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Bernd Nilius
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Mammalian Transient Receptor Potential (TRP) Cation Channels

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Preface

When we, in 2007, edited the first issue on transient receptor potential channel in the *Handbook of Experimental Pharmacology*, we were all very excited by the progress in this field although only one decade after cloning the first TRP channel had passed. At this time, somewhat less than 5,000 papers were published on TRP channel (January 1, 1960, until December 31, 2006). If we check now the period (January 1, 2007, until January 13, 2014), additional 9,300 papers can be found in a PubMed search.[1] Needless to say, the general interest on these 28 members of the Trp gene family which encode ion channels is nearly exponentially growing. Therefore, it seemed to be indicated, although many excellent books on TRP channels have been published meanwhile, to jump into a new adventure editing a comprehensive source book in this successful Springer Handbook series again on the same topic. This is not only an update of the 2007 book but also an impressive introduction of novel areas which TRP channels have entered. The 2007 view that TRP channels are mainly cell sensors with an intriguing variability concerning the modes of activation has dramatically extended into the evolutionary field, the structural approach, and especially the advent of the important role of TRP channels in hereditary and acquired diseases. Important new data concerning the role of TRP channels in intracellular compartments are included. We also refer to the still controversial topic how TRP channels are involved in store-dependent Ca^{2+} entry. Indeed, the TRP field expansion did not lose the fast speed. It is extending into so far unexpected areas. The *gain of knowledge* has reached such an extent that we have not been able to restrict the source book into a single volume; rather, we had to agree on a two-volume publication. In the first volume, we go through all the known TRP channels. Leading experts in the field summarize features of individual TRP channels starting with the description of the gene, expression patters, associated proteins, biophysical and biochemical function properties, and transgenic animal models and closing with cellular TRP functions, dysfunctions, and their role in diseases. The second volume starts with a chapter on sensor properties and functions

[1] The used search string was ("transient receptor potential" OR trpa* OR trpc* OR trpm* OR trpp* OR trpv* OR PKD* OR stim1 OR stim2 OR orai1 OR orai2 OR orai3 OR trpa*). Note that this search included also the main players of store-operated Ca^{2+} entry, because of the still often reported links to TRP as also discussed in Volume 2.

of TRP channels. This was highlighted in the 2007 book but is not very much extended. Surprising new features are reported, e.g., new insights into thermo- and light-sensing, novel roles or TRPs in taste perception and chemesthesis, and especially their functional importance as chemosensors for gasotransmitters, including oxygen sensing, which was evidenced only in the last 5 years. In the second part, more general topics related to TRP functions and features are discussed such as channel structure; TRPs as targets of pharmacological modulation, including a wealth of natural compounds; and the exciting discovery of novel channel toxins. New aspects are discussed concerning the role of TRPs as important players in the physiology of reproduction and in neural networks which control reproductive behavior opening a *TRP window* into neuroendocrinology, i.e., their role in hormone-secreting cells. We finish this book with some critical remarks on the current state of TRP research, controversies, and surprises.

We hope that this book will guide a large reader community through the fascinating world of the TRP channel family from basic science to pathophysiology and disease. May this voluminous source/textbook also help to establish interactions between the fundamental and clinical research and the research in drug discovery and development! We are convinced that this book is "translational" in the best meaning of this word. Despite the many advances in the understanding of the molecular mechanisms and function features of TRP channel, there is still a tremendous need for more in-depth understanding of the structure of TRP channels, their implementation in diverse signal cascades, and more mechanistic insight into channel function at the molecular and systemic level, as well as the need for identifying selective pharmacological tools and new therapeutic targets and developing new treatment options. We hope this book stimulates further research. Finally, we may conclude that we might be still in a period of the end of the beginning rather than the beginning of the end! The editors wish to thank all authors for excellent contribution and also Wilma McHugh (Springer) for all expert support and very helpful editorial advice!

Leuven, Belgium — Bernd Nilius
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Contents for Volume I

Part V

TRPs as Special Cell Sensors

TRP Channels and Thermosensation

Thomas Voets

Contents

Abstract

Several TRP channels exhibit highly temperature-dependent gating properties, which leads to steep changes in depolarising current upon either cooling or heating. Based on this characteristic feature, these so-called "thermoTRPs" have been widely studied with the aim to elucidate their potential key role as thermosensors in the somatosensory system and to understand the basis of their high thermal sensitivity. In this chapter, I provide a brief critical overview of current knowledge on the role of TRP channels in thermosensing and on the thermodynamic and molecular basis of their steep temperature dependence.

Keywords

Somatosensory system • Temperature dependence • Gating mechanisms • Channel biophysics

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1 Cellular and Molecular Basis of Thermosensation

Temperature sensing is primarily mediated by sensory neurons that have their sensory endings in the skin and mucosa and their cell bodies in the trigeminal (TG) and dorsal root ganglia (DRG) (Damann et al. 2008; Julius and Nathans 2012). Under resting conditions, voltage-gated K^+ channels ensure a negative membrane potential in these cells (Damann et al. 2008). In response to changes in temperature, different types of depolarising channels open in the nerve endings, leading to membrane depolarisation and, when a certain threshold is crossed, action potential initiation via voltage-gated Na^+ channels. These action potentials are then propagated along the sensory neuron and lead to neurotransmitter release onto second-order neurons in the dorsal horn (DRG) or sensory nucleus in the brain (TG) (Damann et al. 2008). The depolarising channels that open in response to thermal stimulation can be considered as the primary molecular sensors of temperature, nature's thermometers, and understanding their molecular nature and modus operandi represents an important goal in sensory physiology. In the last 16 years, several members of the transient receptor potential (TRP) superfamily have been identified as thermosensitive (hence the name "thermoTRPs") and put forward as primary temperature sensors in the somatosensory system (Caterina et al. 1997; Voets et al. 2005; Talavera et al. 2008). In this chapter, I present a critical reappraisal of the role of TRP channels as thermosensors in the mammalian somatosensory system and bring up some considerations on the basis of their temperature sensitivity.

2 ThermoTRPs as Prime Molecular Thermometers(?)

Since all processes in nature exhibit some degree of temperature sensitivity, three discerning criteria have been put forward that may be used to identify "true" thermoTRPs (Voets 2012). As a first criterion, the candidate thermoTRP's biophysical properties must be such that it can produce robust depolarising currents in response to a thermal stimulus. The robustness of the thermal response can be quantified as the Q_{10} value, which is defined as the relative change in current amplitude upon a 10° increase in temperature. A Q_{10} value of more than 5 (or less than 0.2 for of a cold-activated channel) has been put forward as a minimal requirement, although it should be noted that experimental Q_{10} values are strongly dependent on the cellular context and experimental approach. As a second criterion, the candidate thermoTRP must be expressed at sites in the body that face significant variations in temperature, including the skin, mouth, upper airways and oesophagus. As a third and probably most important criterion, there must be in vivo evidence that the thermal sensitivity of a candidate thermoTRP confers thermosensitivity to a physiological process.

With respect to the first criterion, according to the literature, 11 mammalian TRP channels exhibit Q_{10} values >5 or <0.2 when heterologously expressed in cell lines or Xenopus laevis oocytes: currents mediated by TRPV1, TRPV2, TRPV3, TRPV4,

TRPM2, TRPM3, TRPM4 and TRPM5 increase upon warming (Caterina et al. 1997, 1999; Guler et al. 2002; Peier et al. 2002b; Smith et al. 2002; Xu et al. 2002; Talavera et al. 2005; Togashi et al. 2006; Vriens et al. 2011), whereas currents mediated by TRPM8, TRPA1 and TRPC5 increase upon cooling (McKemy et al. 2002; Peier et al. 2002a; Story et al. 2003; Zimmermann et al. 2011). It should be noted, however, that heat-induced activation is observed for mouse and rat TRPV2 but not for the human orthologue (Neeper et al. 2007) and that cold-induced activation of mammalian TRPA1 has been consistently observed by some laboratories (Story et al. 2003; Sawada et al. 2007; Fajardo et al. 2008; Karashima et al. 2009) but not by others (Jordt et al. 2004; Zurborg et al. 2007; Cordero-Morales et al. 2011).

With respect to the second and third criteria, the situation is even much more contentious. This is particularly the case when considering the role of TRP channels as thermosensors in the somatosensory system. Following the cloning of TRPV1 as the first heat-activated TRP channel and the subsequent identification of closely (TRPV2-V4) and more distally related (TRPM8, TRPA1) as differently tuned thermosensors, it was generally assumed for many years that these six channels would be sufficient to cover the entire range of temperatures that the mammalian somatosensory system can discriminate (Voets et al. 2005; Dhaka et al. 2006; Basbaum et al. 2009). Indeed, the TRP channel literature and physiology handbooks are replete with a variation of figures and schemes with these channels as sensors for noxious (TRPA1) and innocuous (TRPM8) cold, warmth (TRPV3 and TRPV4), heat (TRPV1) and extreme heat (TRPV2).

However, with hindsight, it appears that these schemes were somewhat overoptimistic. Actually, with respect to acute thermosensing, the only TRP channel for which there is general agreement in studies from multiple groups is TRPM8, which seems to be crucial for accurately discriminating temperatures between ~15 and ~30° (Bautista et al. 2007; Colburn et al. 2007; Dhaka et al. 2007). For TRPV1, there is consensus that it plays a crucial role in heat sensing under conditions of inflammation (Caterina et al. 2000; Davis et al. 2000). However, whereas some reports show that TRPV1-deficient mice have a deficit in acute heat sensing (Caterina et al. 2000), others studies have not been able to replicate this (Davis et al. 2000). For TRPV3 and TRPV4, initial studies reported clear behavioural deficits in warmth sensing at temperatures between ~25 and ~35° (Lee et al. 2005; Moqrich et al. 2005). However, more recent studies indicate that the alteration in temperature preference in TRPV3-deficient mice is highly dependent on the genetic background of the mice (Huang et al. 2011; Miyamoto et al. 2011). Moreover, TRPV3/TRPV4 double knockout mice on a C57BL6 background exhibited thermal preference and heat avoidance behaviour that was virtually indistinguishable from that of wild-type C57BL6 mice, even when TRPV1 function was eliminated (Huang et al. 2011). Although heat activation and expression in sensory neurons is widely documented for mouse TRPV2 (Caterina et al. 1999; Neeper et al. 2007), TRPV2-deficient mice show no indication of altered thermosensation (Park et al. 2011). In the case of TRPA1, there are unexplainable differences between labs, not only with respect to cold activation in vitro but also concerning its role

in vivo. Indeed, whereas some laboratories report significant deficits in noxious cold sensing in TRPA1-deficient mice (Kwan et al. 2006; Karashima et al. 2009), others found no acute thermosensing phenotype whatsoever (Bautista et al. 2006; Knowlton et al. 2010).

More recently, evidence has been presented that TRPC5 (as a cold sensor) (Zimmermann et al. 2011) and TRPM3 (as a heat sensor) (Vriens et al. 2011) may play a role in acute thermosensing in mice, but it seems wise to await further independent confirmation of these interesting findings. TRPM2 and TRPM4 are expressed in sensory neurons (Vandewauw et al. 2013), but there is no evidence so far that they confer relevant thermosensitivity to somatosensation or any other physiological process. There is also strong evidence that TRPM5 confers thermosensitivity to taste (Talavera et al. 2005), but its expression level in somatosensory neurons is probably too low to play any significant role in (acute) thermosensation (Vandewauw et al. 2013).

Ongoing research in this field may cause the list of thermoTRPs to further grow (or shrink). It also seems warranted to envisage that important aspects of thermosensation may depend on thermosensitive conductances that are not mediated by TRP channels, such as K^+ channels (Noel et al. 2009), TMEM16A (Cho et al. 2012), Stim-Orai (Xiao et al. 2011) and probably more.

3 Temperature-Dependent Gating: Thermodynamic Considerations

Steep temperature-dependent activation of an ion channel can, in principle, be based on either intrinsic steep temperature dependence of the channel itself or, alternatively, on the steep temperature sensitivity of another cellular component (e.g., enzyme, membrane, cytoskeleton) that directly or indirectly regulates the activity of the channel. Without excluding a contribution of the latter mechanism, there is a growing body of strong evidence that the temperature sensitivity of at least some thermoTRPs, including TRPM8 and TRPV1, is largely preserved in cell-free patches and even upon purification and reconstitution in artificial lipid membranes (Zakharian et al. 2009; Cao et al. 2013). This indicates that in these channels temperature sensitivity is an intrinsic property of the channel's structures that determine gating and permeation.

In general, the ion flux through an open (TRP) channel pore exhibits mild thermal sensitivity, comparable to the temperature dependence of ionic diffusion in aqueous solution, with typical Q_{10} values <2 (Voets 2012). Consequently, research has focused on the origin of temperature-sensitive channel gating. However, despite significant efforts and a growing number of papers, we are still far from fully understanding the thermodynamic processes and structural rearrangements that underlie the highly temperature-sensitive gating of thermoTRPs.

In the TRP channel literature, there are widely diverging global views on thermosensitive gating of TRP channels. On the one hand, thermosensitivity of

TRP channels has been explained using a simple two-state model, where the actual channel gating step is temperature sensitive and thermosensitivity is not confined to a specific part of the channel (Voets et al. 2004, 2005; Clapham and Miller 2011). On the other hand, models have been put forward in which the origin of thermosensitivity is confined in one or few restricted domains of the channel, which act as thermosensor "modules" and are allosterically coupled to the channel gate (Brauchi et al. 2004; Latorre et al. 2007; Matta and Ahern 2007). Both extreme views can be easily criticised; for instance, a two-state model is obviously an (over)simplification, given the extensive evidence (e.g., from kinetic and single-channel analyses) for the existence of multiple open and closed states, whereas restricting thermosensitivity to delineated channel domains disregards the fact that every atom in the channel protein is affected by temperature.

Irrespective of the model, the behaviour of steeply temperature-dependent TRP channels implies that the equilibrium between closed and open states is highly thermosensitive. In thermodynamic terms, the equilibrium between two global states, for instance, the closed and open state of a channel or the inactive and active state of a thermosensor module, is given by

$$K_{\mathrm{eq}} = \exp\left(\frac{-\Delta G}{RT}\right),$$

where K_{eq} represents the ratio between the open and closed state of the channel (or between the active and inactive conformation of the thermosensor module), ΔG the free energy change, R the universal gas constant and T the temperature in Kelvin (Fig. 1a).

If we disregard effects of mechanical or electrical forces, ΔG is given by

$$\Delta G = \Delta H - T\Delta S,$$

where ΔH represents the difference in enthalpy (in J mol^{-1}) and ΔS the difference in entropy (in J mol^{-1} K^{-1}) between the two states.

When $\Delta G = 0$, it follows that $K_{\mathrm{eq}} = 1$, which means that 50 % of the channels (or thermosensor modules) are in the open state (active conformation). This occurs at the temperature for half-maximal activation (T_{50}), which is given by

$$T_{50} = \frac{\Delta H}{\Delta S}.$$

Assuming that T_{50} has a realistic (i.e., positive) value, it follows that ΔH and ΔS must have the same sign. When ΔH and ΔS are both positive, ΔG decreases upon heating. This leads to a heating-induced shift of the equilibrium towards the open state/active conformation, as would be the case in a heat-activated (TRP) channel. Oppositely, when ΔH and ΔS are both negative, ΔG decreases upon cooling. This leads to a cooling-induced shift of the equilibrium towards the open state/active conformation, as would be the case in a cold-activated (TRP) channel (Fig. 1b).

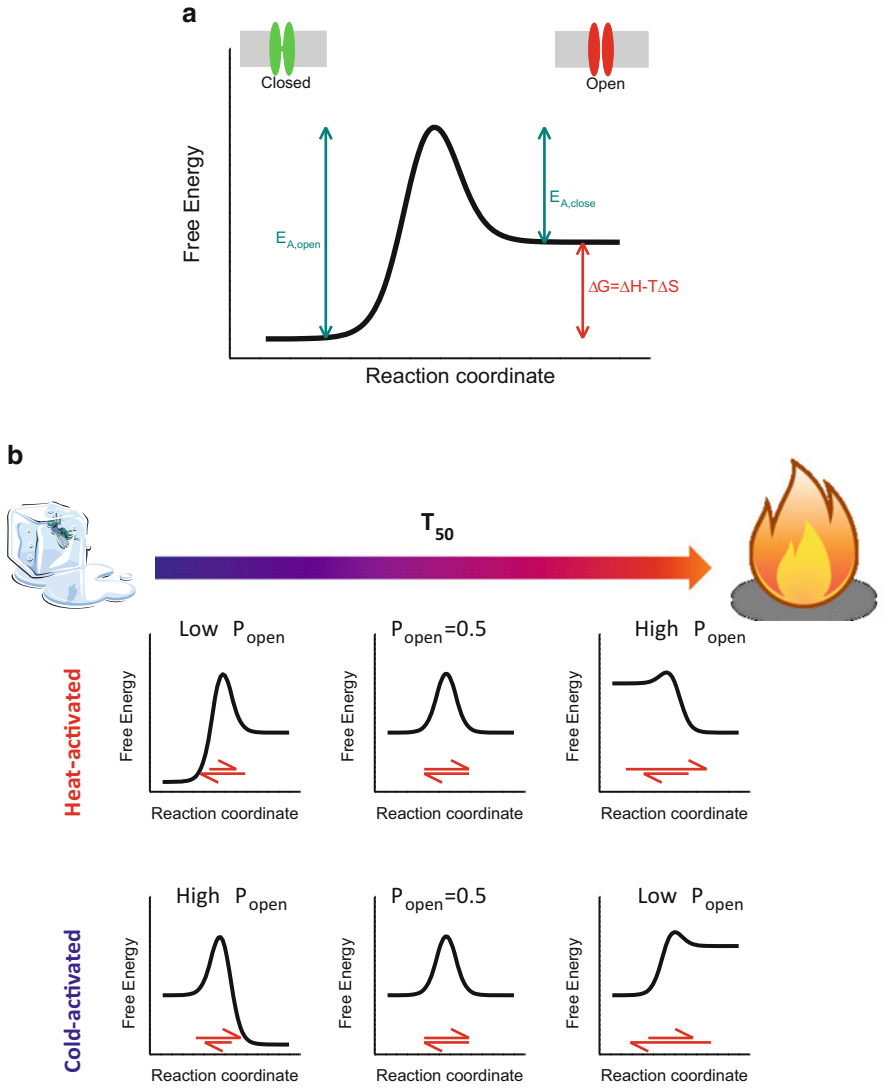


Fig. 1 Energy diagrams describing the gating process of temperature-sensitive channels. (**a**) General scheme. ΔG, the difference in Gibbs free energy between the close and open states determines the equilibrium, whereas the activation energies for opening ($E_{A,open}$) and closing ($E_{A,close}$) determine the opening and closing rates (indicated by the length of the *red arrows* in panel **b**). (**b**) Influence of temperature on the energy diagram in heat- and cold-activated channels

Energy diagrams such as those shown in Fig. 1 also allow understanding the effect of temperature on the gating kinetics of cold- and heat-activated TRP channels. In the case of a heat-activated channel, increasing temperature causes a

destabilisation of the closed state. This reduces the activation energy for channel opening ($E_{A,open}$), which leads to a steep increase of the channel opening rate in response to heating, whereas channel closing rates are only mildly temperature dependent. In contrast, in the case of a cold-activated channel, decreasing temperature causes a stabilisation of the open state. This increases the activation energy for channel opening ($E_{A,close}$), which leads to a steep decrease of the channel closing rate in response to cooling, whereas in this case channel opening rates are only mildly temperature dependent (Voets et al. 2004).

At very low open probabilities, the temperature dependence of channel gating, expressed as $Q_{10,gating}$, is directly related to ΔH, according to

$$Q_{10,\,\mathrm{gating}} \approx 10^{4.34\times\frac{\Delta H}{RT^2}}.$$

It should be noted that the Q_{10} for current through a thermoTRP is determined not only by $Q_{10,gating}$ but also by $Q_{10,permeation}$, which quantifies the temperature dependence of ion flux through the open channel pore. Taking this into account, we arrive at

$$Q_{10} = Q_{10,\,\mathrm{gating}} \times Q_{10,\,\mathrm{permeation}}.$$

For ion diffusion through ion channels, $Q_{10,permeation}$ generally has values between 1.1 and 2, thus providing a minor contribution to the high Q_{10} of heat-activated TRP channels and counteracting the cold activation of cold-activated TRP channels.

Several studies have provided measurements of ΔH and ΔS for different temperature-sensitive TRP channels, based on steady-state current measurements at different temperatures and voltages or kinetic analyses of currents in response to heat and/or voltage jumps. These studies indeed consistently show $\Delta H > 0$ and $\Delta S > 0$ for the opening of heat-activated TRP channels and $\Delta H < 0$ and $\Delta S < 0$ for the opening of cold-activated TRP channels. Estimates for ΔH range between ± 150 and ± 400 kJ mol^{-1} and for ΔS between ± 500 and $\pm 1{,}200$ J mol^{-1} K^{-1}. (Note that I use the SI unit joules (J) rather than the commonly used calorie (cal) as unit for energy; 1 cal = 4.184 J.)

So how can one interpret ΔH and ΔS for a channel gating reaction? A positive value for ΔH, as found in heat-activated channels, indicates that the opening of the channel is an endothermic reaction, absorbing heat from the surroundings. This is, for instance, the case when gating involves the disruption of internal interactions in the channel, such as salt bridges, cation–π interactions or hydrogen bonds. Oppositely, opening of cold-activated channels has a negative ΔH, indicative of the formation of stabilising internal interactions. As a yardstick, typical ΔH values for disruption of a single hydrogen bond in a protein are in the order of ~5 kJ mol^{-1}, whereas ΔH values for disruption of cation–π interactions or salt bridges are typically 2–4-fold higher (Jackson 2006).

A positive value of ΔS, as found in heat-activated channels, indicates that gating leads to an increase in the degrees of freedom of the channel, for instance, due to

exposure of amino acid side chains upon unfolding of a close-packed protein domain. Oppositely, opening of cold-activated channels has a negative ΔS, indicating a reduction in the degrees of freedom in the open state. Published estimates of ΔS for protein unfolding are in the order of 20 mol^{-1} K^{-1} per amino acid residue (Jackson 2006).

Understanding the molecular basis of thermosensitivity in (TRP) channels comes down to understanding the (sub)molecular events that underlie these changes in enthalpy and entropy when the channel transits between closed and open states. Temperature-sensitive TRP channels are tetramers consisting of between ~3,500 and 4,500 amino acids. Moreover, ΔH and ΔS refer to the complete system, which includes not only the channel protein but also interacting water molecules and lipids. As such, there is ample "room" for local or global conformational changes to accommodate the changes in enthalpy and entropy. For instance, it has been estimated that membrane channels typically contain 0.29 hydrogen bonds per amino acid residue (Joh et al. 2008). If this holds through for TRP channels, a change in the number of hydrogen bonds during gating of only a few percent would suffice to explain the typical ΔH values.

In addition, it has been pointed out that ΔH and ΔS should not necessarily be viewed as constants, but vary with temperature whenever gating is accompanied by changes in heat capacity (ΔC_p, expressed in kJ mol^{-1} K^{-1}) (Clapham and Miller 2011):

$$\Delta H(T) = \Delta H_0 + \Delta C_p(T - T_0),$$

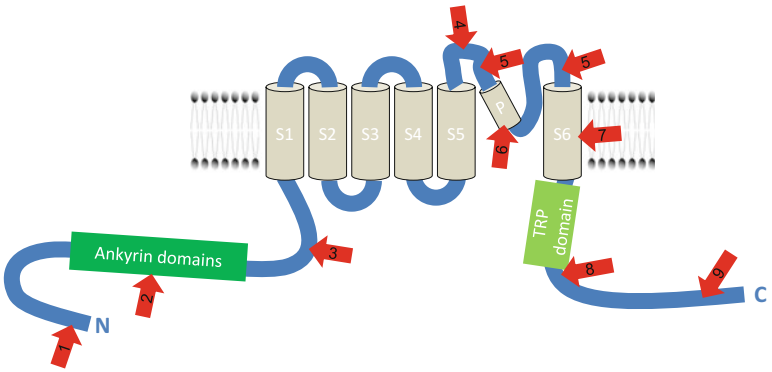
and

$$\Delta S(T) = \Delta S_0 + \Delta C_p \ln(T/T_0),$$

where ΔH_0 and ΔS_0 represent the change in enthalpy and entropy at a reference temperature T_0. If ΔC_p is large, ΔH and ΔS will change significantly with temperature and may even change sign, which implies that a channel is both cold and heat activated. Although this is an interesting and provocative idea, there is currently no evidence for such behaviour in TRP channels. In contrast, several studies indicate that ΔH remains fairly stable within the biologically relevant temperature range (Voets et al. 2004, 2007; Yang et al. 2010; Yao et al. 2010a, 2011).

4 (No) Lessons from Structure–Function Studies?

There is a steadily growing number of studies aimed at understanding the structural basis of temperature sensitivity in TRP channels. In most cases, these studies started from the assumption that TRP channels are modular machines that contain clearly delineated functional regions and even transplantable thermosensing modules. Several approaches were taken to pursue such modules, including "brute force" methods consisting of random mutagenesis followed by medium- to high-



1) N terminal thermosensitivity-reducing region (*Drosophila* TRPA1) (Kang et al. 2012)
2) Portable heat-sensitive modules within the ankyrin-repeat-rich domain (rattlesnake TRPA1) (Cordero-Morales et al. 2011)
3) Membrane proximal domain modular thermal sensor (human TRPV1 and TRPV2) (Yao et al. 2011)
4) Pore turret (part of the thermo-sensing apparatus of mouse TRPV1) (Yang et al. 2010)
5) Pore-loops (required for temperature sensitivity in human TRPV1 and TRPV3) (Grandl et al. 2008, 2010)
6) Pore helix (dictates thermosensitivity in *Drosophila* TRPA1) (Wang et al. 2013)
7) Sixth transmembrane (required for temperature sensitivity in human TRPV3) (Grandl et al. 2008)
8) Minimal C-terminal portion able to turn TRPM8 into a heat receptor (human TRPV1) (Brauchi et al. 2007)
9) Distal C terminal (structural basis for thermal sensitivity in human TRPV1) (Vlachova et al. 2003)

Fig. 2 Schematic overview of regions that have been implicated in thermosensitivity in the TRP channels TRPV1, TRPV2, TRPV3, TRPM8 and TRPA1

throughput functionality screens, site-directed mutagenesis aimed at disturbing the functionality of putative functional domains, characterisation of orthologues and splice variants with distinct and/or opposite thermosensitivities and analysis of chimeric channels obtained by swapping putative thermosensing modules between closely or distantly related TRP channels (Vlachova et al. 2003; Brauchi et al. 2006, 2007; Grandl et al. 2008, 2010; Yang et al. 2010; Cordero-Morales et al. 2011; Yao et al. 2011; Kang et al. 2012; Zhong et al. 2012; Wang et al. 2013). The main outcomes of such studies performed on mammalian isoforms of TRPV1, TRPV3 and TRPM8, as well as on TRPA1 orthologues from mammals, snakes and Drosophila, are schematically illustrated in Fig. 2.

Although most individual studies claim identification of specific domain required for thermosensation, the overall picture that emerges from these studies is fuzzy, with studies inculpating specific domains in the N- and C-terminal cytosolic loops, transmembrane domains and pore region. Moreover, at least in the case of TRPV1 and TRPA1, there are also some apparently conflicting data between studies. For instance, Cordero-Morales et al. (2011) reported that the N-terminal cytoplasmic domain of heat-sensitive TRPA1 from *Drosophila* and rattlesnake and specific ankyrin repeats therein contain transplantable heat-sensitive modules that can confer heat sensitivity to heat-insensitive human TRPA1. In contrast, Wang et al. (2013) reported that heat sensitivity was conferred to the human TRPA1 channel upon transplantation of the pore region, but not the N-terminal part of *Drosophila* TRPA1. Further complexity is added by the surprising finding that a specific part of Drosophila TRPA1 acts as a suppressor

of heat sensitivity (Kang et al. 2012). Similarly, whereas some studies strongly implicate the pore region and pore turret in setting the thermosensitivity of TRPV1 (Grandl et al. 2010; Yang et al. 2010), other studies dismiss this possibility and rather implicate the domain between ankyrin repeats and the first transmembrane helix (Yao et al. 2010b, 2011). The reasons for these discrepancies are not always obvious, but may reflect differences in methodology, the lack of robustness of some of the parameters that are used to quantify thermal sensitivity and possibly artefacts caused by the harshness of some experimental protocols to evoke thermal responses from TRP channel-(over)expressing cells.

It may be concluded that, in contrast to voltage-gated Na^+, K^+ or Ca^{2+} channels, in which the voltage sensor is mainly contained within the fourth transmembrane (S4) domain, or to ligand-gated channels such as the cys-loop and ionotropic glutamate receptors, in which the ligand binding site is located in the extracellular domain, thermosensing does not seem to be generated in a clearly delineated and conserved domain of TRP channels. Possibly, the significant difference in enthalpy between closed and open states arises from multiple submolecular rearrangements occurring in different and diffuse areas of the channel.

Obviously, detailed understanding of the molecular basis of thermosensing in TRP channels is therefore still far ahead. Deeper insight may be obtained by meticulous analysis, using standardised patch-clamp assays and/or calorimetry, of the thermodynamic consequences of thousands of point mutations. Once TRP channel structures in closed and open configuration will be available, it may also become feasible to calculate the contribution of domains, residues and atoms to the thermodynamic properties of TRP channels.

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