Biochimica et Biophysica Acta 1793 (2009) 979–984

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Review

The influence of calcium signaling on the regulation of alternative splicing

Joachim Krebs

NMR-based Structural Biology, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany

ARTICLE INFO

Article history:
Received 29 October 2008
Received in revised form 27 November 2008
Accepted 3 December 2008
Available online 24 December 2008

Keywords:
Alternative splicing
Calcium signaling
Plasma membrane calcium pump
CaMKIV
ALG-2
RBM22

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.

E-mail address: jkrebs@nmr.mpibpc.mpg.de.

0167-4889/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamcr.2008.12.006

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

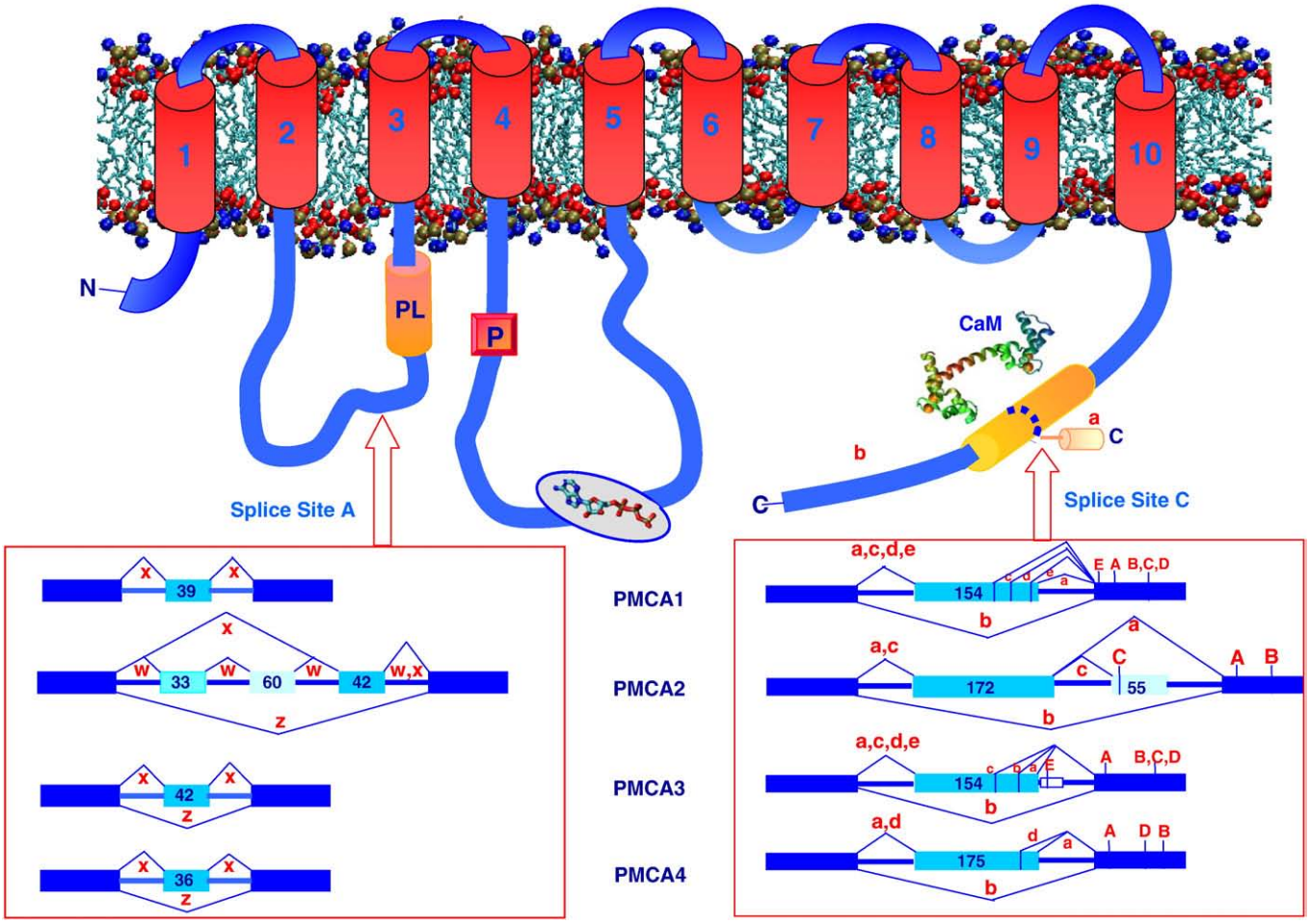

Fig. 1. Topology domains and splicing variants of the PMCA isoforms. The 10 putative transmembrane domains of the pump are numbered and indicated by red boxes. Splice sites "A" (first cytosolic loop) and "C" (C-terminal tail) are indicated by red arrows. Splice site "C" lies within the calmodulin-binding domain (yellow cylinder; defined by the structural model of CaM = calmodulin). The exon structure of the different regions affected by alternative splicing is shown for each of the 4 different *PMCA* genes. Constitutively spliced exons are indicated as dark blue boxes, alternatively inserted exons are shown in light blue; the resulting splice variants are labeled by their lower case symbols, the positions of the translation stop codons for each splice form are indicated by the corresponding capital letters. In PMCA3, splice variant "e" results from a read-through of the 154-nt exon into the following intron (indicated as small open box). The sizes of alternatively spliced exons are given as nucleotide numbers. PL = phospholipid binding domain; P = location of the aspartyl-phosphate formation. The Figure was adopted from Fig. 1 of chapter 7 by C. Ortega, S. Ortolano and E. Carafoli in: Calcium, a matter of life or death (J. Krebs and M. Michalak, eds.) pp. 179–197, published by Elsevier, Amsterdam, 2007; with permission from the publishers.

and 42 nt which can be alternatively either inserted or excluded. With respect to the discussion below only 2 splice variants are of interest here: variant "w" which includes all 3 exons and variant "x" which includes only the 42 nt exon (see Fig. 1). In 1996 Zacharias and Strehler [51] reported that by depolarizing IMR32 neuroblastoma cells alternative splicing occurred at splice site A of PMCA 2 due to the intracellular rise of Ca^{2+}, i.e. next to PMCA2w also the variant "x" was expressed. On the other hand, in non-perturbed cells the "x" variant of splice site A could only be detected in PMCA1,3,4, whereas in PMCA2 only the "w" variant was expressed indicating cell-specific differences in the splice variants of the different PMCAs. Next to PMCA2w which was always expressed PMCA2x could be detected already after 1 minute of depolarizing the cells with a high concentration of KCl [51]. This expression of the splice variant PMCA2x was only induced by a transient rise of intracellular Ca^{2+}, i.e. in the presence of the calcium chelator BAPTA the expression of PMCA2x was suppressed. Of further interest was the notion that in the presence of a serine/threonine kinase inhibitor the transient rise of intracellular Ca^{2+} did not induce the PMCA2x splice variant [51] indicating that a kinase – possibly a calcium-dependent kinase – played an important role by transmitting this signal transduction pathway. In this context it is of interest that phosphorylation/dephosphorylation is an essential mechanism for the function of various spliceosomal proteins (for a recent review see [52]).

A significantly different splicing pattern of the PMCA isoforms was reported by Guerini et al. [53]. These authors investigated the expression of PMCAs in cerebellar granule neurons during differentiation. By comparing synaptosomal preparations from cerebella of 3-day-old with those from 3-week-old rats the authors found a marked increase in the expression of all 4 PMCA proteins. Remarkable was the finding that the increase in PMCA1 expression was mainly due to the isoform PMCA1a (see Fig. 1) which includes the 154 nt exon within the calmodulin-binding domain at splice site "C" leading to a truncated version of PMCA1 due to a frame shift of the reading frame. In contrast to the observation made by Zacharias and Strehler [51] Guerini et al. [53] did not observe a change of alternative splicing at site "A" after depolarizing granule cells indicating cell-specific differences of Ca^{2+}-dependent regulation of alternative splicing of the different PMCA isoforms. A further cell-specific difference between neuroblastoma and cerebellar granule cells in culture was the notion that the Ca^{2+}-dependent induction of PMCA2x in neuroblastoma cells occurred within minutes. This is in contrast to the much longer incubation time of granule cells under membrane depolarizing conditions (obtained by applying high KCl concentrations) to observe the isoform switch of

PMCA1a. Furthermore it was interesting to observe that PMCA4 was downregulated by depolarizing conditions in cultured granule cells even if it was clearly detectable in adult cerebellum indicating the presence of PMCA4 in other cell types of the cerebellum [53]. These changes in the expression of the different PMCAs and their isoforms due to alternative splicing induced under prolonged depolarizing conditions which led to morphological changes typical for mature granular neurons was clearly a Ca^{2+}-dependent process. Addition of nifedipine, a specific inhibitor of L-type (Ca_v1) Ca^{2+}-channels, completely abolished both the up-regulation of the PMCA1a, 2 and 3 and the down-regulation of the PMCA4 isoforms. On the other hand, the cytosolic Ca^{2+} could be also increased by applying N-methyl-D-aspartic acid, an activator of glutamate-operated Ca^{2+} channels. Using this protocol similar observations could be made with respect to the expression pattern of PMCA isoforms during the differentiation process of granular cells [53].

These changes in the expression pattern of PMCA during the maturation process of the granular cells may reflect the demand of specific functional aspects of the calcium pump. Of the 4 PMCA isoforms PMCA2 which is particularly abundant in brain has the highest affinity for calmodulin [54]. This could provide cells with the possibility to respond with a higher sensitivity even at low Ca^{2+} concentrations. On the other hand, Ca^{2+}-dependent alternative splicing at site "C" (Fig. 1) generated PMCA1a, an isoform with lower affinity for calmodulin in analogy to the well characterized PMCA4a isoform [55,56], but a higher basal, e.g. calmodulin-independent activity [56]. Recently, Caride et al. [57] studied in detail the difference in activation kinetics between PMCA isoforms 4a and 4b. These authors made the interesting observation that binding of calmodulin to PMCA4a is faster than to PMCA4b even if the affinity of calmodulin for PMCA4a is lower than for 4b. From this observation the authors concluded that the affinity of the calmodulin-binding domain for its receptor sites was higher for PMCA4b than for 4a and therefore the binding kinetics of calmodulin for PMCA4b was slower. As a consequence it can be envisioned that PMCA4a is more efficient than PMCA4b by reducing cytosolic Ca^{2+} concentrations after a spike. If similar conclusions can be drawn for PMCA1a then the Ca^{2+}-dependent up-regulation of the latter isoform due to alternative splicing could confer to the maturating granular cells a more effective protein with higher Ca^{2+}-pumping capacity.

If the view is correct that granule cells have an advantage during development and differentiation to increase the expression of the spliced isoform PMCA1a, the question arises how the cells regulate alternative splicing to select for the PMCA1a isoform (see Fig. 1). As mentioned before Black and co-workers [31–33] provided evidence that by depolarizing GH3 pituitary cells the rise of intracellular Ca^{2+} activated CaMKIV which through a CaMKIV-responsive element (CaRRE) was able to suppress the STREX exon of the calcium-activated potassium channel [31] or exon 21 of the NMDA receptor 1 in P19 cells [33]. By screening the human genome the Lab of Black identified a CaMKIV-dependent consensus sequence in a number of genes including PMCA [33]. The CaRRE of PMCA was described as CACATGTA which corresponds exactly to a sequence of the second internal splice donor site of exon 21 of PMCA1 [43]. Thus by depolarizing granular cells activated CAMKIV could suppress splicing of exon 21 of PMCA1 at the second cryptic splicing site by recognizing the CaRRE of that site. If one assumes that secondary structure of the pre-mRNA also could influence alternative splicing (for a recent review see [58]) one could envision that CAMKIV could also suppress the first internal splice site of exon 21 of PMCA1 thereby enhancing the expression of the PMCA1a isoform as detected in differentiating granular cells [53]. In this context it is of interest to point out that CAMKIV is particularly enriched in the granular cells of the cerebellum [30] which were the primary cellular source to characterize the enzyme [59].

Furthermore it should be pointed out that by comparing the primary structures of the different *PMCA* genes of human and rat origin a CaRRE could be identified only within PMCA1 underlining the selectivity of the spliced isoform of PMCA1a. Since CaMKIV is a protein kinase it now would be important to identify the target of phosphorylation, possibly a spliceosomal protein which coordinates the splicing event of PMCA1a.

6. RBM22, a regulator of alternative splicing, interacting with ALG-2 in a calcium-dependent manner

The calcium-binding protein ALG-2 was discovered by the group of D'Adamio in 1992 [60]. ALG-2 belongs to the family of penta EF-hand Ca^{2+}-binding proteins [61], e.g. these proteins contain five EF-hand domains. We and others reported on the intracellular distribution of ALG-2 and provided evidence for a significant nuclear concentration of ALG-2 [62–64]. In order to evaluate the reason for the nuclear translocation of the cytoplasmic protein ALG-2 we undertook a detailed screening for possible partners by using a yeast two-hybrid screening with ALG-2 as bait [65]. We identified the RNA-binding protein RBM22 as a new target for the interaction with ALG-2. To confirm such an interaction between the two proteins *in vivo* we performed a detailed study preparing fluorescent constructs by using the monomeric red fluorescent protein (mRFP) to label ALG-2 and the enhanced green fluorescent protein (EGFP) to label RBM22 [65]. Confocal microscopy of NIH 3T3 cells transfected with either the ALG-2 or RBM22 fluorescent expression constructs alone revealed that the majority of ALG-2 was localized in the cytoplasm whereas RBM22 was located in the nucleus [65]. In contrast, when cells were co-transfected with expression vectors encoding both fusion proteins ALG-2 was now detected in the nucleus indicating that RBM22 was instrumental for the translocation of ALG-2 to the nucleus [65]. Similar findings have also been made with zebrafish embryos during development [65]. The interaction between RBM22 and ALG-2 seems to be Ca^{2+} dependent. If NIH 3T3 cells were cotransfected with RBM22-EGFP and ALG-2-mRFP, the latter being the double mutant E47Q, E114Q preventing Ca^{2+}-binding at the high affinity Ca^{2+}-binding sites 1 and 3 [66], ALG-2 concentration in the nucleus was significantly reduced (data not shown).

RBM22 is a highly conserved RNA-binding protein, human and mouse homologues are identical proteins [65]. Next to the RNA-binding domain it contains an unusual zinc finger of the type Cx8Cx5Cx3H and 2 conserved sumoylation consensus sequences (see [65] for details). RBM22 was identified by 2 Laboratories as an auxiliary protein of the spliceosome of unknown function [67,68]. By a genome wide screening to identify essential proteins involved in the regulation of alternative splicing RBM22 was identified as one of the essential regulators of alternative splicing of the complex *Drosophila* gene *Dscam* [69], it also influenced the heart development of *Drosophila* [70], the development of zebrafish [71] and it influenced the regulation of the cell cycle in HeLa cells [72]. These findings underline the important regulatory property of RBM22 and by interacting with ALG-2 Ca^{2+}-dependent signaling may influence alternative splicing and cell division during development.

Spliceosomes are formed in a dynamic process on nascent pre-mRNAs, and they disassemble after introns are excised and exons are ligated. Spliceosomal components at rest and not engaged in splicing accumulate in morphologically distinct structures such as interchromatin granule clusters or speckles [73,74]. Recent studies [75] seem to indicate that modifying components of the spliceosome by the small ubiquitin-like modulator protein (SUMO) may influence their localization to nuclear bodies like speckles. As shown in Fig. 2 if the wild type protein RBM22 is labeled with GFP it distributes evenly over the nucleus (Fig. 2A) reflecting its dynamic behaviour. However, if the lysines of the 2 sumoylation consensus sites are mutated to arginines, i.e. K170R and K324R, RBM22 seems to accumulate in speckle-like structures (Fig. 2B). This results in a 2–3 times enlargement of those structures indicating an interruption of the dynamic distribution of RBM22 within the nucleus. This may prevent to execute its regulatory role at the spliceosome. Similarly, it has been suggested that protein

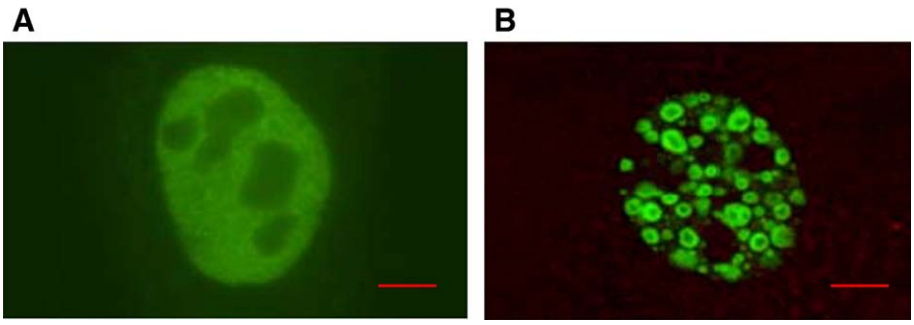

Fig. 2. Nuclear images of transfected NIH 3T3 cells. Cells were transfected with expression vectors containing cDNA encoding EGFP constructs of either wild type RBM22 (RBM22-EGFP; (A) or a double mutant of RBM22 replacing K170 and K324 by R to prevent sumoylation (B). Images were taken with a filter for green fluorescence (excitation at 488 nm). The bar indicates 5 μm.

phosphorylation is an important mechanism to recruit splicing factors from speckles for active sites of pre-mRNA splicing [76].

In conclusion, as outlined in the present review data are accumulating to indicate an influence of Ca^{2+}-signaling on the regulation of alternative splicing either through a Ca^{2+}-dependent enzyme such as CaMKIV which may phosphorylate a spliceosomal component thereby influencing the regulation of alternative splicing or by interacting of a Ca^{2+}-binding protein such as ALG-2 with a regulatory component of the spliceosome such as RBM22. In the future it will be important to characterize the targets by which Ca^{2+}-signaling could control the regulation of alternative splicing thereby influencing basic cellular processes during development.

Acknowledgements

The continuous support of my colleagues Dr. Stefan Becker and, especially, Dr. Christian Griesinger, Head of the NMR based Structural Biology Department at the Max Planck Institute for Biophysical Chemistry, Göttingen, Germany, is gratefully acknowledged. Dr. Ernesto Carafoli is thanked for the permission to reproduce Fig. 1.

References

[1] D.L. Black, Mechanisms of alternative pre-messenger RNA splicing, Annu. Rev. Biochem. 72 (2003) 291–336.
[2] B.J. Blencowe, Alternative splicing: new insights from global analyses, Cell 126 (2006) 37–47.
[3] V. Stolc, Z. Gauhar, C. Mason, G. Halasz, M.F. van Batenburg, S.A. Rifkin, S. Hua, T. Herreman, W. Tongprasit, P.E. Barbano, H.J. Bussemaker, K.P. White, A gene expression map for the euchromatic genome of *Drosophila melanogaster*, Science 306 (2004) 655–660.
[4] J.M. Johnson, J. Castle, P. Garrett-Engele, Z. Kan, P.M. Loerch, C.D. Armour, R. Santos, E.E. Schadt, R. Stoughton, D.D. Shoemaker, Science 302 (2003) 2141–2144.
[5] A.J. Lopez, Alternative splicing of pre-mRNA: developmental consequences and mechanisms of regulation, Annu. Rev. Genet. 32 (1998) 279–305.
[6] E.T. Wang, R. Sandberg, S. Luo, I. Khrebtukova, L. Zhang, C. Mayr, S.F. Kingsmore, G. P. Schroth, C.B. Burge, Alternative isoform regulation in human tissue transcriptomes, Nature 456 (2008) 470–476.
[7] Q. Pan, O. Shai, L.J. Lee, B.J. Frey, B.J. Blencowe, Deep surveying of alternative splicing complexity in the human transcriptome by high-throughput sequencing, Nat. Genet. 40 (2008) 1413–1415.
[8] J.F. Caceres, A.R. Kornblihtt, Alternative splicing: multiple control mechanisms and involvement in human disease, Trends Genet. 18 (2002) 186–193.
[9] B.J. Blencowe, Exonic splicing enhancers: mechanism of action, diversity and role in human genetic diseases, Trends Biochem. Sci. 25 (2000) 106–110.
[10] W.G. Fairbrother, R.F. Yeh, P.A. Sharp, C.B. Burge, Predictive identification of exonic splicing enhancers in human genes, Science 297 (2002) 1007–1013.
[11] A. Andreadis, M.E. Gallego, Nadal-Ginard, Generation of protein isoform diversity by alternative splicing: mechanistic and biological implications, Annu. Rev. Cell Biol. 3 (1987) 207–242.
[12] C.W. Smith, B. Nadal-Ginard, Mutually exclusive splicing of alpha-tropomyosinexons enforced by an unusual lariat branch point location: implications for constitutive splicing, Cell 56 (1989) 749–758.
[13] N.J. Proudfoot, A. Furger, M.J. Dye, Integrating mRNA processing with transcription, Cell 108 (2002) 501–512.
[14] D.L. Bentley, Rules of engagement: cotranscriptional recruitment of pre-mRNA processing factors, Curr. Opin. Cell Biol. 17 (2005) 251–256.
[15] K.M. Neugebauer, On the importance of being cotranscriptional, J. Cell Sci. 115 (Pt. 20) (2002) 3865–3871.
[16] M. Sheng, G. McFadden, M.E. Greenberg, Membrane depolarization and calcium induce c-fos transcription via phosphorylation of transcription factor CREB, Neuron 4 (1990) 571–582.
[17] R.P. Matthews, C.R. Guthrie, L.M. Wailes, X. Zhao, A.R. Means, G.S. McKnight, Calcium/calmodulin-dependent kinas types II and IV differentially regulate CREB-dependent gene expression, Mol. Cell. Biol. 14 (1994) 6107–6116.
[18] H. Enslén, P. Sun, D. Brickey, S.H. Soderling, E. Klamo, T.R. Soderling, Characterization of Ca2+/calmodulin-dependent protein kinase IV. Role in transcriptional regulation, J. Biol. Chem. 269 (1994) 15520–15527.
[19] M. Sheng, M.E. Thompson, M.E. Greenberg, CREB: a Ca(2+)-regulated transcription factor phosphorylated by calmodulin-dependent kinases, Science 252 (1991) 1427–1430.
[20] Z. Sun, P. Sassone-Corsi, A.R. Means, Calspermin gene transcription is regulated by two cyclic AMP response elements contained in an alternative promoter in the calmodulin kinase IV gene, Mol. Cell. Biol. 15 (1995) 561–571.
[21] P. Sun, L. Lou, R.A. Maurer, Regulation of activating transcription factor 1 and the camp response element-binding protein by Ca2+/calmodulin-dependent protein kinases type I, II, and IV, J. Biol. Chem. 271 (1996) 3066–3073.
[22] C.K. Miranti, D.D. Ginty, G. Huang, T. Chatila, M.E. Greenberg, Calcium activates serum response factor-dependent transcription by a ras- and elk-1-independent mechanism that involves a Ca2+/calmodulin-dependent kinase, Mol. Cell. Biol. 15 (1995) 3672–3684.
[23] P. Pognonec, K.E. Boulukos, J. Gesquiere, D. Stehelin, J. Ghysdael, Mitogenic stimulation of thymocytes results in the calcium-dependent phosphorylation of c-ets-1 proteins, EMBO J. 7 (1988) 977–983.
[24] C.L. Fisher, J. Ghysdael, J.C. Cambier, Ligation of membrane Ig leads to calcium-mediated phosphorylation of the proto-oncogene, Ets-1, J. Immunol. 146 (1991) 1743–1749.
[25] J. Krebs, R.L. Means, P. Honegger, Induction of calmodulin kinase IV by the thyroid hormone during the development of rat brain, J. Biol. Chem. 271 (1996) 11055–11058.
[26] Y.Y. Liu, G.A. Brent, A complex deoxyribonucleic acid response element in the rat Ca^{2+}/calmodulin-dependent protein kinase IV gene 5′-flanking region mediates thyroid hormone induction and chicken ovalbumin upstream promoter transcription factor 1 repression, Mol. Endorinol. 16 (2002) 2439–2451.
[27] K.F. Jensen, C.A. Ohmstede, R.S. Fisher, N. Sahyoun, Nuclear and axonal localization of Ca2+/calmodulin-dependent protein kinase type Gr in rat cerebellar cortex, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 2850–2853.
[28] H. Bito, K. Deisseroth, R.W. Tsien, CREB phosphorylation and dephosphorylation: a Ca(2+)- and stimulus duration-dependent switch for hippocampal gene expression, Cell 87 (1996) 1203–1214.
[29] G.A. Gonzalez, M.R. Montminy, Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at serine 133, Cell 59 (1989) 675–680.
[30] J. Krebs, Calmodulin-dependent protein kinase IV: regulation of function and expression, Biochim. Biophys. Acta 1448 (1998) 183–189.
[31] J. Xie, D.L. Black, A CaMKIV responsive RNA element mediates depolarization-induced alternative splicing in ion channels, Nature 410 (2001) 936–939.
[32] J. Xie, C. Jan, P. Stoilov, J. Park, D.L. Black, A consensus CaMKIV-responsive RNA sequence mediates regulation of alternative exons in neurons, RNA 11 (2005) 1825–1834.
[33] J.A. Lee, Y. Xing, D. Nguyen, J. Xie, C.J. Lee, D.L. Black, Depolarization and CaMKinase IV modulate NMDA receptor splicing through two essential RNA elements, PLoS Biol. 5 (2007) e40 10.1371/journal.pbio.0050040.
[34] J. Colomer, A.R. Means, Physiological roles of the Ca2+/CaM-dependent protein kinase cascade in health and disease, Subcell. Biochem. 45 (2007) 169–214.
[35] F. Di Leva, T. Domi, L. Fedrizzi, D. Lim, E. Carafoli, The plasma membrane Ca2+ ATPase of animal cells: Structure, function and regulation, Arch. Biochem. Biophys. 476 (2008) 65–74.
[36] P.L. Pedersen, E. Carafoli, Ion motive ATPases. I. Ubiquity, properties, and significance for cell function, Trends Biochem. Sci. 12 (1987) 146–150.
[37] P.L. Pedersen, E. Carafoli, Ion motive ATPases. II. Energy coupling and work output, Trends Biochem. Sci. 12 (1987) 186–189.

[38] P. James, M. Maeda, R. Fischer, A.K. Verma, J. Krebs, J.T. Penniston, E. Carafoli, Identification and primary structure of a calmodulin binding domain of the Ca^{2+} pump of human erythrocytes, J. Biol. Chem. 263 (1988) 2905–2910.
[39] E.E. Strehler, D.A. Zacharias, Role of alternative splicing in generating isoform diversity among plasma membrane calcium pumps, Physiol. Rev. 81 (2001) 21–50.
[40] M. Brini, Plasma membrane Ca(2+)-ATPase: from a housekeeping function to a versatile signaling role, Pflugers Arch. (in press), doi:10.1007/s00424-008-0505-6.
[41] A.K. Verma, A.G. Filoteo, D.R. Stanford, E.R. Wieben, J.T. Penniston, E.E. Strehler, R. Fischer, R. Heim, G. Vogel, S. Mathews, M.A. Strehler-Page, P. James, T. Vorherr, J. Krebs, E. Carafoli, Complete primary structure of a human plasma membrane Ca^{2+} pump, J. Biol. Chem. 263 (1988) 14152–14159.
[42] G.E. Shull, J. Greeb, Molecular cloning of two isoforms of the plasma membrane Ca2+-transporting ATPase from rat brain. Structural and functional domains exhibit similarity to Na+, K+ - and other cation transport ATPases, J. Biol. Chem. 263 (1988) 8646–8657.
[43] E.E. Strehler, M.A. Strehler-Page, G. Vogel, E. Carafoli, mRNAs for plasma membrane calcium pump isoforms differing in their regulatory domain are generated by alternative splicing that involves two internal donor sites in a single exon, Proc. Natl. Acad. Sci. U. S. A. 86 (1989) 6908–6912.
[44] E. Zvaritch, P. James, T. Vorherr, R. Falchetto, N. Modyanov, E. Carafoli, Mapping of functional domains in the plasma membrane Ca2+ pump using trypsin proteolysis, Biochemistry 29 (1990) 8070–8076.
[45] R. Falchetto, T. Vorherr, E. Carafoli, The calmodulin-binding site of the plasma membrane Ca2+ pump interactr with the transduction domain of the enzyme, Protein Sci. 1 (1992) 1613–1621.
[46] T.P. Stauffer, H. Hilfiker, E. Carafoli, E.E. Strehler, Quantitative analysis of alternative splicing options of human plasma membrane calcium pump genes, J. Biol. Chem. 268 (1993) 25993–26003.
[47] T.P. Keeton, S.E. Burk, G.E. Shull, Alternative splicing of exons encoding the calmodulin-binding domains and C termini of plasma membrane Ca(2+)-ATPase isoforms 1, 2, 3, and 4, J. Biol. Chem. 268 (1993) 2740–2748.
[48] T.P. Keeton, G.E. Shull, Primary structure of rat plasma membrane Ca(2+)-ATPase isoform 4 and analysis of alternative splicing patterns at splice site A, Biochem. J. 306 (Pt. 3) (1995) 779–785.
[49] E. Carafoli, T. Stauffer, The plasma membrane calcium pump: functional domains, regulation of the activity and tissue specificity of isoform expression, J. Neurobiol. 25 (1994) 312–324.
[50] P. Brandt, R.L. Neve, Expression of plasma membrane calcium-pumping ATPase mRNAs in developing rat brain and adult brain subregions: evidence for stage-specific expression, J. Neurochem. 59 (1992) 1566–1569.
[51] D.A. Zacharias, E.E. Strehler, Change in plasma membrane Ca^{2+}-ATPase splice-variant expression in response to a rise in intracellular Ca^{2+}, Curr. Biol. 6 (1996) 1642–1652.
[52] S. Stamm, Regulation of alternative splicing by reversible protein phosphorylation, J. Biol. Chem. 283 (2008) 1223–1227.
[53] D. Guerini, E. Garcia-Martin, A. Gerber, C. Volbracht, M. Leist, C. Gutiérrez-Merino, E. Carafoli, The expression of plasma membrane Ca^{2+} pump in cerebellar granule neurons is modulated by Ca^{2+}, J. Biol. Chem. 274 (1999) 1667–1676.
[54] H. Hilfiker, D. Guerini, E. Carafoli, Cloning and expression of isoform 2 of the human plasma membrane Ca^{2+} ATPase. Functional properties of the enzyme and ist splicing products. J. Biol. Chem. 269 (1994) 26178–26183.
[55] A. Enyedi, A.K. Verma, R. Heim, H.P. Adamo, A.G. Filoteo, E.E. Strehler, J.P. Penniston, The Ca^{2+} affinity of the plasma membrane Ca^{2+} pump is controlled by alternative splicing, J. Biol. Chem. 269 (1994) 41–43.
[56] B.S. Preiano', D. Guerini, E. Carafoli, Expression and functional characterization of isoforms 4 of the plasma membrane calcium pump, Biochemistry 35 (1996) 7946–7953.
[57] A.J. Caride, A.G. Filoteo, J.T. Penniston, E.E. Strehler, The plasma membrane Ca^{2+} pump isoform 4a differs from isoform 4b in the mechanism of calmodulin binding and activation kinetics. Implications for Ca^{2+} signaling, J. Biol. Chem. 282 (2007) 25640–25648.
[58] P.J. Shepard, K.J. Hertel, Conserved RNA secondary structures promote alternative splicing, RNA 14 (2008) 1463–1469.
[59] C.A. Ohmstede, K.F. Jensen, N.E. Sahyoun, Ca2+/calmodulin-dependent protein kinase enriched in cerebellar granule cells. Identification of a novel neuronal calmodulin-dependent protein kinase. J. Biol. Chem. 264 (1989) 5866–5875.
[60] P. Vito, E. Lacana, L. D'Adamio, Interfering with apoptosis: Ca(2+)-binding protein ALG-2 and Alzheimer's disease gene ALG-3, Science 271 (1996) 521–525.
[61] M. Maki, S.V.L. Narayana, K. Hitomi, A growing family of the Ca2+-binding proteins with five EF-hand motifs, Biochem. J. 328 (1997) 718–720.
[62] J. Krebs, R. Klemenz, The ALG-2/AIP-complex, a modulator at the interface between cell proliferation and cell death? A hypothesis, Biochim. Biophys. Acta 1498 (2000) 153–161.
[63] J. Krebs, P. Saremaslani, R. Caduff, ALG-2: a Ca^{2+}-binding modulator protein involved in cell proliferation and in cell death, Biochim. Biophys. Acta 1600 (2002) 68–73.
[64] Y. Kitaura, S. Matsumoto, H. Satoh, K. Hitomi, M. Maki, Peflin and ALG-2, members of the penta-EF-hand protein family, form a heterodimer that dissociates in a Ca2+-dependent manner, J. Biol. Chem. 276 (2001) 14053–14058.
[65] P. Montaville, Y. Dai, C.Y. Cheung, K. Giller, S. Becker, M. Michalak, S.E. Webb, A.L. Miller, J. Krebs, Nuclear translocation of the calcium-binding protein ALG-2 induced by the RNA-binding protein RBM22, Biochim. Biophys. Acta 1763 (2006) 1335–1343.
[66] L. Subramanian, J.W. Crabb, J. Cox, I. Durussel, T.M. Walker, P.R. van Ginkel, S. Bhattacharya, J.M. Dellaria, K. Palczewski, A.S. Polans, Ca2+ binding to EF hands 1 and 3 is essential for the interaction of apoptosis-linked gene-2 with Alix/AIP1 in ocular melanoma, Biochemistry 43 (2004) 11175–11186.
[67] K. Hartmuth, H. Urlaub, H.P. Vornlocher, C.L. Will, M. Gentzel, M. Wilm, R. Luhrmann, Protein composition of human prespliceosomes isolated by a tobramycin affinity-selection method, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 16719–16724.
[68] J. Rappsilber, U. Ryder, A.I. Lamond, M. Mann, Large-scale proteomic analysis of the human spliceosome, Genome Res. 12 (2002) 1231–1245.
[69] J.W. Park, K. Parisky, A.M. Celotto, R.A. Reenan, B.R. Graveley, Identification of alternative splicing regulators by RNA interference in *Drosophila*, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 15974–15979.
[70] Y.O. Kim, S.J. Park, R.S. Balaban, M. Nirenberg, Y. Kim, A functional genomic screen for cardiogenic genes using RNA interference in developing *Drosophila* embryos, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 159–164.
[71] A. Amsterdam, R.M. Nissen, Z. Sun, E.C. Swindell, S. Farrington, N. Hopkins, Identification of 315 genes essential for early zebrafish development, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 12792–12797.
[72] R. Kittler, G. Putz, L. Pelletier, I. Poser, A.K. Heninger, D. Drechsel, S. Fischer, I. Konstantinova, B. Habermann, H. Grabner, M.L. Yaspo, H. Himmelbauer, B. Korn, K. Neugebauer, M.T. Pisabarro, F. Buchholz, An endoribonuclease-prepared siRNA screen in human cells identifies genes essential for cell division, Nature 432 (2004) 1036–1040.
[73] D.L. Spector, Macromolecular domains within the cell nucleus, Annu. Rev. Cell. Biol. 9 (1993) 265–315.
[74] A.I. Lamond, D.L. Spector, Nuclear speckles: a model for nuclear organelles, Nat. Rev. Mol. Cell Biol. 4 (2003) 605–612.
[75] M. Alvarez, X. Estivill, S. de la Luna, DYRK1A accumulates in splicing speckles through a novel targeting signal and induces speckle disassembly, J. Cell Sci. 116 (2003) 3099–3107.
[76] K.E. Handwerger, J.G. Gall, Subnuclear organelles: new insights into form and function, Trends Cell. Biol. 16 (2006) 19–26.