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Review

# A review of TRP channels splicing

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**Abstract**

Ion channel functional diversity can be achieved at the structural level by means of three main mechanisms: (1) transcriptional regulation and processing of mRNA, (2) heteromerization of different pore-forming channel subunits and (3) incorporation of regulatory subunits to the functional channel complex. In this review article we will focus on one of these mechanisms, alternative pre-mRNA splicing, in the context of the TRP superfamily of cation channels. For this purpose, the basic principles governing pre-mRNA splicing will be introduced and comprehensive tables classifying only published spliced-variants of TRP channels will be presented.
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*Keywords:* TRP; Channel; Splicing; Calcium; Diversity

## Contents



## 1. The plasticity of splicing generates a new concept of gene

The protein coding sequences of eukaryotic genes (exons) are typically interrupted by much longer non-coding intervening sequences (introns) that are removed during the RNA splicing process to produce mRNA. A splice event, that very often occurs while RNA is still being transcribed, removes one intron and joins the adjacent exons end to end, to give a shorter RNA product.

There are at least two potential advantages for the existence of introns: in an evolutive scale, it facilitates recombination of exons from different genes to produce new proteins with new functions, and in a given organism it allows the production of different proteins from the same gene. Moreover, cells use splicing to control gene expression [1].

Once it was thought that the organism complexity was given by the transcriptome size. The low number of genes predicted in our genome (30,000) made us turn back to the posttranscriptional

*Abbreviations:* aas, aminoacids; ANK, ankirin repeat; bp, base pairs; CaM, calmodulin binding site; DAG, diacylglycerol; DRG, dorsal root ganglia; 5′,6′-EET, 5′,6′-epoxyeicosatrienoic acid; EST, expressed sequence tag; GPCR, G protein coupled receptor; hnRNPs, heterogeneous nuclear ribonucleoproteins; $IP_3R$, inositol triphosphate receptor; Kb, kilobase; N.D., not described; NS, nervous system; nt, nucleotides; PIP2, phosphatidylinositol 4,5-bisphosphate; PKD, polycystic kidney disease; PTC+ mRNAs, premature stop codons in mRNAs; RUST, regulated unproductive splicing and translation; snRNA, small nuclear RNA; snRNP, small nuclear ribonucleoproteins; SR proteins, serine/arginine-rich proteins; TM, transmembrane domain; TNFα, tumor necrosis factor α; TRP channels, transient receptor potential channels; TRPC, TRP channels of the classical or "canonical" subfamily; TRPM, TRP channels of the melastatin subfamily; TRPML, TRP channels of the mucolipin subfamily; TRPV, TRP channels of the vanilloid subfamily; TRPP, TRP channels of the polycystin subfamily

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and posttranslational processes to explain functional variability. Looking through transcriptome databases one realizes than many (if not most) of genes are alternative spliced and some times it is difficult to differentiate which one is the canonical transcript just by its abundance or tissue-wide occurrence, or by containing (only) the constitutive (versus alternative) exons, or perhaps by the length of the transcript. It would be more realistic conceiving genes as trains with multiple, different and interchangeable wagons (exons), with practically no canonical exons in their structure.

Alternative splicing is a complex multifactorial process regulated on the *cis* side by the splice sites, splice enhancers and silencers and determinants of pre-mRNA secondary structures at the RNA level; and at the DNA level by promoters, transcriptional enhancers and the pol II elongation rate. On the *trans* side it is controlled by the abundance, cellular localization and phosphorilation status of regulatory proteins (different for each tissue), together with other transcription and elongation factors. The degeneracy of the splicing regulatory sequences, together with the availability of the combined factors mentioned above and the weak RNA–protein interactions, make possible to fine-tune alternative splicing in a reversible way and in response to external stimuli such as pH, temperature, metal ion and osmotic conditions, among others [2].

## 2. The splicing machinery

The splicing machinery, also known as spliceosome, is a large RNA–protein complex which consists of five types of small nuclear RNA (snRNA) and more than 50 protein species [3], that combine to form small nuclear ribonucleoproteins (snRNP). During splicing, the spliceosome recognizes sequentially three positions on the RNA through dynamic base-pairing with the snRNAs: the 5′ splice site, the branch point in the intron sequence that forms the base of the excised lariat, and the 3′ splice site. These three positions are quite conserved: introns almost always start with GU and end with AG, and the branch point is generally an A, 40 nucleotides before the AG dinucleotide at most.

However, the selection of the splicing sites is not error-proof: These splicing regulatory sequences degenerate, and the spliceosome is unable to distinguish between two or more alternative pairings of 5′ and 3′ splice sites. Nevertheless, additional sequence elements located in the exon or in adjacent intronic elements assist the recognition process by binding to regulatory proteins [4]. These proteins can be subdivided into serine/arginine-rich (SR) proteins and heterogeneous nuclear ribonucleoproteins (hnRNPs), that in general bind weakly to poorly conserved RNA sequences, e.g., the enhancer and silencer splicing elements, respectively, allowing high flexibility in the splicing process that can be modulated by their concentration or their fosforilation [5].

Most alternative splicing events can be classified into five basic splicing patterns: cassette exons (where an exon is optional), alternative 5′ splice sites (where the optional 5′ splicing site is inside the 5′ exon), alternative 3′ splice sites (where the optional 3′ splicing site is inside the 3′ exon), mutually exclusive cassette exons (where the inclusion of one exon implies the exclusion of the adjacent one) and retained introns (where the splice site is inside the intron). Usually, alternative splicing changes the coding sequence, but some other splicing occurs at the 5′ or 3′ untranslated regions affecting the processing or the stability of mRNA. Thus, the splicing of a pre-mRNA molecule can be thought of as a delicate balance between competing splice sites—a balance that can easily be tipped by regulatory proteins.

## 3. TRP variants and their functional implications

Based on expressed sequence tag (EST) analysis, 50% of the human transcriptome is alternatively spliced [6]. Alternative splicing, once considered an anomaly, is in fact important to cellular and systemic homeostasis. Changes in coding sequences due to alternative splicing can have profound consequences on the regulation and activity of channels, as shown in Tables 1–4, where structural and functional details of only published spliced TRP channels are presented. Comprehensive description of all different TRP members are outside the scope of this article and the reader is referred to excellent detailed reviews on TRP channels [7–9] and information available at the following address: http://clapham.tch.harvard.edu/master.php?page_type=resources.

Splicing events might change the properties of the channel, as is the case of TRPM3α1 and α2 regarding their conductance and permeability properties [10]. It is also the case of TRPV1b [11], TRPM2 isoform 2 [12] and TRPC6 variant B [13], which show differential activation compared with their respective canonical forms. In some cases the splicing variant shows stronger activation, as for TRPC4β in response to phospholipase C-coupled receptor; in fact, the hTRPC4α is considered a dominant negative modulator of the β isoform [14]. Other variants that also function as dominant negatives of the primary transcript when forming heteromultimeric channels are the smTRPC2 [15], TRPV1β [16] and TRPM2-S [17] variants, among others. It is also interesting the observation that in the case of TRPV1(VAR), the potentiation or inhibition of the TRPV1 full-length, depends on the cell line where they are co-expressed [18], stressing the tissue-specificity of splicing variants.

Nevertheless, many of the splice variants are not functional or might not even be efficiently translated, and it could be considered as negligible populations of incomplete or aberrantly spliced transcripts. Recently, we have started to better understand alternative splicing as a regulatory process, contributing to biological complexity not only by proteome expansion, but also through its ability to control the expression of functional proteins. This can be accomplished by the production of non-functional isoforms of the gene through the alteration of domains necessary for channel opening, membrane localization or association of TRPs (as it could be the case for TRPV4 variants B, C and E, TRPM5 splice 2 and most of TRPM6 variants; see tables for full details), but another way is the alternative splicing as an inducer of nonsense-mediated mRNA decay (NMD) through the incorporation of premature stop codons in mRNA (PTC+ mRNAs) that are recognized by a surveillance system, and the transcripts are targeted for destruction rather than translation into proteins [19,20]. This could be the case for TRPC1γ

Table 1
Summary of published TRPC splice variants with key references

| TRPC subfamily | Isoform | Splice event | Structural characteristics | Functional properties/activation | Tissue expression | References |
|---|---|---|---|---|---|---|
| **TRPC1** | **Canonical** | **RefSeq: NM_003304, 12 exons** | **UniProt: P48995** | **Heteromeric channels: GPCR-activated Monomeric?**[b] | **Widely distributed** | **[23]** |
| | TRPC1short,b | Deleted exon 3 | Deleted part of ANK2 | Functional[a] | Aortic endothelia, NS, insulinoma cell line, undifferentiated stem cells, different tissues | [24–28] |
| | TRPC1A,β | | | | | [29][a] |
| | TRPC1 γ | Deleted exon 2 | Frame shift | Not efficiently expressed | Insulinoma cell line, different tissues | [26] |
| | TRPC1δ | Deleted exon 2 and 3 | Frame shift | Not efficiently expressed | Insulinoma cell line, different tissues | [26] |
| | TRPC1 γ1 | 121bp insertion exon 9–10 | Truncation before TM5 | N.D. | Pregnant miometrium cell line | [30] |
| | TRPC1 γ2 | 55bp insertion exon 9–10 | Truncation before TM5 | N.D. | Pregnant miometrium cell line | [30] |
| **TRPC2** | **Canonical, rodent Human pseudogene** | **RefSeq: NM_011644, 20 exons** | **UniProt: Q9R244-1** | **DAG-activated** | **NS, kidney, heart, lung, skeletal muscle, testis, liver.** | **[38]** |
| | smTRPC2 | Only exons 3 to 7 are expressed; first 33 bp differ | Lacks all TM and the pore | Dominant negative of TRPC2 | NS, kidney | [15] |
| | TRPC2B, clone 17 | Starts at exon 3; first 33 bp as smTRPC2 | Shorter cytoplasmic N terminus | Functional[a] | Testis, brain library | [31][a] |
| | | | | | | [15,32] |
| | TRPC2α | Starts at exon 7 | Shorter cytoplasmic N terminus; no CaM domain | Functional[a] | Testis, brain, heart | [32,33] |
| | | | | | | [15][a] |
| | TRPC2β | Different start codon from α | 4aas more than α at the N end; no CaM domain | Non-functional when heterologously expressed[a] | Only in vomeronasal organ[c] | [33][a] |
| | | | | | | [32] |
| **TRPC3** | **Canonical** | **RefSeq: NM_003305, 12 exons** | **UniProt: Q13507** | **GPCR and/or DAG-activated** | **NS, reproductive systems. Megacaryocites** | **[34]** |
| | hTRPC3β | Insertion of 48 nt between exons 8–9 | Insertion in carboxi- terminal domain | N.D. | Pregnant miometrium cell line | [30] |
| | TRPC3sv | Lost part of exons 1 and 2 | Deletion Ank repeat 1 and 2 | Functional | Multiple tissues | [35] |
| **TRPC4** | **Canonical** | **RefSeq: NM_016179, 11 exons** | **UniProt: Q9UBN4** | **GPCR-activated Dominant negative of β isoform** | **NS, Heart, smooth muscle, kidney, panchreas, eye, epithelia** | **[36]** |
| | TRPC4 β, splice1, mSTRPC4S | Deleted 252bp in exon 11 | Deleted part of the $IP_3R$ binding domain | Stronger response to receptor activation than alpha[a] | HEK 293, T cells, smooth muscle, different tissues | [37–39] |
| | | | | | | [14][a] |
| | rTRPC4γ | Deleted last 54bp in exon 3 | Keeps cytoplasmic N terminus and TM1 and 2 | Dominant negative effect | NS and heart | [40] |
| | bCCE 1Δ514 | Translation starts at aa 514 | Keeps TM5, TM6 and C terminus | N.D. | Only in adrenal gland[c] | [41] |

Table 1 (*Continued*)

| TRPC subfamily | Isoform | Splice event | Structural characteristics | Functional properties/activation | Tissue expression | References |
|---|---|---|---|---|---|---|
| **TRPC6** | **Canonical** | **RefSeq: NM_016179, 11 exons** | **UniProt: Q9UBN4** | **GPCR and/or DAG-activation Modulated by tyr kinase** | **Heart, vessels, panchreas, brain, kidney, eye, male reproductive system** | **[42]** |
| | Variant 2 | Deleted exon 3 and 4 | Lost part of N-terminal cytoplasmic domain | N.D. | Placenta | [42] |
| | Variant 3 | Deleted exon 4 | Lost part of N-terminal cytoplasmic domain | N.D. | Placenta | [42] |
| | Variant B | Deleted part of exon 1 | Deletion before ANK domain. | Not activated by DAG | PC12 | [13] |
| | Variant C | Deleted as B plus exon 8 | Deletion before ank domain and after TM6 | Not processed correctly | PC12 | [13] |
| **TRPC7** | **Canonical** | **RefSeq: NM_020389, 11 exons** | **UniProt: Q9HCX4** | **GCPR and/or DAG-activation** | **Panchreas, SN, kidney, spermatocytes, [c]canine smooth muscle** | **[43]** |
| | Splice 1, TRPC7γ | Deleted exons 3 and 4 | Deleted TM1 and adyacent amino-terminal region | Non-functional[a] | Murine smooth muscle[c] | [39] [43][a] |
| | Splice 2, TRPC7β | Deleted exon 4 | Deleted TM1 | Non-functional[a] | Murine smooth muscle[c] | [39] [43][a] |
| | TRPC7δ | Keeps exon 1 and 2 | Truncated before TM1 | N.D. | Murine library | [43] |

The main characteristics of the "canonical" member of each subfamily is provided: RefSeq (http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?val=RefSeq) and Uniprot numbers (http://www.ebi.uniprot.org/uniprot-srv/uniProtView.do?proteinAc) for the human orthologs, main activation mechanisms and expression patterns. Variants are compared with their respective NCBI references for the human full-length, except in the case of TRPC2, for which murine references are listed. Most of the variants are also included in the Uniprot full-length URL. Abbreviations: aas, aminoacids; ANK, ankirin repeat; bp, base pairs; CaM, calmodulin binding site; DAG, diacylglycerol; GPCR, G protein coupled receptor; $IP_3R$, inositol triphosphate receptor; NS, nervous system; nt, nucleotides; N.D., not described; TM, transmembrane domain.

[a] Functional analysis.

[b] It is not entirely clear if monomeric channels are functional.

[c] Species or tissue specific expression.

Table 2
Summary of published TRPV splice variants with key references

| TRPV subfamily | Isoform | Splice event | Structural characteristics | Functional properties | Tissue expression | References |
|---|---|---|---|---|---|---|
| **TRPV1** | **Canonical (TRPV1α)** | **RefSeq: NM_018727, 15 exons** | **UniProt: Q8NER1** | **Vanilloids, heat, eicosanoids and proton activated** | **Nervous and renal systems, mouth, tongue, ovary** | **[44–46]** |
| | VR.5′sv | Translation starts at exon 6; exon 7 is spliced | Deletion of the N cytoplasmic domain except part of ANK3 | Not activated by capsaicin, resiniferatoxin, protons or temperature[a] | Lower expression levels. Same distribution | [47][a]<br>[48,49] |
| | TRPV1β | Deleted last 30 bp in exon 7 | Deletion of 10 aas between ANK3 and TM1 | Dominant negative of α | DRG, skin, stomach, tongue | [16] |
| | hTRPV1b | Deleted exon 7 | Deletion of 60 aas from cytoplasmic N domain, including part of ANK3 | Activated by temperature, not by capsaicine or protons | Trigeminal neurons | [11] |
| | TRPV1(VAR) | Frameshift by 101bp insertion between exons 5 and 6. | Only produces a truncated cytoplasmic N domain with one ANK repeat | When coexpressed in HEH293 potentiates TRPV1, but in COS-7 is dominant negative | Multiple kidney tissues | [18] |
| **TRPV4** | **Canonical (TRPV4 A)** | **RefSeq: NM_147204, 15 exons** | **UniProt: Q9HBA0** | **Osmotic and mechanical activation: 5,6-EET-mediated; heat, PKC and pH activated** | **Nervous and renal systems, endothelia, epithelia, hair cells, keratinocytes, smooth muscle, spleen, immune cells** | **[50–52]** |
| | TRPV4 B | Deleted exon 7 | Deletion of 60 aas of cytoplasmic N domain, including part of ANK3 | Non-functional, no heteromerizes | Human aortic endothelial and airways epithelial cells | [53,54] |
| | TRPV4 C | Deleted exon 5 | Deletion of 47 aas of cytoplasmic N domain, including part of ANK1 and 3 | Non-functional, no heteromerizes | Human airway epithelial cells | [54] |
| | TRPV4 D | Deleted 34 aas inside exon 2 | Deletion at the cytoplasmic N domain, upstream the ANK repeats | Functional | Human airway epithelial cells | [54] |
| | TRPV4 E | Deleted exons 5 and 7 | Deletions of variants B plus C | Non-functional, no heteromerizes | Human airway epithelial cells | [54] |

Abbreviations: DRG, dorsal root ganglia; 5′,6′-EET, 5′,6′-epoxyeicosatrienoic acid.
[a] Functional analysis.

Table 3
Summary of published TRPM splice variants with key references

| TRPM subfamily | Isoform | Splice event | Structural characteristics | Functional properties/activation | Tissue expression | References |
|---|---|---|---|---|---|---|
| **TRPM1** | **Canonical** | **RefSeq: NM_002420, 27 exons** | **UniProt: O75560** | **Channel activity?** | **Eye, melanocytes** | **[55,56]** |
| | TRPM1-S | Short 1,8 Kb cDNA | 500 aas of the N-terminal segment | Dominant negative? | Human melanocyte and mainly metastasic melanoma cell lines | [57] |
| | | | | | | [58][a] |
| | (189-1,078 | Deleted exons 4 to 20 | Deleted TM1 to 5 | Not described | Murine CNS and eye | [59] |
| | TRPM1-L | Insertion of 6 aas between exon 22 and 23 | Insertion of 6 aas in the pore region | Not described | Rat cDNA | [59] |
| **TRPM2** | **Canonical** | **RefSeq: NM_003307, 32 exons** | **UniProt: O94759** | **$H_2O_2$, ADP-ribose and TNFα activated** | **NS, lung, eye, immune system** | **[60,61]** |
| | TRPM2 isoform 2 | Deleted part of exon 11 and exon 27 | Lost part of the citosolic C terminus | Activated by $H_2O_2$ but not by ADP ribose | Neutrophil granulocites and HL-60 cells | [12] |
| | TRPM2 S, isoform 3 | Deleted exons 17 onwards | Only keeps the amino-terminal end until TM2 | Dominant negative | Human bone marrow | [17,62] |
| **TRPM3** | **Canonical, hTRPM3f, mTRPM3α1** | **RefSeq: NM_206945, 24 exons** | **RefSeq: Q9HCF6-1** | **Constitutively active?** | **NS, kidney, testis** | **[63]** |
| | | | | Monovalent cations selective | | |
| | hTRPM3.2, hTRPM3a (mTRPM3(2) | Deleted exon 7 (human) | Deleted part of cytoplasmic N terminus | Divalent cations selective. | Human kidney Murine eye and NS | [63] |
| | | | | Blocked by monovalent cations[a] | | [10][a] |
| | TRPM3 isoform 3, b (equiv. mTRPM3(3) | Deleted exon 7; insertion of 12 aas between exon 13 and 14 (human) | Deletion and insertion in cytoplasmic N terminus | N.D. | Human kidney | [10,63] |
| | | | | | Murine eye and NS | |
| | TRPM3 isoform 4, d (equiv. mTRPM3(5) | Deleted exons 7 and 15 (human) | Deletions in cytoplasmic N terminus | N.D. | Human kidney | [10,63] |
| | | | | | Murine eye and NS | |
| | TRPM3 isoform 5, e (equiv. mTRPM3(4) | Deleted exons 7 and 15; insertion of 12 aas between exon 13 and 14 | Deletions and insertion in cytoplasmic N terminus | N.D. | Human kidney | [10,63] |
| | | | | | Murine eye and NS | |
| | TRPM3 isoform 6, c (equiv. mTRPM3(1) | Deleted exon 7; insertion of 12 aas between exons 22 and 23 (human) | Deleted part of cytoplasmic N terminus. Insertion in the pore region | Constitutively active? | Human kidney | [63] |
| | | | | Monovalent cations selective[a] | Murine eye and NS | [10][a] |
| | TRPM3 isoform 10 | Deleted exon 7 and most of 26; 7 last aas changed in exon 26 | Deleted part of the cytoplasmic N and C terminus; change C terminus sequence | Constitutive $Ca^{2+}$ entry modulated by extracellular osmolarity | Human fetal brain and kidney | [64] |

| | | | | | | |
|---|---|---|---|---|---|---|
| **TRPM4** | **Canonical (hTRPM4b)** | **RefSeq: NM_017636; 25 exons** | **UniProt: Q8TD43** | **$Ca^{2+}$-activated, high $Na^{+}$ permeability, voltage-dependent, $PIP_2$ modulated** | **Widely expressed** | **[65–67]** |
| | mTRPM4b | Deleted part of exon 2 | Deleted TM2 | Non-functional; dominant negative effect | Murine brain and panchreas islets | [68] |
| | hTRPM4a | Starts in the middle of exon 5 (Met $^{175}$) (Met $^{187}$ in mouse) | Deleted amino-terminal unique region 1 | Constitutively active | Human and murine different tissues | [65,67] |
| | mTRPM4 (pM4 27 and 28) | | | $Ca^{2+}$ permeable[a] | | [58][a] |
| | hTRPC4c | Starts in exon 11 | Deleted amino-terminal unique region 1–4 | N.D. | Human different tissues | [67] |
| **TRPM5** | **Canonical** | **RefSeq: NM_014555, 24 exons** | **UniProt: Q52LU2** | **$Ca^{2+}$ and GPCR-activated** Monovalent cations selective Voltage-dependent, $PIP_2$ modulated | **Spleen, intestine, pancreas prostate, lung, kidney, taste receptors** | **[69–71]** |
| | TRPM5 splice variant 2 | Deleted exon 18; premature STOP codon | Protein truncated after TM4 | N.D. | Human different tissues | [72] |
| **TRPM6** | **Canonical** | **RefSeq: NM_017662, 39 exons** | **UniProt: Q9BX84** | **Unknown activators High $Mg^{2+}$ permeability, $Mg^{2+}$-inhibited** | **Intestine, NS, lung, kidney, testis** | **[73,74]** |
| | Four different mutations that affect splicing | Mutations in intron 16, 18, 23 and 25 | Disruption of the protein sequence | Alteration in magnesium homeostasis | In four human familial hypomagnesemia with secondary hypocalcemia | [74,75] |
| | TRPM6a | Alternative first exon, 1A | Alternative N terminus | Only functional when associated to TRPM7 | Human kidney, testis, $HEK_{293}$ cells and lung cell lines | [73] |
| | TRPM6b | Alternative first exon, 1B | Alternative N terminus | Non-functional when expressed alone | Human kidney, testis, $HEK_{293}$ cells and lung cell lines | [73] |
| | TRPM6c | Alternative first exon, 1C | Alternative N terminus | Non-functional when expressed alone | H510 cells and human testis | [73] |
| | TRPM6t | Contains an exon 36B with a STOP codon | Different ( kinase domain; protein truncated | N.D. | Testis specific | [73] |
| | M6-kinase 1 | Deleted exons 14 to 28 | Only retains the N terminus and the ( kinase domain | N.D. | N.D. | [73] |
| | M6-kinase 2 | Deleted exons 13 to 30 | Only retains the N terminus and the ( kinase domain | N.D. | N.D. | [73] |
| | M6-kinase 3 | Deleted exons 7 to 34 | Only retains the N terminus and the ( kinase domain | N.D. | N.D. | [73] |
| **TRPM8** | **Canonical** | **RefSeq: NM_024080, 25 exons** | **UniProt: Q7Z2W7** | **Cold, menthol, icilin, $PIP_2$ Voltage-dependent** | **Liver, NS, sensory ganglia, prostate** | **[76,77]** |
| | TRPM8b | Transcription starts in exon 5b | Alternative N terminus | N.D. | Human prostate cancer | [59] |

The TRPM3 isoforms 7, 8 and 9 are not published, but they can be found at www.ebi.uniprot.org/entry/Q9HCF6. There are more short isoforms of TRPM1 not well described structurally that can be found in [57].
Abbreviations: CNS, central nervous system; Kb, kilobase; PIP2, phosphatidylinositol 4,5-bisphosphate; TNFα, tumor necrosis factor α.

[a] Functional analysis.

Table 4
Summary of published TRPP and TRPML splice variants with key references

| TRPP/ML subfamily | Isoform | Splice event | Structural characteristics | Functional diversity/activation | Tissue expression | References |
|---|---|---|---|---|---|---|
| **TRPP3** | **Canonical, (PKD2L1, PCL)** | **RefSeq: NM_016112, 16 exons** | **UniProt: Q9POL9** | **Unknown activators, $[Ca^{2+}]_i$ ?** | **NS, Kidney, retina, testis, skeletal muscle, liver** | **[78–80]** |
| | PCL-LV | Deletion within exon 12 | Deleted 29 aas of putative Ca-binding EF-hand motif | Increased $Ca^{2+}$-dependent activation | Liver | [80] |
| | Isoform 2 PCL-TS, PKDL(Δ15) | Deletion of exon 15 | 45 aas shorter at the C-terminus | Functional[a] | NS, Testis, eye, liver, lung, kidney | [80][a]<br>[78] |
| | Isoform 3 PKDL(Δ5) | Deletion of exon 5 | 75 aas deletion of part of the first extracellular loop and part of TM2 | N. D. | Only in lung, liver and human transformed lymphoblasts[b] | [78] |
| | Isoform 4 PKDL(Δ456) | Deletion of part of exon 4 and 6, and exon 5 complete. | 120 aas deletion including TM2 and TM3 | N.D. | Only in human transformed lymphoblasts[b] | [78] |
| **TRPP5** | **Canonical, (PKD2L2)** | **RefSeq: NM_014386, 14 exons** | **UniProt: Q9NZM6** | **N.D.** | **Testis, NS, heart, kidney, HepG2 cells** | **[81,82]** |
| | Isoform 2, PKD2L2b | Deleted exon 2 | Deleted 34 aas including part of cytoplasmic N domain and part of TM1 | N.D. | Only in transformed lymphoblasts[b] | [82] |
| | Isoform 3, PKD2L2a | Deleted part of exon 3; frameshift and premature STOP codon | Frameshift from aa 45 and STOP at aa 47. Truncation after TM1 | N.D. | In all tissues tested but not in HEK cells | [82] |
| | Isoform 4, PKD2L2c | Deleted exon 2 and part of 3; frameshift and premature STOP codon | Truncation at aa 24. Only keeps cytoplasmic N terminus. | N.D. | NS, muscle and SK-N-SH cells | [82] |
| **TRPML1** | **Canonical** | **RefSeq: NM_020533, 14 exons** | **UniProt: Q9GZU1** | **Unknown activators, inhibited by low pH** | **NS, Intestine, lymph node, heart, testis, muscle, liver, lung** | **[83–85]** |
| | Isoform 2, ML4 2037bp | ORF starts at nt 81; part of exon 5 deleted; different sequence from aa 36 to 227 | Deleted first 35 aas at N terminus, different sequence until TM2 | N.D. | cDNA clones from the UK Human Genome Mapping Project Resource Centre | [83] |
| | Splice-acceptor mutation in intron 3 | Deleted exon 4 | Frameshift; retains only the first 21 aas | The aberrant splicing causes mucolipidosis type IV | N.D. | [83–85] |

Abbreviations: PKDL, polycystic kidney disease like.
[a] Functional analysis.
[b] Species or tissue specific expression.

and δ, as well as TRPC6 variant C (see tables). This process, termed regulated unproductive splicing and translation (RUST), is conserved in all eukaryotes in which it has been examined (reviewed in [21]).

This unproductive splicing, that some times is noise from data contamination, biological errors that give rise to diseases (e.g., TRPM6 and human familial hypomagnesemia with secondary hypocalcemia or TRPML1 and mucolipidosis type IV, see tables) or evolutive changes, can also represent an additional layer of regulation of gene expression, at a reasonably biological cost, considering that roughly 90% of the transcribed sequence is spliced out as introns and discarded in humans [22]. In fact, many RUST events that are implicated in the correct functioning of a specific tissue, regardless of the transcript activity, are now being considered into the *functional splice forms* category.

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