Voltage Sensing in Thermo-TRP Channels

ARTICLE *in* ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY · JANUARY 2011

Impact Factor: 1.96 · DOI: 10.1007/978-94-007-0265-3_28 · Source: PubMed

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Chapter 28
Voltage Sensing in Thermo-TRP Channels

Sebastian Brauchi and Patricio Orio

Abstract Membrane voltage, ligand binding, mechanical force and temperature can all induce conformational changes that open ion channel pores. A key question in understanding ion channel function is how the protein domains involved in sensing stimuli (sensors) communicate with the pore to gate its opening and closing. TRP channels are considered six-transmembrane cation-permeable channels, distant relatives of voltage-gated potassium channels (Kv), which are known to be activated by membrane depolarization. Understanding the molecular nature of thermo-TRP channel gating offers a fair challenge to biophysicists. This chapter will summarize our present knowledge on the effect of voltage and temperature during thermo-TRP channel activation.

28.1 TRP Channel Family and Thermo-TRPs

Mammalian TRP channel proteins are polymodal cation channels with essential roles in cellular sensing. Other than a loose sequence homology, predicted channel architecture, and a common poor cation selectivity, there are no particular features defining the TRP family. TRP channels are grouped by homology into six subfamilies named C, M, V, A, P, and ML, for canonical, melastatin related, vanilloid binding, ankyrin repeat, polycystin, and mucolipin, respectively [1]. By integrating multiple stimuli they supply signal amplification through calcium permeation and membrane depolarization. Cooperativity intrinsic to TRP channels may result in allosteric coupling of distinct activation stimuli. A good example of the allosteric nature of TRP channels would be the case of temperature-activated TRP channels (*thermo-TRPs*) [2]. Mammalian thermo-TRPs correspond to a subgroup of 9 TRP channels which are expressed in sensory nerve endings and in skin, characterized

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M.S. Islam (ed.), *Transient Receptor Potential Channels*, Advances in Experimental Medicine and Biology 704, DOI 10.1007/978-94-007-0265-3_28,

by their distinctive high temperature-dependence. Thermo-TRP channels elicit different dynamic ranges for their activation profile, are activated by different natural compounds, and are also known because of their participation in nociceptive pathways. One interesting feature of thermo-TRP channels is the presence of members from at least three different TRP families (V, M, and A). Whereas TRPV1–4, TRPM2, TRPM4, and TRPM5 are heat-activated, TRPM8 and TRPA1 are activated by cold [3]. The first member of these channels that was cloned was TRPV1 [4], and the temperature activation of TRPA1 remains controversial.

28.2 The Process of Channel Gating

Based on our understanding of canonical voltage-gated ion channels, it is predicted that membrane depolarization should induce a conformational change in the Voltage Sensor of TRP channels, where positively charged amino acids (and/or dipoles) contained within the trans-membrane region can be affected by such changes of membrane potential. However, despite this preconceived paradigm of voltage-dependence, the location of the voltage sensor in TRP channels remains undefined. Nevertheless, whatever the location and nature of the voltage sensor within the channel structure, some voltage-dependent conformational change lead to channel gating, opening the conduction pathway. Once TRP channels open, they allow the flux of cations down their electrochemical gradient.

Channel gating process can be understood as a process of distribution of particles (p_1 and p_2) in different energy levels, therefore can be described by the Boltzmann equation:

$$\frac{p_2}{p_1} = \exp\left(-\frac{u_2 - u_1}{k_b T}\right) \tag{28.1}$$

Where $u_2 - u_1$ represents the energy difference between the levels, k_b is Boltzmann's constant and T is the absolute temperature. For the case of ion channels, the Boltzmann equation will allow us to describe the distribution of the open or closed conformations at equilibrium (open probablity, P_o= open/(open + closed)). If the free energy (ΔG) for transition between the closed and open state is expressed in terms of electrical energy of an electrical particle (with charge z) responding to a change in membrane potential by moving across the electric field (V), the open probability will correspond to:

$$P_O = \frac{1}{1 + \exp\left(-\frac{zF\Delta V}{RT}\right)} \tag{28.2}$$

where F and R correspond to the Faraday constant and the gas constant respectively.

At resting membrane potentials, the closed state of voltage-sensitive channels is lower in energy than the open state, and is therefore the preferred conformation of the protein. Energy provided from external forces (e.g. agonist binding, temperature,

voltage) will allow for the population to reach conformational states that otherwise would be less likely. At depolarized membrane voltages, the conformational energy for the open state is lower, allowing the stabilization of the channel in its open conformation.

TRP channels are tetramers composed by 6 transmembrane protein subunits, resembling *Shaker* type voltage-dependent potassium (Kv) channels. By analogy to Kv channels, the working hypothesis is that each TRP channel subunit may be divided into two major functional domains, a VSD-like domain (S1–S4) and a pore domain (S5–S6) that contains the actual channel gate. In Kv channels, the voltage sensing domain (VSD) is connected to the pore domain through the S4–S5 linker, which likely functions to transfer the necessary mechanical energy from VSD movement to the gated pore.

28.3 Voltage Dependence

Voltage-gated ion channels play a pivotal role in muscle contraction, neuronal excitability, and secretion. The VSD is the fundamental feature of voltage-gated ion channels for sensing transmembrane potential, and has been studied for more than 50 years [5] at the levels of both biophysics and protein structure [6, 7]. The main feature of VSD is the array of positively charged amino acids in the fourth and sometimes the second transmembrane segment [8–10]. These charges sense and induce a protein movement in response to a changing electrical field [5, 11]. For the case of classical voltage-dependent ion channels, in which VSD movements are highly coupled to gate opening, voltage-sensitivity is very strong (e.g. *Shaker* type Kv channels) and the Boltzmann distribution changes over a narrow range of voltage. Recent findings have demonstrated that a similar structural strategy for voltage sensing is used not only by voltage-dependent channels, but also in voltage-dependent enzymes [12] and voltage-dependent proton channels [13, 14].

Predicted by Hodgkin and Huxlex in 1952, the presence of the "activating particles", was first measured almost twenty years later [15, 16]. Generally speaking, gating currents correspond to a nonlinear capacitive current transient explained by the movement of a charged particle within the membrane; thus measuring the number of electrical charges associated to the gating process provides a descriptor of its voltage-dependency. Strongly voltage dependent channels such as *Shaker* K^+ channels can displace up to 12 effective electrical charges during activation of a single ion channel [17, 18]. Arginine residues located at the S4 segment of *Shaker* K^+ channels were proven to be the charges responsible for this voltage-dependence [9, 19]. For the case of *Shaker* K^+ channels the first four arginines in the S4 helix account for most of the displaced gating charge and during channel activation they move through the entire electric field. Gating current recordings are one of the important gaps present in TRP channel biophysics, however, some efforts have been made to surmount this obstacle. Equivalent gating charges can be obtained from conductance vs. voltage data using the form of the Boltzmann function described on Eq. (28.2). Using this method, the calculated number of gating charges for TRPV1

and TRPM8 is 0.6–0.8e [20, 21]. Similar weak voltage dependence ($z\delta \sim 0.4$) was also observed for TRPA1 channels [22]. Such weak voltage dependence is in contrast with the strong voltage dependence observed in Kv channels, suggesting that the inner workings of the voltage-sensing mechanism may not be exactly the same. However, regardless of mechanism, thermo-TRPs are voltage-activated channels [20, 21, 23], therefore they should contain a voltage sensor.

Because of their apparent structural similarity with Kv channels [24, 25], dogma dictates the existence of an S1–S4 voltage-sensor-module. Following this logic, Voets et al. [27] used the limiting slope analysis [18, 26] to calculate 0.85 effective gating charges associated with TRPM8 channel activation. Since TRP channels are tetramers, this finding implies that each voltage sensor only contributes about 0.2 electronic charges coupled to channel opening. The predicted S4 domain of TRPM8 channels contains 2 arginines, one of them conserved throughout the TRPM channel sub-family, and a histidine residue that, depending of local pH and pKa may contribute to the total gating charge (Fig. 28.1). Neutralization of charges contained in TRPM8s' S4 (R842) and in the S4–S5 linker (K856) reduces the total gating charge to 0.62 suggesting that these residues may participate in the voltage sensor activation process [27]. This result is somewhat unexpected since according to the crystal structure of Kv1.2 [7] the S4–S5 linker is located outside the electric field, suggesting that K856 would unlikely to contribute to the total gating charge. This result may be explained only if the tertiary structure of TRPM8 differs from Kv1.2, allowing the S4 helix to be longer (e.g. more tilted or kinked), thereby placing K856 inside the electric field. The only structural data availble for TRP channels came from cryo-EM studies. There are notorious differences between the cryo-EM structures available. While TRPVs seems similar to Kv1.2, TRPMs and TRPCs looks awkwardly bulky and bigger [3, 24]. Thus, there is no reason to dismiss the possibility that the structure of TRPM8 is different from the known structure of Kv channels.

The results obtained by Voets et al. [27] support the use of a two-state model (see *allosteric models section*), however, this model does not explain the single channel electrical activity that is characterized by bursts separated by long resting periods [23, 28]. The most simple kinetic model able to account for this type of channel gating will require more than one closed state and at least two open states [23]. If this is the case, the determination of the effective gating charge using the limiting slope may not be the best experimental approach since this method does not provide an accurate determination of the total charge when describing kinetic models containing more than one open state connected by voltage-dependent transitions [18].

Based on single channel recordings, the voltage dependence of TRP channels appears to be intrinsic to the channel-forming protein. A closer inspection of the predicted S4 segment of these channels reveals the presence of only one basic residue in TRPV1, TRPV3, and TRPV4 and possibly three in TRPM8. TRPA1 is also voltage-dependent despite the fact that it does not have a single positively charged residue in its S4. Although it is possible that the weak voltage dependence of thermo-TRPs is caused by the low density of positive charges in the S4 domain, the need for a plausible alternative model seems to be imperative because these observations strongly suggest that the actual location of the voltage sensor in TRP channels is a well kept secret.

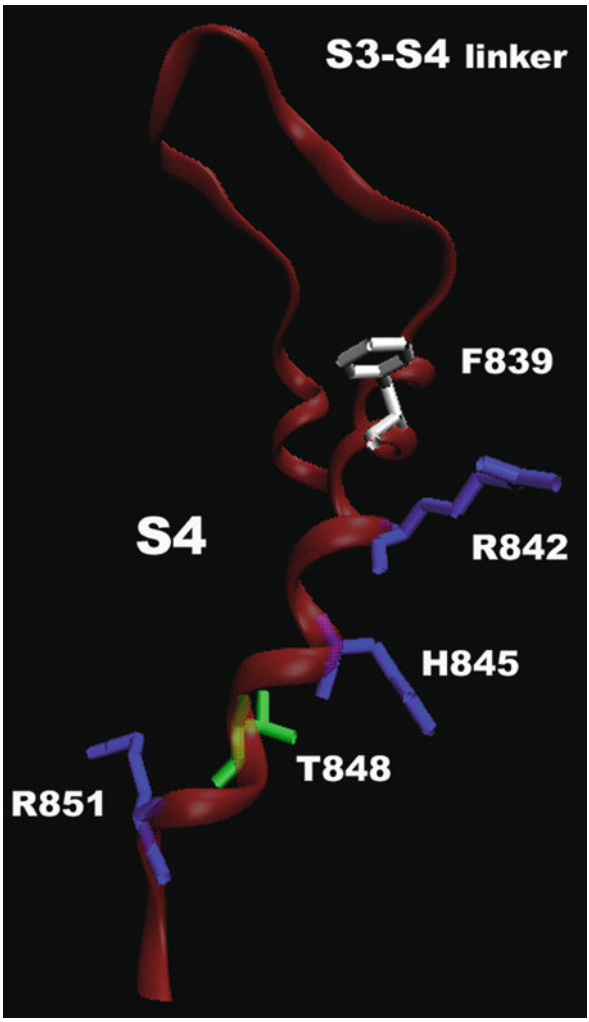

Fig. 28.1 Topology of TRPM8 S4 helix. The model shows the localization of charged amino acids in the TRPM8 S4 segment. F839 and T848 correspond to voltage-sensing arginines in Kv channels. TRPM8 homology model was built using the crystal structure of Kv1.2 (PDB:2A79) as template. Intra- and extracellular loops were relaxed using a Monte Carlo (MC) protocol implemented in ICM. The initial minimization was followed by a short molecular dynamics simulation (1 ns). The assembly of the system and figures employed VMD (Visual Molecular Dynamics; http://www.ks.uiuc.edu/Research/vmd/)

28.4 Temperature Activation

Changes in temperature affect the protein conformational landscape, therefore ion channel gating. Temperature-activated TRP channels have Q_{10}[1] values up to 30, whereas Q_{10} values for non-temperature-dependent enzymes or ion channels is about 2. Changes in ambient temperature are well correlated with changes in the firing rate of somatosensory neurons [29]. Temperature activation of thermo-TRP channels allows Na^+ and Ca^{2+} to enter cells, resulting in depolarization of sensory

[1] Change in enzyme activity over a change of 10ºC.

neurons and triggering of action potentials. This is again a process in which the energy provided to the channel (in the form of heat) changes the distribution of open and closed states according to Eq. (28.1). Due to the stochastic nature of the gating process, the widespread concept of threshold for activation lacks meaning. However, there are temperature ranges at which different thermo-TRP channels exhibit significant changes on their open probability, from cool (24–10ºC; TRPM8), to warm (>30ºC; TRPV3, TRPV4), to hot (>40ºC; TRPV1).

For the case of TRPV1 and TRPM8, temperature produces large (>100 mV) leftward shifts of the voltage activation curve upon heating and cooling, respectively [20, 21] (Fig. 28.2). Thermodynamic parameters such as the overall changes in enthalpy (H) and entropy (S) associated with temperature- and voltage- dependent channel opening can be obtained considering a simple two-state model:

$$C \underset{k_{-1(T,V)}}{\overset{k_{1(T,V)}}{\rightleftharpoons}} O$$

Where k_1 and k_{-1} correspond to activation and deactivation rate constants, respectively. C and O can represent a collection of closed and open states, respectively, as this thermodynamical analysis is independent of the kinetic activation scheme and the number of states or transitions. It is easy to visualize the thermodynamic meaning of the observed temperature-dependent shift in the open probability vs. voltage curve (Fig. 28.2) since:

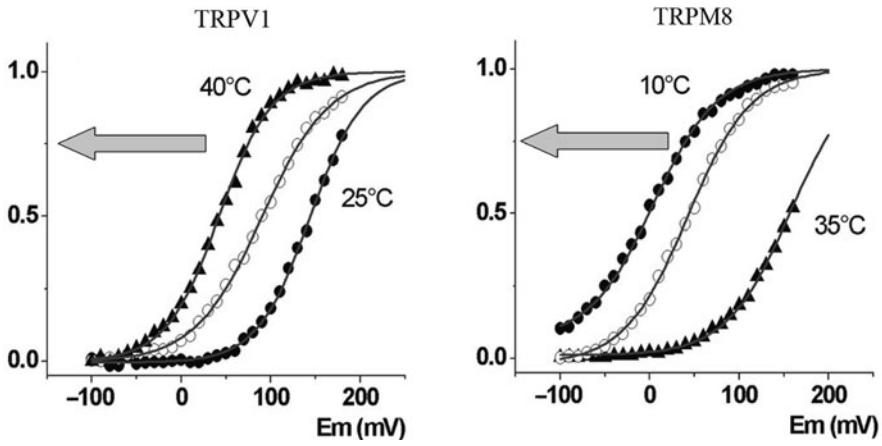

Fig. 28.2 Voltage vs normalized Conductance relationships are shifted upon heating and cooling. Plots showing the normalized conductance (G/Gmax) in function of voltage (Em) at different temperatures for cells expressing TRPV1 (*Left*) or TRPM8 (*Right*). Solid lines correspond to the best fit to Boltzmann functions. The arrow indicates the direction of curve shifting upon temperature activation

$$K_{eq} = \frac{k_1}{k_{-1}} = \exp\left(-\frac{\Delta G}{RT}\right) \tag{28.3}$$

Substitution into Eq. (28.2) results in a predicted relation between the equilibrium constant (K_{eq}) and the open probability.

$$P_O = \frac{1}{1 + K_{eq}^{-1}} \tag{28.4}$$

If we consider that ΔG corresponds to the transitional energy for the close-to-open reaction [30] according to:

$$\Delta G = \Delta H - T\Delta S - zF\Delta V \tag{28.5}$$

And assuming that for the temperature-dependent transition the term $[-zF\Delta V]$ is negligible, the enthalpy and entropy changes (ΔH and ΔS, respectively) associated with the open transition can be calculated from the slope of the ln K vs. of 1/T plot, according to the van't Hoff equation:

$$\ln K_{eq} = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \tag{28.6}$$

Voets et al. [20] have proposed that the temperature-dependent activities of TRPV1 and TRPM8 can be explained by the effect of temperature on the voltage-dependent gating. According to this, because the gating charge (z) in thermo-TRPs is small, temperature changes promote large shifts of the voltage-activation curves when compared with a channel with strong voltage dependence such as *Shaker*.

However, it can be argued that what makes thermo-TRPs temperature-dependent is not their modest gating charge, but rather the enormous entropic change and its compensation by a large change in enthalpy, rendering small values for the net free energy change (ΔG) that can be easily overcome to transit between the closed and open state. It would not be unreasonable to state that having such small voltage dependence allows TRP channels to be sensitive to other stimuli. A strongly coupled voltage-sensor would lock the channel on the closed state at non-permisive potentials, therefore, different protein rearrangements originated by the activation of alternative sensor modules (e.g. temperature or agonist binding) will not be translated on pore openings. Therefore a weak-voltage-dependence is likely necessary for the polymodal regulation observed for TRP channels. This is explained by a relation for the change in P_o with temperature presented by Latorre et al. [23]:

$$\frac{\partial P_O}{\partial T} = \frac{\Delta H - zFV}{\frac{RT^2}{4}\cosh^2\left(\frac{\Delta G}{2RT}\right)} \tag{28.7}$$

This function tends to zero when $\Delta G \to \infty$, and tends to $4(\Delta H\text{-}zFV)/(RT^2)$ when $\Delta G \to 0$. As thermo-TRP channels exhibit relatively small ΔG values during

the closed-open transition, from this relation is clear that to ensure large changes in P_o, the only value that has to be large is ΔH [23]. This, in turn, implies that the entropy term has to be large in order to obtain a small change in the free energy. The absolute magnitude for the transitional enthalpy and entropy during TRP channel temperature-dependent gating has been estimated to be about 50–300 kcal/mol [20, 21, 31].

A reasonable explanation for the effect of temperature on thermo-TRP channels would be the presence of a temperature-sensing domain suffering large structural rearrangements upon temperature changes. Another case in which a highly temperature-dependent process takes place only because the large enthalpic change is compensated by a large entropic change keeping ΔG relatively small is protein denaturation [32, 33]. While heat denaturation arises from the fact that disordered conformations become accessible upon heating, cold denaturation is usually attributed to a weakening of the hydrophobic effect caused by the temperature-dependent structure of bulk water [34–36]. It has been previously proposed that the large negative entropy (about –200 kcal/mol; [21, 31]) observed during the temperature-activation process of TRPM8 channels may be linked to a net loss of hydrophobic interactions in the closed-to-open temperature-dependent transition [21, 23]. Therefore it is possible that during the process of opening, an exposure of aliphatic and aromatic groups to bulk water takes place.

Using fluorescence resonance energy transfer, in combination with electrophysiological recordings, and site directed mutagenesis, Yang et al. [31] showed that conformational rearrangements of the turret, a structure located on the external mouth of the pore domain (Fig. 28.3), are essential for temperature-dependent activation [31]. This result is somewhat supported by recently published results in which pore mutations near the turret region either ablate or severely affect temperature dependent gating [37–39]. However, the results presented by Yang et al. have been severely questioned and this controversy remains to be solved [48, 49].

28.5 Allosteric Models and Thermo-TRP Channel Activation

A contentious issue regarding voltage activation of TRMP8 and TRPV1 is the strictness of coupling between the voltage sensor and the pore gate. A strict coupling implies that whenever the voltage sensor(s) is (are) activated the channel will open, and this basic assumption underlies two-state models of channel activation. On the other hand, allosteric coupling assumes that activation of voltage sensor(s) does not lead directly to channel opening but rather to an increase of the open state probability. These two models have profound differences in their mechanistic interpretations and the prediction of channel behavior under certain conditions. Allosteric coupling has been proposed and demonstrated for several voltage-activated channels, remarkably the hyperpolarization- and cyclic nucleotide-gated (HCN) channels [43] and the high conductance voltage- and calcium-activated potassium (BK) channel [44]. A common feature of these channels, also shared by thermo-TRP channels, is the activation by voltage *and* another

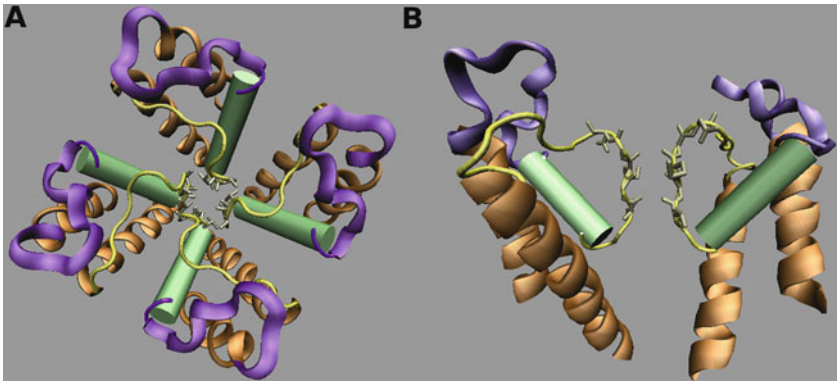

Fig. 28.3 TRP channels Pore Turret has been suggested as an structural part of the temperature activation mechanism. (**a**) An upper view of the TRPV1 pore. Purple shade ribbon highlights the location of the pore turret segment mutated by Yang et al. 2010. (**b**) A side view of the TRPV1 pore just showing two subunits, highlighting the location of the turret (purple ribbon) with respect to the selectivity filter (yellow sticks), and the pore helix (green cartoon). TRPV1's pore homology model was built using the crystal structure of Kv1.2 (PDB:2A79) as template [40]. The assembly of the system and figures employed VMD

stimulus (e.g. agonist binding). Also, their voltage dependence is much lower than that of typical voltage-activated channels, reflected in shallower conductance vs. voltage relationships.

One structural condition for a channel to be thought as allosterically activated is that its sensors have to be different protein domains. Although different domains have been proposed as part of the voltage and temperature sensors [27, 31], they are far from being well described. A modular structure has been proposed for thermo-TRP channels [2, 40], with evidence suggesting the existence of different activation domains for voltage, temperature, and PIP_2 in TRPM8. However, this is not sufficient as evidence for allosteric gating, as the classical (e.g., Shaker related) voltage-activated channels also have a modular structure [41] but their limiting slope for voltage-dependent activation suggests a strict coupling between the voltage sensors and the gate [17].

An allosteric gating scheme can be thought as two or more unconnected state transitions whose rate (and equilibrium) constants are modified depending on the state of the other equilibria. Figure 28.4a depict the simplest example of a 2-state pore allosterically gated by a 2-state voltage sensor. When the voltage sensor is in the resting state, (C_R or O_R), the channel opens with a (probably very low) equilibrium constant L. When the sensor is in the activated state (C_A or O_A), this equilibrium constant is multiplied by an allosteric factor D, thus incrementing the probability of the open state. Conversely, when the pore gate is in the open state, the equilibrium constant for voltage sensor activation (J) is multiplied by the same factor D, thus fulfilling the microscopic reversibility principle.

Additional gating mechanisms of the channel, such as inactivation or other sensors for different stimuli, can be added either as additional states or as another

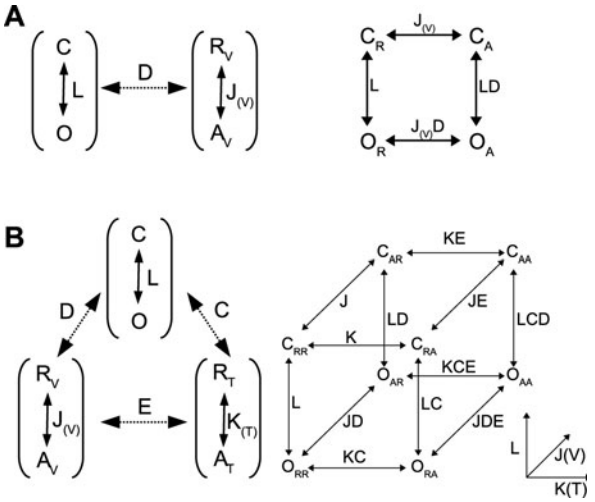

Fig. 28.4 Allosteric models for channel activation. (**a**) allosteric activation by voltage. *Left,* two independent equilibriums that interact allosterically. R_V and A_V represent the resting and activated state of the voltage sensor. C and O are the closed and open conformation of the pore gate. Only the equilibrium constant for voltage sensor activation, J, is voltage dependent. *Right*, when the combinations of the two equilibriums are considered, a 4-state diagram results. Cn and On represent closed and open conformations of the pore, respectively, with the voltage sensors in the 'n' state. The probability of being in any open state is: $P_O = \left(1 + \frac{1+J}{L(1+JD)}\right)^{-1}$ (**b**) Allosteric activation by voltage and temperature. A third equilibrium is added where R_T and A_T represent the resting and active conformations of the temperature sensor, respectively. Two new allosteric factors are introduced (C and E) to account for the possible interactions between structures. The expanded representations needs to be rendered in 3D (right). In this scheme, the probability of being in any open state is: $P_O = \left(1 + \frac{1+J+K+JKE}{L(1+JD+KC+JKCDE)}\right)^{-1}$

equilibrium that will interact allosterically. Figure 28.4b depicts the model proposed by Brauchi et al. [21] for the activation by voltage and temperature, both in its allosteric depiction and its expanded form that takes the shape of a cube. The number of states of the channel as a whole, as depicted in Fig. 28.4b, grows quickly when other sensors for stimuli such as temperature or chemical agonists are included, and soon cannot be easily depicted in 2 dimensions. For instance, Matta and Ahern [42] expanded the model to include activation by ligands and the expanded form needs four dimensions to be drawn with all the possible transitions [42]. However, when seen in its allosteric depiction (Fig. 28.4b, left), it is realized that a relatively small number of parameters are required to describe the proposed intramolecular interactions with simplicity. Also, the probability of the open state can be predicted with straightforward mathematical expressions and kinetic modeling can be simplified.

The experimental behavior of a channel predicted by an allosteric gating scheme has several features that have been described and extensively studied in BK and HCN channels [43, 44]. They include the existence of several open states evidenced as multiple mean open times or multiple exponentials in current relaxations, and

the movement of gating charges between open states. However, the most notorious prediction is that there will be a minimum open probability that can be reached by hyperpolarization (given by the equilibrium constant L) and a maximum open probability upon depolarization (given by the product LD). Depending on the values of L and D this may or may not be evident from macroscopic current recordings. For instance, the BK channel shows a maximum P_O almost equal to 1 ($LD \gg 1$) and there is no minimum open probability because the equilibrium constant L has a weak voltage dependence that has been shown to be independent of the voltage sensors. Still, this phenomenon is evident only at $P_O < 10^{-6}$ [45].

When a third sensor is added to an allosteric gating scheme, its activation should modify the maximum P_O achievable by voltage sensor activation (Fig. 28.5). This led Brauchi et al. [21] to postulate an allosteric gating scheme for TRPM8, to account for recordings demonstrating that cold increases the maximum P_O. This was not noticed by Voets et al. [20] but they based their analysis and fitting only on macroscopic current recordings while Brauchi et al. measured actual P_O values with single channel recordings. Later, Matta and Ahern [42] reported the same for both TRPM8 and TRPV1 (in the case of TRPV1, heating increases maximum P_O) also with the support of single channel recordings. Moreover, Matta and Ahern showed that the minimum P_O of both channels is dramatically increased by the presence of chemical agonists (menthol for TRPM8, capsaicin and resiniferatoxin for TRPV1). The analysis presented by Voets et al. [27] for the activation of TRPM8 by agonists led them to propose an allosteric gating scheme for the effect of menthol, while retaining a 2-state mechanism for the activation by voltage (implying a strict coupling). Again, they did not determine actual values of P_O and based their conclusions on normalized macroscopic G/V curves.

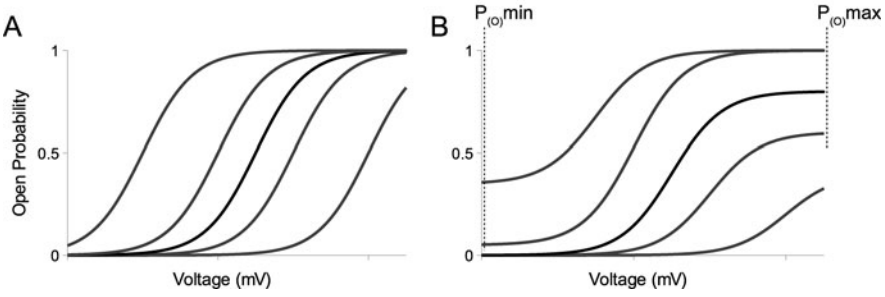

Fig. 28.5 Different behavior of strict coupling versus allosteric coupling. (**a**) A two-states model for voltage activation (strict coupling between sensor and gate) will always have maximum $P_{(O)} = 1$ and minimum $P_{(O)} = 0$. Another agonist or activator can only change the $V_{1/2}$ of the G/V relationship shifting the curve to left or right. (**b**) On the other hand, with allosteric activation by voltage minimum and maximum $P_{(O)}$ is not restricted to 0 and 1. In the model depicted in Figure 28.4b, when the temperature sensor is resting ($K_{(T)} << 1$) the minimum $P_{(O)}$ (when $J_{(V)} << 1$) is $(1+1/L)^{-1}$ and the maximum open probability that can be reached by depolarization ($J_{(V)} >> 1$) is $(1+1/LD)^{-1}$. On the contrary, when the temperature sensor is fully active ($K_{(T)} >> 1$) the minimum $P_{(O)}$ is $(1+1/LC)^{-1}$ and the maximum is $(1+1/LCD)^{-1}$. The values of L, C and D will determine whether these probabilities are significantly different from 0 or 1 for a macroscopic current analysis, but nevertheless they should always be measurable with single channel recordings

The debate between strict and allosteric coupling has other arguments than maximum and minimum open probabilities; Latorre et al. [2] showed that activation by voltage of TRPM8, despite being very fast, shows a brief delay after the onset of a depolarizing pulse. Moreover, this delay is shortened by a depolarizing pre-pulse. This phenomenon, known as the Cole-Moore effect, cannot be observed in a two-state scheme for voltage activation and is a clear indication of multiple closed states [46]. More evidence supporting the existence of multiple open and closed states comes from single channel dwell time analysis in the TRPV1 channel [28] and the bursting nature of the single channel activity [23].

Thus, it appears difficult to sustain a strictly coupled 2-state mechanism for voltage-activation of thermo-TRP channels. Though it has a tempting simplicity that reproduces very well the macroscopic G/V relationships and their modulation by temperature and agonists, the debate between strict and allosteric coupling of the voltage sensor and the pore gate goes beyond the best fit of the experimental data. If the purpose of the model is to reproduce the channel behavior (to be used in a conductance-based neuron model, for instance) then the choice will be the simplest model. But when it comes to draw mechanistic or structural conclusions, then the model should be challenged at every thinkable condition, even at the extremes. Evidence for allosteric coupling has arisen even in the family of Kv channels, thought to have the strictest coupling between the voltage sensor and the pore gate [47], and it would not be surprising that allosteric coupling is the norm that in classical voltage-activated channels was tuned to an almost strict coupling. However in channels activated by more than one stimuli allosteric coupling may be more convenient, because the dynamic range for the effect of each agonist can be independently tuned.

28.6 Final Words

In summary, it is clear that different stimuli act separately to modulate thermo-TRP channel activity therefore these channels act as signal integrators that sum the energies imparted by voltage, temperature, ligand binding, and pH to open the channel pore. To fulfill their role as integrators a loose pore is needed, since strict coupling between any of these stimuli to the gate will probably impede the others to open the channel pore.

Acknowledgments SB work is funded by FONDECYT grant 11070190. PO work is funded by FONDECYT 11090308 and DIPUV 51/2007 (U. de Valparaiso) grants. We thank Dr. H. Kurata for his critical reading of the manuscript.

References

1. Ramsey IS, Delling M, Clapham DE (2006) An introduction to TRP channels. Annu Rev Physiol 68:619–647
2. Latorre R, Brauchi S, Orta G, Zaelzer C, Vargas G (2007) Thermo TRP channels as modular proteins with allosteric gating. Cell Calcium 42:427–438

3. Latorre R, Zaelzer C, Brauchi S (2009) Structure-functional intimacies of transient receptor potential channels. Q Rev Biophys 42:201–246
4. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389: 816–824
5. Hodgkin AL, Huxley AF (1952) A quantitative description of membrane current and itsapplication to conduction and excitation in nerve. J Physiol 117:500–544
6. Bezanilla F (2000) The voltage sensor in voltage-dependent ion channels. Physiol Rev 80:555–592
7. Long SB, Tao X, Campbell EB, MacKinnon R (2007) Atomic structure of a voltage-dependent k+ channel in a lipid membrane-like environment. Nature 450:376–382
8. Noda M, Shimizu S, Tanabe T, Takai T, Kayano T, Ikeda T, Takahashi H, Nakayama H, Kanaoka Y, Minamino N et al (1984) Primary structure of electrophorus electricus sodium channel deduced from cDNA sequence. Nature 312:121–127
9. Seoh SA, Sigg D, Papazian DM, Bezanilla F (1996) Voltage-sensing residues in the S2 and S4 segments of the shaker k+ channel. Neuron 16:1159–1167
10. Jiang Y, Lee A, Chen J, Ruta V, Cadene M, Chait BT, Mackinnon R (2003) X-ray structure of a voltage-dependent k channel. Nature 423:33–41
11. Swartz KJ (2008) Sensing voltage across lipid membranes. Nature 456:891–897
12. Murata Y, Iwasaki H, Sasaki M, Inaba K, Okamura Y (2005) Phosphoinositide phosphatase activity coupled to an intrinsic voltage sensor. Nature 435:1239–1243
13. Sasaki M, Takagi M, Okamura Y (2006) A voltage sensor-domain protein is a voltage-gated proton channel. Science 312:589–592
14. Ramsey IS, Moran MM, Chong JA, Clapham DE (2006) A voltage-gated proton-selective channel lacking the pore domain. Nature 440:1213–1216
15. Armstrong CM, Bezanilla F (1973) Currents related to movement of the gating particles of the sodium channels. Nature 242:459–461
16. Schneider MF, Chandler WK (1973) Voltage dependent charge movement of skeletal muscle: a possible step in excitation-contraction coupling. Nature 242:244–246
17. Schoppa NE, McCormack K, Tanouye MA, Sigworth FJ (1992) The size of gating charge in wild-type and mutant shaker potassium channels. Science (80) 255:1712–1715
18. Sigg D, Bezanilla F (1997) Total charge movement per channel. the relation between gating charge displacement and the voltage sensitivity of activation. J Gen Physiol 109: 27–39
19. Aggarwal SK, MacKinnon R (1996) Contribution of the S4 segment to gating charge in the shaker K+ channel. Neuron 16:1169–1177
20. Voets T, Droogmans G, Wissenbach U, Janssens A, Flockerzi V, Nilius B (2004) The principle of temperature-dependent gating in cold- and heat-sensitive TRP channels. Nature 430:748–754
21. Brauchi S, Orio P, Latorre R (2004) Clues to understanding cold sensation: thermodynamics and electrophysiological analysis of the cold receptor trpm8. Proc Natl Acad Sci USA 101:15494–15499
22. Karashima Y, Talavera K, Everaerts W, Janssens A, Kwan KY, Vennekens R, Nilius B, Voets T (2009) TRPA1 acts as a cold sensor in vitro and in vivo. Proc Natl Acad Sci USA 106:1273–1278
23. Latorre R, Vargas G, Orta G, Brauchi S (2007) Voltage and temperature gating of thermoTRP channels. In: Liedtke W, Heller S (eds) TRP ion channels function in sensory transduction and cellular signaling cascades. CRC Taylor & Francis, London, pp 287–302
24. Gaudet R (2008) P channels entering the structural era. J Physiol 586:3565–3575
25. Moiseenkova-bell VY, Stanciu LA, Serysheva II, Tobe BJ, Wensel TG (2008) Structure of TRPV1 channel revealed by electron cryomicroscopy. Proc Natl Acad Sci USA 105:7451–7455
26. Almers W (1978) Gating currents and charge movements in excitable membranes. Rev Physiol Biochem Pharmacol 82:96–190

27. Voets T, Owsianik G, Janssens A, Talavera K, Nilius B (2007) TRPM8 voltage sensor mutants reveal a mechanism for integrating thermal and chemical stimuli. Nat Chem Biol 3:174–182
28. Liu B, Hui K, Qin F (2003) Thermodynamics of heat activation of single capsaicin ion channels vr1. Biophys J 85:2988–3006
29. Iggo A (1969) Cutaneous thermoreceptors in primates and sub-primates. J Physiol 200: 403–430
30. Lecar H, Ehrenstein G, Latorre R (1975) Mechanism for channel gating in excitable bilayers. Ann NY Acad Sci 264:304–313
31. Yang F, Cui Y, Wang K, Zheng J (2010) Thermosensitive trp channel pore turret is part of the temperature activation pathway. Proc Natl Acad Sci USA 107:7083–7088
32. Privalov PL (1989) Thermodynamic problems of protein structure. Annu Rev Biophys Chem 18:47–69
33. Privalov PL. Thermodynamics of protein folding. 1997; 447–474
34. Gursky O, Atkinson D (1996) High- and low-temperature unfolding of human high-density apolipoprotein a-2. Protein Sci 5:1874–1882
35. Privalov PL, Griko YV, Venyaminov S, Kutyshenko VP (1986) Cold denaturation of myoglobin. J Mol Biol 190:487–498
36. Griko YV, Venyaminov S, Privalov PL (1989) Heat and cold denaturation of phosphoglycerate kinase (interaction of domains). FEBS Lett 244:276–278
37. Myers BR, Bohlen CJ, Julius D (2008) A yeast genetic screen reveals a critical role for the pore helix domain in TRP channel gating. Neuron 58:362–373
38. Grandl J, Hu H, Bandell M, Bursulaya B, Schmidt M, Petrus M, Patapoutian A (2008) Pore region of TRPV3 ion channel is specifically required for heat activation. Nat Neurosci 11:1007–1013
39. Grandl J, Kim SE, Uzzell V, Bursulaya B, Petrus M, Bandell M, Patapoutian A (2010) Temperature-induced opening of TRPV1 ion channel is stabilized by the pore domain. Nat Neurosci 6:708–714
40. Brauchi S, Orta G, Mascayano C, Salazar M, Raddatz N, Urbina H, Rosenmann E, Gonzalez-Nilo F, Latorre R (2007) Dissection of the components for pip2 activation and thermosensation in trp channels. Proc Natl Acad Sci USA 104:10246–10251
41. Yellen G (1998) The moving parts of voltage-gated ion channels. Quart Rev Biophys 31:239–295
42. Matta JA, Ahern GP (2007) Voltage is a partial activator of rat thermosensitive TRP channels. J Physiol 585:469–482
43. Altomare C, Bucchi A, Camatini E, Baruscotti M, Viscomi C, Moroni A, DiFrancesco D (2001) Integrated allosteric model of voltage gating of HCN channels. J Gen Physiol 117:519–532
44. Horrigan FT, Aldrich RW (2002) Coupling between voltage sensor activation, Ca2+ binding and channel opening in large conductance (BK) potassium channels. J Gen Physiol 120: 267–305
45. Horrigan FT, Cui J, Aldrich RW (1999) Allosteric voltage gating of potassium channels i. mslo ionic currents in the absence of Ca2+. J Gen Physiol 114:277–304
46. Colquhoun D, Hawkes AG (1981) On the stochastic properties of single ion channels. Proc R Soc Lond B Biol Sci 211:205–235
47. Lu Z, Klem AM, Ramu Y (2002) Coupling between voltage sensors and activation gate in voltage-gated K+ channels. J Gen Physiol 120:663–676
48. Yao J, Liu B, Qin F (2010) Pore turret of thermal TRP channels is not essential for temperature sensing. Proc Natl Acad Sci USA 107(32):E125
49. Yang F, Ciu Y, Wang K, Zheng J (2010) Reply to Yao et al.: is the pore turret just thermoTRP channels' appendix? Proc Natl Acad Sci USA 107(32):E126–E127