Ca^{2+}-sensitive transcriptional regulation: direct DNA interaction by DREAM

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1. ABSTRACT

Calcium is a major regulator of cell function, acting as a second messenger to relay signals from the cell surface to other parts of the cell. It plays an integral role in contraction of muscle cells and it regulates cell growth and proliferation, as well as cell death (1). The present review will discuss how Ca^{2+} mediates these functions through the regulation of gene expression. This can be accomplished by Ca^{2+}-sensitive protein kinases as well as phosphatases which activate transcription factors. It can also be mediated by direct interaction of Ca^{2+}-sensitive proteins with regulatory elements within the DNA sequence itself. Special attention will be given to recent advances in research in the down-stream regulatory element (DRE) and its Ca^{2+}-sensitive modulator DREAM (downstream regulatory element antagonist modulator; also named calsenilin or K^{+} channel interacting protein 3 (KChIP3)) (2).

2. INTRODUCTION

The Ca^{2+}-sensitivity of transcriptional regulatory factors is mediated by EF-hand Ca^{2+}-binding motifs (3). This allows Ca^{2+}-binding proteins to act as both a Ca^{2+} buffers and Ca^{2+} sensors.   The EF-hand domain is composed of two perpendicular α-helices connected by a loop, i.e. a helix-loop-helix motif, that may be repeated 2-12 times within a protein's amino acid sequence (2, 4, 5). Upon Ca^{2+} binding, the EF-hand undergoes a conformational change from a closed to an open conformation resulting in activation of regulatory domains, as is the case with calmodulin (5). There are more than 600 EF-hand motif containing proteins (2), and a number of these proteins affect gene transcription in response to changes in intracellular Ca^{2+} concentration. For example, activation of the Ca^{2+}/calmodulin-dependent protein kinase (CaMK) signaling cascade results in activation of cAMP response-element (CRE) binding protein (CREB) to

activate transcription (6). The Ca^{2+}-dependent phosphatase calcineurin dephosphorylates nuclear factor of activated T-cells (NFAT), a transcription factor which is then transported to the nucleus to regulate transcription (7). More recently, the Ca^{2+}-binding protein DREAM has been identified as a transcriptional repressor that is directly sensitive to nuclear Ca^{2+} concentrations (8). Together, these Ca^{2+}-sensitive transcriptional regulators represent a significant role of Ca^{2+} in the regulation of cellular function.

3. INFLUENCE OF Ca²⁺-DEPENDENT KINASES AND PHOSPHATASES

A well-documented example of Ca^{2+}-sensitive transcriptional regulation is the CaMK signaling cascade. This family of proteins, including CaMKI/IV and CaMKII, is activated by the binding of Ca^{2+} to calmodulin, followed by activating phosphorylations by CaMK kinase or by auto- or transphosphorylation (6). CaMKs exert their transcriptional influence via CREB. All three of the CaMKs can bind CREB and phosphorylate its activating site, Ser-133, *in vitro*, although CaMKII can phosphorylate an additional site, Ser-142 (9), while CaMKIV is the major activator of CREB (10). Once activated, CREB not only binds to CRE to induce transcription, but also interacts with numerous other transcription factors, including p53 (11), c-Jun (12), and c-*fos* (13). CREB also binds to the Ca^{2+} response element (CARE), which is structurally similar to CRE and is also found in c-*fos*. The effects of CREB binding are extremely widespread. A genome-wide search to identify putative CREB targets, followed by chromatin immunoprecipitation of over 16,000 human gene promoter regions (Hu19k promoter microarray) identified ≈3,000 promoter sites occupied by CREB *in vivo* (14).

Another transcription factor, NFAT is regulated in a Ca^{2+}-dependent manner by the protein phosphatase calcineurin. In this case, increase in intracellular Ca^{2+} by phospholipase C mediated store-operated Ca^{2+} entry and binding to calmodulin leads to activation of calcineurin (15). Calcineurin then dephosphorylates multiple serine residues of NFAT, which is then translocated to the nucleus and binds to kappa-B sites via Rel homology regions to regulate gene transcription (7).

4. DIRECT TRANSCRIPTIONAL REGULATION BY Ca²⁺-SENSITIVE DOWNSTREAM REGULATORY ELEMENT ANTAGONISTIC MODULATOR

While the transcriptional regulation described above is dependent on kinase or phosphatase activity, there is another mode by which Ca^{2+} regulates gene transcription more directly yet still mediated in the nucleus via a downstream regulatory element (DRE). The DRE site was first identified in the prodynorphin gene which is highly expressed in the brain (8). Prodynorphin is the precursor of dynorphin, which plays a role in memory acquisition and pain sensation (16, 17). Four cAMP response elements have been identified within the prodynorphin gene (18). CREB binding to the third CRE of the prodynorphin promoter represses gene expression, while binding of AP-1

to this site activates transcription (18). Through mutation analysis of the human prodynorphin promoter region, Carrión *et al.* identified a DRE consensus sequence (PuNGTCAPuPuG; see reference 8). Derepression of prodynorphin at the DRE site was required for protein kinase A- (PKA) dependent transcription (8). A 110-kD protein complex was also identified that specifically bound to the DRE site, and whose binding was reduced upon PKA stimulation (8). Therefore, DRE functions as a transcriptional silencer. In a subsequent study, Carrión *et al.* identified the DRE modulatory protein by screening a human caudate cDNA expression library using a double-stranded DRE oligonucleotide as a probe (19). A 284 amino acid protein with a predicted molecular weight of 31,800 was identified, which was named the DRE-binding protein DRE-antagonist modulator (DREAM). DREAM binds to DRE as a homotetramer (corresponding to the 110 kDa protein described above; reference 8) with high affinity, and as a monomer with low affinity. Four Ca^{2+}-binding EF-hand motifs were identified within the amino acid sequence of DREAM, with high sequence homology to recoverin and recoverin-like binding proteins vilip1, and hippocalcin (20, 21). Functionality of these EF-hands was confirmed, as release of intracellular Ca^{2+} by caffeine resulted in derepression of DRE by DREAM. DREAM also repressed expression of the Ca^{2+}-sensitive proto-oncogene c-*fos* (22), which also contains a DRE site (8).

5. Ca²⁺ BINDING TO DREAM RESULTS IN DEREPRESSION OF DRE

The DNA binding properties of DREAM have been further explored in order to elucidate its Ca^{2+}-sensitive transcriptional properties. Osawa and colleagues demonstrated that DREAM binds three Ca^{2+} ions in its C-terminal EF-hand domains (23). This is consistent with previous reports that the first of DREAM's four EF-hand motifs is non-functional (24). Ca^{2+} binding results in conformational changes in DREAM structure which disrupts tetramer formation (Figure 1) (23). Ca^{2+}-bound DREAM dimers have a lower DNA binding affinity and do not bind to the DRE site, which accounts for the Ca^{2+}-mediated derepression (Figure 1B) (23). Ca^{2+}-free DREAM binds to DNA via residues in both its basic N-terminal and Ca^{2+}-binding C-terminal regions (23). Interestingly, Mg^{2+} also binds DREAM in the nucleus, and is required for sequence-specific DNA binding (Figure 1A) (25, 26).

In addition to regulation by other ions, DREAM activity is sensitive to PKA activity via interaction with cAMP response element modulator (alpha CREM; reference 23). Two leucine-charged residue-rich domains (LCDs) within alpha CREM and DREAM result in binding of the two proteins, blocking interaction of DREAM with DRE and resulting in derepression of prodynorphin (27). DREAM also interacts with CRE function by blocking the binding of CREM and CREB via LCD domains *in vitro* (28). This effect is abolished by Ca^{2+}-binding to DREAM. Additionally, the binding of DREAM to the CREB LCD domain normally occupied by CREB binding protein (CBP) results in lower CBP activity. These examples
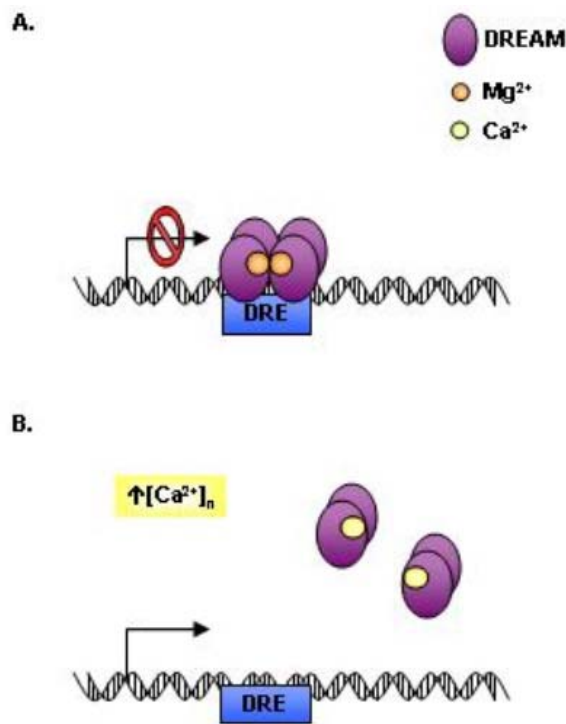
Figure 1. Ca^{2+} binding to DREAM results in derepression of DRE. A. DREAM forms a tetramer that binds to the DRE motif downstream of the promoter to block transcription. Tetramer formation is stabilized by Mg^{2+} binding in the second EF-hand. B. Increase in nuclear Ca^{2+} $((Ca^{2+})_n)$ results in Ca^{2+} binding to DREAM, disrupting the tetramer. This results in derepression of the DRE site.

represent multiple effects of Ca^{2+} through crosstalk between DRE and CRE-mediated transcriptional regulation.

6. DOWNSTREAM EFFECTS OF DREAM REGULATED GENE EXPRESSION

In addition to its role as a transcriptional regulator, DREAM can also affect cellular Ca^{2+} homeostasis. In cerebellar granule cells, DREAM represses the expression of the Na^+/Ca^{2+} exchanger NCX3, which is relieved by increased intracellular Ca^{2+} in a similar manner to regulation of prodynorphin as described above (29). Overexpression of EF-hand mutated DREAM that constitutively binds to DRE results in down-regulation of NCX3 and a subsequent increase in intracellular Ca^{2+} is associated with a loss of cell viability.

DREAM is also highly expressed in the thyroid, where DRE regulates Ca^{2+} homeostasis via calcitonin (30). In this case, two DRE sites are found upstream of the calcitonin promoter, while it is located downstream of the prodynorphin promoter (30). This implicates DREAM in a feedback mechanism whereby increase in the plasma Ca^{2+} concentration and subsequent increase in intracellular Ca^{2+} in thyroid cells results in derepression of calcitonin by

DREAM. Increased calcitonin secretion can then decrease plasma Ca^{2+} by inhibiting bone Ca^{2+} resorption (31).

In the pancreas, DREAM is expressed in both alpha- and beta-cell nuclei (32). Ca^{2+}-mediated derepression of prodynorphin occurs in these cells as shown previously in neuroblastoma cells (8). Interestingly, prodynorphin expression in beta-cells is also glucose sensitive, and in alpha-cells, upregulation of glucagon secretion is mediated by dynorphin stimulation of kappa-opiod receptors (32). This indicates that DREAM may play a significant role in glucose metabolism.

7. NON-TRANSCRIPTIONAL ACTIVITY OF DREAM

DREAM has additional functions outside of the nucleus, where it was independently identified as calsenilin (33) and potassium-channel interacting protein-3 (KChIP3; reference 34). The first 256 amino acids of DREAM are identical to a protein originally identified as calsenilin. It interacts with the C-terminal region of presenilin-1 and -2 in the endoplasmic reticulum (ER; reference 35). Presenilin mutations are involved in early-onset, autosomal dominant, familial Alzheimer's disease, causing increased production of 42 peptide length amyloid β (Aβ42; reference 36). Leissring and colleagues demonstrated that calsenilin reverses presenilin-mediated enhancement of Ca^{2+} signaling (35). Calsenilin also triggers apoptosis in neuroglioma cells by increasing ER Ca^{2+} stores (37), and contains a cleavage site for caspase-3 (38). This provides a potential link between calsenilin's Ca^{2+}-sensitive function and Aβ42-mediated neuronal cell death in Alzheimer's disease (39).

DREAM/calsenilin/KChIP3's interaction with A-type voltage-gated potassium channels demonstrates another facet of this multi-functional protein (34). KChIP3 knockout is associated with increased long-term potentiation in hippocampal neurons of the dentate gyrus by decreasing potassium channel current density (37). This provides a link between KChIP3 and memory formation. Another protein, KChIP2, has high homology to KChIP3 and is highly expressed in mammalian heart (34). In this scenario, KChIP2 knockout leads to loss of the transient outward K^+ current in cardiomyocytes and prolonged ST segment elevation accompanied by a high susceptibility to arrhythmias (40).

In summary, this important set of studies identified DREAM as the first transcriptional modulator directly affected by Ca^{2+} in the nucleus rather than via kinase or phosphatase activity or protein-protein interactions. It also implicates DREAM in the regulation of genes not containing the DRE site via its repression of the transcription factor c-*fos*.

8. DISCUSSION

The significance of Ca^{2+} in the regulation of cellular function cannot be understated. It influences many second messenger systems and acts as a second messenger

itself. Ca^{2+} also regulates the life and death of cells, influencing growth and proliferation as well as apoptosis (1). Many of these important functions are carried out by Ca^{2+}-binding proteins that influence the transcriptional regulation of a host of genes. The ability of Ca^{2+}-sensitive protein kinases and phosphatases to affect activity of transcription factors has long been established (15). More recently, Ca^{2+} has been shown to directly bind to and regulate transcription factors in the nucleus, as exemplified here by the DRE modulatory protein DREAM (2). This unique mode of transcriptional regulation may serve as a treatment target in a number of cell types. For example, DREAM regulation of the endogenous opiod dynorphin may play a role in central sensitization of nociceptors (41). This may make it a target in pain management, as evidenced by analgesia described in DREAM KO mice (42, 43). DREAM-mediated regulation of dynorphin may also be a target for modulation of glucagon secretion in diabetes (32). In addition, DREAM regulation of genes that effect Ca^{2+} handling presents a novel mechanism by which cells maintain Ca^{2+} homeostasis. This presents a new level of significance for Ca^{2+}-sensitive transcriptional regulation.

9. ACKNOWLEDGEMENTS

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Abbreviations: CaM: calmodulin; CaMK: Ca^{2+}/calmodulin-dependent protein kinase; KChIP: potassium channel interacting protein; CRE: cAMP response element; CREB: CRE binding protein; CBP: CREB binding protein; DRE: downstream regulatory element; DREAM; downstream regulatory element antagonistic modulator

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