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Transcriptional regulation and processing increase the functional variability of TRPM channels

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Abstract Mammalian TRP channels display heterogeneous biophysical properties and are involved in a variety of signal transduction pathways. To carry out their diverse biological functions and to adapt these functions to changes of the environment, mechanisms to regulate their molecular structure are required. Transcriptional regulation and post-transcriptional RNA processing represent essential instruments to generate TRP channel variants with modified properties. TRP variants are expressed depending on the tissue and developmental state. They can show distinct biophysical properties and mechanisms of activation, and thereby determine channel function and malfunction in certain human diseases. In this review, we give an overview of the variants of a given TRP gene, with the focus on the TRPM subfamily, and discuss their relevance with respect to their function under physiological and pathological conditions.

Keywords TRP channel variants · Transcriptional regulation · Promoter · Alternative splicing · Post-transcriptional processing · Transient receptor potential

Introduction

Transient receptor potential (TRP) channels were first described in *Drosophila melanogaster* as gene products involved in signal transduction of fly photoreceptors. The search for mammalian homologs of these proteins finally identified members of the classical (TRPC) subfamily and today we know at least 28 members of the TRP superfamily, subdivided into TRPC, melastatin-related (TRPM) and vanilloid receptor related (TRPV) channels (Montell et al. 2002). In addition, TRPA, mucolipins (TRPML) and poly-

cystins (TRPP) can be included. All TRP channels share structural similarity with six putative transmembrane segments (TM) and a predicted pore region between TM5 and TM6. Some amino acid motifs are well conserved throughout the superfamily whereas the overall amino acid sequence homology of distantly related TRP channels is rather low. Thus, it is no surprise that the mechanisms of activation, the ion permeation properties and the patterns of expression are quite diverse. Correspondingly, the biological functions of TRP channels are heterogeneous ranging from pain-perception, taste- and mechanosensation up to regulation of vasorelaxation. In the current view TRP proteins form tetrameric channel complexes and at least closely related members of one subfamily are capable to build heteromeric channels. By combinations of different TRP channel subunits, which build a common ion-conducting pore, the diversity of native TRP related channels may be widely increased.

The latter mechanism is not the only way how functional diversity of TRP channels can be accomplished. There is growing evidence that transcriptional regulation and alternative mRNA processing also contributes to the diversity of TRP channels. At present, variants have been described for a large number of TRP proteins. Most of them originate from alternative splicing (Table 1). TRP channel variants might be able to modulate functional properties of TRP channel complexes or to impair their functions. Accordingly, knowledge of these variants is essential, since they might provide mechanisms to adapt the properties of a given TRP channel to different functions and changes of the cellular environment. Furthermore, comparison of such variants may allow identification of structural domains essential for TRP function. This might be an advantage compared to synthetically generated mutants because the presence or absence of a specific part of the protein in a variant might define a naturally occurring phenotype. The biophysical and functional features of TRPM channels have been reviewed recently (Fleig and Penner 2004) and in an accompanying article of this issue (Harteneck 2005). In this review we now summarize what is currently known about splicing and transcriptional regulation of TRPM

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Table 1 Selected literature reporting alternative processing of TRPC, TRPV, and TRPM transcripts

Gene	Reference
TRPC 1	(Sakura and Ashcroft 1997; Chang et al. 1997; Wang et al. 1999; den Dekker et al. 2001; Yang et al. 2002; Antoniotti et al. 2002)
TRPC 2	(Berg et al. 1997; Wissenbach et al. 1998; Vannier et al. 1999; Hofmann et al. 2000; Chu et al. 2002, 2004; Yildirim et al. 2003)
TRPC 3	(Ohki et al. 2000; Yang et al. 2002)
TRPC 4	(Freichel et al. 1998; Walker et al. 2001, 2002; Mery et al. 2001; Schaefer et al. 2002; Yang et al. 2002; Qian et al. 2002; Satoh et al. 2002)
TRPC 6	(Philipp et al. 2000; Zhang and Saffen 2001)
TRPC 7	(Walker et al. 2001)
TRPV1*	(Schumacher et al. 2000; Sanchez et al. 2001; Xue et al. 2001; Wang et al. 2004)
TRPM1	(Fang and Setaluri 2000; Xu et al. 2001; Zhiqi et al. 2004); this manuscript
TRPM2	(Wehage et al. 2002; Zhang et al. 2003a)
TRPM3	(Lee et al. 2003)
TRPM4	(Launay et al. 2002; Nilius et al. 2003; Murakami et al. 2003)
TRPM5	(Prawitt et al. 2000)
TRPM6	(Schlingmann et al. 2002; Walder et al. 2002; Chubanov et al. 2004)
TRPM8	This manuscript

*For a comparison of TRPV1 splice variants see review by B. Niemeyer (2005)

channels and supplement the published data by unpublished observations obtained in our lab.

Materials and methods

Northern blots Five to ten micrograms of polyA⁺-RNA were hybridized under stringent conditions to [α^{32} P] dCTP-labeled cDNA fragments of TRPM1-Long (nucleotides 1–1,325 and nucleotides 3,350–5,030) or to a 230-bp fragment encoding amino acid residues Glu⁶³⁷ to Arg⁷¹³ of TRPM8a (Acc. no Q7Z2W7). The filters were exposed to X-ray film with intensifying screens for 21 days and hybridized again with a 239 bp cDNA fragment of the human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as control.

Cloning of TRPM1 splice variants Oligonucleotide primers up1 (rat/mouse: 5'TCC TTC TGA GCT GAC TGA GCC), up 2 (rat/mouse: 5'GCG GGA CCA GCT TCT AGT TAC), down 1 (rat/mouse: 5'GGG CCA AAG ACG AAG ATC TGG) and down 2 (rat: 5'CCA TTA GCA TTC AGT TTC CGG G; mouse: 5'CAT CAG CAC TCA GTT TCC GCG) were used to amplify mouse and rat cDNA fragments after oligo dT₍₁₈₎ primed reverse transcription of total RNA from mouse or rat brain. For amplification we used the Long expand PCR Kit (Roche, Mannheim, Germany) and the following conditions: 2 min at 94°C, 10 cycles (94°C, 10 s;

62°C, 30 s; 68°C, 5 min), 25 cycles (94°C, 10 s; 62°C, 30 s; 68°C, 5 min + 20 s/cycle) and 7 min at 68°C. Fragments were subcloned and sequenced on both strands. The number of clones analysed are indicated in Fig. 2.

Cloning of TRPM8 splice variants Oligonucleotide primers 5'ATG AAA TCC TTC CTT CCT GTC and 5'TTA TTT GAT TTT ATT AGC AAT CTC TTT CAG were used to amplify human cDNA fragments after oligo dT₍₁₈₎ primed reverse transcription of RNA obtained from prostate cancer tissues (stage pT3b) of four patients. Fragments were amplified and subcloned using the following conditions: 2 min at 94°C, 35 cycles (94°C, 30 s; 58°C, 30 s; 72°C, 4 min) and 10 min at 72°C. Five independent clones were sequenced on both strands.

TRPM1

Melastatin (TRPM1, MLSN) is the founding member of the TRPM family. Its first initial was chosen to rename all eight relatives "TRPM" (Montell et al. 2002). Although it was the first TRPM to be cloned, there are no published reports about its biophysical properties. Originally, it has been identified by differential cDNA display in mouse B16 melanoma cell lines in search for genes whose expression correlates with the aggressive metastatic behavior of the cells (Duncan et al. 1998). A 2,722 bp transcript encoding 542 amino acid residues (aa) was cloned (see Fig. 2c), which was highly expressed in poorly metastatic cells but strongly reduced in a highly metastatic line. This correlation suggested that this protein may serve as a tumor suppressor protein but this idea could not be further substantiated. Nevertheless, TRPM1 may have clinical importance since down-regulation of TRPM1 mRNA in primary cutaneous tumors may serve as a prognostic indicator in patients with melanoma (Deeds et al. 2000; Duncan et al. 2001; Miller et al. 2004). The TRPM1 mouse variant was initially not recognized as member of the TRP family since it included only the aminoterminal part of a TRP protein and lacked a transmembrane region and the characteristic "Trp"-domain (see Fig. 2c). However, a human ortholog with 1,533 amino acids in length (Gene accession no. AF071787) was identified in a hybridization screen of a retinal cDNA library (Hunter et al. 1998; see Fig. 2c). The human and mouse genes map to chromosome 15q13-q14 and to chromosome 7, respectively, and show a similar organization (see Fig. 2a). The mouse gene was reported to consist of 27 exons with the initiation codon in exon 3 located 345 nucleotides downstream of the transcription start site (Hunter et al. 1998). However, this 5' untranslated region shows sequence homology to other TRPM coding sequences suggesting that additional exons might exist, which may encode an expanded TRPM1 amino terminus. Exon 14 is the final exon in the mouse 2,722 bp transcript and so far no mouse transcripts that correspond to the longer human form have been isolated. Directly upstream of the transcription initiation site a proximal promoter region (see Fig. 2a) contains transcriptional regulatory elements unique to genes

with restricted melanocytic expression pattern (Hunter et al. 1998; Miller et al. 2004; Zhiqi et al. 2004). This promoter is capable to drive reporter gene expression in mouse and human melanoma cells. Recently, it could be shown that MITF is an essential transcription factor for melanocytic development and a major transcriptional regulator of TRPM1 expression through its interaction within the proximal promoter region (Miller et al. 2004; Zhiqi et al. 2004). RT-PCR analysis of mouse (Duncan et al. 1998) and Northern blots of rat tissues (Fig. 1) revealed TRPM1 transcripts in eye and melanoma cells but failed to detect transcripts in lung, heart, liver, spleen, kidney and intestine. Additionally, long TRPM1 transcripts seem to be expressed in human B- and T-cell lines and in monocytes (Perraud et al. 2004). Northern blot analysis of pigmented human SK-MEL-19 and 23c22 melanocytes showed a variety of long (5.4 kb) and short (1.3, 1.8, and 4.0 kb) transcripts, whose expression was upregulated after induction of cell differentiation (Fang and Setaluri 2000). In addition, transcripts of 0.8, 1.7, 2.3, and >3.0 kb were generated constitutively by alternative splicing and may include 3' terminal exons. Thus, multiple transcripts were generated and it has been suggested that TRPM1 expression can be regulated at the level of both transcription and mRNA processing (Fang and Setaluri 2000). The inducible short transcripts were predicted to encode short human TRPM1 variants (MLSN-S) of ~500 amino acids which—similar to the 542 aa mouse melastatin—include only the N-terminal segment but lack any transmembrane domain of the long

(1,533 aa) TRPM1 protein (MLSN-L). Although such short variants have never been identified in human tissues, this prediction inspired the idea that MLSN-S may represent a cytoplasmic isoform, which regulates MLSN-L (Xu et al. 2001). It has been shown that overexpression of 500 aminoterminal amino acids of TRPM1 altered the membrane localization of the long variant presumably by direct interaction of the two variants. In addition, MLSN-S seemed to suppress MLSN-L mediated Ca^{2+} entry. Therefore, it has been hypothesized that control of translocation through interaction between MLSN-S and MLSN-L represents a mode for regulating ion channel activity (Xu et al. 2001).

TRPM1 transcripts are expressed in several human melanocytic lines (Fig. 1). Whereas short transcripts of ~1.3 and ~1.8 kb and transcripts larger than 10 kb are present in SK-MEL-28 cells only a ~1.8 kb mRNA is abundant in MeWo cells. These patterns differ to those described for human SK-MEL-19 and SK-MEL-23c22 melanocytes (Fang and Setaluri 2000), indicating that alternative splicing of TRPM1 strongly depends on the cell subtype. In addition, we found TRPM1 mRNA of ~6.5, ~8.0 and ~10 kb in rat eye were it may be abundant in the pigmented epithelium of the retina. Finally, large TRPM1 transcripts are expressed (~7 kb, ~10 kb, >12 kb) in kidney epithelial cells from dog (MDCK2) but not in human embryonic kidney cells (HEK-293) showing that its expression is not limited to pigmented epithelial cells from skin and eye.

By database analysis we identified a new TRPM1 transcript cloned from a mouse retinal library (Acc no. AK044507). As shown in Fig. 2c this 2,008 nt mRNA is predicted to encode 461 amino acids with overlapping sequence homology to mouse and human melastatin but extending these sequences at the aminoterminal end by 116 amino acid residues. Just as the whole TRPM1 gene, the coding nucleotides are well conserved in the mouse and human genes and in the rat gene, which is located on chromosome 1q22 and consists of 28 exons (Fig. 2a). The additional 116 amino acid residues are encoded by an additional exon 1a and by exons 2 and 3. Exons 2 and 3 are non-coding in mouse and human melastatin sequences when they are expressed from the proximal promoter (Fig. 2a). Exon 1a is located ~33.5 K upstream to a region homologous to the human and mouse proximal promoter (AF 084519) which is responsible for TRPM1 expression in melanocytes. We suggest that a second distal promoter may exist, which drives expression of aminoterminal extended variants of TRPM1. Using a RT-PCR based strategy (Fig. 2b) and primer up1 deduced from the genomic sequence upstream of the initiation codon of AK044507 in combination with primer down1 located in exon 10, we cloned cDNA fragments from mouse brain and eye which confirmed the sequence of AK044507. Using primer up1 in combination with primer down2, which is located at a position corresponding to the translation stop of human MLSN (AF071787), we failed to amplify cDNA fragments longer than ~2,200 bp. We suppose that secondary mRNA structures may inhibit reverse transcription of longer transcripts. However cloning of these ~2,200-bp amplification products identified a new TRPM1 variant from mouse called TRPM1 $_{\Delta 189-1,078}$ (Fig. 2b, c). In this variant

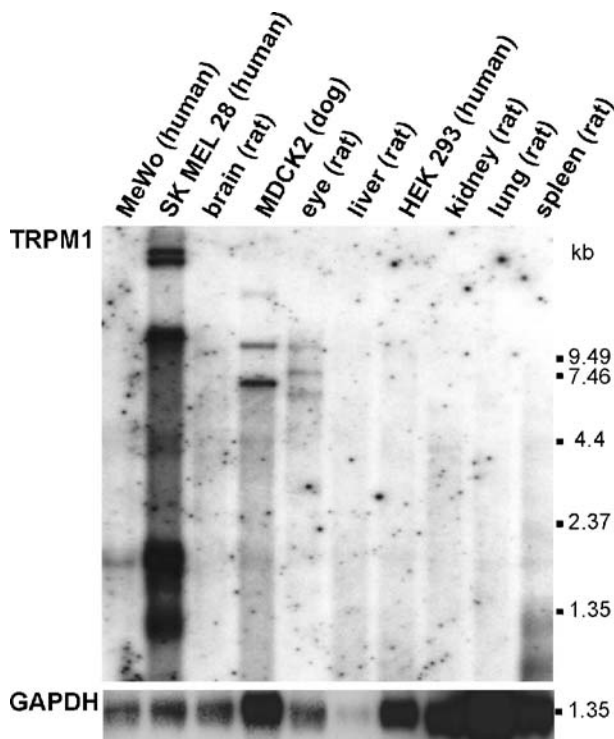


Fig. 1 TRPM1 transcripts of different length are expressed in human, rat, and dog tissues. PolyA⁺-RNA was hybridized to labeled cDNA fragments of TRPM1-Long (upper panel). The lower panel shows a control hybridization of the same blot with the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA.

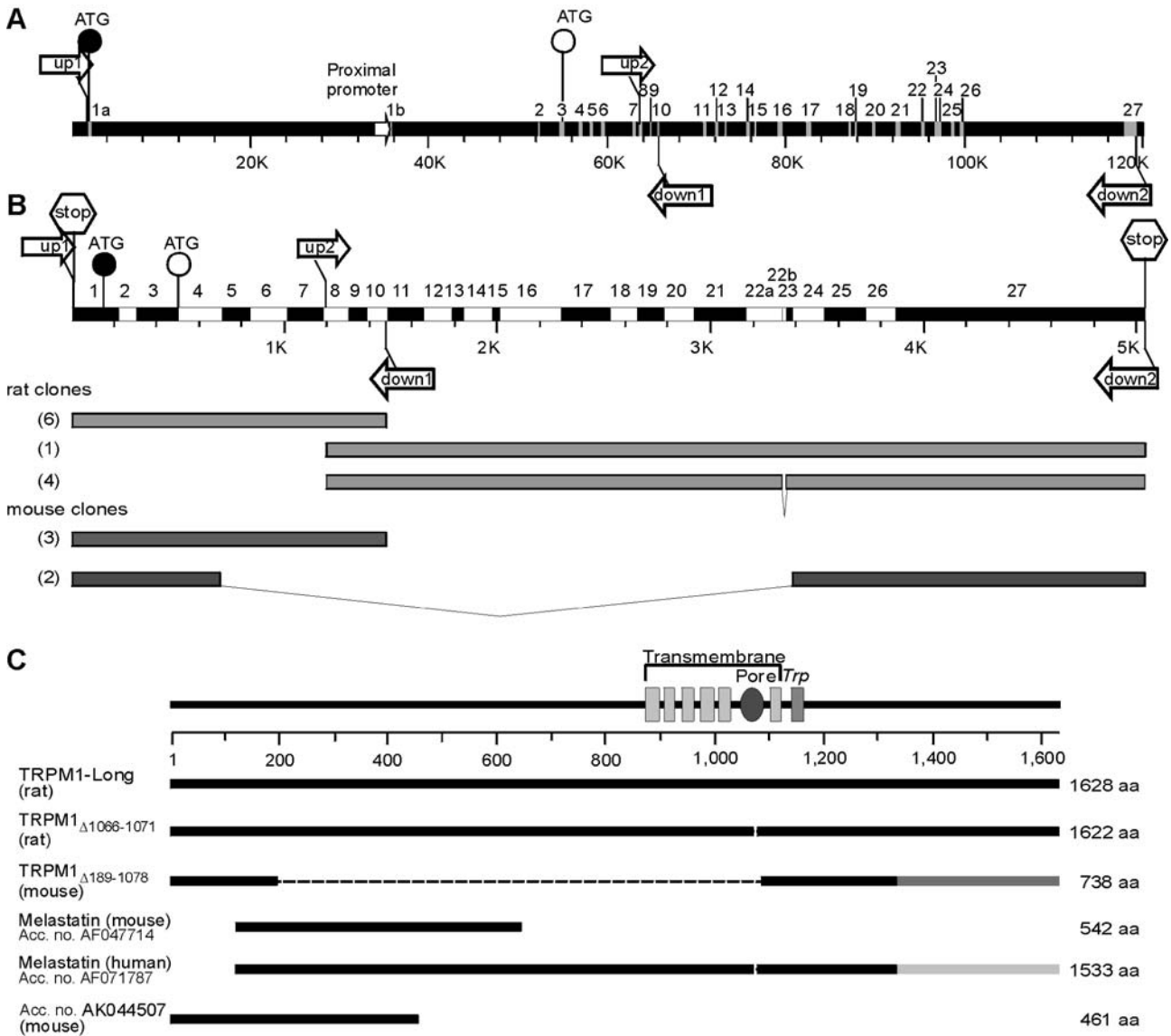


Fig. 2 Identification of TRPM1 splice variants. **a** Scheme of the rat TRPM1 gene consisting of 28 exons (gray boxes), highly similar to the mouse and human genes. Splice donor and splice acceptor sites are invariant in all intervening intron sequences (black boxes). As in **b**, the location of an initiation codon (ATG) corresponding to mouse and human melastatin (AF047714 and AF071787) is indicated by an open circle. A hollow arrow marks a region homologous to the human and mouse proximal promoter (AF084519). This promoter is 5' flanking to exon 1b present in the mouse and human melastatin mRNA (AF047714, AF071787) shown in **c**. An additional start ATG (closed circle) present in the alternative exon 1a of AK044507 (**c**) and of the obtained rat and mouse clones shown in **b** is located 33.5 K upstream. Just as in **b**, positions of primers up1, up2, down1, and down2 used to amplify mouse and rat cDNA (**b**) are indicated.

b Cloning strategy of rat (light gray bars) and mouse (dark gray bars) TRPM1 cDNAs. Number of clones sequenced as indicated in parentheses. Stop codons in frame are shown. In two mouse clones exon 4 is spliced to exon 24. In one rat clone splicing to an alternative splice donor site within exon 22 leads to the extension of exon 22 by 18 nucleotides (exon 22b). **c** Scheme of different TRPM1 splice variants from rat, mouse, and human with 461–1,628 amino acids (aa) in length. Black bars represent regions with >90%, dark gray bars with 80–90%, and light gray bars with 50–60% sequence identity. Dotted lines indicate missing domains compared with TRPM1-Long. Sequences of TRPM1-Long, TRPM1 Δ 1066–1071, and TRPM1 Δ 189–1078 have been deposited in the GeneBank database. Trp TRP domain.

exon 4 is spliced to exon 24. It spans a reading frame of 738 amino acid residues. Similar to AK044507 it contains 116 additional residues at the aminoterminal as well as carboxy-terminal end homologous to the human variant AF047714 (Fig. 2c). This new variant indicates that also in mouse tissues carboxyterminal extended TRPM1 variants are expressed.

With the primer combinations up1/down1 and up2/down2 we amplified overlapping cDNA fragments from rat brain and eye (Fig. 2a, b). The predicted new TRPM1 splice variant TRPM1-Long (TRPM1-L) contains 1,628 amino acid residues. A stop codon upstream and in frame of the initiation codon (Fig. 2b) indicates that the reading frame is complete. Apart from the extended aminoterminal end, this

variant is highly homologous to human and mouse sequences with more than 90% amino acid sequence identity (black bars in Fig. 2c). However, the last ~300 amino acid residues show considerable sequence divergence, unexpected for orthologous proteins. Whereas mouse and rat sequences are 89% identical within this region (dark gray bars in Fig. 2c), the human sequence shows only ~50% identity (light grey bars in Fig. 2c) and several additional short sequence insertions (not shown), indicating that in this region alternative splicing may occur, too. TRPM1-L contains six additional amino acid residues compared to a further identified variant, called TRPM1 $_{\Delta 1,066-1,071}$ (Fig. 2b, c) and compared to the human variant AF071787. Their presence arise by the usage of an alternative splice donor site within the intron between exon 22 and exon 23 leading to an extended exon 22b. Interestingly, these residues are located within the putative pore region of TRPM1. This finding may have special importance, since it may designate alternative splicing as a mechanism to adapt the permeation properties of the channel. We do not know the physiological significance of the new variants. However our findings may be a step forward to understand TRPM1 channels and might explain, why there is only little known about the functional properties of this first identified member of the TRPM subfamily.

TRPM2

The TRPM2 gene (LTRPC2) was originally identified on human chromosome 21q22.3 within a region which is linked to a variety of human genetic disorders (Nagamine et al. 1998). However, the hope to identify a gene which is linked to a human disease has not yet been fulfilled. When overexpressed in HEK-293 cells the TRPM2 cDNA encodes non-selective cation channels of 1,503 amino acids which can be activated by ADP-ribose, β -NAD or H₂O₂ (Perraud et al. 2001; Sano et al. 2001; Hara et al. 2002). TRPM2 is unique for its C-terminal NudT9-homology domain (NudT9-H) which shows considerable homology to NudT9 ADP-ribose hydrolase (ADPRase). This domain has been shown to be essential for channel activation by ADP-ribose (Perraud et al. 2001). The channel is thought to be involved in pathways which respond to oxidants and might play a role as a Ca²⁺ permeable channel that mediates cell-death induced by oxidative stress. Comprehensive reviews about the structure and function of TRPM2 (Perraud et al. 2003) and their potential role in immune cells (Perraud et al. 2004) have been published recently. Furthermore, a review on the regulation of TRPM2 channels in neutrophil granulocytes by ADP-ribose is included in this issue (Heiner et al. 2005).

Northern blots unmasked TRPM2 transcripts of different length in human brain areas (Nagamine et al. 1998) and in mouse spleen (Hara et al. 2002). Amplification of two adjacent cDNA segments from HL-60 neutrophils identified two alternative splice events (Wehage et al. 2002). Splicing internal to exon 11 led to the absence of amino acid residues 538–557 within the aminoterminal and skipping of

exon 27 was connected to the loss of residues 1,292–1,325 within the carboxyterminus of the protein. Additionally, two amino acid exchanges (S1088N and D1291E) were detected (Wehage et al. 2002). Thus transcripts missing a part of exon 11 and/or the complete exon 27 may exist, although their relative abundance in blood leukocytes, HL-60 cells and neutrophil granulocytes seemed to be rather low (Wehage et al. 2002; Perraud et al. 2003). Recombinant TRPM2 proteins carrying one or both deletions were overexpressed in HEK-293 cells and showed intriguing properties. In contrast to TRPM2 these variants did not respond to ADP-ribose indicating that both regions may be essential for channel activation. Most interestingly, while the C-terminal variant TRPM2 $_{\Delta 1,292-1,325}$ lost its activity when exposed to ADP-ribose it retained the ability to gate in response to H₂O₂. This result is in line with the idea of an independent mechanism of TRPM2 activation by H₂O₂ and argues against a model in which NAD and its breakdown product ADP-ribose serve as diffusible mediators of oxidant-induced gating. However, the mechanisms underlying oxidant induced gating are under debate.

An additional splicing event has been detected in human bone marrow cells (Zhang et al. 2003a). Amplification of five partial cDNA fragments together covering the complete TRPM2 open reading frame detected an alternative splice acceptor site within intron 16 which encodes a stop codon. The deduced protein called TRPM2-S was predicted to contain the complete aminoterminal and the first two transmembrane spanning domains. Although it is not yet clear, if such a variant exists in vivo, its heterologous expression in HEK-293 cells gave further insights into the structure–function relationship of TRPM2. TRPM2-S co-immunoprecipitated with the long variant (TRPM2-L) and both variants seemed to be localized in the plasma membrane indicating that the aminoterminal part of TRPM2 is sufficient for protein–protein interaction. Expression of TRPM2-S suppressed H₂O₂ induced Ca²⁺ influx through TRPM2-L as well as oxidant induced cell death suggesting that this variant modulates TRPM2 channel activity and thereby providing an instrument through which cells can control their reaction to damaging oxidants.

TRPM3

TRPM3 is the closest relative of TRPM1. It is expressed in human kidney, testis and in the central nervous system but seemed to be absent in a variety of other human tissues (Grimm et al. 2003; Lee et al. 2003). Two publications described the cloning and functional properties of human TRPM3 (Grimm et al. 2003; Lee et al. 2003) but the data are inconsistent in view to molecular as well as functional aspects. Using 5' and 3' RACE strategies Grimm and colleagues identified three overlapping cDNA fragments from fetal brain and kidney together predicting a protein of 1,325 amino acid residues (Grimm et al. 2003). HEK-293 cells overexpressing TRPM3_{1,325} showed constitutive Ca²⁺ entry, which was increased during treatment with hypotonic extracellular solutions. Electrophysiological analysis re-

vealed that TRPM3_{1,325} build nonselective Ca²⁺-permeable channels sensitive to the osmolarity of the extracellular medium (Grimm et al. 2003) and recent results indicated that TRPM3_{1,325} can be activated by some sphingolipids (Grimm et al. 2005). TRPM3_{1,325} seemed neither to be activated by phospholipase C (PLC) coupled receptors, Ca²⁺ store depletion (Grimm et al. 2003), fatty acids nor lipid metabolites from the phospholipase A2 pathway (Grimm et al. 2005). Due to its osmosensitivity and its presence in the kidney it has been suggested to play a role in renal Ca²⁺ homeostasis.

Another publication described a human protein of 1,554 amino acid residues designated TRPM3a (Lee et al. 2003). TRPM3a is in part identical to the protein described by Grimm and colleagues but differ in its amino- and carboxyterminus. Whereas 153 amino terminal residues present in TRPM3_{1,325} are missing, TRPM3a possess additional 382 amino acids at the C-terminal end. TRPM3a also showed constitutive Ca²⁺ entry when expressed in HEK-293 cells but in contrast to TRPM3_{1,325} this Ca²⁺ influx was augmented after stimulation of PLC coupled muscarinic receptors and after passive Ca²⁺ store depletion with thapsigargin. Northern blots showed TRPM3 transcripts of different length (Grimm et al. 2003; Lee et al. 2003) and five additional splice variants, TRPM3b–f, have been isolated from human kidney varying in size from 1,544 to 1,579 amino acid residues but none of them was identical to TRPM3_{1,325}. However, these data showed that TRPM3 is subject to frequent alternative splicing, implying that the functional differences described so far may be caused by the different primary structure of the variants. Hopefully, future comparative analysis of TRPM3 splice variants will illuminate their different functional behavior and will probably allow deeper insights into the functional domains of TRPM3.

TRPM4/5

Originally human TRPM4 has been described as a protein of 1,040 residues (Xu et al. 2001) but later it turned out, that this protein is a N-terminal truncated variant of a longer protein with 1,214 residues designated TRPM4b (Launay et al. 2002; Nilius et al. 2003). The human TRPM4 gene consists of 25 exons located at chromosome 19q13.33. TRPM4_{1,040} (TRPMa) arises by splicing of exon 2 to an alternative splice acceptor site within exon 5. Ca²⁺ imaging experiments showed elevated Ca²⁺ influx in HEK-293 cells upon transient transfection of TRPM4a suggesting that this variant might build a Ca²⁺ influx pathway (Xu et al. 2001). This finding is somewhat surprising, since both variants share the same pore structures and extensive analysis characterized the longer variant TRPM4b as a Ca²⁺-activated but Ca²⁺-impermeable monovalent cation channel (Launay et al. 2002) whose activity is voltage-dependent (Hofmann et al. 2003; Nilius et al. 2003), blocked by ATP⁴⁻ (Nilius et al. 2004b) and modulated by decavanadate (Nilius et al. 2004a). Most interestingly, a mutant highly similar to TRPM4a co-immunoprecipitated with TRPM4b, had dominant negative effects on endogenous Ca²⁺-activated currents in lympho-

cytes and modulated the oscillatory pattern of the intracellular Ca²⁺ concentration after T-cell activation (Launay et al. 2004). A third human variant TRPM4c has been cloned from prostate (Nilius et al. 2003). This variant starts with a truncated exon 11, which is alternatively spliced to exon 12. It contains 678 amino acid residues starting at Met⁵³⁷ of TRPM4b. Although functional data are lacking, this variant might also build functional channels since it possesses the complete transmembrane region and a putative pore.

The mouse TRPM4 gene is localized in chromosome 7. B2 and displays a similar organization as the human gene with 25 exons. The mouse TRPM4b cDNA has been cloned from heart encoding a protein of 1,213 residues (Nilius et al. 2003) with 83% amino acid sequence identity to human TRPM4b. In addition, two shorter splice variants were identified which start at Met¹⁸⁷. One of them displays 1,027 amino acids identical to TRPM4b. The other one is predicted to have 592 residues since an insertion in the transcript results in a shift of the reading frame and a premature stop. This variant lacks all transmembrane spanning domains and the carboxyterminus of TRPM4b. Two further splice variants have been reported (Murakami et al. 2003). Unfortunately they were also called TRPM4a (Acc. no. AB112658) and TRPM4b (AB112657) but should not to be confused with the above mentioned variants. Due to the strategy used for cloning of the transcripts, they both start within exon 16 and seem to be encoded by 5' incomplete cDNA clones. Whereas AB112658 is identical to 488 carboxyterminal residues of TRPM4b starting at Met⁷²⁶ within the first transmembrane segment, AB112657 lacks additional 66 residues of transmembrane segment 2 which are encoded by a part of exon 17. AB112658 still appeared to mediate Ca²⁺ entry whereas AB112657 was non-functional.

The closest relative of TRPM4 is TRPM5 (MTR1). Similar to TRPM4 it is a Ca²⁺-activated, monovalent selective cation channel (Hofmann et al. 2003; Liu and Liman 2003; Prawitt et al. 2003) which is involved in taste transduction (Perez et al. 2002; Zhang et al. 2003b). It has been initially identified in search for genes causal for Beckwith–Wiedemann syndrome (Prawitt et al. 2000) but so far it is not clear whether TRPM5 is involved in a disease. 4.5 kb TRPM5 transcripts with a putative open reading frame encoded by 24 exons are expressed in a variety of tissues. Alternative splicing leads to two predicted proteins of 872 or 1,165 residues (Prawitt et al. 2000). The longer form has been extensively studied in a variety of functional studies but the properties of the shorter one have not been studied so far.

TRPM6/7

TRPM6 (Chak2) and TRPM7 (Chak1, LTRPC7, TRP-PLIK) are unique among ion channels because they contain a α -kinase domain at their C-terminus. The emerging roles of TRPM6/TRPM7 channel kinase signal transduction complexes are reviewed in an accompanying article of this issue (Gudermann et al. 2005). Both channels are thought to be involved in Mg²⁺ transport (reviewed by Montell 2003). They

are known to build heteromultimeric structures (Chubanov et al. 2004) but they differ in their expression pattern. TRPM7 is abundant in various tissues (Runnels et al. 2001) but TRPM6 is mainly expressed in kidney and intestine, where it is responsible for Mg^{2+} (re)absorption (Schlingmann et al. 2002; Walder et al. 2002; Voets et al. 2004b). TRPM6 gives an excellent example for the physiological importance of correct mRNA processing: As reported by two independent groups (Schlingmann et al. 2002; Walder et al. 2002)

several mutations leading to missplicing of TRPM6 transcripts are associated with an autosomal recessive hereditary disorder called hypomagnesemia with secondary hypocalcemia (HSH). This disease is characterized by very low magnesium and calcium serum levels and HSH-patients suffer from generalized convulsions, muscle spasm, and tetany. These findings also document the first example that TRPM dysfunction is responsible for a human disease. In addition, multiple TRPM6 mRNA isoforms encoding proteins

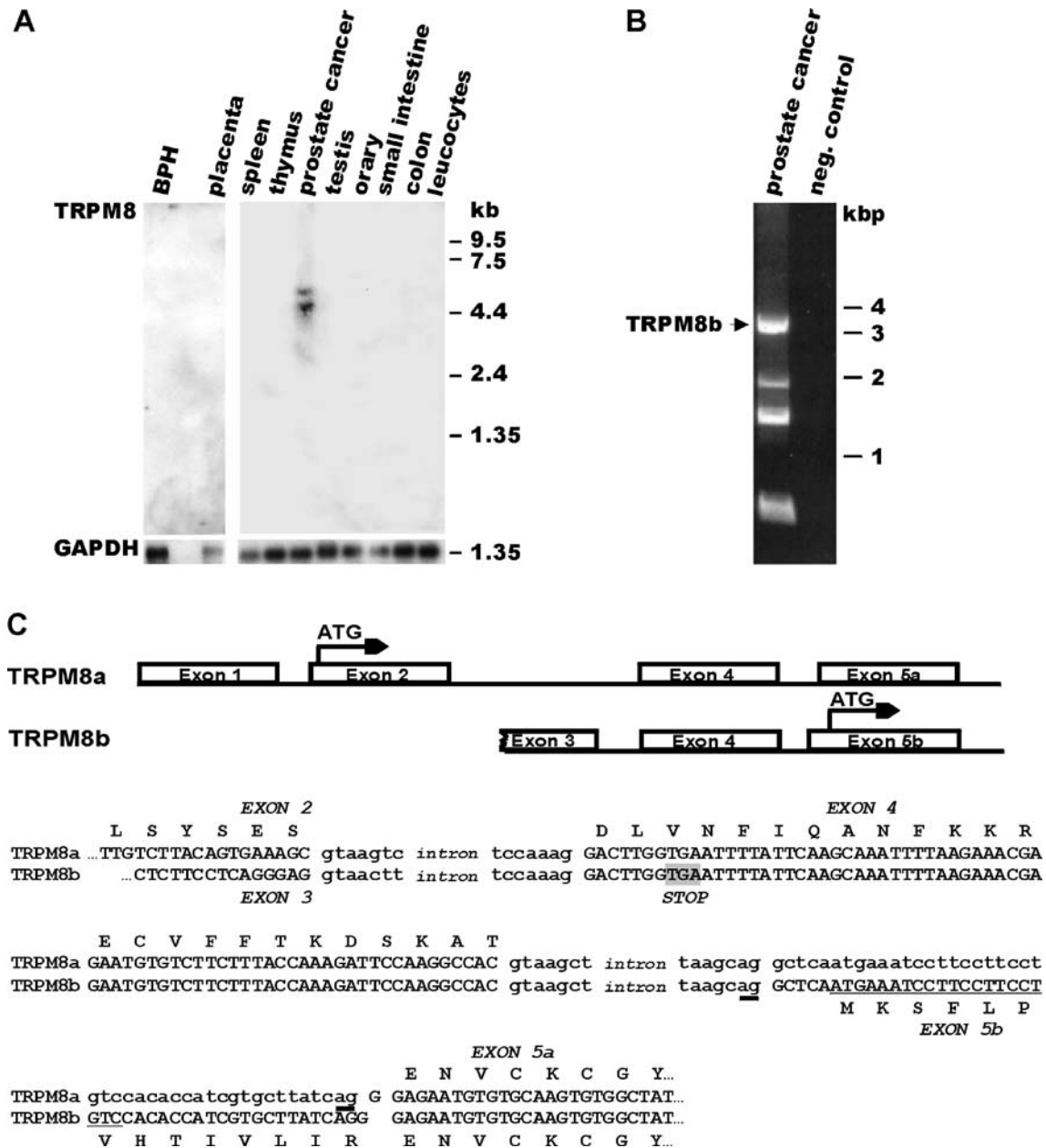


Fig. 3 Identification of TRPM8 splice variants in human prostate cancer. **a** Northern blot analysis identifies expression of different TRPM8 transcripts in prostate cancer, but not in benign prostate tissue (BPH). Lower panel shows control hybridizations of the same blots with the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA. **b** RT-PCR amplification of TRPM8b fragments. The longest fragment (arrow) encodes the complete TRPM8b cDNA. **c** Schematic structure of a part of the TRPM8 gene. The lower panel shows nu-

cleotide sequences of exons (upper case) 2, 3, 4, 5a, and 5b including intron borders (lower case) and the encoded amino acid sequences of TRPM8a and TRPM8b. Alternative splice acceptor sites (ag) are marked, a stop codon in frame to the translation initiation site of TRPM8b is highlighted in gray. Nucleotides of a TRPM8b specific primer are underlined. The sequence of TRPM8b has been deposited in the GeneBank database.

of 569–2,022 residues were identified (Chubanov et al. 2004). TRPM6a, TRPM6b and TRPM6c differ only in their aminoterminal end and derive from alternative use of exons 1a, 1b, and 1c located within a 700-bp region on chromosome 9q22. Obviously the TRPM6 gene harbors a promoter with alternative transcription initiation sites. These variants seemed to have individual expression patterns. TRPM6a and TRPM6b are present in kidney, testis and cells derived from lung but TRPM6c is absent in kidney cells. Furthermore, three splice variants lacking the transmembrane region were described in the same manuscript (Chubanov et al. 2004). They were called M6-kinases, since their aminoterminal end is still homologous to melastatin and their carboxyterminus carries the kinase domain. The identification of these variants implies that the function of the kinase domain is not restricted to ion channel modulation. Finally, splicing in exon 36 has been reported, leading to a premature stop codon and a C-terminal truncation. The resulting variant was designated TRPM6t since it seems to be specifically expressed in testis (Chubanov et al. 2004).

TRPM8

TRPM8 (formerly CMR1 or Trp-p8) comprises 1,104 amino acids and is expressed in a subset of sensory neurons which can be stimulated by cold temperatures (McKemy et al. 2002; Peier et al. 2002). TRPM8 also responds to menthol and icilin and shows voltage dependent gating but a link between temperature sensitivity and voltage dependence is controversially discussed (Voets et al. 2004a; Brauchi et al. 2004). TRPM8 is expressed in malign prostate cancer cells (Tsavaler et al. 2001) but in benign prostate hyperplasia (BPH) TRPM8 transcripts are not detectable (Fig. 3a). The human TRPM8 gene consists of at least 27 exons located at chromosome 2q37. Several human expressed sequence tags (EST CA394721, BX283818, BE274448, BE390627) encode an altered aminoterminal of TRPM8. We identified a splice variant of TRPM8 called TRPM8b, which differ from the 1,104 aa protein (now defined as TRPM8a) in its aminoterminal (Fig. 3b, c). We used a primer specific for TRPM8b deduced from the exon 5b in combination with a primer located downstream of the reading frame of both variants to amplify 3,165-bp fragments from prostate cancer (Fig. 3b, arrow). Amplification products were subcloned and sequencing identified TRPM8b with 1,054 amino acids in length. The shorter amplification products (Fig. 3b) are not yet characterized but might encode further TRPM8 splice forms. Figure 3c shows the part of the TRPM8 gene which encodes the alternative aminoterminals of TRPM8a and TRPM8b. Exon 1 and exon 3 are non-coding. Translation of TRPM8a starts in exon 2, the one of TRPM8b in exon 5b. In TRPM8a exons 1, 2, 4, and 5a are fused. In TRPM8b exons 3 and 4 are fused. An alternative splice acceptor site within the following intron leads to an extension of exon 5 (exon 5b) by 47 nucleotides. In the resulting transcript of variant b the first ATG is in frame to an upstream located stop codon (Fig. 3c). Starting with the amino acid sequence ENV in exon 5 the reading frames of

both proteins are identical. The functional significance of the new variant in prostate cancer cells is still unclear. However, TRPM8b might serve as a dominant negative regulator of TRPM8a similar as suggested for amino- and carboxyterminal truncated variants of TRPM4 (Launay et al. 2004), TRPM2 (Zhang et al. 2003a), and TRPM1 (Xu et al. 2001).

Conclusions

Genomic database resources have largely facilitated the identification of most of the TRP genes in the last 10 years by similarity screens. However, because transcriptional regulation and post-transcriptional mRNA processing are hardly predictable from genomic information, we need now to decipher the TRP transcriptome. Two major aspects have to be considered: First, comparative analysis of different variants can help us understand structure–function relationships of TRP channel proteins. Second, as some examples already show, TRP variants are differentially expressed and might serve particular functions in different cell types. Thus, to understand the physiological role and the molecular identity of native TRP channels it is necessary to determine the subset of TRP variants expressed in a specific kind of cell. Hitherto, we know little about alternative TRP transcripts. Some variants are already identified but there are presumably more. Some of them may only be accidental and with unknown significance. Therefore, future studies should initially show if alternative transcripts are abundant and if the encoded proteins are really present in the cell before we draw conclusions about their physiological relevance.

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