Role of Cyclic AMP in the Control of Cell-Specific Gene Expression

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Genes have to be expressed in specific cell types at appropriate times of development dependent on external signals. CAMP signaling occurs in all cells, thus raising the question of how this signal transduction pattern is integrated into mechanisms determining cell-specific gene expression. We have analyzed expression of the tyrosine aminotransferase gene as a model to study the basis of this cell type specificity of hormone induction. We found that cell-type-specific expression is generated by combined action of CAMP signal-dependent and liver cell-specific transcription factors. The interdependence of the CAMP response element and an element determining liver cell specificity enables a gene to respond to an ubiquitous signal in a cell-specific manner. (Trends Endocrinol Metab 1993;4:204–209)

Extracellular signals influence gene expression by utilizing a limited number of intracellular signaling pathways. Certain hormones (for example, steroids and thyroid hormone) associate with cytoplasmic or nuclear receptor proteins, which are activated upon ligand binding to sequence-specific transcription factors [reviewed by Beato (1989)]. On the other hand, neurotransmitters, growth factors, and peptide hormones bind specifically to cell-surface receptors. This binding leads to increases in the levels of intracellular Ca²⁺ and/or synthesis of second messengers, which in turn may activate protein kinases and phosphatases within the cell (Berridge 1993). Alternatively, binding may initiate signal transduction by activation of membrane receptors, which either have intrinsic tyrosine kinase activity or the ability to recruit cytosolic tyrosine kinases (Schlesinger and Ullrich 1992). Either cascade can then lead to functional modifications of intracellular target proteins.

One of the best-studied examples of membrane-generated signal transduction is the activation of protein kinase A (PKA). Ligand binding to a membrane receptor activates, via G proteins, adenylate cyclase, which catalyzes an increase in the levels of CAMP. PKA is composed of two regulatory (R) and two catalytic (C) subunits [reviewed by Taylor et al. (1990)]. Binding of two cAMP molecules to each regulatory subunit of the holoenzyme liberates the C subunit, the active form of the enzyme, by reversible dissociation of the inactive R,C₂ complex. At present, four R subunits (R₁α, R₁β, R₂α, and R₂β) and three C subunits (Cα, Cβ, and Cγ) have been identified (Taylor et al. 1990). The regulatory subunits α are constitutively expressed in most tissues, whereas the regulatory subunits β are predominant in brain, testis, and ovary. In contrast to the holoenzyme, which is cytoplasmic and/or membrane associated, the free C subunit possesses a potential nuclear localization signal and can be rapidly and reversibly translocated to the nucleus (Meinkoth et al. 1990).

Stimulation of PKA clearly has pleiotropic effects, as the C subunit can phosphorylate a plethora of different proteins. Phosphorylation of target proteins that are present in many cell compartments can positively or negatively affect their activity. We restrict this review to the role of CAMP-induced modification of transcription factors. Since the role of cAMP in gene regulation has been extensively studied in liver, we primarily discuss examples of transcriptional regulation of genes involved in gluconeogenesis and glycolysis.

- **CAMP Activates a Family of Sequence-Specific Transcription Factors**

Recent work has shown that many CAMP-regulated genes have in their control region a short perfect or imperfect palindromic sequence termed CRE (cAMP-responsive element). Such a CRE was first identified in the somatostatin promoter by Montminy et al. (1986), and a protein binding to that sequence, a CRE-binding protein, CREB, was described (Montminy and Bilezikjian 1987). Cloning of the cDNA for CREB identified it as a member of the bZip family (Hoeffler et al. 1988, Gonzalez et al. 1989) of transcription factors that have a characteristic leucine zipper adjacent to a basic domain. These transcription factors have been shown to interact as dimers with palindromic binding sequences. The sequence of the leucine zipper domain (Zip) specifies formation of homo- or heterodimeric, whereas the basic DNA-binding domain (b) contacts the DNA [reviewed by Kerppola and Curran (1991)].

It later became clear that several CRE-binding proteins exist, called ATFs for activating transcription factors (Hai et al. 1989), which form a subfamily of bZip proteins. This subfamily is related to the API subfamily (Ziff 1990) that binds to the TPA response elements (TRE), a palindromic sequence highly related to the CRE, which transduces signals via the protein kinase C pathway (among others). Only a few members of the ATF family appear to be involved in cAMP signaling. Besides CREB, these are ATF1 (Hai et al. 1989, Hurst et al. 1991) and CRE-modulating protein or CREM (Floates et al. 1997, Del-
Figure 1. A protein-kinase-A (PKA) recognition site is conserved in CREB, CREM, and ATF1. Conserved amino acids are indicated by asterisks. The PKA recognition sequence is boxed, with the characteristic amino acids highlighted. The phosphorylated serine residue is in CREB, CREM, and ATF1.

Phosphorylation by PKA is necessary for transcriptional activation of these factors (Gonzalez and Montminy 1989, Lee et al. 1990, Hurst et al. 1991, Foulkes et al. 1992) and, in the case of CREB, also increases CRE binding (Nichols et al. 1992). The crucial role of CREB phosphorylation is underscored by the recently elucidated attenuation of CREB activity by dephosphorylation by protein phosphatase 1 (Hagihara et al. 1992). Similarly, in the extinguished state of hepatoma x fibroblast hybrids (see below), inactivity of cAMP-controlled genes was strongly correlated with lack of CREB phosphorylation by PKA (Boshart et al. 1991). Furthermore, in experiments in which a nonphosphorylatable transdominant-negative CREB mutant was expressed in transgenic mice under the control of the pituitary-specific GH gene promoter, a marked mutant phenotype was observed (Struthers et al. 1991). The transgenic mice showed a strong depletion of somatotrophic cells in the pituitary anterior lobe and developed as dwarfs. The mutant CREB acts as a transdominant-negative inhibitor by competitive inhibition of DNA binding of phospho-CREB and other CRE-binding proteins, or by forming inactive heterodimers.

- Interdependency of cAMP-Responsive and Cell-Specific Elements Generates a Signal-Dependent Switch

While the cAMP-PKA signaling pathway is operative ubiquitously and members of the CREB family activated by this signaling pathway are present in most cells, many of the cAMP-controlled genes are expressed in only one or a few cell types. For example, the enzyme tyrosine aminotransferase (TAT), which can be induced by glucocorticoids and cAMP, is expressed exclusively in liver parenchymal cells (Hargrove and Granner 1985). How is this cell-specific restriction achieved in view of the ubiquitous activity of the cAMP signal-transducing system? Detailed analysis of the control elements of the TAT gene by gene transfer experiments and by in vitro and in vivo protein-DNA-binding studies have helped to clarify this question.

Expression of the TAT gene is controlled by three liver-specific enhancers. One enhancer region (at -3.6 kb) mediates the cAMP response (Boshart et al. 1990), while the enhancer at -2.5 kb is responsive to glucocorticoids (Jamieson et al. 1987). A detailed analysis of the cAMP-responsive enhancer by clustered point mutations demonstrated that it consists of two essential elements [see Figure 2 (Boshart et al. 1990)], one of which is an imperfect CRE palindrome. cAMP administration leads to transcriptional activation of TAT that closely correlates with increased protein-DNA interaction at the CRE in vivo, as revealed by genomic footprinting (Weil et al. 1990). CREB binds to the TAT-CRE, as shown with CREB purified from rat liver and brain or generated by expression in Escherichia coli (Nichols et al. 1992). The other important element of the -3.6 kb enhancer interacts with the liver-enriched transcription factor HNF4 (Nitsch et al. 1993b), a member of the steroid receptor superfamily that controls the expression of several liver-specific proteins (Sladec et al. 1990). Either element, tested in front of a heterologous promoter, is inactive, but their combination restores cell-specific and cAMP-dependent expression as observed in the native TAT gene enhancer [Figure 3, Boshart et al. 1990]. This combination of two interdependent modules generates a sensitive switch, the activity of which depends on both the presence of the cell-specific factor and the amount of active CREB. This organization of cell-specific and signal-dependent elements should be contrasted with that of constitutively active enhancers. For example, mutational analysis of the SV40 enhancer showed that its activity is generated by multiple elements, none of which is essential for overall activity (Zenke et al. 1986, Fromental et al. 1988).

Analysis of control elements in other cAMP-inducible genes has also revealed this principle of modular organization of interdependent elements for generation of enhancers that operate as a cell type-specific switch. A few well-studied examples are briefly discussed.

The regulation of phosphoenolpyruvate carboxylase gene expression, which in many respects resembles TAT gene regulation, has been recently reviewed in great detail (Lucas and Granve 1992, McGrane et al. 1992). PEPCK is expressed in liver, kidney, and fat cells in dependence of cAMP and glucocorticoid, which upregulate, and of insulin, which downregulates its transcription. The regulatory region extending from -460 to +73 contains CREs and GREs (glucocorticoid response element) intermingled with binding sites for the liver-enriched factor HNF1 and members of the C/EBP family. On the other hand, L-type pyruvate kinase is negatively controlled by glucagon and cAMP. Experiments in transgenic mice have demonstrated that the sequences required for liver-specific and hormone-controlled expression reside between positions -183 and -11. This region contains binding sites for the liver-enriched factors HNF1 and HNF4, and an element that, in cooperation with the HNF4-binding site, confers repression by cAMP and induction by insulin and glucose (Bergot et al. 1992, Cuff et al. 1992). Interestingly, the latter element shows no homology with a CRE. The somatostatin gene is expressed in brain, in endocrine cells of the pancreas, and in the gastrointestinal tract. Pancreas-specific expression depends on an upstream sequence with a composite structure, which binds to the pancreas-specific transcription factor Isl-1 (Vallejo et al. 1992a, Leonard et al. 1992) and to a CRE-binding protein (Vallejo et al. 1992b). This upstream enhancer element interacts synergistically with a downstream CRE first identified by Montmny et al. (1988). The α-subunit glycoprotein is expressed in placental trophoblast cells and in the anterior pituitary, coordinately
Mutational analysis of the enhancer of the TAT gene by clustered point mutations shows that synergism of two essential elements is required for cell-specific and cAMP-dependent expression. The TAT upstream sequence from -3651 to -3660 is shown. The position of the CRE is indicated by arrows, and the position of the HNF4 binding site is overlined. The positions of the clustered point mutations are shown by brackets. Hepatoma cells were transfected with either the wild-type (wt) construct TATCAT or with the 17 mutants (Boshart et al. 1990). CAT activity is given as arbitrary units, setting wt expression to 100.

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We believe that the switchlike properties of the combination of cAMP-responsive and cell-specific elements represent a widely used principle in the control of gene activity. The glucocorticoid-dependent enhancer of the TAT gene also illustrates such an organization. The glucocorticoid receptor synergizes with members of the HNF3 and C/EBP families (Nitsch et al. 1993b). The nature of these enhancers enables a better understanding of the perinatal activation of gluconeogenic genes like TAT and PEPCK (Greengard 1970). Before birth, owing to low fetal levels of glucocorticoids and glucagon/cAMP, the enhancers are inactive, even though all components required for activation are already present. The inactivity of the CRE may be further enforced by high levels of free PKA-RI subunit (see below) and of insulin in the prenatal period, which antagonizes the activity of the CRE (Ganss and Weih unpublished). The postnatal hypoglycemia leads to elevation of glucagon and glucocorticoid levels, lowers insulin concentration, and thereby strongly induces synthesis of TAT and other gluconeogenic enzymes.

**The CRE Is a Target for Extinction of Gene Expression in Somatic Cell Hybrids**

The crucial role of CREs for the cell specific expression of cAMP-regulated genes is underlined by analysis of the ex.

Figure 2. Mutational analysis of the enhancer of the TAT gene by clustered point mutations shows that synergism of two essential elements is required for cell-specific and cAMP-dependent expression. The TAT upstream sequence from -3651 to -3660 is shown. The position of the CRE is indicated by arrows, and the position of the HNF4 binding site is overlined. The positions of the clustered point mutations are shown by brackets. Hepatoma cells were transfected with either the wild-type (wt) construct TATCAT or with the 17 mutants (Boshart et al. 1990). CAT activity is given as arbitrary units, setting wt expression to 100.

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Figure 3. Interdependence of a CRE and a liver cell-specific element—the basis of the cAMP-dependent enhancer. A combination of oligonucleotides containing either wild-type or mutated CRE and HNF4 motifs was cloned in front of the HSV-thymidine kinase promoter and assayed for CAT activity after transfection in hepatoma or fibroblast cells in the absence or presence of cAMP. ND, not determined.
distinct phenomenon (Boshart et al. 1991, Nitsch et al. 1993a). Extinction of gene activity in somatic cell hybrids is a multifactorial process that leads to the loss of cell-specific gene products of either parent after fusion of distinctly differentiated somatic cells (Davidson 1974, Weiss et al. 1988, Gourdeau and Fournier 1990). In rat hepatoma x mouse fibroblasts hybrids, a gene causing TAT extinction was localized on a distinct part of the human chromosome 17 (Killary and Fournier 1984, Leach et al. 1989). This locus, called TSEI (tissue-specific extinguisher), drastically reduces the expression of several liver-specific genes that are all regulated by cAMP (Thayer and Fournier 1989, Ruppert et al. 1990). Remarkably, repression by TSEI is reversible by cAMP, which suggests that TSEI may directly interfere with cAMP-dependent signaling. The target of TSEI repression has been identified as the CRE (Boshart et al. 1990). Functional antagonism of TSEI and cAMP was observed at the levels both of in vivo protein binding to the CRE and of PKA-specific phosphorylation of CREB (Boshart et al. 1990).

Further experiments have shown that the regulatory subunit RIIa of PKA is identical with TSEI (Boshart et al. 1991, Jones et al. 1991). Both the level of cAMP and the levels of TSEI-RIa and the other regulatory subunits determine the equilibrium between inactive holoenzyme and free R and C subunits. In nonstimulated hepatoma cells, where TSEI-RIa is expressed at a very low level, the basal level of cAMP leads to only low activation of PKA. In hybrid cells, high levels of TSEI-RIa sequester the low level of cAMP present in nonstimulated cells, thus shifting the equilibrium toward the inactive R,C2 configuration. An increase in cAMP concentration then activates PKA by liberation of C subunits (Figure 4).

As already mentioned, the TAT gene belongs to a group of genes that are upregulated around birth. It is appealing to speculate that the TSEI-RIa gene is prenatally active in hepatocytes and that its expression is downregulated around birth, thus allowing expression of cAMP-dependent gluconeogenic enzymes. Consistent with this speculation, TSEI activity in a hepatoma line showing fetal characteristics is high compared with differentiated hepatoma cells (Gourdeau et al. 1989). This finding has been strengthened by direct determination of RIa mRNA levels in fetal and differentiated hepatoma cells (Faust, Boshart, Schutz, and Weiss unpublished).

- Conclusion

Modular organization of control regions offers a versatile and economical mechanism to create a large number of differentially regulated enhancers by combinatorial use of a much smaller number of regulatory units. The combination of ubiquitously active, hormone-dependent elements with cell-specific elements generates enhancers whose expression is restricted by the presence of cell-specific proteins and that are tuned by the activity of the hormone-dependent transcription factor. Transgenic experiments to show that this principle is important during developmental activation of liver genes are under way.

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References


