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TRPM2 Splice Variants in Hematopoietic Proliferation and Susceptibility to Cell Death

B.A. Miller and W. Zhang

Weis Center for Research, Geisinger Clinic, Danville, PA, USA

TRPM2 is a calcium-permeable channel and a member of the transient receptor potential (TRP) superfamily, which share six putative transmembrane domains. This channel is part of a physiological pathway through which oxidative stress and tumor necrosis factorα (TNFα)induce susceptibility to cell death. TRPM2 is activated by micromolar levels of H2O2 and other agents which produce reactive oxygen species, resulting in an increase in the intracellular free oxygen concentration. Alternative splice variants of TRPM2 have been identified including a short isoform (TRPM2-S), which consists of the TRPM2 N terminus and the first two predicted transmembrane domains. Inclusion of a stop codon (TAG) at the splice junction between exons 16 and 17 results in deletion of the four C-terminal transmembrane domains, the putative calcium pore, and the entire C-terminus. Heterologous expression of full length TRPM2 (TRPM2-L) in 293T cells resulted in susceptibility to H2O2-induced cell death, which correlated with elevation of the intracellular calcium concentration. In contrast, TRPM2-S expression inhibited calcium influx through TRPM2-L and susceptibility to cell death induced by H2O2 in cells expressing TRPM2-L [1]. Experiments with confocal microscopy demonstrated that TRPM2-L and TRPM2-S colocalize on the plasma membrane. Immunoprecipitation experiments demonstrated that TRPM2-L and TRPM2-S directly associate, suggesting that this may be a mechanism through which TRPM2-S modulates TRPM2-L activation [1]. Here, the physiological role of TRPM2-L and TRPM2-S in the proliferation and susceptibility to cell death of hematopoietic cells was examined. This approach was taken because TRPM2-S was cloned from human bone marrow, and TRPM2-L and TRPM2-S are widely expressed in primary hematopoietic cells and cell lines. Exposure of the human monocytic cell line U937 to H2O2 or TNFα results in significantly reduced cell viability. TRPM2-L or TRPM2-S were overexpressed by retroviral infection of U937 cells stabley expressing ecotrophic retroviral receptor. The retrovirus used also expresses GFP, and high efficiency of cell infection was documented by GFP fluorescence. TRPM2 expression was confirmed by RT-PCR and Western blotting. (1) The effect of increased expression of TRPM2-L or TRPM2-S on cell proliferation was examined using a colorimetric method to identify metabolically active cells (CellTiter 96 Aqueous One Cell Proliferation Assay, Promega, Madison, WI). For untreated U937 cells, increased expression of TRPM2-L had no significant influence on cell proliferation compared to control cells. In contrast, increased expression of TRPM2-S in U937 cells resulted in significantly greater numbers of proliferating cells compared to cells infected with empty retrovirus or expressing TRPM2-L. (2) The effect of increased TRPM2-L or TRPM2-S expression on cell viability following treatment with H2O2 or TNFαwas examined by trypan blue exclusion. Treatment of U937 cells expressing increased levels of TRPM2-L with H2O2 or TNFα resulted in a significant decrease in cell viability, compared to cells infected with retrovirus alone. In contrast, increased TRPM2-S expression resulted in significantly enhanced cell viability after treatment with H2O2 or TNFα, compared to control cells infected with empty retrovirus or TRPM2-L. (3) The effect of increased TRPM2-L or TRPM2-S expression on induction of apoptosis by H2O2 or TNFα was examined by Alexa Fluor 488/annexin V staining. In U937 cells overexpressing TRPM2-L, treatment with H2O2 or TNFα resulted in an increase in cells undergoing apoptosis, compared to cells infected with retrovirus alone. Expression of TRPM2-S reduced the number of apoptotic cells observed following treatment with H2O2 or TNFα. These data suggest that TRPM2-L is an important modulator of hematopoietic cell death and that TRPM2-S can antagonize this pathway. To further explore the physiological role of TRPM2 in hematopoietic cell survival, endogenous TRPM2 was down regulated by infection of U937 cells with retrovirus (pSuppressorRetro, Imgenex, San Diego, CA) expressing RNAi oligonucleotides targeted to TRPM2. Infection with retrovirus and decreased expression of TRPM2 mRNA were confirmed by RT-PCR. Reduced expression of TRPM2 in U937 cells was associated with significantly enhanced cell viability after treatment with H2O2 or TNFα, compared to control cell infected with empty retrovirus or retrovirus with RNAi targeted to luciferase. These data demonstrate that TRPM2 isoforms are important modulators of hematopoietic cell survival following oxidative stress and exposure to TNFα. They also suggest that the stoichiometry of TRPM2-L and TRPM2-S expression is an important determinant of hematopoietic cell fate.

Zhang, W*et.al.*(2003) *J.B.C.***278**, 16222-16229.

SA8

The role of TRPM2 in oxidative stress-mediated cell death

S. Goransson

Department of Anatomy, University of Cambridge, Cambridge, UK

Regulation of the intracellular calcium concentration is of critical importance in the determination of cell fate. Oxidative stress, through the production of oxygen metabolites including hydrogen peroxide, causes an increase in intracellular calcium, which results in cell injury, apoptosis, or necrosis. TRPM2, a member of the transient receptor potential (TRP) protein superfamily, is a widely expressed calcium-permeable cation channel that can be activated by micromolar levels of hydrogen peroxide and other reagents that produce reactive oxygen species. This channel is thought to be part of a physiological pathway through which hydrogen peroxide and TNFα may induce cell death.I demonstrate here, using RT-PCR, that TRPM2 mRNA is present in a human microglial cell line, C13. To elucidate the role of TRPM2 in oxidative stress-induced cell death in this line, cell death assays and Western analysis were used in combination with RNA silencing. In all assays there was no apparent differences between cells transfected with a random non-sense siRNA compared to non-transfected cells. TRPM2 specific siRNA successfully rescued C13 cells from concentrations of hydrogen peroxide that were otherwise fatal to these cells. Toxicity was determined by the methyl thiazole tetrazolium (MTT) assay as well as by flow cytometric analysis (FACS) of membrane phosphatidylserine redistribution. In a proportion of the cells phosphatidylserine flip was observed without an impairment of membrane integrity (propidium iodide remained excluded from the cells). This would be indicative of apoptosis. The cell death rescue was dependant on both the concentration of hydrogen peroxide and the time the cells were left to recover after a 45 minute

treatment. Both endogenously produced and exogenously added hydrogen peroxide can activate the mitogen-activated protein (MAP) kinase superfamily. In this study, hydrogen peroxide added to intact C13 cells caused an increase in the activity of one such subfamily, the extracellular signal-regulated kinases (ERKs) as determined by an elevation of the phosphorylated forms. The increase was rapid but transient and more importantly, 30 minutes following hydrogen peroxide application, ERK activity had markedly declined below basal levels. In addition hydrogen peroxide also caused a decrease in Akt activity with an associated increase in glycogen synthase kinase 3αβ (GSK3αβ) activity. All of these signalling events have been shown to induce apoptosis and are therefore likely to contribute to the hydrogen peroxide-induced cell death in the C13 cell line. Knock-out of TRPM2 using siRNA technology completely prevented the depression below basal of the ERK activity and induced a partial rescue of the attenuated Akt activity. This would suggest that the hydrogen peroxide-mediated apoptosis, via the TRPM2 signalling cascade, involves both the ERK and PI3K-Akt pathways.

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SA9

Molecular analysis of oxidant-mediated TRPM2 activation

A.M. Scharenberg[1], A. Perraud[1], C. Takanishi[1], M. Smith[1] and B. Shen[2]

[1]Pediatrics/Immunology, University of Washington, Seattle, WA, USA and [2]Dept. of Basic Science, Fred Hutchinson Cancer Center, Seattle, WA, USA

TRPM2, a dual function protein with ion channel and enzymatic domains, allows calcium entry to the cytosol in response to oxidative stress (1,2). In order to understand the mechanism through which TRPM2 channel gating is induced in response to cellular oxidant exposure, we have taken a combined approach involving structural, biochemical and electrophysiologic studies. The results from these studies suggest the existence of a novel oxidant-stress activated biochemical pathway which is localized to mitochondria, highly conserved evolutionarily, and which results in the production, release to the cytosol, and subsequent accumulation of free ADP-ribose sufficient to gate TRPM2 channels. Thus, production and release of free ADP-ribose represents a 2nd messenger mechanism through which mitochondria coordinate their function with cytosolic biochemistry during oxidative stress.

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SA10

Characterization of TRPM3

C. Harteneck, C. Grimm, R. Kraft, K. Grosse and G. Schultz

Institut fuer Pharmakologie, Charite Campus Benjamin Franklin, Berlin, Germany

Proteins of the TRP family form nonselective Ca^{2+}-permeable cation channels. Their activation mechanisms are not completely understood. In particular, the functional properties of TRPM channels, i. e. the members of the melastatin-like TRP subfamily, are still under investigation. Lee et al. (2003) reported a regulation of TRPM3 activity by intracellular calcium stores. These authors cloned cDNAs from human kidney and described six splice variants between 1544 and 1579 amino acids, all starting at the same position as published melastatin does. Recently, we showed the activation of heterologously expressed human TRPM3 by reduction of extracellular osmolarity (Grimm et al., 2003). We cloned the cDNAs of human and mouse TRPM3 from kidney and brain, respectively. The orthologous cDNAs code for a TRPM3 variant with an N-terminal extension of 153 amino acids compared to melastatin and a shorter C-terminus, resulting in a protein of 1325 amino acids. The presence of a very characteristic N-terminal protein motif, found selectively in the sequences of TRPM-channels, makes quite sure that we study a protein identical to the natively expressed TRPM3. By performing 3-prime RACE reactions, we cloned further variants of the TRPM3 protein. The variant with an extended C-terminal end and a length of 1707 amino acids was chosen for expression experiments. In transiently transfected HEK293 cells, we studied the activation mechanism of both TRPM3 variants. We tested for the known stimuli, i.e. spontaneous activity, store-dependent regulation, activation by hypoosmolarity and other extracellular stimuli. In these experiments, the long TRPM3 variant was expressed as determined by Western blot and fluorescence analysis, but did not respond to any of the stimuli used. The short TRPM3 variant (1325 aa) showed spontaneous and hypoosmolarity-induced activity. An activation by intracellular calcium stores, however, was not detectable. These data confirm earlier results of an involvement of TRPM3 in renal osmo-regulation.

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SA11

TRPM4 and TRPM5: comparing siblings

B. Nilius, J. Prenen, N. Ullrich, T. Voets and G. Droogmans

Physiology, KU Leuven, B-3000 Leuven, Belgium

TTRPM4 and TRPM5 are structurally related cation channels (40% identity) forming a subgroup within the melastatin (M) family of "transient receptor potential" (TRP) channels. Both channels are widely expressed in excitable and non-excitable cells. TRPM5 is highly expressed in taste receptor neurons, and mutations in this channel might be associated with the Beckwith-Wiedemann syndrome. We present here functional data of TRPM4 and TRPM5 channels (human and mouse) after heterologous expression in HEK293 cells. A comparison of

activation properties and modulation of both channels might unravel functionally important structural determinants of these channels. Both channels require intracellular Ca^{2+} for activation, but are impermeable for Ca^{2+}. Activation of TRPM4 by Ca^{2+} is transient and requires higher (μM) Ca^{2+} concentrations than for TRPM5. The Ca^{2+} dependence of TRPM5 activation is also steeper than that of TRPM4. Exposure to constantly elevated $[Ca^{2+}]_i$ induced a decrease of the currents through both channels. TRPM4 but not TRPM5 reactivates after a delay of some minutes. Reactivation is accelerated when cells were depleted of ATP under hypoxic/glucose free conditions. Deletions in the N-terminus including possible Ca^{2+}/calmodulin binding sites result in non-functional channels. However, C-terminal truncations form functional channels with a substantially reduced Ca^{2+}- activation, pointing to a possible Ca^{2+} sensor located in the C-terminus. TRPM4 and TRPM5 are voltage-dependent. They inactivate rapidly at negative potentials and activate more slowly at positive potentials. This voltage-dependent behaviour results in a striking outward rectification of the steady state current. Voltage dependence of activation is of the Boltzmann-type, i.e. positive potentials increase the fraction of open channels and negative potentials decrease it (Nilius et al. 2003). Activation of TRPM5 at positive potentials is faster and shifted towards more positive potential as compared with TRPM4. Voltage dependence is not due to block by divalent cations or to a voltage-dependent binding of intracellular Ca^{2+} to an activator site. A kinetic scheme for the voltage dependence of TRPM4 will be presented, which can explain kinetic and steady state properties of both channels as well as the current decrease during repetitive stimulation. Mutations of lysine/arginine residues in transmembrane helices of TRPM4, which are conserved in TRPM5, change the voltage dependence but also the concentration range for activating Ca^{2+}. These residues are conserved in TRPM4,5, and 8 but not in TRPM7 and resemble the voltage sensor in Shaker potassium channels.ATP, ADP, AMP, AMP-PNP and adenosine induce a fast and reversible block of currents through TRPM4. Block by ATP is not affected by intracellular Mg^{2+}. IC_{50} for block by ADP and ATP^{4-} is ~2 μM. GTP, UTP and CTP do not induce a similar block. TRPM5 is insensitive to ATP. TRPM4 has three Walker B motifs and an ABC-transporter signature motif, which are not conserved in TRPM5. Mutations of the ABC motif induce a dramatic inactivation of TRPM4 currents at positive potentials, indicating that this region modifies the kinetic behaviour of TRPM4 and might explain kinetic differences between TRPM4 and TRPM5.Spermine blocks currents through TRPM4 and TRPM5 (IC_{50} of ~60 μM). Possible aspartate and glutamate interaction sites for polyamines, flanking the pore region, are conserved in both channels.In conclusion, TRPM4 and TRPM5 are more than only Ca^{2+} activated cation channels. They are characterized by an intrinsic voltage sensing-mechanism, which may be functionally important in excitable tissues generating plateau-like or bursting action potentials. The most dramatic difference between TRPM4 and TRPM5 channels, among the several significant quantitative differences, is the sensitive block of TRPM4 channels by ATP, which does not occur in TRPM5.

Nilius, B., Prenen, J., Droogmans, G., Voets, T., Vennekens, R., Freichel, M., Wissenbach, U., Flockerzi, V.Voltage dependence of the Ca2+ activated cation channel TRPM4 Journal of Biological Chemistry 278: 30813-30820, 2003

SA12

An essential role of TRPM6 for body magnesium homeostasis revealed by mutations in patients with Hypomagnesemia with Secondary Hypocalcemia

K.P. Schlingmann[1], S. Waldegger[1], V. Chubanov[2], T. Gudermann[2] and M. Konrad[1]

[1]Pediatrics, Philipps-University, Marburg, Germany and [2]Pharmacology, Philipps-University, Marburg, Germany

Magnesium is the second most-prevalent intracellular cation involved in a great variety of physiological and biochemical processes such as protein and nucleic acid synthesis, modulation of membrane transporters or signal transduction. Body magnesium is mainly distributed in bone and in the intracellular compartments of muscle and soft tissues. Serum magnesium levels are maintained at almost constant values. Homeostasis depends on the balance between intestinal absorption and renal excretion. Over the last years, a number of hereditary disorders of magnesium homeostasis have been clinically characterized. The genetic analysis of patients affected by these rare diseases represents a unique opportunity to gain insights into the physiology of cellular magnesium transport at the molecular level. Hypomagnesmia with Secondary Hypocalcemia (HSH) is characterized by extremely low serum magnesium levels and low serum calcium levels. Patients usually present within the first months of life with cerebral convulsions, muscle spasms or tetany. Untreated, this disorder may be fatal or result in permanent neurological damage. Relieve of clinical symptoms and normocalcemia are achieved by the administration of high oral doses of magnesium. Pathophysiological studies in affected patients pointed to a primary defect in intestinal magnesium transport. In some patients an additional renal magnesium leak was suspected. A gene locus for HSH was mapped to chromosome 9q22 and further refined to a critical interval of approximately 1cM. By using a positional candidate gene approach, we identified the TRPM6 gene within this region and detected mutations in TRPM6 in patients with HSH.The gene product TRPM6 is a new member of the long transient receptor potential channel or TRPM family and a close relative to TRPM7, a magnesium and calcium permeable ion channel. Unlike other TRP channels, TRPM6 and TRPM7 share the unique feature of a c-terminal alpha-kinase enzyme domain.By in-situ hybridization and RT-PCR, expression of TRPM6 was detected along the intestine as well as in the distal convoluted tubule, the main site of active transcellular magnesium reabsorption in the kidney. So far, we studied 12 families with typical clinical HSH. The mutational analysis of the TRPM6 gene in affected patients yielded mutations mostly truncating the TRPM6 protein, however one point mutation (S141L) was identified for which the pathomechanism remained unknown. Clinically, the observation of inappropriately high fractional excretion rates of magnesium in the presence of severe hypomagnesemia clearly indicates an additional role of renal magnesium wasting for the pathophysiology of HSH. Upon heterologous expression, TRPM6 alone failed to form functional channel complexes, as it was retained intracellularly. Considering the ability of oligomer formation demonstrated for other members of the TRP family and the overlapping expression pattern of TRPM6 with its closest relative TRPM7, we hypothesized that the two homologous TRPMs may be functionally linked and both

together be involved in the process of epithelial magnesium absorption.In fact, coexpression of TRPM6 with TRPM7 in xenopus oocytes resulted in an amplification of TRPM7-evoked currents. Western blot analysis of oocyte lysates supported the functional data as the TRPM6 protein, when co-expressed with TRPM7, was detectable in the membrane fraction. In contrast, the naturally occurring (S141L) mutation resulted in a loss of TRPM7-like current amplification and in an intracellular retention of the mutant TRPM6 protein.These results were confirmed by the observation of a TRPM7-dependant trafficking of TRPM6 to the plasma membrane in HEK293 cells. Consistently, the functional assessment of Mn2 entry into HEK cells by fura-2 fluorescence quenching showed an increase in TRPM7-evoked Mn2 influx upon co-expression with TRPM6. Finally, a direct and specific protein-protein interaction between TRPM6 and TRPM7 was shown by FRET (fluorescence resonance energy transfer) analysis. In line with the functional data obtained in xenopus oocytes, TRPM7 failed to promote a membrane insertion of the mutant (S141L)TRPM6 suggesting that the S141L mutation prevented the assembly of TRPM6/TRPM7 heteromultimers.In summary, these findings indicate a crucial role of TRPM6 for epithelial magnesium transport and suggest an important contribution of TRPM6/TRPM7 heteromultimerization for the formation of functional magnesium channels.

SA13

The cold and menthol receptor TRPM8: its role in cold sensing and cold adaptation

G. Reid

Department of Animal Physiology and Biophysics, Faculty of Biology, University of Bucharest, Bucharest, Romania

We sense the temperature of our skin and surroundings using specific thermoreceptors, responding either to skin cooling or warming. Until a few years ago the molecular basis of thermal sensing was completely unknown. The discovery by Cesare and McNaughton in 1996 of an ionic current activated by noxious heat in cultured dorsal root ganglion (DRG) neurones revealed the first of a new family of temperature-gated ion channels, which we now know are all TRP channels. Here I will concentrate on one member of the family, the cold- and menthol-activated ion channel TRPM8, and its properties in native DRG neurones.Cold-sensitive DRG neurones are scarce - only 7 % of the total - and for this reason we pre-selected them using $[Ca^{2+}]_i$ imaging. In neurones showing a sudden sharp increase in $[Ca^{2+}]_i$ during cooling, we found an inward current activated by cold and sensitised by menthol, which looked like a "mirror image" of TRPV1's activation by heat and sensitisation by capsaicin, and seemed an obvious candidate for a specific cold transduction mechanism (Reid & Flonta, 2001). The channel underlying this cold- and menthol-activated current turned out also to be a member of the TRP family, namely TRPM8 (McKemy *et al.*, 2002; Peier *et al.*, 2002).We had two major questions on discovering this current: firstly, to what extent could it account for cold transduction in intact thermoreceptors?; secondly, is it due to an ion channel that is directly activated by cold and menthol?To understand its function, we first looked at which of the known properties of intact cold receptors could be accounted for by this current. Intact cold receptors are sensitised by menthol and by low $[Ca^{2+}]_o$; the effect of menthol is antagonised by high $[Ca^{2+}]_o$ and by warming; and intact cold receptors adapt to sustained cooling with a gradual reduction in their firing rate. The cold- and menthol-activated current shares all these properties, leading us to propose that it is probably the major transduction mechanism in innocuous thermoreceptors (Reid & Flonta, 2001).Excised patches from cold-sensitive DRG neurones contain a nonselective cation channel that is activated by cold and sensitised by menthol, accounting for the macroscopic current (Reid & Flonta, 2002). This confirmed that cold and menthol are acting on the same target, and that the channels involved are activated directly by cold and menthol and not by way of a soluble intracellular second messenger. However, channels in excised outside-out patches do not behave identically to those in intact neurones. Firstly, they are much less sensitive to cold; secondly, they no longer adapt to cold but instead show a sustained activity that depends only on temperature and does not change with time. We concluded that, whereas the temperature sensor and the menthol binding site are intrinsic to the channel (or an accessory subunit whose association with the channel resists patch excision), the adaptation mechanism is not contained within the channel itself but depends on something that is supplied by the cell (Reid & Flonta, 2002).The observations above indicate that the temperature threshold of cold receptors depends essentially on the modulation of TRPM8 and not only on its intrinsic thermal sensitivity. This matches well with our everyday sensory experience - water at 20 °C feels very cold when we jump into it on a hot summer day, but very warm when we have just been throwing snowballs - and suggested that a shift in TRPM8's cold activation threshold (as distinct from a simple decline in the current on sustained cooling) could account for cold receptor adaptation. During cold pulses of varying duration and intensity, we found that cooling indeed shifts the activation threshold of the cold- and menthol-activated current towards lower temperatures. Adaptation was strongly inhibited by removing extracellular Ca^{2+} or chelating intracellular Ca^{2+}, and thermal threshold could be lowered without cooling simply by raising $[Ca^{2+}]_i$ (Reid *et al.*, 2002). We concluded that the thermal threshold of cold receptors is modulated by Ca^{2+} entry through TRPM8, implying a feedback regulation of cold receptor sensitivity. Further work to be presented at this meeting shows that cold adaptation depends on membrane integrity but not on an intact cytoplasm. All procedures accord with the principles of UK legislation.

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SA14

TRP channels and thermosensation

A. Patapoutian

Cell Biology, TSRI, La Jolla, CA, USA

Recent years have seen great advances in the molecular description of sensory neurobiology. Of the five popularly characterized senses

- sight, hearing, taste, smell, and touch - touch is among the most varied and least understood. Within this modality is the ability to sense mechanical forces, chemical stimuli, and temperature, and the molecules that mediate this ability have been a long-standing mystery. Categorized according to their mechanism of gating, ion channels can be activated by signals such as specific ligands, voltage, or mechanical force. Another type of signal, temperatures above 43oC, was shown to activate the ion channel TRPV1 (VR1), a member of Transient Receptor Potential (TRP) family of cation channels. These high temperatures are perceived as painful or noxious by many organisms. Interestingly, TRPV1 is also activated by capsaicin, the active ingredient of chili peppers. A second TRP, TRPV2 (VRL1) was soon found to respond to yet higher temperatures (53oC). Building on these initial findings, my lab focused on identifying additional temperature-activated ion channels. Although other members of the TRP family of channels were not known to have thermosensing roles when we started this project three years ago, we postulated that novel members were likely to be functionally related to TRPV1 and TRPV2. Taking advantage of the ongoing human genome project, we mined for additional TRP channels. The next step was to systematically screen these new TRPs for expression in tissues relevant for temperature sensation and to assay the activity of these channels in heterologous systems either by imaging intracellular calcium levels or by electrophysiology. As hoped, our work has led to the characterization of a novel warm-activated TRP channel, TRPV3 (33oC threshold) and two novel cold-activated TRP channels, TRPM8 (25oC threshold) and ANKTM1 (17oC threshold). TRPM8 is also the receptor for the compound menthol, providing a molecular explanation of why mint flavors are typically perceived as refreshingly cooling. Together these temperature-activated channels represent a new sub-family of TRP channels that we have dubbed thermoTRPs. The remarkable ability of the thermoTRPs to confer temperature sensitivity to a variety of cell types suggests that they are either direct sensors of temperature or are activated via a ubiquitous temperature-activated signaling mechanism. In agreement with a role in initiating temperature sensation, most of the thermoTRPs are normally found in subsets of Dorsal Root Ganglia (DRG) neurons. These sensory neurons convey information about the environment through specialized neurites that extend to the skin from cell bodies in the vertebral column. In fact, a single DRG neuron nerve ending marks a small spot (<1mm in diameter) on the skin surface that often senses a narrow range of temperature stimuli. Recording directly from DRG nerve fibers has helped classify some of these neurons as hot-, warm-, or cool-responsive. Still other neurons, called polymodal nociceptors, sense noxious thermal (cold and hot) and mechanical stimuli. These physiological studies strikingly correlate with results of our detailed expression analysis of the thermoTRPs in DRG neurons. We have found neurons that express only TRPV1, only TRPM8, or both TRPV1 and ANKTM1. However, a surprisingly distinct expression pattern was observed for TRPV3, the warm receptor. High levels of TRPV3 are only observed in skin keratinocytes in the mouse, suggesting that skin cells might be able to "sense" temperature and then communicate this information to DRG neurons. How temperature information is coded from the skin to the spinal cord and brain is not well understood. The need for thermosensation exists in all organisms. Therefore we have asked whether non-vertebrates also use thermoTRPs to sense temperature. We have recently shown that the Drosophila sequence orthologue of ANKTM1 is an ion channel activated by warm temperatures, suggesting an evolutionary conserved role of TRP channels in temperature sensing. This leads to the question of whether all temperature-activated ion channels are members of the TRP family. Conversely, not all TRPs are thermosensitive. However, a wide-ranging set of experiments in different species points to a recurring role of TRP channels in diverse sensory functions. By identifying the proteins that likely initiate the molecular cascade leading to temperature perception, we now have the opportunity not only to probe the basic foundation of our sense of temperature but to extend these insights into important areas of human health such as pain pathophysiology.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.