Submembraneous microtubule cytoskeleton: biochemical and functional interplay of TRP channels with the cytoskeleton

Chandan Goswami and Tim Hucho

Department for Molecular Human Genetics, Max Planck Institute for Molecular Genetics, Berlin, Germany

The microtubule cytoskeleton plays a role in a variety of cellular aspects such as division, morphology and motility, as well as the transport of molecules and organelles toward and from the cell membrane. Although all these phenomena affect the plasma membrane, however, most of the microtubule filaments do not reach to the lipid membrane region, partially due to a thick hindering cortical actin network. However, recent studies indicate that a small number of dynamic microtubules can extend rapidly to the cell membrane. Although most contacts are established only transiently, there are membranous regions in which the plus end of these pioneering microtubules is stabilized. Stabilization appears to be mediated by the interaction with various membrane proteins, which often are part of large protein complexes. The dynamic properties and the complexity of tubulin as an interacting protein in large complexes at the membrane just are beginning to be unravelled. One apparent function is to serve as a scaffold protein and modulator of transmembrane signalling.

Cytoskeletal components in signalling complexes at membranes

The cytoplasmic domains of transient receptor potential (TRP) channels recruit large complexes of proteins, lipids and small molecules. Depending on the
preparation method, these structures have been referred to as ‘signalplex’, i.e. complexes involved in signalling events [1], or ‘channelsome’, i.e. complexes formed around functional ion channels [2], and/or as ‘lipid raft complexes’, i.e. complexes localized to this membranous subdomain [3]. Proteomic studies of ‘signalplexes’ or ‘channelsomes’ purified from cell lines, as well as from brain, give both direct and indirect evidence for the presence of the cytoskeleton as well as ion channels. Scaffolding adaptors like inactivation-no-afterpotential D [4–6], Na+⁄H+ exchanger regulatory factor [7] and ezrin/moesin/radixin-binding phosphoprotein 50 [8] are also found, some of which interact directly with ion channels, e.g. TRP channels [9], but also contain binding motifs for cytoskeletal proteins [8,10]. Accordingly, cytoskeletal proteins such as spectrin, myosin, drebrin and neurabin, as well as tubulin and actin [1,2,6,11] are confirmed components in signalplexes and channelosomes.

Complementary proteomic studies of purified lipid rafts reveal the presence of several cytoskeletal proteins [12,13] such as α- and β-tubulin, tubulin-specific chaperone A (a folding protein involved in tubulin dimer assembly), KIF13 (a kinesin) [12], actin, unconventional myosin II and unconventional myosin V [14]. Similarly, proteomics studies of the ‘membrane cytoskeleton’, a submembranous fraction, which is attached to the cytoskeleton [15], and of cytoskeleton-associated proteins in general [16], indicate the presence of lipid raft membrane proteins as well as cytoskeletal proteins. Together, these varying studies give strong evidence that cytoskeletal proteins are part of signalling complexes including transmembrane proteins and are involved in their organization at membrane.

**Structural features of TRP channels**

The TRP family of ion channels is named after the *Drosophila melanogaster* *trp* mutant, which is characterized by a transient receptor potential in the photoreceptors in response to light [17]. In the meantime, orthologues and paralogues of TRP channels have been described in organisms ranging from simple eukaryotes to human. They share a high degree of homology in their amino acid sequence. TRP channels are formed by monomers with six transmembrane regions that assemble into tetramers, which form the functional cation-permeable pore. The most conserved region is the sixth transmembrane domain, which constitutes most of the inner lining of the ion channel pore. The N- and C-termini of TRP channels are located in the cytoplasm and, depending on the respective TRP channel, consist of various functional domains like ankyrin repeats, Ca2+-sensing EF hands, phosphorylation sites, calmodulin-binding sites and a so-called ’TRP box’. Based on their sequence, the mammalian TRP family is differentiated into six subfamilies, namely TRP canonical (TRPC), TRP vanilloid (TRPV), TRP melastatin (TRPM), TRP polycystin (TRPP), TRP mucolipin (TRPML) and TRP ankaryin (TRPA) ion channels [18]. All TRP channels investigated to date are involved in the detection and/or transduction of physical and chemical stimuli.

**TRPV1 and the cytoskeleton**

**Physical interaction of TRPV1 with the cytoskeleton**

TRPV1 is the founding member of the vanilloid subfamily of TRP channels and detects several endogenous agonists (e.g. N-arachidonoyl-dopamine) and noxious exogenous stimuli, such as capsaicin (the main pungent ingredient of hot chilly) and high temperature (> 42 °C) [19,20]. TRPV1 is a nonselective cation channel with high permeability for Ca2+. In recent years, TRPV1 has gained extensive attention for its involvement in signalling events in the context of pain and other pathophysiological conditions including cancer [21–27].

The interaction of TRPV1 with tubulin was first identified through a proteomic analysis of endogenous interactors enriched from neuronal tissue [28]. The interaction was then confirmed by biochemical approaches including co-immunoprecipitation, microtubule co-sedimentation, pull-down and cross-linking experiments. In contrast to the tubulin cytoskeleton, the physical interaction of TRPV1 with actin or neurofilament cytoskeleton has not been observed to date [28,29].

The C-terminus of TRPV1 (TRPV1-Ct) is sufficient for the interaction with tubulin while the N-terminus of TRPV1 (TRPV1-Nt) apparently does not interact [28]. Using deletion constructs and biotinylated peptides, the tubulin-binding region located within TRPV1-Ct was mapped to two short, highly basic regions (amino acids 710–730 and 770–797) [29]. If an α-helical conformation is assumed, these two regions project all their basic amino acids to one side, thus potentially enabling interactions with negatively charged residues (Fig. 1). Indeed, correspondingly, the C-terminal over-hanging region of tubulin contains a large number of negatively charged glutamate (E) residues in a stretch characterized as unstructured region of the tubulin and referred as E-hook. These E-hooks are known to be essential for the interaction of tubulin
with various microtubule-associated proteins such as MAPs, Tau, as well as others. Indeed, binding of TRPV1-Ct with tubulin was abolished when the E-hooks containing over-hangs were removed by protease treatment [29]. The tubulin-binding region of TRPV1 apparently is under high evolutionary pressure as its sequence is highly conserved in all TRPV1 orthologues [29]. Also between homologues, the distribution of basic amino acids composing the tubulin-binding regions is conserved even though the overall amino acid conservation is rather limited. Based on these data an interaction of tubulin with TRPV2, TRPV3 and TRPV4 (Fig. 2) can also be predicted. These TRPV1 homologues have the highest conservation of basic charge distribution within the tubulin-binding sequences. Indeed, in the meantime we could confirm this for TRPV2 and TRPV4 (unpublished observation).

TRPV1 preferably interacts through its C-terminal domain with β-tubulin and to a lesser extend also with α-tubulin thereby forming a high-molecular weight complex [29]. This suggests stronger binding of TRPV1 to the plus end rather than the minus end of

![Fig. 1. Characteristic of the tubulin-binding motifs located at the C-terminus of TRPV1. (A) The extreme C-terminus of both α- and β-tubulin contains highly negatively charged amino acids (indicated in red) and is mostly unstructured. (B) The basic amino acids (indicated in blue) that are located within the tubulin-binding regions of TRPV1 are located at one side of the putative helical wheel, where it can interact with the acidic C-terminus of tubulin.

![Fig. 2. Conservation of the tubulin-binding regions in TRPV1 orthologues and homologues. (A) The tubulin-binding region is conserved in mammals. The conserved basic amino acids are shown in blue and are indicated by an asterisk (*). NCBI accession numbers: rat (NP-114188), mouse (CAF05661), dog (AA71314), human (NP_542437), guinea pig (AAUj43730), rabbit (AA34448), chicken (NP_969903) and pig (CAD37814). (B) TRPV1 homologues (based on sequences from rat species only) were aligned using CLUSTAL. The distribution of basic amino acids (in blue) located within the first tubulin-binding motif is partially conserved. NCBI accession numbers: TRPV1 (NP-114188), TRPV2 (AAH98215), TRPV3 (NP-001020928), TRPV4 (NP-076460), TRPV5 (AAV31121) and TRPV6 (Q0R186).]
microtubules as the plus ends of microtubule protofila-
ments are decorated with β-tubulin. It is therefore
tempting to speculate that TRPV1 may act as a micro-
tubule plus-end-tracking protein (+TIP) [30]. This
speculation is corroborated by the recent observation
that despite their differences in primary amino acid
sequences, the crystal structures of microtubule-bind-
ing regions of different classes of +TIP proteins such
as Stu2p, EB1 and Bim1p contain a common motif of
at least two α helices with positively charged residues
at the surface [31]. The tubulin-binding ability of
TRPV1-Ct is supported by the predicted structural
models also [32,33]. This is particularly due to the fact
that the tubulin-binding regions are predicted to con-
tain α helices. Fragile histidine triad protein (FHIT), a
tumour suppressor gene product has high sequence
homology with TRPV1-Ct and the crystal structure of
FHIT was used as a template for predicting the struc-
ture of TRPV1-Ct [32]. Remarkably, FHIT also binds
to tubulin [34].

Different post-translationally modified tubulin, like
tyrosinated tubulin (a marker for dynamic microtu-
bules), detyrosinated tubulin, acetylated tubulin, poly-
glutamylated tubulin, phospho (serine) tubulin and
neurone-specific β-III tubulin (all markers for stable
microtubules) interact with TRPV1-Ct [29]. This implies
that TRPV1 interacts not only with soluble tubulin, but
also with assembled microtubules in various dynamic
states. And indeed, the interaction of TRPV1-Ct also
with polymerized microtubules could experimentally
been proven [28]. In addition to sole binding, TRPV1-
Ct exerts a strong stabilization effect on microtubules,
which becomes especially apparent under microtubules
depolymerising conditions such as presence of noco-
dazol or increased Ca$^{2+}$ concentrations [28].

TRPV1 channels are nonselective cation channels. Therefore, the role of increased concentration of Ca$^{2+}$
on the properties of TRPV1–tubulin and/or TRPV1–
microtubule complex is of special interest. Tubulin
binding to TRPV1-Ct is increased by increased Ca$^{2+}$
concentrations [28]. Interestingly, the microtubules
formed with TRPV1-Ct in the presence of Ca$^{2+}$
become ‘cold stable’ as these microtubules do not depo-
lymerise further at low temperature [28]. The exact
mechanism how Ca$^{2+}$ modulates these physicochemi-
ical properties in vitro are not clear. In this regard, it is
important to mention that tubulin has been shown to
bind two Ca$^{2+}$ ions to its C-terminal sequence [35–38]
and thus Ca$^{2+}$-dependent conformational changes of
tubulin [39] may underlie the observed effects of Ca$^{2+}$.

The biochemical data of direct interaction as well as
microtubule stabilization find their correlates in cell
biological studies. Transfection of TRPV1 in dorsal
root ganglia-derived F11 cells results in co-localization
of TRPV1 and microtubules and accumulation of
endogeneous tyrosinated tubulin (a marker for dynamic
microtubules) in close vicinity to the plasma membrane
[28] (Fig. 3). As suggested by its preference to bind to
the plus-end-exposed β-tubulin, TRPV1 apparently sta-
bilizes microtubules reaching the plasma membrane
and thereby increases the number of pioneering micro-
tubules within the actin cortex (Fig. 4). But stabiliza-
tion induces even stronger changes. The overall
cellular morphology is altered dramatically by massive
induction of filopodial structures in neuronal as well as
in non-neuronal cells [40] (Fig. 4). The mechanism for
this is currently under investigation and apparently
also includes alterations in the actin cytoskeleton. But,
co-localization of TRPV1 with tubulin was observed
all along the filopodial stalk and, of note, including
the filopodial tips [40]. Tubulin and components attrib-
uted to stable microtubules (like acetylated tubulin
and MAP2ab) were also observed within these thin
filodopial structures [40].

**TRPV1-activation induced microtubule
disassembly**

In contrast to the stabilization of microtubules at rest-
ing state, activation of TRPV1 results in rapid disas-
sembly of microtubules irrespective of the investigated
cellular system (Fig. 3) [41,42]. Again, the underlying
mechanism of TRPV1 activation-mediated cytoskele-
ton remodelling is largely unknown. In F11 cells,
TRPV1 activation leads to an almost complete destruc-
tion of peripheral microtubules, whereas microtubules
close to the microtubule-organizing centre, a structure
composed of γ-tubulin and stable microtubules at the
perinuclear region, remain intact (Fig. 3). Also, the
integrity of other cytoskeletal filaments like actin and
neurofilaments is not affected by activation of TRPV1
[41]. Potentially, TRPV1 activation may even increase
the amount of polymerized actin [43].

Effects caused by the activation of a nonselective
cation channel are suggestive of mediation by the
influx of, for example, Ca$^{2+}$. Indeed, high Ca$^{2+}$
concentrations have the potential to depolymerize micro-
tubules in vitro and in vivo [44,45] through either
‘dynamic destabilization’, i.e. a direct effect of Ca$^{2+}$
on microtubules, or indirectly by a calcium-induced
but signal-cascade-dependent depolymerization [46].
Also, chelating extracellular Ca$^{2+}$ with EGTA and
depletion of intracellular Ca$^{2+}$ stores with thapsigargin
cannot prevent TRPV1-activation-mediated microtu-
bule disassembly [41,47]. Thus, TRPV1-activation-
induced microtubule disassembly is apparently not a
direct effect of high Ca\textsuperscript{2+} concentrations. Even combined EGTA and thapsigargin, treatment cannot exclude small changes in local Ca\textsuperscript{2+} concentration. Therefore, these small changes in Ca\textsuperscript{2+} might trigger an enzymatic cascade leading to depolymerization. This view is also supported by previous studies demonstrating that a small amount of calmodulin can cause massive microtubule depolymerization in the presence of catalytic amounts of Ca\textsuperscript{2+}, but not in the complete absence of Ca\textsuperscript{2+} [45,48–50]. Subsequent activation of Ca\textsuperscript{2+}-dependent proteases may also trigger proteolysis of structural proteins as a downstream effect [51].

Another potential mechanism that can lead to rapid disassembly of microtubules might be the phosphorylation of microtubule-associated proteins (MAPs). We observed fragmented microtubules all over the cytoplasm after TRPV1 activation, which suggest that specific microtubule-severing proteins like katanin, fidgetin and spastin are probably also involved in this process (Fig. 3) [52–54]. Prolonged stimulation of TRPV1 activates through high Ca\textsuperscript{2+} concentrations among others caspase 3 and 8, which leads eventually to cell death [55–59]. In general, extensive fragmentation of the cellular cytoskeleton and programmed cell death correlate well. However, in response to short-term stimulation of TRPV1 we have not observed any fragmented tubulin bands in western blot analysis [41]. Last, but not least, TRPV1 activation-mediated inhibition of protein synthesis and endoplasmic reticulum fragmentation may also have impact on the microtubule integrity [42].

Implications of TRPV1-induced cytoskeleton destabilization

TRPV1 affects biological functions, like cell migration and neuritogenesis, that are largely dependent on the cytoskeleton [42,60,61]. Indeed, rapid disassembly of dynamic microtubules by TRPV1 activation has a strong effect on axonal growth, morphology and migration. TRPV1 is endogenously expressed already at an early embryonic stage and localizes to neurites...
and growth cones (Fig. 4) [47,62]. Activation of TRPV1 results in rapid disassembly of microtubules within neurites (and also at growth cones) while keeping the actin cytoskeleton intact and functional. This destroys the balance between the anterograde force (generated by microtubule cytoskeleton) and the retrograde force (generated by actin cytoskeleton) that determines the axonal morphology and the net neurite growth [63,64]. Sudden loss of polymerized microtubules results in retraction of growth cones and formation of varicosities all along the neurites (Fig. 5). Long-term low-level TRPV1 activation by an endogenous ligand results in shortening of neurites in primary neurons [47]. But as endogenous expression of TRPV1 is widespread and not restricted to neuronal cells, activation of TRPV1 increases the motility of non-neuronal cells like HepG2 and dendritic cells [42,65]. In agreement with the role of TRPV1 in cell motility, dendritic cells from trpv1 -/- animal show less migration than wild-type [65].

Differential activation of TRPV1 complexes can create an asymmetry in the microtubular organization. Thus, activation of TRPV1 in a specific cellular region may result in the disassembly of microtubules, thereby facilitating the retraction of that part of the cell, thus creating a trailing edge. By contrast, stabilization of microtubules at TRPV1-enriched plasma membranes may facilitate a cell to extend at this region, marking the leading edge and initiating cell migration [66].

In contrast to a strong and long-term activation of TRPV1, which affects microtubules globally, mild and localized short-term activation may affect parts of the cytoskeleton differently. Thus, growth cones may be helped to avoid a repulsive guidance cue. Reciprocally, stabilization effect of TRPV1-enriched membranes on the plus ends of microtubules may help a growth cone to steer towards an attractive cue (Fig. 4). A similar mechanism by which other TRP channels can regulate the growth cone attraction, repulsion or retraction has been described [67,68]. Although not tested, TRPV1 may potentially regulate the sperm motility as the presence of TRPV1 at the sperm acrosome and throughout the tail has been reported [69]. Short-term and low-level activation may increase sperm motility whereas...
robust activation may cause a non-motile sperm due to complete disassembly of microtubules at the sperm tail.

**TRPV4 and the cytoskeleton**

TRPV4 is a member of the TRPV subfamily and a close homologue of TRPV1. It is activated by endogenous endovanilloids, by temperatures of > 37 °C, and by both hypo- and hyperosmotic stimuli. In many studies, the synthetic ligand 4α-PDD is alternatively employed [70]. TRPV4 is involved in mechanosensation of the normal and the sensitized neuron [71]. To date, the evidence for the functional, as well as physical, interaction of TRPV4 with cytoskeletal components is mostly indirect. For example, TRPV4 has been shown to be important for the development of taxol-induced mechanical hyperalgesia suggesting a functional link of TRPV4 with microtubule cytoskeleton [72]. Often, activation of TRPV4 is correlated to cellular changes, which in turn are known to involve cytoskeletal rearrangement such as cell volume in regulatory volume decrease [72–74] and cell motility due to changes in lamellipodia dynamics [60]. However, the extent to which the changes in the cytoskeleton are induced by TRPV4 directly is mostly unknown. Biochemical and cell biological data are sparse and patchy. The distance between actin and TRPV4 in a live cell was measured by FRET to be < 4 nm [75] assuring, that these two components have the potentiality to interact with each other. In addition, TRPV4 has been identified by yeast two-hybrid screen to interact with MAP7, an interaction confirmed by immunoprecipitation as well as pull-down experiments [76]. This interaction is dependent on the C-terminal amino acids 798–809. Interestingly, the C-terminal cytoplasmic domain of TRPV4 also contains a partially conserved putative tubulin-binding site [29].

**Physical and functional interaction of other TRP channels with cytoskeletal components**

Physical links of several TRP channels other than TRPV1 and TRPV4 with the cytoskeleton have been established. For example, β-tubulin interacts directly with TRPC1 [77]. Two other members of the TRPC family, TRPC5 and TRPC6, are also interacting with cytoskeletal proteins [1]. These also include actin and tubulin as they are confirmed components of the purified ‘signalplex’ [1]. TRPC5 interacts with stathmin 2, a microtubule cytoskeleton-binding protein [78]. Direct physical and functional interplay with both the microtubule and actin cytoskeleton has also been described for TRPP channels (see minireview by Chan et al. in this series).

Apart from the direct interaction, many of these TRP channels are localized to the microtubule and actin cytoskeleton-enriched structures like filopodia, cilia and growth cones, indicating a potential association and complex signalling with the cytoskeleton. Again, activation of these TRP channels correlates...
with cytoskeleton-dependent morphological changes. For example, both rat and human pulmonary arterial endothelial cells express TRPP1, the activation of which leads to a change in cell shape due to reorganization of cortical actin network [79]. Likewise, *Xenopus* TRPN1 (NOMPC) localizes to microtubule-based cilia in epithelial cells, including inner ear hair cells [80].

Almost all of the TRPC channels have been reported to localize to growth cones [67,77,81–84]. In all cases, activation of TRPC channels regulates growth cone morphology and motility in response to chemical guidance cues. TRPC1 modulates the actin cytoskeleton by modulating ADF/cofilin activity via LIM kinase [85]. It is involved in growth cone turning in response to Netrin 1 [82,83]. TRPC4 is upregulated after nerve injury and is important for neurite outgrowth [81]. TRPC3 and TRPC6 are important for growth cone turning in response to brain-derived neurotrophic factor [84]. Likewise, TRPC5 expression results in increased length of neurites and filopodia [78].

In addition to data on the interaction of TRP channels with the cytoskeleton, there are few examples suggesting that the activity of the TRP channel is influenced by the cytoskeleton. Mostly, alteration of the cytoskeleton results in inhibition of TRP channel opening. For example, the requirement for a functional cytoskeleton in the activation of TRP channels in store-mediated Ca\(^{2+}\) entry and/or store-operated Ca\(^{2+}\) entry has been reported [86–88]. In human platelets, physical coupling of hTRP1 with inositol 1,4,5-triphosphate receptor (IP3R), a prerequisite step for store-mediated Ca\(^{2+}\) entry, depends on the degree of polymerized actin [86,87]. Disruption of the actin cytoskeleton by cytochalasin D also prevents phosphatidylinositol 4,5-bisphosphate (PIP2)-mediated inhibition of TRPC4α [89]. Another example has been reported from primary human polymorphonuclear neutrophil cells, in which reorganization of actin results in the internalization of endogenous TRPC1, TRPC3 and TRPC4 from plasma membrane to the cytosol, which correlates well with the loss of store-operated calcium entry [88]. Accordingly, pre-disruption of the actin cytoskeleton by cytochalasin D rescues the loss of store-operated calcium entry, indicating that the actin dynamics are important for this TRPC-mediated store-operated calcium entry. Apparently, an intact actin cytoskeleton is also essential for strong agonist-mediated activation of TRPC7. Thus, pharmacological disruption of the actin cytoskeleton results in reduced agonist-induced activation [90]. All these examples suggest that the cytoskeleton can indeed act as modulators of TRP channel function.

### TRP channels and myosin motors

Nonconventional myosin motors and TRP channels are often localized within specific subcellular regions such as filopodia or ciliary tips. These two groups of proteins also share a special genotype–phenotype correlation as abnormal expression/function of these myosins or TRPs gives rise to similar pathophysiological conditions like deafness, blindness, and syndromes affecting the function of other tissues and/or organs. For example, in case of deafness, several nonconventional myosin motors (myosin I, IIA, IIIA, VI, VIIA and XV) are important for either development of the stereocilia of hair cells in the inner ear or proper localization of TRP channels at the tip of these stereocilia, which is crucial for the activity of these cells [91,92]. Reciprocally, mutations and abnormal expression/function of several TRP channels (TRPML1, TRPML2, TRPML3, TRPV4, TRPV5 and TRPV6) also lead to deafness [93–97]. In a similar manner, both myosins and TRP channels are causally involved in blindness. Recently it has been reported that translocation of eGFP-tagged TRP-like channels to the rhabdomeral membrane in *Drosophila* photoreceptors is myosin III dependent [98]. Apart from the above genetic interactions, TRP channels interact directly with myosins. Using a proteomic screen, myosin was identified to bind to TRPC5 and TRPC6 [1]. Another study showed that myosin IIa is directly phosphorylated by TRPM7, a cation channel fused to an alpha-kinase [99]. This phosphorylation in turn regulates cell contractility and adhesion. Notably, TRPM7 phosphorylates positively charged coiled-coil domain of myosin II [100].

In some cases, similar cellular phenotypes also suggest a functional link between TRP channels and nonconventional myosin motors. For example, we observed that ectopic expression of TRPV1 induces extensive filopodial and neurite-like structures in neuron-derived F11 cells as well as in non-neuronal cells (e.g. HeLa, Cos and HEK 293 cells) [40]. Interestingly, TRPV1 expression induces club-shaped filopodia with a bulbous head structure that contains negligible amount of F-actin but accumulates TRPV1 [40]. This phenotype resembles the dominant negative effect of the expression of the non-conventional myosin II, III, V, X and XV [101–112]. This renders the observation that TRPV1 expression induces drastic upregulation of endogenous myosin IIa and IIIa tempting suggestive [40]. In addition, the subcellular distribution of myosins is markedly changed from a uniformly cytoplasmic to a strongly clustered localization mostly at the cell periphery [40]. In another study, cardiac-specific overexpression of TRPC6 in transgenic mice resulted
in an increase expression of beta-myosin heavy chain [113]. Such phenomena strongly suggest the cooperative role of myosins and TRP channels in development as well as proper function of ciliary and filopodial structures.

The molecular mechanisms behind the increased expression of these myosins are poorly understood. In some cases, TRP channels apparently increase myosin expression by regulating transcription factors [113]. As myosins are also susceptible to protease-mediated degradation; a higher level of endogenous myosin might also imply less proteolytic degradation. It is therefore worth exploring how TRP channels affect the distribution, function and endogenous level of myosins.

Modulation of TRP ion channels activities by the cytoskeleton

TRP channels can be modulated by Ca\(^{2+}\)-dependent or Ca\(^{2+}\)-independent mechanisms. Desensitization can be initiated by the Ca\(^{2+}\)-influx through the channel itself and is manifested through phosphorylation–dephosphorylation of the TRP channel and/or by the Ca\(^{2+}\)-dependent interaction with calmodulin at the C-termini of, for example, TRPV1 and TRPV4 [114–121]. As an example of Ca\(^{2+}\)-independent mechanisms, the channel inactivation through physical interaction of the TRPC channel with the cytoplasmic protein homer has been described. TRPC mutants lacking the homer-binding site become constitutively active [9]. This latter example spurs one to hypothesize whether other scaffolding proteins than homer, such as actin and/or tubulin, can regulate TRP channel properties though to date the experimental evidence is only circumstantial. Modulation of TRPV4 by a cytosolic component is suggested, as the channel can be activated by heat only if analysed using whole-cell recordings and not in excised patches of cell-free membranes [122,123]. In turn, the involvement of cytoskeletal components in the regulation of TRPV4 channel activity has been demonstrated experimentally by the addition of cytoskeletal regulating drugs. The microtubule stabilizer taxol reduces TRPV4-dependent currents while the microtubule-disrupting agents colchicine and vin-cristine as well as actin cytoskeleton regulating drugs like phallloidin (a stabilizer) or cytochalasin B (a destabilizer) do not alter the TRPV4-mediated current [76]. In the same manner, mechanosensitive ion channel activity in cultured sensory neurons appears to depend largely on the status of the cytoskeleton. Thus, disruption of actin or microtubule cytoskeleton by pharmacological agents greatly reduces the activity of mechanosensitive channels [124]. However, if the modulation of TRP channels occurs through direct interaction with the cytoskeleton remains to be proven.

In addition to purely circumstantial evidence, few studies attempted the establishment of a direct modulatory role of the cytoskeleton, the best of which was performed on TRPP channels [125,126]. Montalbetti and co-workers isolated syncytiotrophoblast apical membrane vesicles from human placenta, and performed single-channel electrophysiological experiments of polycystin channel 2 (PC2) on reconstituted lipid bilayers. This system eliminates all factors except the channel-associated complex. Biochemical analysis revealed the presence of actin, the actin-related components \(\alpha\)-actinin and gelsolin, tubulin including acetylated \(\alpha\)-tubulin, and the kinesin motor proteins KIF3A and KIF3B in these membranes [125,126]. PC2 channels interact directly with KIF3. Disruption of actin filaments with cytochalasin D or with the actin-severing protein gelsolin activates the channel. This activation can be inhibited by the addition of soluble monomeric G-actin with ATP, which induces actin polymerization. This indicates that actin filaments, but not soluble actin, are an endogenous negative regulator of PC2 channels. Also microtubules regulate PC2 channel function only in opposing manner. Depolymerization of microtubules with colchicine rapidly inhibits the basal level of PC2 channel activity, whereas polymerization and/or stabilization of microtubules from soluble tubulin with GTP and taxol stimulates the PC2 channel activity [125]. Involvement of the microtubule cytoskeleton in the regulation of PC2 channel has also been described in vivo in primary cilia of renal epithelial cells [127]. In that system, addition of microtubule destabilizer (colchicine) rapidly abolished channel activity, whereas the addition of microtubule stabilizers (taxol) increased channel activity [127]. Similar results were obtained using reconstituted lipid bilayer system, which reveals that both spontaneous activity and the opening probability of TRPP3 ion channels is increased by the addition of \(\alpha\)-actinin, demonstrating that this channel can be indeed modulated by cytoskeleton [128].

TRPV1, TRPV4 and the cytoskeleton in pathophysiological conditions

The importance of TRP channels in the development of disease becomes increasingly evident. In particular, TRPV channels are involved in various aspects of pain such as inflammatory pain, cancer pain and neuropathic pain, as well as other diseases including allergy, diabetes and cough [129]. Indeed, data tie TRPV1 and TRPV4 to the status of the cytoskeleton in models of pain. For
example, rapidly dividing cancer cells are pharmacologically targeted by modulators of the microtubule cytoskeleton such as taxol, vincristin and their derivatives. But in addition to the deleterious effect of these agents on the cancer, if applied systemically over the long-term they are highly potent inducers of strong neuropathic pain [130–132]. Systemic vincristine treatment strongly alters the cytoskeletal architecture [133,134]. On a shorter timescale, inflammatory signalling pathways leading to sensitization in a healthy animal are dependent on both the ‘integrity’ and the ‘dynamics’ of the microtubule cytoskeleton [135,136]. Short-term modulation of the cytoskeleton abolishes inflammatory mediator-induced sensitization [135]. The precise mechanism by which vinca-drugs and taxoid-group-containing molecules influence pain is not clear. Vincristine by itself is known to form tubulin paracrystals [137]. Taxol can also form crystals, which can masquerade as stabilized microtubules and can rapidly incorporate tubulin dimers [138]. In addition, a unique type of straight GDP–tubulin protofilament forms in the presence of taxol [139]. Whether these uncommon altered physical forms of tubulins/microtubules are important for pain development remains a central question. However, more subtle effects like differential binding properties to other proteins might play a role.

In addition, the involvement of several TRP channels in the development of cancer and cancer pain is increasingly prominent [22,140–142]. Endogenous expressions of some TRP channels are either upregulated or downregulated in different tumours, cancerous tissue and also in different cancerous cell lines. For example, TRPV1 is overexpressed in bone cancer, prostate cancer and pancreatic cancer [143–146]. Along the same lines, TRPV4, which is involved in mechano-sensation, has been shown to be essential for the development of chemotherapy-induced neuropathic pain in the rat [72].

TRP channels also share functional links with the cytoskeleton by other means, namely cytotoxicity and cell death. For example, activation of TRPV1 leads to the inhibition of protein synthesis and endoplasmic reticulum fragmentation [42]. Prolonged stimulation with capsaicin induces apoptosis in TRPV1 expressing neurons by activating different caspase pathways (mainly caspase 8, caspase 3) [55–58,147–153]. However, whether the cytotoxicity and cell death described above is due to disassembly of microtubule has not been tested. Loss of TRPV1-expressing neurons/cells from specific tissues are functionally linked with the development of patho-physiological conditions. For example, loss of TRPV1 positive neurons in liver is linked with diabetes [154].

However, the deleterious effect of TRP channels on the specific subtype of neurons or cells has some clinical advantages. In fact, retraction and degeneration of a subset of sensory neurons (specifically TRPV1-expressing neurons), which involve events that affect the integrity of the cytoskeleton, forms the basis for the analgesic effect of topical capsaicin-cream treatment [155]. Resiniferatoxin (RTX), a potent agonist of TRPV1 has been used successfully to eliminate pancreatic cancerous cells because TRPV1 is highly expressed in this pancreatic cancer conditions [143–146]. Such clinical application of agonists specific for different TRP channels may, therefore, turn out to be effective and has full potential as chemotherapeutics. Based on this approach, recently use of TRP agonists as therapeutics is becoming popular in ‘TRPpathies’ or ‘channelopathies’ [156].

**Concluding remarks**

The last few years have seen rapid progress in the study of TRP channels as well as other ion channels in the context of both actin and the microtubule cytoskeleton. The presence of the microtubule cytoskeleton at the membrane is now beyond doubt [157]. The role of microtubule plus-end-binding proteins for specific sorting and targeting of different ion channels, receptors to specific regions of the membrane is well established [158]. However, the functional implications of this remain one of the current challenges. We find the role of the cytoskeleton to be both direct and indirect. In particular, the inside-out modulation of ion channels emerges as a peculiarly novel aspect with wide ranging consequences for both the pathological and the general homeostatic state. Because the large extended structure of the cytoskeleton is able to potentially integrate signalling events from very distant sides, single signals as well as associative stimuli will have to be investigated. In addition, the cytoskeleton has been proven to be both a target of signalling cascades and to initiate them itself. Thus it has the potential to recruit feedback and feed-forward regulation of a number of cellular effects. This renders the cytoskeleton as an interesting target for therapeutic approaches with respect to the TRP channels with much to discover.

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TRP channels and cytoskeleton regulate each other

C. Goswami and T. Hucho


