in Table I. Direct binding of purified AP-2 to the AP-2 element(s) in the promoter-regulatory region of the metallothionine IIa, growth hormone, and proenkephalin genes has been demonstrated (21). Although footprinting of the tyrosine aminotransferase gene with purified AP-2 has not been reported, an unidentified nuclear factor(s) does bind over both AP-2 sequences in vitro (23). While the AP-2 element has not been subjected to the same detailed analysis as the CRE, the two share several features. First, more than one copy is present in the 5’-flanking regions of several genes (Table I); second, the AP-2 element can act as a basal enhancer, increasing the rate of gene transcription in the absence of any hormonal stimulation (21, 22). The AP-2 element, however, distinguishes itself from the CRE by its ability to mediate both cAMP and phorbol ester induction of gene transcription. One exception to our classification system is the proenkephalin gene, which contains both CRE (~97 to −90) and AP-2 (~80 to −65) elements (6). Deletion analysis of the promoter-regulatory region of this gene suggests that both sequences confer cAMP responsiveness, although the lack of a direct effect of phorbol ester on gene transcription (6) makes an unequivocal assessment of the role of the AP-2 element difficult.

Identification of Factors Involved in the Regulation of Gene Transcription by cAMP

The identification of sequences within the promoter-regulatory region of cAMP-sensitive genes is the first step in the isolation and characterization of regulatory molecules which alter transcription. These sequences can act as targets for nuclear proteins which are isolated and tested for specificity of binding by several methods, including DNase I footprinting. These techniques have been widely used as an assay for the identification and purification of a variety of transcription factors (24, 25). Footprinting of the promoter-regulatory regions of the genes for α-hCG (9) and glucocorticoid receptor (26) with nuclear proteins has been reported. The gene for α-hCG contains a 18-bp sequence that is directly repeated within the promoter-regulatory region. These sequences were shown by footprinting analysis to contain two functional CREs and to bind a nuclear protein(s) (9). Montminy and Bile-

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The mechanism and a Model for cAMP Stimulation of Gene Transcription

The precedent established for the cAMP regulation of gene transcription in E. coli has a strong appeal based on its simplicity and the conservation of certain elements of the system between prokaryotes and eukaryotes. As mentioned above, there is no evidence to support a direct role for the RII subunit of protein kinase in the regulation of gene transcription in eukaryotes. Several laboratories have reported that the RII subunit enters the nucleus and has provided evidence that it binds to chromatin (14, 32). The only evidence for direct binding of the RII subunit to DNA in vitro is the report by Constantinou et al. (33), indicating that the RII subunit of protein kinase has the properties of a topoisomerase. However, the topoisomerase activity of the RII subunit was restricted to the protein isolated from rat liver, and at this writing, the mechanism(s) involved in this effect have not been resolved. Virtually all of the evidence available to date supports the role of the C subunit of protein kinase as the proximal intermediate in the effect of cAMP on gene transcription. For example, the cAMP regulatory element from the somatostatin gene is not responsive to cAMP when it is transfected into PC12 cells which are deficient in type II cAMP-dependent protein kinase (7). Also, Grove et al. (34) have co-transfected cells with a plasmid containing the gene which codes for an inhibitor of the catalytic domain of cAMP-dependent protein kinase, together with a cAMP-inducible gene. Expression of the inhibitor gene reduced the cAMP induction of the co-transfected gene by 90-95%.

The most direct experimental evidence supporting an effect of the C subunit of protein kinase on gene transcription is the recent demonstration by Nakagawa et al. (35) that this subunit could stimulate the transcription of the gene for the urokinase-type plasminogen activator in vitro. This stimulation occurred only when an extract from the homologous tissue (kidney) was added to the in vitro transcription system. More important, the C subunit of protein kinase stimulated transcription of the urokinase gene to a greater extent than cAMP itself. These findings provide the first direct evidence that phosphorylation events are central to the mechanism by which cAMP enhances gene transcription. Whether the RII subunit of protein kinase modulates these events remains to be determined.

**Minireview: Cyclic AMP and Induction of Gene Transcription**

Despite the obvious risks in proposing a model for cAMP stimulation of gene transcription in such a rapidly moving field, such an exercise may be of some value in focusing the available experimental data. A starting point for any model is the multiple levels of control of gene transcription exerted by cAMP. For example, many of the genes listed in Table I are transcribed in a tissue-specific manner. Expression of a gene, such as tyrosine aminotransferase, may be controlled by the level of its methylation or by alterations in chromatin structure (23). Thus, transcription of the gene can be negligible, despite the presence in}

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**Fig. 1. Models for mechanism of cAMP-stimulated transcription.** The above model is based on the following considerations. 1) There are at least two cis-acting sequences, and their respective binding proteins, which are responsive to cAMP; 2) both may act to enhance basal transcription; 3) cAMP does not affect the binding activity of either protein; 4) the main effector of cAMP is the catalytic subunit of cAMP-dependent protein kinase. The above model illustrates only the CRE and its binding protein, but it also pertains to AP-2 and its binding site. A, the CRE binding protein (stippled triangle) binds to the CRE. This binding stimulates basal transcription by interacting with proximal promoter elements such as the TATA box binding factor (diagonal lined box) and/or RNA polymerase II (Pol II) (38). Elevation of cAMP induces phosphorylation of the CRE binding protein, which further stimulates transcription by one of at least two general mechanisms (B and C). B, phosphorylated CRE binding protein recruits more transcription factors such as pol II and/or increases the interaction between these factors. C, phosphorylated CRE binding protein interacts with a non-DNA binding protein (solid box) allowing the formation of a higher order protein complex.
cells of various transcriptional factors. Another mechanism controlling the expression of a gene in a specific tissue is the presence in these tissues of either positive or negative regulatory factors (20). The tissue specificity of AP-2 is an example of such a control (22). Finally, factors such as cAMP may accelerate the rate of basal transcription of a gene by modulating the activity of transcription factors already bound to target sequences in the promoter-regulatory region of the gene.

Maniatis et al. (20) have described a model for the regulation of gene transcription in which inducible enhancer elements can be controlled by positive or negative regulatory elements, either alone or in combination. In the case of cAMP-regulated genes, transcription factors appear to bind to the CRE and the AP-2 sites in a cAMP-independent manner, and thus resemble basal transcription factors. In this case, cAMP-inducible enhancer elements may represent a class of environmentally altered enhancers which are regulated by a rapid modification of proteins already bound to their specific DNA elements. These proteins will probably contain a DNA binding domain and a transcriptional activation domain, the latter being sensitive to specific metabolic signals. Such a domain separation has already been demonstrated for yeast transcription factors Gal 4 (36) and GCN 4 (37). In our model, these proteins maintain a basal rate of gene transcription, which is dependent on their concentration in a specific tissue. Rapid changes in the intracellular level of cAMP would then lead to a modification of the transcriptional activation domains of the CRE binding protein or the AP-2, already bound to their respective elements in the promoter-regulatory region of the gene. This modification in the binding protein could alter transcription in several ways (Fig. 1). First, it could increase the binding of basic transcription factors such as RNA polymerase II (pol II). Second, it could increase protein-protein interactions between the CRE binding protein/AP-2 and other transcription factors, such as the TATA binding factor and pol II (38). Third, the cAMP-induced phosphorylation of the transcriptional regulatory domain in the binding protein could lead to protein-protein interactions with another non-DNA binding protein, forming a higher-order complex with the basic transcriptional factors. This complex would then, in some as yet undefined manner, increase the rate of transcription of the gene.

This model applies only to those situations in which cAMP rapidly stimulates gene transcription and does not involve a direct effect of the R subunit of cAMP-dependent protein kinase. Although the model considers only cAMP and the subsequent phosphorylation of basal enhancer binding proteins, it may be more generally applicable to other types of regulation of gene transcription. For example, the induction of type I collagen gene transcription by transforming growth factor β, which is mediated through the binding site for nuclear factor 1 (39), may also fit the proposed model. Of course, the model is a speculative one and is based mainly on the existing experimental evidence, and it would have to be altered as new information is generated for several of the genes now under intensive study. That the CRE can act as a basal enhancer element has not as yet been rigorously established. Also, nuclear proteins dependent on cAMP for their phosphorylation remain to be isolated and characterized. The newly described in vitro transcription system, which is sensitive to cAMP (35), could be a valuable tool in testing a model of the type proposed in this review. However, this system must be more specific. Rapid changes in the intracellular level of cAMP in order to establish its general usefulness in delineating specific regulatory mechanisms. Clearly, our model for the regulation of gene transcription shares common features with schemes proposed by other investigators (20, 38).

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