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Review

The influence of calcium signaling on the regulation of alternative splicing

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ABSTRACT

In this review the influence of calcium signaling on the regulation of alternative splicing is discussed with respect to its influence on cell- and developmental-specific expression of different isoforms of the plasma membrane calcium pump (PMCA). In a second part the possibility is discussed that due to the interaction of the calcium-binding protein ALG-2 with a spliceosomal regulator of alternative splicing, RBM22, Ca^{2+}-signaling may thus influence its regulatory property.

1. Introduction

External signals to activate cells often result with a change of the cellular environment. To maintain viability cells must be able to respond. This requires that cells must have the ability to change their functions rapidly due to a given stimulus. Such cellular responsiveness is accomplished through activation of signal transduction cascades transmitting signals from the cell surface to internal cellular machineries, often accompanied by significant alterations of the protein composition of cells. Such changes in cellular expression of proteins can occur through a variety of mechanisms regulating transcription, translation or post-translational modifications, but recently growing evidence documented the importance of signal-induced changes in the pattern of alternative splicing as an important means to mediate biologically relevant cellular responses.

Activation of cells due to an external signal often results in up to a 100fold rise in the intracellular free Ca^{2+} concentration due to the uptake of extracellular Ca^{2+} or the release of Ca^{2+} from intracellular stores. These changes of the free Ca^{2+} concentration can cause significant oscillations of Ca^{2+} in the cytosol providing the possibility of signal transduction for a number of different cellular activities such as metabolism, protein phosphorylation and dephosphorylation, fertilization, cell proliferation, division, gene expression and apoptosis, to name a few. Many of these functions are accomplished through the interaction of Ca^{2+} with specific proteins resulting in modulations of protein–protein interactions due to conformational changes of the Ca^{2+}-receptors. This review will focus on the increasing evidence for an existing interface built up between the regulation of alternative splicing and signal transduction pathways, with special emphasis on calcium signaling.

2. Alternative splicing: general remarks

Alternative splicing is a dominant property of higher organisms to produce multiple proteins from a single gene [1,2]. It has been calculated that more than 40% of *Drosophila* genes [3] and over two thirds of mouse or human genes [4] give rise to alternatively spliced pre-mRNAs. These numbers might be still underestimated since in recent years it became evident that many isoforms are either tissue specific or are developmentally regulated [5]. Indeed, in 2 recent publications by Wang et al. [6] and by Pan et al. [7] it was documented by analyzing different tissues and cell lines that about 95% of human genes containing multiple exons undergo alternative splicing. However, the regulation of alternative splicing is still poorly understood. For splicing to occur introns must be distinguished from exons, for which a prerequisite is the pairing of the splice sites and their recognition by the spliceosome with high precision. In order to change the content of exons of a given mRNA thereby influencing the function of the encoded protein in a specific cellular environment, alternative splicing permits fluctuation in the precise pairing of the splice sites thus giving rise to alternative protein products. But how is this process regulated? So far it seems clear that binding of spliceosomal subunits to the pre-mRNA strongly influences the decision which splice sites will be joined [1]. Such binding of spliceosomal subunits to the pre-mRNA is a dynamic and highly ordered process to build the active spliceosome which can be promoted or inhibited by splicing factors binding to adjacent parts of the pre-mRNA thereby influencing the selection of the splice sites.

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Next to the choice of the correct splice sites there exist additional exonic and intronic regulatory sequences which bind specific factors influencing splice-site selection as either exonic or intronic splicing enhancers or silencers. These elements are required for the regulation of alternative splicing [8–10].

Besides of constitutive exons which are always spliced into the final mRNA, several subgroups of alternative splicings have been described: (1) cassette exons which are either included or excluded from the mRNA [11], or, in the case of multiple cassette exons, they are mutually exclusive [12], i.e. the final mRNA always includes one of several possible exons; (2) 5′ splice site or (3) 3′ splice site selection.

3. Transcription and splicing

For a long time it has been assumed that transcription and pre-mRNA splicing are independent processes until it became evident that splicing and polyadenylation reactions are often coupled to transcription [13,14], depending on the time RNA polymerase II needs to synthesize the transcription unit and to release the nascent pre-mRNA [15]. Considering the size of many genes with numerous introns of the size of several kbs, cotranscriptional splicing of the pre-mRNA appears to be a rational concept.

It is well documented that one of the consequences of elevated calcium in the cell, especially in the nucleus, is the induction of gene expression [16], due to Ca^{2+}-dependent transcription factors such as CREB [17–19], CREMτ [17,20], ATF-1 [21], SRF [22], ETS-1 [23,24] which are among the best substrates for the Ca^{2+}-calmodulin dependent kinase IV (CaMKIV). CaMKIV which itself is induced by the thyroid hormone T3 during neuronal development [25,26] has been localized to the nucleus [17,27,28], and thus has direct access to transcription factors to regulate their function in a Ca^{2+}-dependent manner such as CREB which was originally identified as a cAMP-dependent transcription factor which could be activated by PKA-dependent phosphorylation [29]. Since later studies provided evidence that this transcription factor could also be activated in a Ca^{2+}-dependent manner [16,19] CREB now stands for cAMP/calcium-responsive element binding protein which is activated due to phosphorylation at Ser133 (for a review see [30]).

4. Ca^{2+} signaling and alternative splicing

Since Ca^{2+} is an important signal to induce transcription it is perhaps not surprising if Ca^{2+} signaling is also involved in the regulation of alternative splicing due to a possible coupling between transcription and splicing as described before. In a number of papers the group of Black provided evidence that the Ca^{2+}-calmodulin dependent kinase IV is directly involved in Ca^{2+}-dependent regulation of alternative splicing [31–33]. In 2001 Xie and Black [31] provided evidence that CaMKIV suppresses the splicing of the STREX exon of the calcium-activated potassium channel. This channel plays an important role in shaping the action potential of excitable cells, and its splicing pattern is highly regulated. Inclusion of the STREX exon confers higher Ca^{2+} sensitivity to the calcium-binding domain of the channel. By depolarizing GH3 pituitary cells it was noticed that splicing of the STREX exon was reduced by 50% [31]. Since it is well documented that cellular depolarization stimulates the activity of calmodulin-dependent kinases and subsequently Ca^{2+}-dependent gene expression [34] Xie and Black showed in elegant experiments [31] that only CaMKIV, but not CaMKI or II specifically decreased the inclusion of the STREX exon into the mRNA of the channel. The authors identified a CaMKIV-responsive RNA element (CaRRE) mediating the alternative splicing of the pre-mRNA. In later studies [32,33] Black and his co-workers extended these observations and showed that the alternative splicing of the STREX exon is also controlled in neurons by the CAMKIV pathway by using a primary cerebellar neuron culture [32]. In this study the authors identified a CaMKIV-dependent consensus sequence (CACATNRTTAT) in a number of genes within the human genome which responded to CAMKIV [32].

The plasma membrane calcium pump (PMCA) plays a key role in regulating the intracellular Ca^{2+} concentration in eukaryotic cells [35]. The enzyme which belongs to the P-type class of ion-transporting ATPases [36,37] is ubiquitous, and its major regulating activator is calmodulin due to direct interaction with the pump [38]. In mammals four different genes (*PMCA 1–4*) have been identified [35] which can give rise to a plethora of different isoforms due to alternative splicing (for review see [35,39,40]). *PMCA 1* and *4* are considered as housekeeping pumps since they can be found in all tissues, whereas *PMCA2* is mainly detected in brain and heart, *PMCA3* in brain and skeletal muscles [35]. By determining the first primary structure of PMCA from human and rat sources [41,42] it became evident that alternative splicing may play an important role in establishing the diversity of PMCA isoforms. This became even more obvious when Strehler et al. [43] analyzed one of the splice sites in more detail.

To date it is well documented that supplementary exons of the 4 *PMCA* genes are alternatively transcribed in the proximity of the two main regulatory domains of the PMCA ([39]; see Fig. 1). Of the two regions of alternative splicing splice site A is located upstream of the phospholipids binding domain [44] within the first intracellular loop of the calcium pump and downstream of a sequence interacting with the C-terminal part of the calmodulin-binding domain [45], whereas splice site C is found within the calmodulin-binding domain at the C-terminal cytosolic tail of the protein thereby influencing the strength of interaction between calmodulin and the calcium pump [39,40]. As will be discussed below in more detail this diversity of spliced isoforms not only influences the function of the enzyme severely by interfering with two important regulatory sequences of the enzyme, but also enables the protein to control Ca^{2+} homeostasis in a cell- and tissue-specific manner.

5. Regulation of alternative splicing of PMCA isoforms

By comparing cDNA clones from a fetal skeletal muscle cDNA library with a teratoma cDNA encoding the human plasma membrane Ca^{2+} pump Strehler et al. [43] discovered that these clones contained isoforms of PMCA with an insertion of a 154 base-pair exon which could give rise to either 29, 38 or 51 amino acid insertions within the calmodulin-binding domain (splice site C). These variations occurred due to alternative splicing making differential use of two internal “cryptic” donor splice sites. Isoforms containing either 87 bp (29 amino acids, isoform “c”) or 114 bp (38 amino acids, isoform “d”) insertions did not change the reading frame whereas if the entire exon of 154 bp was inserted (isoform “a”) the reading frame was changed resulting in a pump protein with a shorter C-terminal amino acid sequence due to an early stop codon (see Fig. 1). In isoform “b” the 154 bp exon is excluded. A similar complex pattern of alternative splicing has later been described for all 4 *PMCA* genes of human [46] or rat origin [47,48], not only for site “C”, but also for site “A” as mentioned before (see Fig. 1).

In many different studies it has been documented that the expression pattern of the different PMCA isoforms varies in different tissues, and the expression of many of its splice variants is developmentally regulated [46,49,50]. By far the highest diversity of different isoforms can be found in the brain manifesting their cellular and developmental specificity, but the mechanism behind the regulation of the alternative splicing events of the different PMCAs is poorly understood.

As can be noticed from Fig. 1 splicing at site “A” affects an exon of either 39 nucleotides (nt; PMCA1), 42 nt (PMCA2,3) or 36 nt (PMCA4). In all PMCAs this exon can be either inserted or excluded from the mature mRNA. For PMCA2 the situation is more complex. As shown in Fig. 1 site “A” of PMCA2 includes 3 exons of the size 33, 60