TOPICAL REVIEW

The impact of splice isoforms on voltage-gated calcium channel α_1 subunits

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Semi-conserved exon boundaries in members of the *CACNA1* gene family result in recurring pre-mRNA splicing patterns. The resulting variations in the encoded pore-forming subunit of the voltage-gated calcium channel affect functionally significant regions, such as the vicinity of the voltage-sensing S4 segments or the intracellular loops that are important for protein interaction. In addition to generating functional diversity, RNA splicing regulates the quantitative expression of other splice isoforms of the same gene by producing transcripts with premature stop codons which encode two-domain or three-domain channels. An overview of some of the known splice isoforms of the α_1 calcium channel subunits and their significance is given.

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There are only 10 genes in the human genome that encode pore-forming α_1 subunits of voltage-gated calcium channels. In combination with accessory subunits, these 10 α_1 subunits must mediate such diverse functions as: (i) intracellular calcium homeostasis, (ii) regulation of gene expression, and (iii) coupling of membrane potential changes to various downstream processes like neurotransmitter release or muscle contraction. Nature has chosen alternative pre-RNA splicing as a thrifty means to generate the required functional and structural diversity of the α_1 subunits. This paper gives an overview of recurrent patterns in calcium channel α_1 subunit RNA splicing and their functional significance. Reviews giving a general overview of a calcium channel family including information on splicing (Catterall, 2000; Lacinova *et al.* 2000; Abernethy & Soldatov, 2002; Perez-Reyes, 2002) or focusing especially on splicing in the N-type channel (Lipscombe *et al.* 2002) are available for further study.

Calcium channel α_1 subunits

Voltage-gated calcium channels have been functionally differentiated according to their inactivation properties into either transient (T-type) or long lasting (L-type) currents. Additionally, N (neuronal), P (Purkinje cell), Q (granular cell) and R (toxin-resistant) channels can be distinguished depending on their tissue expression pattern and toxin sensitivity, respectively. Based on the phylogeny underlying these pharmacological and biophysical differences, Ertel *et al.* (2000) have suggested a more uniform nomenclature for the α_1 subunits of calcium channels which is now commonly used (Fig. 1).

The α subunit of the channel, contains as a basic motif a tetrameric association of four domains each containing a series of six transmembrane α-helical segments, numbered S1–S6, which are connected by both intracellular and extracellular loops (Fig. 2). It comprises the ion-conducting pore and determines the main characteristics of the cation channel complex such as its ion selectivity, voltage sensitivity, pharmacology and binding characteristics for endogenous and exogenous ligands. The voltage sensitivity of cation channels is conveyed by the S4 segments, which are thought to move outward upon depolarization causing the channels to open. Calcium flows through the ion conducting pore, which is thought to be lined by the S5–S6 loops of all four domains. Whereas the localization of the activation gate may well be within the pore, the inactivation gate has not been unambiguously localized in calcium channels (for review see Catterall, 2000).

In order to form a functional calcium channel complex, the α_1 subunit coassembles with at least three accessory subunits encoded by two gene families: an intracellular β subunit encoded by a *CACNB* gene, and an extracellular α_2 subunit linked by a di-sulphide bond to the membrane-anchoring δ subunit both of which are encoded by

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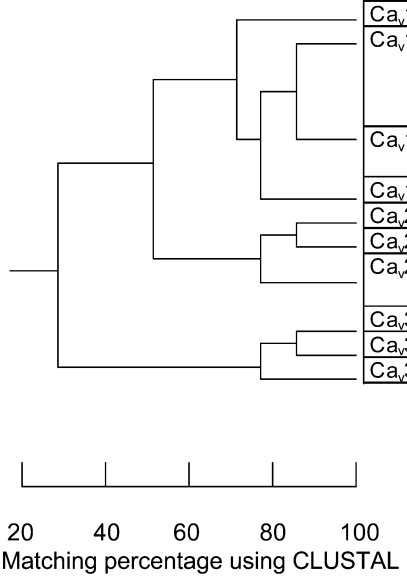
a *CACNA2D* gene. In skeletal muscle, an additional accessory transmembranal γ subunit is part of the channel complex (Kang & Campbell, 2003; Wolf *et al.* 2003). In neuronal channels, its contribution to the channel complex is a matter of debate because coexpression did not regularly yield a functional change (Moss *et al.* 2003). The contribution of accessory subunit-mediated modulation to calcium channel diversity is reviewed elsewhere (Walker & De Waard, 1998; Birnbaumer *et al.* 1998). Briefly, the accessory α_2/δ and β subunits increase the current amplitude (Brice *et al.* 1997), accelerate inactivation kinetics and facilitate gating (Singer *et al.* 1991), and shift the voltage dependence of inactivation in the hyperpolarizing direction (Singer *et al.* 1991).

Splicing of *CACNA1* transcripts

In order to obtain mature mRNA which can be directly translated into a protein sequence, the non-coding regions corresponding to introns of the DNA must be removed from the precursor pre-mRNA by the splicing process. The machinery performing this task, the spliceosome, recognizes introns by typical nucleotide sequences within the intron and adjacent exons (Wu & Krainer, 1999; Singh, 2002; Black & Grabowski, 2003). The probability for splicing out a sequence at any position in the pre-mRNA depends, among others, on the combination of different nucleotide sequences in key positions and the tissue-dependent spliceosome composition available. Therefore, the mRNA population resulting from one gene is not homogeneous in a given cell type, but rather shows the result of a variety of combinations of different single splicing events, each of which is present in a number of transcripts proportionate to its splicing probability.

In calcium channel transcripts, combinations of four types of alternative splicing are found: (i) splicing at alternative intron sequences near the 5′ intron end causing a possible elongation or shortening of the preceding exon (so-called alternative splice donor), (ii) splicing at alternative intron sequences near the 3′ intron end causing a possible elongation or shortening of the following exon (so-called alternative splice acceptor), (iii) optional splicing within an intron to retain an optional exon (so-called cassette exon) by use of less probable acceptor and donor splice signals flanking this exon, and (iv) alternative splicing of more than one cassette exon within a large intron that may result in a variety of combinations of these exons including the obligatory exclusion of one another. So far, cases of alternative donor and acceptor sites within an exon resulting in splicing out of central portions of that exon have not yet been described in *CACNA1* transcripts.

Voltage-gated calcium channels are thought to have evolved by multiple gene duplication from a common ancestral channel gene encoding a one-domain potassium channel (Strong *et al.* 1993; Nelson *et al.* 1999; Anderson & Greenberg, 2001). The intron–exon boundaries or



Protein	Gene	Chromosome	Primary tissues	Calcium current
$Ca_v1.1$ (a_{1S})	*CACNA1S*	1q32	skeletal muscle	L-type
$Ca_v1.2$ (a_{1C})	*CACNA1C*	12p13.3	heart smooth muscle brain, heart, pituitary, adrenal	L-type
$Ca_v1.3$ (a_{1D})	*CACNA1D*	3p14.3	brain, pancreas, kidney, ovary, cochlea	L-type
$Ca_v1.4$ (a_{1F})	*CACNA1F*	Xp11.23	retina	L-type
$Ca_v2.1$ (a_{1A})	*CACNA1A*	19p13	brain, cochlea, pituitary	P/Q-type
$Ca_v2.2$ (a_{1B})	*CACNA1B*	9q34	brain, nervous system	N-type
$Ca_v2.3$ (a_{1E})	*CACNA1E*	1q25-31	brain, cochlea, retina, heart, pituitary	R-type
$Ca_v3.1$ (a_{1G})	*CACNA1G*	17q22	brain, nervous system	T-type
$Ca_v3.2$ (a_{1H})	*CACNA1H*	16p13.3	brain, heart, kidney, liver	T-type
$Ca_v3.3$ (a_{1I})	*CACNA1I*	22q12.3-13-2	brain	T-type

Figure 1. Calcium channel nomenclature (modified according to Ertel *et al.* 2000)

gene structures are therefore not only conserved from one *CACNA1* gene to another (Huang *et al.* 1990; Barry *et al.* 1995; Wu & Krainer, 1999; Lipscombe *et al.* 2002), but also from one species to another, for example *Drosophila versus* man (Peixoto *et al.* 1997). The gene structure within the *CACNA1* gene family is generally well conserved at coding regions for segments S1–S5 of all four domains (Fig. 2*A*). The remaining regions, especially those coding for the domain interlinkers and the S6 segments of all four domains, show less conserved gene structure suggesting

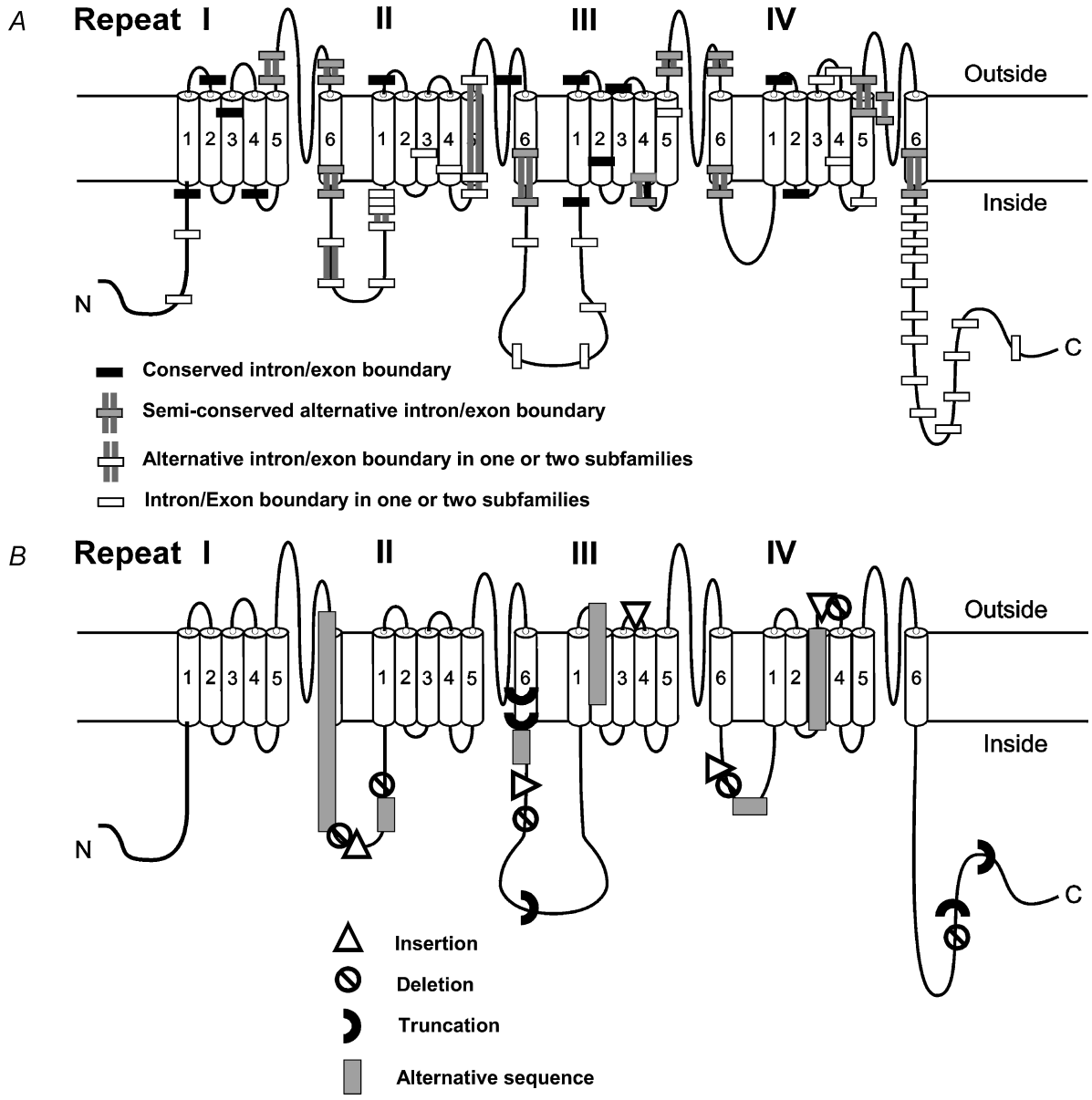


Figure 2 . Scheme of the voltage-gated calcium channel α_1 subunit

The α_1 subunit of voltage gated calcium channels consists of four domains (repeats) of six transmembrane segments connected by intracellular loops. *A*, conservation of the gene structure at the protein level as determined by protein alignments encoded by all exons of all 10 members of the *CACNA1* family. Each protein region encoded by an exon is delineated by bars. White, black and grey bars indicate degree of conservation as noted in the figure. The following human reference sequences were used at NCBI: $Ca_v2.1$: NP_075461; $Ca_v2.2$: NP_000709; $Ca_v1.2$: NP_000710; $Ca_v1.3$: NP_000711; $Ca_v2.3$: NP_000712; $Ca_v1.4$: NP_005174; $Ca_v3.1$: NP_061496; $Ca_v3.2$: NP_066921; $Ca_v3.3$: NP_066919; and $Ca_v1.1$: NP_000060. *B*, regions of the protein that are affected by alternative splicing. Note that the changes now reflect the protein level only (i.e. deletions leading to frame shifts and early truncations are marked as truncation only). The diversity of the primary protein sequence due to insertions, deletions, truncations and alternative sequences is marked by the various symbols.

that the encoded protein areas are important for generating functional diversity. These less conserved coding regions coincide with the regions in which alternative splicing produces several different transcripts derived from a single gene (Fig. 2*B*). These transcripts generate proteins with similar function and sequence but different expression patterns, the so-called splice isoforms.

S3–S4 loops

The extracellular loops between S3 and S4 may influence S4 voltage sensor function because of their close vicinity to the S4 segments, which must move upon depolarization (Bezanilla, 2002). Effects on the voltage sensor function could therefore be achieved by alternative splicing in these areas while leaving the S4 itself unaffected. In the L-type Ca^{2+} channel family, the IS3–S4 loop of skeletal and cardiac channels helps to determine activation kinetics as has been demonstrated by the study of chimeras (Nakai *et al.* 1994). However, it is unlikely that this mechanism is taken advantage of in splicing regulation of IS3–S4 because it would require intraexonic splicing for all 10 hitherto known *CACNA1* genes in which distal IS3, the whole IS3–S4 loop, and proximal IS4 are all encoded by one exon. For domain II, a similarly unlikely pattern of splicing would be required, but for domains III and IV, all 10 *CACNA1* genes have an intron between genomic regions encoding S3 and S4. It is therefore not surprising that these regions are able to produce different splice isoforms. In domain III however, the only known variant is one with the insertion of 12 bases by cassette exon 24a leading to addition of four amino acid residues, SFMG, to IIIS3–S4 in the N-type $Ca_v2.2$ channel which does not, however, seem to have significant impact on the kinetics or voltage dependence of gating (Lin *et al.* 1999; Stea *et al.* 1999). This leaves only the variants generated by alternative splicing of regions encoding IVS3–S4 to be of functional significance.

In domain IV, the insertion of only six bases by a supplementary cassette exon 31a in both P/Q-type $Ca_v2.1$ and N-type $Ca_v2.2$ channels has a distinct functional effect even though it leads to the introduction of only two amino acid residues, NP or ET. The presence of ET in the N-type $Ca_v2.2$ channels results in a rightward shift of voltage dependence of activation and slowed activation kinetics (Lin *et al.* 1997, 1999). Given that exon 31a is preferentially expressed in the peripheral but not in the central nervous system, this could lead to facilitated calcium entry selectively in cerebral neurones (Lin *et al.* 1999). In P/Q-type $Ca_v2.1$ channels, NP slows activation and inactivation and decreases affinity to ω-agatoxin IVA (Bourinet *et al.* 1999; Hans *et al.* 1999; Krovetz *et al.* 2000). The NP variant is generally thought to be present in the Q-type (low ω-agatoxin IVA affinity), while the variant lacking NP is thought to decisively contribute to the P-type (high affinity) calcium channel, even though secondary modifications and accessory subunits may also be contributing to the functional characteristics of P-type and Q-type (Mermelstein *et al.* 1999). Expression patterns suggest that the more rapidly gating P-type calcium channel is important for not only cerebellar Purkinje cells, but also pancreatic β cells (Ihara *et al.* 1995; Ligon *et al.* 1998).

In L-type channels, IVS3–S4 is altered by the splicing out of a short exon encoding part of this loop: for the skeletal muscle $Ca_v1.1$ channel it is exon 29 coding for TFLASSGGLYCLGGGCGNV, for the cardiac $Ca_v1.2$ channel exon 33 coding for PAEHTQCSPSM, and for the neuronal $Ca_v1.3$ channel exon 32 coding for PSENIPLPTATPG (Barry *et al.* 1995; Safa *et al.* 2001). In the cardiac $Ca_v1.2$ channel, this exon deletion is additionally accompanied by the replacement of exon 31 by exon 32 (exon 31 and 32 are mutally exclusive exons in the current nomenclature; Abernethy & Soldatov, 2002) encoding an altered sequence for part of IVS3 and IVS3–S4 (Perez-Reyes *et al.* 1990; Snutch *et al.* 1991; Diebold *et al.* 1992; Yu *et al.* 1992; Abernethy & Soldatov, 2002). The 11 amino acid IVS3–S4 deletion is present in several tissues making up about 12% of all transcripts while the IVS3 variation is tissue dependent and differentially regulated during development (Feron *et al.* 1994). For the skeletal muscle $Ca_v1.1$ channel, the 19 amino acid IVS3–S4 deletion makes up 10% of transcripts in adult muscle, but over 66% in regenerating muscle (unpublished data). For these three channels, the functional significance of these variants has not yet been clarified.

II–III loop

The task of the intracellular loop connecting domains II and III is to mediate interaction with effector proteins such as the calcium release channel for skeletal muscle excitation–contraction coupling (Dulhunty *et al.* 2002) or synaptic proteins such as syntaxin and SNAP25 (soluble attachment proteins of NSF – *N*-ethylmaleimide-sensitive fusion protein) or proteins for neuronal excitation–exocytosis coupling (Catterall, 1999; Mochida *et al.* 2003). Therefore, a functional modulation brought about by change or removal of these regions by alternative splicing may be expected. For example, in N-type $Ca_v2.2$ calcium channels, the first part of this loop may be altered by introducing an additional cassette following exon 18 (Pan

& Lipscombe, 2000). This exon 18a is expressed in adult sympathetic ganglia, but not very abundantly in most of the regions of the neocortex except for monoaminergic neurones (Ghasemzadeh *et al.* 1999). This suggests that even though the putative synaptic protein binding site is not directly altered by alternative splicing, the 18a-encoded region may contribute to the targetting of the isoform to distinct synapses (Lipscombe *et al.* 2002). Likewise, even though the binding site of the β subunit is not affected, different β isoforms act differentially on the 18a variants (Scott *et al.* 1996; Pan & Lipscombe, 2000). Functionally, exon 18a causes a right-shift of the voltage-dependence of steady-state inactivation which may directly lead to hyperexcitability by affecting neurotransmitter release or to decreased excitability by activating Ca^{2+}-dependent K^+ channels indicating a possible higher degree of excitability of those neurones in which it is expressed (Pan & Lipscombe, 2000). In addition to the 18a variant, exons 19–21 may be spliced out with or without modification of the preceding splice donor site (Kaneko *et al.* 2002). These isoforms show not only the right shift of the steady-state inactivation curve, but also accelerated recovery from inactivation (Kaneko *et al.* 2002). Additionally, the sensitivity to ω-conotoxin GIVA is reduced, the reason for which could be the preferred binding of the toxins to the inactivated state, which is destabilized by the deletions (Lipscombe *et al.* 2002).

In the closely related P/Q $Ca_v2.1$ channels, exon 17, which corresponds to exon 18 of $Ca_v2.2$, is alternatively spliced at the splice donor site of intron 17 leading to a potential insertion/deletion of nine bases coding for VEA (Soong *et al.* 2002). No functional studies have yet been performed so that a possible effect on the synaptic protein binding site cannot be decided. However, rat and rabbit splice isoforms with sequence differences in the region encoded by human exon 19 showed different affinity to SNAP-25 and syntaxin (Kim & Catterall, 1997; Rettig *et al.* 1996) and different cellular expression patterns (Sakurai *et al.* 1996). This supports the idea of isoform-specific targeting as in the $Ca_v2.2$ channels (Catterall, 1999). Recently, exactly this region encoded by exon 19 was shown to mediate the synaptic protein interaction site (Mochida *et al.* 2003) confirming these assumptions. The II–III interlinker of the P/Q $Ca_v2.1$ channels also shows single amino acid substitutions caused by RNA editing which have been described in the mouse homologue affecting residues 886 and 1085, but the functional significance has not been clarified yet (Tsunemi *et al.* 2002).

In R-type $Ca_v2.3$ channels, exon 19, corresponding to exon 19 of $Ca_v2.1$ channels, can be spliced out, which results in the absence of calcium-dependent slowing of inactivation and acceleration of recovery from inactivation (Pereverzev *et al.* 2002). This isoform with decreased sensitivity to calcium influx shows differential distribution in murine cerebellum, the islets of Langerhans and kidney (Vajna *et al.* 1998; Grabsch *et al.* 1999). Even though the targeting to synapses has not been studied, the specific tissue distribution in other cell types may indicate changed protein interaction. Lastly, comparable to the N-type $Ca_v2.2$ channels and despite little sequential homology in II–III linker, in the T-type $Ca_v3.1$ channels, exon 16 may be spliced out leading to a right shift of steady state inactivation curve and slowing of inactivation kinetics (Mittman *et al.* 1999*a*; Chemin *et al.* 2001).

C-terminus

The C-terminus makes up a third of the channel protein and is not very well conserved within the *CACNA1* gene family suggesting it to be a region of functional specialization. It is encoded by 3–14 exons, and the protein contains several regulatory elements such as binding sites for calcium, calmodulin and G-proteins (for reviews see Catterall, 2000; Hering *et al.* 2000; Abernethy & Soldatov, 2002; Perez-Reyes, 2002). Additionally, in $Ca_v2.2$ channels, the C-terminus is important for targeting the channels to synapses (Maximov & Bezprozvanny, 2002). This region is also relevant for a disease as indicated by the occurrence of a CAG repeat expansion in the coding region of $Ca_v2.1$ channels responsible for spinocerebellar atxia type 6 (Zhuchenko *et al.* 1997). The presence of at least two C-termini of different lengths is generally accepted for most calcium channels and is even now found in the NCBI database routinely. Perhaps the most well known are the P/Q $Ca_v2.1$ isoforms that are generated by alternative splice donor sites at the 5′ end of exon 47 resulting directly in a stop codon or insertion of five bases leading to a frame shift and an elongation of the C-terminus by 244 amino acids (Zhuchenko *et al.* 1997). Functionally, these isoforms and an additional one of intermediate length did not differ significantly when coexpressed with four different β subunits (Tsunemi *et al.* 2002). In the related N-type $Ca_v2.2$ channels of chicken, a very similar 5-bp insertion is present leading to an elongated C-terminus (Lu *et al.* 2001). In human N-type $Ca_v2.2$, a deletion of 187 bp in exon 46 has been described which changes the open reading frame and results in a 102-amino acid shorter translational product (Williams *et al.* 1992).

In all three types of Ca_v2 channels, there are one to two exons coding for distal parts of the C-terminus that may potentially be spliced out (exons 43 and 44 in P/Q-type $Ca_v2.1$, exon 46 in the N-type $Ca_v2.2$, and exon 45 in R-type $Ca_v2.3$). Generally, the shorter the C-terminus becomes, the greater the current amplitude and the stronger the calcium dependence of inactivation (Hell *et al.* 1994; Bourin *et al.* 1999; Krovetz *et al.* 2000; Sandoz *et al.* 2001; Soong *et al.* 2002; Pereverzev *et al.* 2002). For these isoforms, the reduction of current amplitude is most likely to be due to a reduced number of channels perhaps either by mRNA destablization or by reduction/hinderance of targeting signals in the C-terminus (Soong *et al.* 2002). It has been hypothesized that because the C-terminus mobility may contribute to removing the calcium–calmodulin complex from the inner mouth of the pore, a shorter C-terminus may contribute to this mobility and accelerate inactivation (Kobrinsky *et al.* 2003).

For L-type channels, variants with shortened C-terminus in heart and skeletal muscle $Ca_v1.2$ and $Ca_v1.1$ have long been known (Beam *et al.* 1992; Gerhardstein *et al.* 2000; Gao *et al.* 2001), but the truncation takes place at the protein level rather than being the result of RNA splicing. In the cardiac $Ca_v1.2$ channel, exons 40–43 show combinations of usage of an alternative splice donor site at the 3′ end of exon 40 or alternative splice acceptor sites at the 5′ ends of exons 42 and 43, inclusion of a supplementary cassette exon following exon 40 or skipping of exon 42 (Klockner *et al.* 1997; Soldatov *et al.* 1997). As in neuronal channels, the shorter the C-terminus becomes, the greater the current amplitude and the greater the calcium-dependent inactivation (Soldatov *et al.* 1997). Likewise, in neuronal $Ca_v1.3$ channels, replacement of exon 41 by a mutually exclusive exon 41a leads to an early stop codon truncating over 500 amino acid residues encoded by exons 42–49, which results in a twofold increase of current amplitude without change of voltage dependence of gating (Safa *et al.* 2001).

Alternatively spliced C-termini in T-type channels have been described for $Ca_v3.3$ (Mittman *et al.* 1999*a*; Murbartian *et al.* 2002). They are produced by combinations of alternative splice acceptor sites in exons 33 and 34 which shorten these exons to different lengths. One of the variants produces a frame shift leading to a premature truncation of the C-terminus. In this variant, it is the presence of the shortened exon 33 in the transcript and not the lack of the subsequent exons that produces the observed functional consequences of slowed activation, accelerated inactivation, and slowed recovery from inactivation (Murbartian *et al.* 2002). The presumed changes to calcium current kinetics may be expected to influence neuronal function in such a manner that the slowly inactivating variants would be liable to sustain firing patterns. Analogous splice variants are present in T-type $Ca_v3.1$-encoding genes affecting exons 34, 35 and 38 (Mittman *et al.* 1999*b*), but functional studies are still lacking.

Two-domain or three-domain truncations

Several two-domain variants consisting of domains I and II exist in L-type calcium channels, possibly a relict of the second gene duplication thought to have taken place during gene evolution. In the cardiac $Ca_v1.2$ channel either exons 17 and 18 or exon 19 is deleted leading to premature stop codons with C-terminal tails of 62 or 19 amino acid residues, respectively, found specifically in cardiac sarcoplasmic reticulum (Wielowieyski *et al.* 2001). The same channel in neuronal and fibroblast tissue shows two additional isoform possibilities: an alternative splice donor site at the 5′ end of exon 15 generating 75% of the transcripts and a rare 12 bp insertion at the 3′ end of exon 16 both leading to premature stop codons in or following IIS6 (Soldatov, 1992; Soldatov, 1994). In rabbit skeletal neonatal muscle, a two-domain channel generated by the splicing of IIS2 onto IVS2 and thus consisting of domain I and chimeric domain IV of the skeletal muscle $Ca_v1.1$ channel has been detected (Malouf *et al.* 1992). None of these have been functionally expressed as yet.

In synaptic membranes, there is evidence for an alternative isoform of the neuronal P/Q-type $Ca_v2.1$ channel consisting of domains I and II at the protein level which may be due to RNA splicing or proteolysis (Scott *et al.* 1998). Later, a deletion of exons 16 and 17 encoding part of IIS6 and the II–III interlinker leading to a frame-shift and generating an early stop codon has actually been described in these channels, but not functionally expressed (Soong *et al.* 2002). A functional hypothesis for the significance of such two-domain channels can be deduced from a study on the closely related N-type $Ca_v2.2$ channels by Raghib *et al.* (2001) who demonstrated that a channel consisting of domains I and II is not functional when expressed alone but only when coexpressed with a construct forming domains III and IV. However, this study demonstrates that coexpression of these isoforms with the full length channel markedly reduced protein quantity and current density mediated by the latter. Similarly, a three-domain channel which lacked the first domain and a part of the second domain by using an alternative promotor does not produce a measurable calcium current but instead,

inhibits the functional expression of the full-length form (Okagaki *et al.* 2001). Therefore, this type of splicing may represent a simple possibility to transiently down-regulate a specific calcium channel without influencing promotor regulation.

Other domain interlinkers and transmembrane segments

The domain I–II interlinker encoded by three to five exons is important for G-protein modulation, inactivation and possibly β subunit interaction (for review see Catterall, 2000). In the trout L-type $Ca_v1.3$ channels, there is a 26 amino acid insertion encoded by an accessory cassette exon 9a for which a human homologue has not yet been described (Ramakrishnan *et al.* 2002); in man, however, exon 11 may be spliced out and/or exon 12 replaced by an alternative exon 12b (Safa *et al.* 2001). In T-type $Ca_v3.3$ channels, there is an alternative exon 9a (Mittman *et al.* 1999*b*). For the P/Q-type $Ca_v2.1$ channels, alternative splice acceptor sites at the 5′ prime end of exon 10 allow insertion of either G or VG residues (Bourinet *et al.* 1999; Soong *et al.* 2002). The isoform containing V showed slowed inactivation but enhanced G-protein inhibition and protein kinase C up-regulation compared to the isoform without V and is thought to also contribute to P-type characteristics of $Ca_v2.1$ calcium currents (Bourinet *et al.* 1999).

In contrast, the domain III–IV interlinker is encoded by only two to three exons and its functional significance is unclear. Even so, in the T-type $Ca_v3.1$ channel gene, an alternative splice donor site of exon 25 has been described that leads to skipping of seven amino acid residues, KAKQMA, and generates a right shift of activation and inactivation and slowing of activation kinetics (Monteil *et al.* 2000; Chemin *et al.* 2002). In the same channel, the skipping of exon 26 leads to an 18-amino-acid deletion with a left shift of inactivation and accelerated activation kinetics. Additional alternative splicing events in this loop occur in neuronal R-type $Ca_v2.3$ channels that may contain a 15 amino acid insertion following exon 29 (Takimoto *et al.* 1997) suggesting an important role of III–IV interlinker isoforms for excitation regulation.

Lastly, regions encoding a few transmembrane segments are alternatively spliced: IS6 of cardiac L-type $Ca_v1.2$ channels encoded by exon 8 or 8a, producing tissue-specific dihydropyridine sensitivity and putative changes of inactivation characteristics (Welling *et al.* 1997; Goodwin *et al.* 1999); IS6 of L-type $Ca_v1.3$ channels encoded by mutually exclusive exons 8a or 8b which result in a six-amino-acid difference in the pore region (Koschak *et al.* 2001); IIIS2 in cardiac L-type $Ca_v1.2$ channels encoded by mutually exclusive exons 21 and 22 which result in a seven-amino-acid difference influencing the voltage-dependent action of dihydropyridines (Soldatov *et al.* 1995); IVS3 in the same channels encoded by mutually exclusive exons 31 and 32 which work as a developmentally regulated switch coinciding with major changes in excitation (Diebold *et al.* 1992); and IVS3 encoded by mutually exclusive exons 31a or 31b which influence dihydropyridine sensitivity (Safa *et al.* 2001).

Perspective

Mutations in voltage-gated calcium channels are responsible for the pathogenesis of several hereditary ion channelopathies such as hemiplegic migraine, periodic paralysis, stationary night blindness, episodic and progressive spinocerebellar ataxias (for review of these see Lehmann-Horn & Jurkat-Rott, 1999) and, only recently reported, absence seizures (Chen *et al.* 2003). Given the above reviewed spectrum of functional and regulatory changes of the α_1 subunits generated by alternative splicing already under normal physiological conditions, a simple mechanism to cause change of function of the voltage gated calcium channels would be to alter the splicing probability and therefore splice isoform distribution. Supporting this hypothesis is the fact that mutations in independent genes are capable of changing ion channel splicing and thus contribute to the phenotype (Charlet *et al.* 2002; Mankodi *et al.* 2002).

Neurotransmitters such as dopamine and glutamate (Berke *et al.* 2001) as well as anti-inflammatory (Vogiagis *et al.* 2001) and antipsychotic (Meshul *et al.* 1996) drugs have been shown to alter splicing patterns of several proteins including ligand-gated ion channels such as glutamate receptors. Even though not yet shown, it is highly likely that voltage-gated calcium channels may also be altered by drug intake, especially if there is an effect on excitability (Fields, 1998; Fass *et al.* 1999; Vigues *et al.* 1999). Therefore, the understanding of function and regulation of the splicing isoforms will be important for determining successful therapeutic strategies in the future, possibly representing an alternative to gene therapy.

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