TRP channels

What’s happening? Reflections in the wake of the 2009 TRP Meeting, Karolinska Institutet, Stockholm

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More than 150 participants from 25 countries gathered in Stockholm during 25th to 27th Sept 2009 to attend the meeting "TRP channels: from sensory signaling to human disease" and enjoyed an international, intensive and vibrant meeting. This meeting shed lights on the recent advances in this field of research in different sectors of biology and identified directions for future research and the areas where TRP channels could be used as potential targets for prevention and treatment of human diseases. The participants of this meeting shared their recent largely unpublished data, state-of-the-art techniques and their critical views which would push research in this field forward in the new decade. Another major outcome of this meeting was the realization that extensive work remains to be done to develop the necessary tools and enhance the quality of research in this area so that the prevailing controversies can be resolved. In this report we summarize the latest scientific excitement, some critical issues, as well as some future directions for research that were addressed and discussed in this meeting.

Background

The Transient Receptor Potential (TRP) family of ion channels is named after the Drosophila melanogaster ion channel that is mutated in the trp gene. This mutant was first reported by Minke.1 Subsequent works by Montell et al. established a solid foundation for this remarkable functional family of ion channels.2-4 TRPs are non-selective cation channels involved not only in a variety of physical and chemical sensation but also in cell signaling, development, and in many pathological conditions.5 Since their discovery, the TRP channels have been investigated by many researchers and these channels have received attention both in basic research as well as in clinical research. The TRP meeting that is the topic of this report took place in the Karolinska Institutet, Stockholm, Sweden from the 25th to the 27th Sept 2009 (Fig. 1). The meeting was organized by Md. Shahidul Islam and Craig Montell. Details of this meeting are available from this site http://trp.islets.se from where the abstracts can be downloaded. As the interest in the TRP channels is growing in an exponential rate, summarizing diverse aspects of the TRP channels discussed in this meeting is a difficult task. Here, we have tried to identify some of the highlights of this meeting and have discussed some of the crucial issues.

Scientific Highlights

Role of TRP channels in thermosensation. The abilities of sensing and differentiating environmental temperatures are important functions performed by almost all organisms. However, different organisms handle this fundamental function by different mechanisms and by recruiting different sets of molecules that can sense different temperatures.6-7 In this context, TRP channels are particularly interesting since some TRP channels can be activated by different temperatures.8-10 Such thermo-sensitive TRP channels are quite selective for different temperature ranges in which they can be activated.11 Such temperature ranges cover from noxious cold to noxious heat. It has, therefore, been speculated that the thermo-sensitive TRP channels are responsible for thermosensation and for the maintenance of the normal body temperature.12-14 However, the molecular mechanisms by which these thermo-sensitive TRP channels recognize different temperatures remain unanswered.15,16 Recent electrophysiologically and knockout studies show that the molecular mechanism of thermosensation can not be solely dependent on the TRP channels. Molecules other than TRP channels are also involved in this process in a more complex manner than it was thought before.17-21 In fact many of the animals lacking TRP channels show normal thermosensation.22,23 On the other hand, some animals genetically lacking molecules other than the TRP channels also manifest altered thermosensation. For example mice lacking P2Y2 receptor reveals abnormal thermal nociception.24 Taking together, these studies indicate that a complex network is operating behind the molecular mechanisms of thermosensation.

Previously it has been shown that swapping the C-terminal cytoplasmic domain of a cold receptor (TRPM8) with a hot receptor (TRPV1) makes the cold receptor recognize high temperature and vice versa.25 This indicated that the C-terminal...
cytoplasmic domain is important for the thermosensitivity/thermoactivation. It has been proposed that in case of the thermosensitive TRP channels, the thermosensitivity is a function of their voltage sensitivity.26-29 Though a lot of investigations have sensitive TRP channels, the thermosensitivity is a function of the activation. It has been proposed that in case of the cytoplasmic domain is important for the thermosensitivity/thermoactivation.

Features of thermal conductance state.

C = f(temperature) ⇋ O_short ⇋ O_long

This model suggests that the 1st step is brief in duration, temperature-sensitive and it triggers the thermo sensor. In contrast, the second step is insensitive to temperature but it stabilizes the conductance state.

Interestingly, they found that in case of a triple mutant of rat TRPV1 (L268K + N652T + Y653T), the long-openings are completely lost. In this regard it is noteworthy that two of these mutants are located between the pore-loop and the 6th transmembrane domain (at the extra-cellular site) of TRPV1. However, the results they obtained with TRPV1 are different from what they found previously with TRPV3. In case of TRPV3, they found that the mutations that are required for heat activation are located in the pore region.31 So it is possible that the outer part of the TRPV1 pore can stabilize the opening. AllostERIC interaction with the intracellular proteins and/or extra-cellular matrix proteins can also affect the pore openings.

Rhodopsin as heat-sensor. This meeting witnessed an alternative hypothesis proposed by Craig Montell regarding the heat-sensing of TRP channels. His co-workers showed that Drosophila melanogaster larvae which carried mutation in TRPA could not distinguish temperature between 18°C (a preferable temperature) and 24°C (a non-preferable temperature).35 Their subsequent studies indicate that the signaling event involved in this PLC-mediated thermo-sensing cascade is very similar to the phototransduction cascade in drosophila. Moreover, they found that the same Gq proteins are involved in the phototransduction as well as in thermosensing events.36 In fact they found that drosophila larvae carrying mutation in Rhodopsin (P332G) actually mimics the phenotype of TRPA mutant in terms of thermo-sensation. This prompted researchers to explore if Rhodopsin, which is involved in light-sensing, is also involved in the process of thermo-sensation. They found that indeed Rhodopsin can sense heat and amplify the thermo-signaling events. Rhodopsin is regulated by the visual cycles, available concentration of the cis-trans isoforms in the retina and also by the enzymes that control retinal metabolism. In addition, several studies with Rhodopsin indicate that its structure-function relationship is complex and its sequence is highly variable in different organisms. The sequence analysis of Rhodopsin indicates the existence of divergent selection pressures where dim light may not be the prime selection force for molecular evolution.37 In this context, it is important to note that many primitive organisms growing in very dark conditions also possess Rhodopsin in their genome. Thus it might be possible that the primitive role of Rhodopsin is actually thermo-sensation rather than the photosensation, and that it might have evolved due to an adaptive pressure to distinguish different temperatures.

The study by Montell and his colleagues raises an important question: does Rhodopsin act as a primitive light sensor or primitive thermo sensor or both? This question also leads to a bigger question: Is there any dedicated and separate molecular machinery...
for sensation of physical stimuli (like temperature and light) or are there overlapping molecular mechanisms that are common for both thermosensation as well as for photosensation? In this context, a recent study from Ward et al. is relevant. C. elegans, a nematode that lives in soil is generally believed to lack photosensation. However, Ward et al. demonstrate that light stimuli elicits a negative phototaxis in this organism in a dose-dependent manner and that cyclic guanosine monophosphate (cGMP)-sensitive cyclic nucleotide-gated (CNG) channel is important for this behavior. This not only reveals conservation in phototransduction between worms and vertebrates but also suggests that animals living in dark environments without light-sensing organs should not be presumed to be light-insensitive. This also indicates that certain second messenger molecules may form a common pathway that can be useful for both phototransduction as well as for thermotransduction.

Recent studies regarding the “gating of thermo-sensitive TRP channels”. Understanding the gating mechanism of thermo-sensitive TRP channels remains a challenge. For example, TRPV1 is characterized by having gating mechanisms, which shows sensitivity to heat. TRPV1 has a very high temperature coefficient (Q10 value) of around 27. This value is much greater than 2, the standard Q10 value known for the majority of biochemical reactions including most of the ion-channel-activities. Experiments with rapid temperature jumps shows that TRPV1 activation is a relatively rapid event in which TRPV1-mediated currents reach a plateau in less than 500 ms. Conversely, it has been shown that lower temperature reduces the probability of channel opening. Both voltage-sensitivity and allosteric modulation have been proposed to be the underlying mechanism behind the gating of the thermo-sensitive TRP channels. For cold channels like TRPM8, it has been shown that menthol and cold-mediated activation takes place through shifts in its voltage-activation curve which causes the channel to open at physiological membrane potentials. Even some specific inhibitors exert their inhibitory effects by shifting the voltage dependence of TRPM8 activation towards more positive potentials.

In this context, results presented by Rosenbaum et al. indicated the importance of the S6 transmembrane segment of the thermosensor channels in gating mechanisms utilized by temperature and capsaicin. To locate the “gate” present in the thermosensor, they introduced Cys residues (point mutations) in S6 segment of the TRPV1 channel and characterized the accessibility of Cys residues to thiol-modifying agents. They demonstrate that the pore-forming S6 segment has helical structures and this segment is responsible for both capsaicin-binding and sensing of different temperatures. There are two constrictions present in the pore: one that impedes the access of large molecules and the other that hampers the access of smaller ions and constitutes an activation gate for these channels. In spite of a great deal of efforts invested by many groups, further understanding of the gating mechanism of the thermosensitive TRP channels remains elusive. One main difficulty is the very short time period that can be considered as the “open-state”, and the presence of multiple intermediate states.

In this regard, progresses made by David Julius and his group are impressive. They isolated a toxin called DkTx from the Earth Tiger Tarantula (Ornithoctonus broueni). The toxin belongs to the Inhibitor-Cystine-Knot (ICK) family of toxins and Vanilolotaxon (VaTx). DkTx can activate rat TRPV1 channel but cannot activate Xenopus TRPV1 and Kv1 (a voltage-sensitive cation channel). Thus, this toxin offers a possibility to perform the chimeric study between rat TRPV1 and Xenopus TRPV1. This study shows that rat TRPV1 mutant A657P is insensitive and Xenopus TRPV1 mutant P663A is sensitive to this toxin. They also found that the recombinant toxin or even a part of this toxin is as effective as the purified toxin. The toxin binds to the pore region of the TRPV1 and the effect of this toxin can be blocked by the ruthidium red. Interestingly, this toxin can be expressed as Histidine tag and the tagged toxin can also be used to pull down/purify the full-length TRPV1. However, the most interesting aspect of this toxin is its effect on the TRPV1 channel. Apparently, the toxin activates TRPV1-expressing cells for a long time, even after the washout, as observed from the Ca2+-imaging assays. Apparently, the toxin fixes rat TRPV1 channel in the open stage for a long time. Thus, the toxin seems to be a conformation-sensitive reagent and/or it stabilizes the “open-stage” of the channel. Therefore, this toxin appears to be an ideal reagent to study the properties of TRPV1 at its “open-state”.

Does membrane structure and composition influence TRP channels? TRP channels are embedded in the lipid bilayer but whether or not membrane environment influences TRP channels is unclear. It is, however, known that some lipids can specifically activate or inhibit some of the TRP channels. For example, sphingolipids and their derivatives can activate TRPM3 and TRPC1. Human TRPM3 is inhibited by cholesterol (abstract # 27, Navlor et al.). Specific lipid binding proteins can also act as specific subunits of TRP channels and can thereby regulate the channel activity to a variable extent. For example, Pirt, a phosphoinositide-binding protein, functions as a regulatory subunit of TRPV1. Even, enzymes that regulate lipid metabolism are known to regulate some TRP channels. For example, phospholipases, PKC and sphingosine kinase are known to modulate the Ca2+-entry via TRP channels. Effect of membrane composition on the TRP channels is also evident from the fact that altering the membrane composition, either by depletion or addition/saturation of membrane cholesterol affects the properties of some TRP channels and/or physiological functions mediated by these channels. The notion that membrane lipid composition and membrane structure (membrane microdomain curvature) can affect TRP channels is favored by many. Indeed, results from Minke’s lab suggested that the “lipid packing” of the membrane as well as the “channel-plasma membrane interface” might be important for the activation of TRP channels (abstract # 41, Minke B). They propose that PLC, which converts PIP2 to diacylglycerol (DAG), induces alteration in the membrane structure and forms a bended curvature in the small lipid membrane microdomain regions. This is due to the fact that DAG is smaller in structure when compared to the PIP2 due to the loss of some head portion. Thus, PLC-mediated changes in the TRP activity might be due to the direct effects of changes in...
induced by TRPC1 is regulated by the presence or absence of TRPC1 in the lipid raft.\(^{67,68}\)

Another example of regulation of TRP channels by lipid structures is TRPM8, which is mainly localized in the cholesterol-rich lipid rafts.\(^{54}\) TRPC3, another TRP channel shows enhanced membrane expression in response to cholesterol. Graziani et al. demonstrated that cholesterol loading activates cellular TRPC3 conductance.\(^{69}\) This cholesterol-induced membrane conductance exhibited a current-to-voltage relationship similar to that observed upon PLC-dependent activation of TRPC3 channels. Shoeb et al. also detected three different TRP channels (TRPC1, TRPC3 and TRPC6) in the sperm membrane rafts (abstract # 6, Shoeb et al.). As lipid rafts have a distinct lipid composition and structure, it is plausible that TRP channels behave differently when they are located outside the lipid rafts and/or when the composition of the rafts are changed. Indeed, such differences have been observed, at least for TRPM8 and TRPV1.\(^{34,54,55,70}\) It has been noted that menthol- and cold-mediated responses of TRPM8 are potentiated when the lipid raft association of the channel is prevented.\(^{64}\) Disruption of lipid rafts shifts the TRPM8 activation threshold to a warmer temperature. These results suggest that the different lipid membrane environments affect the cold sensing properties of TRPM8.

In the case of TRPV1, cholesterol depletion results in significant reduction in the amplitude of the capsaicin current.\(^{55}\) Considering the similarities among these TRP channels, it is tempting to speculate that lipid environment modulates other TRP channels, particularly the TRP channels involved in thermosensation and/or mechanosensation. Not all TRP channels have been tested for their sensitivity to mechanical stimulation, and the number of TRP channels where an ionic conductance can be activated by mechanical force is low. Among all TRP channels, only the TRPV4 has been reported to mediate \(Ca^{2+}\) influx in response to mechanical stimulation. Considering the fact that “mechanical stimulation” is not a well-characterized and well-standardized protocol that can be employed by all investigators, it is at present difficult to predict if and to what extent other TRP channels can respond to mechanical stimulation. Identification of TRP channels that can be activated by mechanical force is of clinical importance since many of the TRPs (for example different TRPC channels) are present in the cardiac and pulmonary systems and they have been implicated in mediating cardiac diseases.\(^{71}\)

In this context, two groups have explored if mechanical pressure can activate TRPC5 and TRPC6 channels.\(^{2,73}\) Gomis et al. demonstrated that application of water pressure through patch pipette induces membrane stretch which activates TRPC5. Interestingly they found that reducing the phosphatidylinositol 4,5-bisphosphate (PIP\(_2\)) levels in the membrane actually abolishes the hypotonicity-evoked activation of TRPC5. Gudermann et al. explored if direct membrane stretch can activate TRPC6.\(^{73}\) They reported that application of pressure on the patch containing TRPC6 does not activate the channel. It has been proposed that stretching and/or alteration of membrane structure is

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**Figure 2.** Membrane alteration-induced activation of TRP channels. (A) Phospholipase C activation converts membrane lipids to inositol 1,4,5-triphosphate (IP\(_3\)) and 1,2-diacyl glycerol (DAG). (B) This conversion induces shortening of certain membrane lipids and thus alters the lipid packing at the inner surface of the membrane micro-domain. (C) Changes in the membrane curvature and in the lipid-TRP channel interactions result in conformational changes leading to the opening of TRP channels. (D) Change in the membrane curvature can also affect the “open channel block” by forcefully removing the metal ions from the channels. This allows further opening of the TRP channels.

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the membrane as well as some indirect effects. These changes include interaction at the membrane-channel interface, the local bending of the membrane, the curvature of the lipid membrane and also the electrostatic properties of the lipid bilayer (Fig. 2).\(^{61,62}\) Furthermore, the alteration of the membrane composition may lead to the removal of open channel block (OCB), a process by which ions bound to the inner side of a channel get released.\(^{57,58}\)

A number of studies have indicated that TRP channels are also localized in specific regions of the plasma membrane micro-domain commonly known as lipid rafts that scaffold several other signaling complexes.\(^{47,63-65}\) For example, TRPM7 in vascular smooth muscle cells is localized in the fraction that corresponds to the caveolae fraction. Immunofluorescence analysis also confirms that TRPM7 co-localizes with flotillin-2, a marker of lipid rafts.\(^{63}\) At very low concentrations, free cholesterol can alter \(Ca^{2+}\) entry via TRPC1, in cholesterol depleted polymorphonuclear neutrophils.\(^{64}\) It has been observed that TRPC1 redistributes into the raft fractions in response to cholesterol. Localization of TRPC1 in the lipid raft in response to ketocholesterol has been observed in THP-1 monocytes.\(^{65}\) Another report indicates that in the cell, the store-operated \(Ca^{2+}\) entry process by which ions bound to the inner side of a channel get released.\(^{57,58}\)
involved in the activation of mechanosensitive TRP channels.74 How mechanical force actually activates these mechanosensitive TRP channels and results in conductance is an important question that remains to be answered.

TRP channels as novel targets for hormone actions. TRP channels have been linked to the development of many pathological conditions including some disease syndromes.75 In this respect, only a few new studies have demonstrated that some hormones indeed regulate several TRP channels both directly, and indirectly by altering their expression level.76 For example, naturally occurring steroid pregnenolone sulfate activates TRPM3 directly.77,78 In contrast, erythropoietin enhances Ca2+-influx in human erythroid cells by indirect activation of TRPC3 channel.79 Although the complex network of different hormones acting on different TRP channels has not yet been explored, at present it appears that TRPV1 is a common target for many hormones. For example, prolactin,80 endothelin81 and neurokinin82 exert regulatory effect on TRPV1. Even the hunger-inducing hormone ghrelin potentiates TRPV1 in supraoptic magnocellular neurones, as the effect of this hormone on miniature excitatory postsynaptic currents is attenuated in TRPV1 knockout mice (trpv1−/−).83 In many cases, both development of the hormone producing tissues and the secretion of hormones are also regulated by TRP channels. Thus, a multidirectional regulation of TRP channels and hormonal actions are important as such cross-talks regulate diverse functions like Ca2+ re-absorption, aging and many other biological processes. In the following paragraphs we have discussed some of the cross talks that have attracted attention in the recent years.

Estrogen and other steroids. According to the classical view, estrogen acts as a transcriptional regulator and it alters the expression of many genes. It is noteworthy that the expression pattern of different TRP channels and steroid receptors show strong correlation. Indeed, a large number of studies indicate that estrogen and other steroids not only regulate the expression of TRP channels but also participate in cross-talks with many TRP channels. For example, expression of TRP4 in bovine aortic endothelial cells is significantly downregulated by application of β-estradiol.84 In contrast, estrogen enhances the expression of TRPV1 channel in c-fibres.85 Interestingly, capsaicin, the agonist of TRPV1 is also known to induce expression of androgen receptor in prostate LNCaP cells suggesting that TRP channels and steroid receptors can regulate each other and can form feedback regulatory loops.86 Such cross talks between estrogen (and other steroids) and TRP channels have clinical implications for several reasons. The expression of some TRP channels are altered in different cancers which are considered as hormone-receptor-positive cancers (these cancers rely on supplies of the steroid hormones to grow) and the channels have been linked to the progression of the cancer tissue to hormone-independence.87 For example, breast cancer epithelial primary culture (hBCE) reveals enhanced expression of TRPC3 and TRPC6.87 It is also reported that TRPM8-specific mRNA is overexpressed in prostate cancer. The expression of TRPM8 requires a functional androgen receptor as trpm8 gene seems to be androgen-responsive.88 This androgen mediated regulation of TRPM8 expression is important for the progression of prostate cancer towards androgen-independence.

Besides regulating the expression of TRP channels, steroids can also elicit some fast responses suggesting that the steroids may act directly on the TRP channels without involving gene transcription.77,78,89 In this respect, only a few studies have examined the mechanisms underlying the rapid action of steroids on TRP channel. These rapid effects of steroids on TRP channels appear to be both indirect and/or direct.

Shoeb et al. reported that the level of TRPC1 within the lipid raft in the capacitated sperm decreases after estrogen treatment (abstract # 6, Shoeb et al.). This suggests that a change in the membrane structure/fluidity may explain the rapid action of steroids. Using renal late distal convoluted tubules (DCT2s) and connecting tubules (CNTs), Praetorius et al. demonstrated that the intracellular free Ca2+ concentration is increased by progesterone (10−11 to 10−9 M) and estrogen (10−9 to 10−7 M) (Hofmeister and Praetorius, abstract # 71, Hofmeister et al.). Interestingly, this increased Ca2+-influx cannot be blocked by the application of classical estrogen receptor inhibitors e.g., mifepristone and fulvestrant, suggesting that the effect is not mediated by the classical estrogen receptors. Further studies suggested that this rapid action of estrogen is due to TRPV5, and that the localization of TRPV5 in the apical membrane is increased by estrogen treatment. This study suggests involvement of a complex cellular signaling cascade that leads to the rapid effect of estrogen. Cao et al. reported similar rapid estrogen signaling events that regulate Mg2+-homeostasis via TRPM6.89 Another exemplary effect of steroids on TRP channels has been presented by Yogi et al. (abstract # 37, Yogi et al.). They reported that aldosterone (100 μM) can stimulate annexin 1 and calpain, downstream targets of TRPM7. Similarly, Navlor et al. reported that TRPM3 can be stimulated by neurosteroid pregnenolone sulphate (Navlor et al. abstract # 27, Navlor et al.). Whether pregnenolone sulphate acts as a direct agonist for TRPM3 is, however, not clear. Interestingly this pregnenolone sulphate-evoked current via TRPM3 can be inhibited by cholesterol. Considering the complexity of steroid bio-synthesis and its degradation, and the diverse ways by which the steroids (also various lipids) can influence several TRP channels, in-depth understanding of these cross talks appears to be important.

Insulin. Previous studies demonstrated that some TRP channels e.g., TRPM2, TRPM6, TRPM7 and TRPC4 may be associated with the development of diabetes.90 Subsequent studies confirmed that many of the TRP channels are expressed in pancreatic β-cells, and insulin secretion can be regulated by these TRP channels.91-93 Even, genetic variants of some TRP channels have been associated with the development of diabetes.95 Recent studies also suggest that insulin can regulate TRPC3 and TRPV1.96,97 In this context, recent works from Nair et al. (abstract # 54, Nair et al.) suggest that TRPM7 is another TRP channel where insulin may have an effect. Using TIRF (Total Internal Reflection Fluorescence) microscope, they demonstrate that trafficking of TRPM7-containing vesicles towards plasma membrane was increased by insulin. They also demonstrate that
insulin-mediated stimulation of TRPM6 current occurred via PI3 kinase and Rac1 signaling.

Parathyroid hormone. It is well known that Parathyroid hormone (PTH) acts as a calcitropic hormone and stimulates Ca\(^{2+}\) re-absorption in bone and renal tissue.\(^98\,99\) However, the precise mechanism by which PTH mediates its actions remains partly unclear. As Ca\(^{2+}\) re-absorption needs involvement of Ca\(^{2+}\) channels, it is speculated that PTH hormones can act on diverse group of Ca\(^{2+}\) channels including some TRP channels. From previous studies it is known that TRPV5 and TRPV6 are expressed in kidney and are involved in Ca\(^{2+}\) re-absorption.\(^100\) Bindels et al. used calmodulin- and Epac-based FRET sensors and demonstrated that PTH-induced rapid Ca\(^{2+}\)-influx in cells that expressed TRPV5.\(^101\) The effect of full-length PTH was mimicked by a fragment of PTH (amino acid residues 1–31 of PTH but not 3–31 can activate TRPV5).

However, if PTH affects TRPV5 exclusively or also affects other TRP channels is not clear. In the absence of information about the tissue-wide expression profile of the PTH receptors and other TRP channels, it is difficult to predict if PTH can affect other TRP channels also. However, it appears that TRPV1 is not under the control of PHT, at least in the peripheral neuronal system. This is due to the fact that in nociceptive myelinated fibers, Parathyroid hormone 2 receptor is present but not in the neurons that express TRPV1.\(^102\)

Klotho. Klotho is a type I membrane glycoprotein that acts as an anti-aging hormone.\(^103\) Extracellular domain of Klotho has two tandem copies of a beta-glucuronidase-like sequence, which can be released as soluble forms after being cleaved by metalloproteinases such as ADAM10 and ADAM17.\(^103\) Bindels et al. demonstrated the role of Klotho in the regulation of TRPV5.\(^104\,110\) They also found that Klotho is expressed in high amount in kidney and co-localizes with TRPV5 (Fig. 3). Moreover, the Ca\(^{2+}\)-uptake is more in cells that are positive for TRPV5 and Klotho as compared to the cells that express only TRPV5. Interestingly, sugar residues seem to be important for TRPV5 activation. This is evident by the fact that salicydase, Enodo-F or Klotho treatment results in the activation of TRPV5.\(^109\,110\) Extracellular soluble Klotho induces deglycosylation of TRPV5. By this effect, it not only stimulates TRPV5 but also accumulates more TRPV5 in the plasma membrane. This results in a prolonged expression of TRPV5 at the plasma membrane. However, if and how TRPV5 is involved in the aging process is yet to be explored. Considering the complexity of hormone biology and the diverse manners by which the hormones influence multiple TRP channels, understanding these complicated networks is important for both basic research as well as for clinical purposes.

Physiological functions of endovanilloids. Most of the ligands (like Capsaicin, RTX, menthol etc.) which are routinely used to characterize the respective TRP channels are exogenous in nature. Therefore, response to these compounds does not reflect the actual regulation of the TRP channels in vivo. The compounds that are present in vivo and can activate TRP channels are the most important as such compounds may directly regulate cellular metabolism and physiology. Among many endogenous compounds, endovanilloids drew attention of many since it has been noted that some of the lipid derivatives containing vanilloid moieties can activate TRPV channels.\(^111\) At present, several endovanilloids (e.g., NADA, OLDA, Oleic acid, NAE) are known to activate TRPV1.\(^112\,113\) Recently, characterization of biochemistry, pharmacology and other regulatory properties of these endovanilloids have gained momentum. The presence of these molecules, their regulatory enzymes and metabolites have been detected and studied in a variety of tissues.\(^111\)

However, it has been noted that these endovanilloids are not very specific and are often active on a number of TRP channels. Endovanilloids are subjected to a complex metabolic regulation. The level of endovanilloids in any given tissue is highly variable and are regulated both by their rate of production and degradation. Synthesis of endovanilloids can be achieved by different pathways and are mainly regulated by GPCRs and/or several kinases. Recent reports indicate that different metabolites of these compounds can also exert diverse actions on several TRP channels. For example, omega-hydroxylated metabolites of NADA can activate TRPV1.\(^116\) However, the identity of all of the endovanilloids within a given tissue, their metabolic regulation and their target receptors are not well established. Due to all these factors and lack of sufficient studies, the physiological functions of endovanilloids are highly debatable. In this meeting, Zygmunt et al. demonstrated that 2-AG (2-Arachidonoylglycerol) is a potent TRPV1 agonist as it activates heterologously expressed TRPV1 in whole cell as well as in isolated membrane patches. They propose that the endovanilloids can act as both analgesic and nociceptive molecule depending on the tissue and the prevailing conditions. Considering that the endovanilloids are under control of metabolic regulation, these compounds can be useful for targeting different TRP channels for clinical purposes. However, in comparison to the usage of exogenous compounds like capsaicin, very little has been done to characterize the endovanilloids and more such studies are needed.

Heteromerization of TRP channels. Generally functional TRP channels are homotetramers by their organization. However, it has been suggested that depending on the degree of sequence homology and the expression pattern, some TRP channels can form heteromers as well. Indeed, some studies have demonstrated that different TRP channels can form hetero-tetramers and these heteromers are functional in terms of ionic conductivity.\(^117\,118\) At present, why and how some TRP channels form heteromers is
Interestingly, the level of melanin expression correlates with the TRPM1 expression in the melanocytes. For example, human melanocytes treated with micro-RNA targeting TRPM1 revealed reduced ratio of melanin with respect to the total protein. Since melanin production is dependent on tyrosinase enzyme, they also checked for the expression level of tyrosinase enzyme in these two different cell lines. They found that the level of tyrosinase is same in these cell lines indicating that the altered level of melanin is due to different level of TRPM1 expression. This work proposes that TRPM1 is an ion channel whose function is critical to normal melanocyte pigmentation. These investigators found that the dark skin has more TRPM1 expression as well as more melanin (with respect to the total protein) as compared to the light skin.

**TRPC3 is the “moon walker.”** Ester Becker screened some mouse mutants and found a line where the animals display motor as well as coordination defects. These animals fail to come back from the end of a static rod and also show loss of cerebellar Purkinje cells. Gene sequencing confirmed that the mutation is in the TRPC3 gene, particularly in exon 7 and cause a T (Thymine) to A (Adenine) mutation. Interestingly, the mutant animal moves backward in the same manner as the pop star Michael Jackson used to do in his famous dance sequence in moon walker album. That is why the mutant line (Mwk<sup>−/−</sup>) received its name as “moon walker”. The mutant animal also does not have uniform steps. The locomotive defect of the mutant animals can be explained by the fact that TRPC3 is expressed in Purkunjee cells at around P14 to P21 days, and mutant channel has a “gain of function”, which is toxic in nature. This is evident by the fact that Mwk<sup>−/−</sup> neurons have defective neurite outgrowth where dendritic branching is severely affected. This mutant line has been considered as a novel mouse model to study the cerebellar ataxia.

**TRPM7 and gene regulation.** The expression of the TRP channels is mostly tissue- and age-specific, indicating that their expression is highly regulated. Clapham et al. showed that there are other possible ways of regulation, e.g., TRP channels can also regulate the expression of other genes. For example, TRPM7 interacts directly with the DEDAF protein which belongs to “polycomb” group of proteins and thus is involved in the chromosomal and transcriptional regulation during development. Interestingly, nuclear localization of DEDAF is reciprocally regulated in the presence of TRPM7, which has a functional kinase at its C-terminal region. Therefore, in case of TRPM7 expression, DEDAF protein stays in the cytoplasm and when there is no expression of TRPM7, the DEDAF localizes to the nucleus.

**TR(I)Ps for smoking.** There are several molecular factors that determine the liking or disliking for smoking. Previous studies suggest that the nicotinic acetylcholine receptor is the main, if not sole, candidate which recognizes and senses nicotine. However, recent data suggest that several TRP channels can also respond to nicotine. Interestingly, effect of nicotine on TRP channels seems to be conserved throughout the animal kingdom. Xu et al. show that the TRP1 and TRP2 in *C. elegans* are involved in the nicotine sensitivity. In this regard, Nilius et al. also showed that nicotine directly activates TRPA1. While the response
to nicotine via the nicotinic acetylcholine receptor is fast, the response to nicotine via the TRPA1 is slow and sustained. These observations may also explain why nicotine patches produce some burning sensation, itching and skin irritation. In addition, Yang et al. predicted that TRPC3 might also be involved in nicotine response (Yang et al. abstract # 73). However, more studies are required in this area.

**Activation of TRP channels by gas.** TRP channels are specialized for the detection of taste, smell, pain, temperature, hormone and pheromone, and are involved in many behavioral as well as other complex functions including olfaction. In this regard, some recent results show that a number of volatile compounds act on TRP channels. In this meeting, Taylor-Clark et al. showed that TRPA1 can be activated by ozone ($O_3$). Similarly, nitric oxide (NO) can induce nociception in mice via TRPV1 and TRPA1. Interestingly, it has been noted that another gas, H$_2$S can also affect TRP channels. A recent study demonstrates that NaHS (donor of H$_2$S) can induce Ca$^{2+}$-influx in TRPA1-expressing CHO cells, but not in non-transfected cells. This result is in line with the fact that many cysteine modifying compounds exerts effects on TRP channels.

**TRP channels and stem cells.** Previous reports also suggest that in vitro expression of TRP channels can alter the cellular morphology as well as function, and may result in cell differentiation. By regulating Ca$^{2+}$ and other second messengers, the TRP channels may contribute to the survival and differentiation of cells and tissues, especially in the early embryonic stages. Indeed, expression of some TRP channels, like TRPV1 in the early embryonic stages has been reported, and the expression of several TRP channels co-relate with the differentiation of the stem cells. Being non-selective Ca$^{2+}$ channels, TRP channels can regulate the Ca$^{2+}$-signaling events including Ca$^{2+}$-waves. These TRP channel-mediated functions are critical for many developmental, neuronal and other functions. For example, TRPA1 is known to increase glutamate release from brain stem cells. TRPM7 is critical for the survival of bone marrow derived mesenchymal stem cells. In this meeting, Valerio et al. presented results which suggest that TRPV2 is expressed in neuroblastoma cells (abstract # 51). They demonstrated that expression of TRPV2 enhances the expression of astrocytic and neuronal markers like GFAP and β-tubulin III. This function of TRPV2 may be responsible for the development of Glioblasoma, a particularly invasive tumor in many patients. Consisting of the fact that expression of some TRP channels are cell- and tissue-specific and activation of particular TRP channels can be achieved by specific physical and/or chemical stimuli, combination of TRP channels and their respective ligands have potential to open up new areas of the stem cell engineering. Unfortunately, only a few studies have so far investigated the involvement of the TRP channels in stem cells.

**New techniques.** This meeting has witnessed the introduction of some novel and innovative techniques that helped characterize the TRP channels. Here we discuss one of these.

**“Urine-imaging” as a substitute of “Ca$^{2+}$-imaging”,** Nilius et al. have shown the effect of TRPV4 gene knock out on the urination pattern by using their so called novel “urine imaging” technique. Urine imaging is basically done by placing a piece of paper towel at the floor of the animal cage and this paper towel indicates all the places where the animal urinates as these specific areas develop spots. Generally, rodents prefer to urinate at the corners of the cage and very rarely at the middle of the cage. Thus, small spots at the middle of the cage indicate the “out-of-control” stage of the animals. Notably, numerical quantification and statistical analysis of the area of the spots and the number of the spots give good estimation of the “urination status” and/or the “urination mode” of the animals. Using this technique, Nilius et al. have shown that TRPV4 knock out mice (trpv4$^-$) urinate often, and also in the middle of the floor, indicating that the animals are mostly in the “out-of-control” stage.

While Ca$^{2+}$-imaging is expensive and needs high-end setups to characterize the function of TRP ion channels at the cellular level, “urine imaging” is a cheaper, quick and robust method which is applicable to the whole animal. The “urine imaging” could supplement the need of the tedious Ca$^{2+}$-imaging to a large extent, provided the function of the TRP channel of interest at the cellular level correlates well with the urination behaviors of the animal. Further modification of this method can improve these kinds of studies. For example, applying some chemical/ enzymatic indicator on the paper towel can develop some color by reacting with the urine. Thus, both quality and quantity of the urine can be judged accurately and directly from the spot size as well as from the color intensity.

**Directions for future research.** In this meeting several aspects of TRP channels were discussed from the view point of application of TRP research for the betterment of human health in general. TRP channels are directly or indirectly involved in pain, and many other diseases. Their functions are essential for normal life. Identification of new compounds that can activate or inhibit respective TRP channels in a given tissue is of paramount importance. Progresses are being made in this area.

For example, TRPM7 knock out animals die in the embryonic stage and it is involved in ischemia-induced heart and brain-damage. Tymianski et al. have screened more than 60,000 compounds and identified “M6”, a compound that acts as a direct inhibitor of TRPM7. The specificity of this compound is under investigation. If proved to be specific, then this compound may have an important role in the treatment of ischemic cardiac diseases, and stroke. Similarly, Varben et al. screened more than 200,000 small compounds and found two compounds that can block TRPC6 (Varben et al. abstract # 68). These compounds have good drug-like properties and represent two distinct chemical scaffolds. Interestingly, these two hits do not have any action on TRPC3, TRPV5 or Na$_V$1.5 and Ikr channels. In this context, it is noteworthy to mention that Andreev et al. identified a 56 residue length polypeptide (named APHC1) from sea anemone (Heteractis crispa) venom, which inhibits capsaicin induced currents in TRPV1. It produces significant antinociception, inhibits hyperalgesia. Being a polypeptide, it has some advantage over small molecules and this polypeptide may be of advantage in the behavior treatment of hyperalgesia. AMTB, a novel
TRPM8 antagonist might be useful for treatment of painful bladder syndrome.148

Development of new generation pesticides. A great hope to control many of the insect-borne disease was raised in this meeting when Montell et al. showed that certain natural compounds have effects on the insect TRP channels but not on TRP channels of higher organisms. For example, “citronellal”, a natural compound can activate insect TRPA but not Xenopus TRPA. So, this compound can be effectively used as insect repellents as these compounds can activate only insect TRP channels. Since the sequence of human TRPA1 is different from that of insect TRPA, this compound or its derivatives can be effectively used to develop potent insect repellents and/or effective pesticides.

Concluding Remarks

In spite of major advances made in the field of research involving TRP channels, there remain several concerns. The research on TRP channels is highly focused on certain popular TRP families like TRPV and TRPC members (Fig. 4A and B). The use of correct nomenclature is also a matter of concern. For example, three different names like VR1, TRPV1 and capsaicin-receptor are still being used to describe the same receptor (Fig. 4C). But the biggest concern lies in the reproducibility of the published results. It has been noted that far too many published results are just not reproducible in other labs. This is particularly true about studies that have relied heavily on “specific” antibodies for characterization of TRP channels. Thus, there is an urgent need to identify factors that determine apparently poor reproducibility of many published results. One potential area of concern is possible lack of responsibility in the scientific reporting. This may affect the whole TRP-channel community as one irresponsible reporting may slow down many other good efforts and may cause loss of significant amount of resources which may hinder the future studies.

To overcome this situation, ideally the materials and methods section should be reported with great details so that the published results can be easily reproduced in other labs. For example, it is possible that apparently minor factors like, materials used for glass coating, which generally varies depending on commercial sources, may affect the response of a TRP channel in a patch-clamp experiment. This is particularly important as TRP channels are highly sensitive to many compounds and can integrate multiple signaling events. In this regard, concerns have been expressed about the authenticity of the commercially available antibodies. Care must be taken to verify the quality of the commercially available antibodies before the results are reported.

Prof. David Clapham proposed to launch a web-site where the quality of the antibodies can be discussed by the actual users. In the wake of this meeting Md. Shahiul Islam’s lab has started developing of a web-site where users can share their experiences on the use of antibodies (not just TRP antibodies) and rate them. An official “TRP channel homepage” is also in the pipe-line.

Until recently, the TRP channel field has been dominated by the electrophysiology and calcium imaging experiments, as the
focus was very much on the “channel function”. At present, the involvement of diverse TRP channels in different signaling cascades as well as in the development of pathological conditions is drawing more attention. In many instances, involvement of TRP channels in pathological conditions cannot be explained only by an altered cation-influx. In fact, a large number of studies indicate that indirect roles of TRP channels (functions other than the cation-influx) may be involved in many processes. It has been noted in this meeting that the TRP channel field is now dealing with the atypical and indirect functions of the channels and their regulations, which are likely to be even more complex and more interesting to study. A significant shift has been made towards the use of endogenous ligands rather than exogenous ligands in order to characterize the TRP channels. However, the progresses with respect to the structural aspects and biophysical properties of TRP channels have been slow and understanding of the conformational changes of the TRP channels is still limited. In future one hopes to see further advancement of TRP channel research in all of these areas.

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44. Salazar H, Jara-Oseguera A, Hernandez-Garcia E, Llorente I, Arias-Olguin II, Soriano-Garcia M, et al. Structural determinants of gating in the TRPV1 channel. Nat Struct Mol Biol 2009; 16:70-4.

45. Ilias LD, Salazar H, Jara-Oseguera A, Nieto-Possadas A, Llorente I, Rangel-Yescas G, et al. The helical character of the S6 segment of TRPV1 channels. Channels (Austin) 2009; 3:311-3.

46. Grimm C, Kraft R, Schultz H, Harteneck C. Activation of the melastatin-related cation channel TRPM3 [corrected] by D-erythro-sphingosine. Mol Pharmacol 2005; 67:798-805.

47. Formigli L, Sassoli C, Squero R, Bini F, Martinesi M, Chellini E, et al. Regulation of transient receptor potential ionotrophic channel 1 (TRP1) by sphingosine-1-phosphate in Cx212 myoblasts and its relevance for a role of mechanotransduction in skeletal muscle differentiation. J Cell Sci 2009; 122:1322-33.

48. Beech DJ, Bahnasi YM, Dedman AM, Al Shawaf E. TRPC channel lipid specificity and mechanisms of lipid regulation. Cell Calcium 2009.

49. Kim AT, Tang ZX, Liu Q, Maag D, Geng YW, J. F. Port, a phosphoinositide-binding protein, functions as a regulatory subunit of TRPV1. Cell 2008; 133:475-85.

50. Gratschev D, Lof C, Heikila J, Bjorkholm A, Sukumaran P, Hinnenken A, et al. Sphingosine kinase as a regulatory entry through sphingoid phospholipase A-1 phospholipase-sphingolipid signaling in thyroid FRTL-5 cells. Endocrinology 2009; 150:5125-34.

51. Hardie RC. Regulation of Drosophila TRP channels by lipid messengers. Novartis Found Symp 2004; 258:160-7.

52. Minke B. The TRP channel and phospholipase C-mediated signaling. Cell Mol Neurobiol 2001; 21:629-43.

53. Rohacs T, Thyagarajan B, Lukacs V. Phospholipase C mediated modulation of TRPV1 channels. Mol Cell Biochem 2008; 313:153-63.

54. Morenilla-Palao C, Pernus M, Mereuget V, Cabedo H, Viana F. Lipid raft segregation modulates TRPM8 channel activity. J Biol Chem 2009; 284:9215-24.

55. Liu M, Huang W, Wu D, Priestley JV. TRPV1, but not P2X, requires cholesterol for its function and membrane expression in rat nociceptors. Eur J Neurosci 2006; 24:1-6.

56. Bergdahl A, Gomez ME, Dreja K, Xu SZ, Adner M, Beech DJ, et al. Cholesterol depletion impairs vascular reactivity to endothelin-1 by reducing stored-outoperated Ca<sup>2+</sup>-channel entry dependent on TRPV1. Circ Res 2003; 93:839-47.

57. Parmas N, Katz B, Lev S, Tzafary V, Dadon D, Gordon-Shaag A, et al. Membrane lipid modulations remove divalent open channel block from TRP-like and NMDA channels. J Neurosci 2009; 29:2371-83.

58. Parmas N, Peters M, Minke B. Linoleic acid inhibits TRP channels with intrinsic voltage sensitivity: Implications on the mechanism of linoleic acid action. Channels (Austin) 2009; 3:164-6.

59. Riske KA, Dobretzeg H.G. Deylcyclig-protein-rich domain formation in giant stearoyl-oleoyl phosphatidylcholine vesicles driven by phospholipase C activity. Biophys J 2003; 85:2351-62.

60. Goldberg AM, Frazier JM, Bruick D, Dickens MS, Flint O, Gettings SD, et al. Framework for validation and implementation of in vitro toxicity tests. In Vitro Cell Dev Biol Anim 1993; 29:688-92.

61. Nilius B, Otianuk G, Voets T. Transient receptor potential channels meet phosphoinositides. EMBO J 2008; 27:2809-16.

62. Chou T, Jaric MV, Sigga ED. Electrorotation of lipid bilayer bending. Biophys J 1997; 72:2042-55.

63. Yogi A, Callera GE, Tores T, Touny RM. Bradykinin regulates calpain and proinflammatory signaling through TRPM7-sensitive pathways in vascular smooth muscle cells. Am J Physiol Regul Integr Comp Physiol 2009; 296:261-7.

64. Kannan KB, Barlos D, Hauser CJ. Free cholesterol alters lipid raft structure and function regulating neuronal Ca<sup>2+</sup> entry and respiratory burst: correlations with calcium channel raft trafficking. J Immunol 2007; 178:5253-61.

65. Lockwood FR, Liu X, Singh BB, Jadowicz L, Weiland S, Ambudkar IS. Assembly of TRP1 in a signaling complex associated with cavelin-scaffolding lipid raft domains. J Biol Chem 2000; 275:11934-42.

66. Berthier A, Lemaire Ewing S, Prunet C, Monier S, Athias A, Besede G, et al. Involvement of a calcium-dependent dephosphorylation of BAD associated with the localization of Trpc-1 within lipid rafts in 7-ko Italia-cholesterol-induced THP-1 cell apoptosis. Cell Death Differ 2004; 11:897-905.

67. Pani B, Ong HL, Liu X, Rauzer K, Ambudkar IS, Singh BB. Lipid rafts determine clustering of STIM1 in endoplasmic reticulum-plasma membrane junctions and regulation of store-operated Ca<sup>2+</sup> entry (SOC). J Biol Chem 2008; 283:17335-40.

68. Durrer JP, Bindslev CM, Workentine TJ, Maignan C, et al. Effect of lipid raft disruption on the cellular cholesterol. J Biol Chem 2009; 284:14788-96.

69. Stayskal J, Moerman E, Oggioni D, et al. Membrane lipid compositions in normal but not in insulin-resistant mouse myocardocytes. Cardiovasc Res 2007; 73:576-85.

70. Datta SK, Datta S, Kondaiah P, et al. A role of mechanotransduction in skeletal muscle differentiation. J Cell Sci 2007; 120:2477-82.
115. Starowicz K, Nigam S, Di MV. Biochemistry and pharmacology of endocannabinoids. Pharmacol Ther 2007; 114:13-33.

116. Rutter AR, Ma QP, Leveridge M, Bonnett TP. Heteromeric organization and co-localization of TRPV1 and TRPV2 in mammalian cell lines and rat dorsal root ganglia. Neuroreport 2005; 16:1735-9.

117. Casaceli J, Schmidt S, DeGray B, Petri ET, Celic A, Foltz-Snegrove E, et al. Analysis of the cytoplasmic interaction between polycystin-1 and polycystin-2. Am J Physiol Renal Physiol 2009; 297:1310-5.

118. Yu Y, Ulbrich MH, Li MH, Baurai Z, Chen ZX, Ong AC, et al. Structural and molecular basis of the assembly of the TRP polymeric complex. Proc Natl Acad Sci USA 2009; 106:11558-63.

119. Schumann F, Hoffmeister H, Bader R, Schmidt M, Rieger T, Willigrot R, et al. NMR-assignments of a cytosolic domain of the C-terminus of polycystin-2. Bioimol NMR Assing 2009; 3:141-4.

120. Kwan HY, Shen B, Ma X, Kwok Y, Huang Y, Man YB, et al. TRPC1 associates with BK(Ca) channel to form a signal complex in vascular smooth muscle cells. Circ Res 2009; 104:670-6.

121. Arai E, Inoue T, Ueno T, Ichiki M, et al. Beta-glucuronidase klotho exclusively activates the TRPA1 receptor by covalent cysteine modification. Proc Natl Acad Sci USA 2009; 106:20097-102.

122. Goswami C, Hulshof T, TRPV1 expression-dependent initiation and regulation of filopodia. J Neurochem 2007; 103:1319-33.

123. Goswami C, Hulshof T. Submembranous microtubule cytoskeleton: biochemical and functional interplay of TRP channels with the cytoskeleton. FEBS J 2008; 275:4684-99.

124. Andalussi-Lijia J, Lanadqvist J, Forby A. TRPV1 expression and activity during retinoic acid-induced neuronal differentiation. Neurochem Int 2009; 55:768-74.

125. Louis M, Zanou N, Van Schoor M, Gailly P. TRPC1 regulates skeletal myoblast migration and differentiation. J Cell Sci 2008; 121:3951-9.

126. Scott G, Leopardi S, Printup S, Madden BC. Filopodia entry at the gate. Pflugers Arch 2005; 451:181-92.