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Minireview

Cyclic AMP signalling and cellular proliferation: regulation of CREB and CREM

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Abstract In eukaryotes, transcriptional regulation upon stimulation of the adenylyl cyclase signalling pathway is mediated by a family of cAMP-responsive nuclear factors. This family consists of a large number of members which may act as activators or repressors. These factors contain the basic domain/leucine zipper motifs and bind as dimers to cAMP-response elements (CRE). The function of CRE-binding proteins (CREB) is modulated by phosphorylation by the cAMP-dependent protein kinase. The ICER (inducible cAMP early repressor) protein is the only inducible member of this family and is a product of the *CREM* gene. The induction of this powerful repressor is likely to be important for the transient nature of cAMP-induced gene expression. CREB proteins have been found to play an important role in the physiology of neuroendocrine functions. In addition, recent results indicate that CREB and CREM could be involved in the proliferation of hepatocytes which follows partial hepatectomy.

Key words: Cyclic AMP; Transcription factor; CREB; CREM; Proliferation

1. Coupling cAMP signalling to gene expression

The function of transcription factors is modulated by specific signal transduction pathways which are activated intracellularly by various signals at the cell surface. Intracellular levels of cAMP are regulated primarily by adenylyl cyclase. This enzyme is in turn modulated by various extracellular stimuli mediated by receptors and their interaction with G-proteins. The binding of a specific ligand to a receptor results in the activation or inhibition of the cAMP-dependent pathway, ultimately affecting the transcriptional regulation of various genes through distinct promoter responsive sites [1]. Increased cAMP levels directly affect the function of the tetrameric protein kinase A (PKA) complex. Binding of cAMP to two PKA regulatory subunits releases the catalytic subunits enabling them to phosphorylate target proteins. An important fraction of the catalytic subunit molecules migrates into the nucleus (Fig. 1). A number of isoforms for both the regulatory and catalytic subunits have been identified suggesting a further level of complexity in this response. In the nucleus, the phosphorylation state of transcription factors and related proteins appears to directly modulate their function and thus the expression of cAMP-inducible genes (Fig. 1). The analysis of promoter sequences of several genes allowed the identification of promoter elements which could mediate the transcriptional response to increased levels of intracellular cAMP [2]. A number of sequences have been identified of which the best characterised is the cAMP-responsive element (CRE). The CRE is constituted by an 8 bp palindromic sequence (TGACGTCA). Several genes which are regulated by a variety of endocrinological stimuli contain CREs in their promoter regions [1,2].

2. cAMP and cellular proliferation: the liver as model system

In the liver, the cAMP-responsive signalling pathway plays an important role in gene regulation. Indeed, it has been shown that modulation of liver-specific gene expression is correlated with increases of cAMP levels [3], modulated expression of G-protein subunits, adenylyl cyclase [4] or PKA [5]. Yet, the involvement of cAMP-responsive transcription factors in liver function is poorly understood. A role for CREB phosphorylation in the regulation of liver gene expression has been postulated [6].

The liver provides a remarkable system to study the phenomenon of cellular proliferation in vivo and thus to analyze the involvement of specific events in gene regulation. In this respect the paradigm of the partial hepatectomy (PH) constitutes an invaluable tool. Indeed, PH triggers coordinated hepatocyte proliferation which ensures complete reconstitution of the liver mass [7–10]. This process has been used extensively as a model system to study mechanisms regulating cell growth and proliferation. Importantly, a multitude of signalling pathways are activated during liver regeneration [3,9]. During the first hours following partial hepatectomy, glucocorticoids are involved in the regulation of gene expression. In addition, it has been shown that glucagon and adrenalin direct an increase in intracellular cAMP levels during the first 2–6 h [4,11]. A second peak of cAMP occurs before the first round of mitosis [4,12]. Various studies have documented associated changes in the expression of PKA subunits [5] and have strongly implicated cAMP in the regulation of hepatocyte proliferation [13]. Importantly, recent results indicate that treatment of hepatoma cells in vitro with cAMP results in a powerful CREB phosphorylation and in an increase of ICER levels [14]. These findings implicate the *CREM* gene as a major player in the cAMP-regulated response of hepatocytes [14]. Thus, in addition to modulating gene expression in response to hormonal stimuli in differentiated cells, these transcription factors have a role in the proliferative events linked to liver regeneration after partial hepatectomy (Fig. 1).

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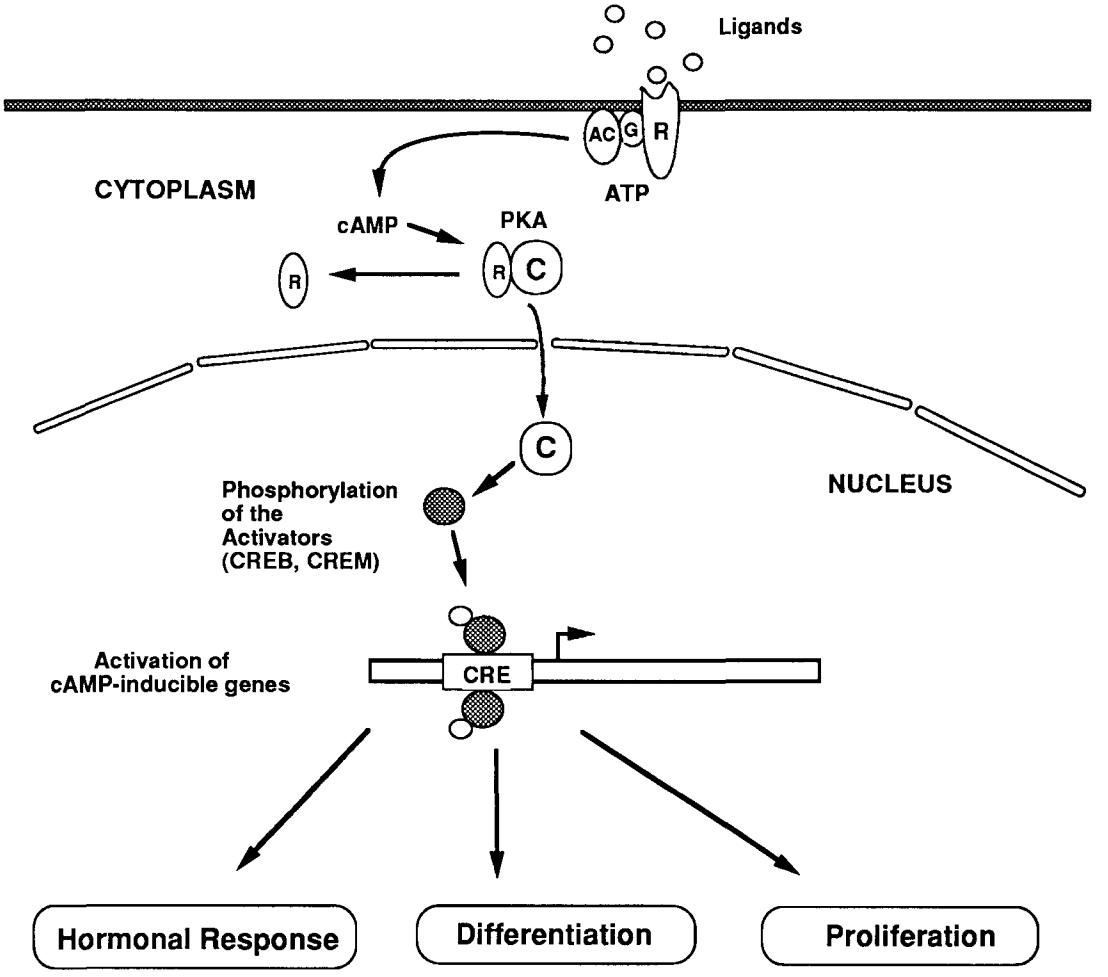

Fig. 1. The cAMP signal transduction pathway. Schematic representation of the route whereby ligands at the cell surface interact with membrane receptors (R) and thereby result in altered gene expression. Ligand binding activates coupled G-proteins (G) which in turn stimulates the activity of the membrane-associated adenylyl cyclase (AC). This converts ATP to cAMP which causes the dissociation of the inactive tetrameric protein kinase A (PKA) complex into the active catalytic subunits (C) and the regulatory subunits (R). Catalytic subunits migrate into the nucleus where they phosphorylate (P) and thereby activate transcriptional activators such as CREB and CREMτ. These then interact with the cAMP response enhancer element (CRE) found in the promoters of cAMP-responsive genes to activate transcription. The products of these genes influence the hormonal response, differentiation and proliferation. In particular, the activation of CREB and ICER in hepatocytes upon partial hepatectomy could be an important event regulating cellular proliferation.

3. CREB and CREM regulation in hepatocytes

Very little is known with respect to the nuclear effectors of the cAMP pathway in hepatocyte proliferation and function in the liver. As mentioned, recent results document dynamic expression of CRE-binding factors in liver and cultured hepatoma cells [14]. Indeed, both CREB phosphorylation and ICER [15,16] expression are powerfully induced following partial hepatectomy. In this model system, an increase in cAMP follows hepatectomy in response to hormonal and proliferative stimulation. This induction is known to be biphasic. The first peak occurs 2–6 h after partial hepatectomy, while the second peak, which precedes DNA synthesis, occurs 10–16 h after partial hepatectomy [4,12]. ICER inducibility coincides with the first peak [14].

In vitro studies with primary hepatocytes have demonstrated that transient cAMP induction increases DNA synthesis in the presence of epidermal growth factor (EGF), while constant cAMP treatment inhibits DNA synthesis [4,17]. Therefore, we speculate that the first peak of cAMP, and thus the associated increase in ICER expression, is linked with the transient upregulation of genes that are expressed in G1 phase (e.g. c-*fos*; [18]), during the first hours following hepatectomy. The second peak of cAMP may be more specifically linked to the regulation of DNA synthesis. Indeed, this scenario would be in agreement with observations by MacManus et al. [12] who have demonstrated that pharmacological inhibition of the first cAMP peak does not affect DNA synthesis, while delay of the second peak is sufficient to retard DNA synthesis [12].

4. Conclusions and perspectives

CREB phosphorylation and the consequent induction of the ICER repressor in the liver beg the question of which genes may be under the control of these transcription factors. Several hepatocyte-specific genes have been postulated to be regulated by the cAMP-responsive signalling pathway [3]. Thus, these results constitute a first step in the elucidation of the mechanisms involved in the regulation of these genes.

ICER induction during liver regeneration deserves special attention. Indeed, this event links, for the first time, the inducibility of the repressor ICER to a proliferative process (Fig. 1). The markedly transient induction of ICER protein following PH [14], which differs from that observed in other systems [19,20], may suggest a distinct function. The involvement of *CREM* gene products in the regulation of *cyclin A* gene and possibly of the cell cycle has been also forwarded [21]. In addition, ablation of the *CREM* gene in the mouse germline by homologous recombination results in an arrest of post-meiotic male germ cell differentiation and an increase in apoptosis [22]. These observations support the notion that the *CREM* gene may have a key role in the differentiation/proliferation decision made by certain cell types in response to activation of specific signalling pathways. The analysis of the physiological and molecular events following PH in the CREM-deficient mice should be then of interest and is likely to shed new light on the multifaceted functions of this transcription factor.

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