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Review

An intelligent nano-antenna: Primary cilium harnesses TRP channels to decode polymodal stimuli

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ABSTRACT

The primary cilium is a solitary hair-like organelle on the cell surface that serves as an antenna sensing ever-changing environmental conditions. In this review, we will first recapitulate the molecular basis of the polymodal sensory function of the primary cilia, specifically focusing on transient receptor potential (TRP) channels that accumulate inside the organelle and conduct calcium ions (Ca^{2+}). Each subfamily member, namely TRPP2 TRPP3, TRPC1 and TRPV4, is gated by multiple environmental factors, including chemical (receptor ligands, intracellular second messengers such as Ca^{2+}), mechanical (fluid shear stress, hypo-osmotic swelling), or physical (temperature, voltage) stimuli. Both activity and heterodimer compositions of the TRP channels may be dynamically regulated for precise tuning to the varying dynamic ranges of the individual input stimuli. We will thus discuss the potential regulation of TRP channels by local second messengers. Despite its reported importance in embryonic patterning and tissue morphogenesis, the precise functional significance of the downstream Ca^{2+} signals of the TRP channels remains unknown. We will close our review by featuring recent technological advances in visualizing and analyzing signal transduction inside the primary cilia, together with current perspectives illuminating the functional significance of intraciliary Ca^{2+} signals.

1. Introduction

The primary cilium is often analogized as the antenna of a cell – detecting stimuli from the extracellular environment and transducing them into specific intracellular functions [1–3]. Each cilium consists of a hair-like protrusion from the plasma membrane, and is supported by a microtubule-based axoneme that emanates out from the mother centriole during the G_0/G_1 state of the cell cycle. To achieve its role as a signal transduction apparatus, the primary cilium is equipped with specialized trafficking machinery that transports a specific collection of ion channels, transmembrane receptors and catalytic enzymes to the ciliary compartment (Fig. 1) ([4,5]). Among these ciliary signaling components are several Ca^{2+}-permeable channels from the transient receptor potential (TRP) superfamily, namely TRPC1, TRPP2, TRPP3 and TRPV4 [6–9]. Each TRP channel is known to possess multiple modes of activation (Table 1). The ciliary localization of these polymodal TRP channels therefore endows the primary cilium with sensitivity to multiple forms of stimuli. Concomitantly, intrinsic signaling properties of the primary cilium could modulate the activity of resident TRP channels to generate local Ca^{2+} signals distinct from the rest of the cell body. The significance of such bi-directional functional regulation between the primary cilium and ciliary TRP channels is shown by the fact that functional loss of these TRP channels (or associated auxiliary subunits) causes developmental defects and renal cystic disorders [7,10–12]. Tremendous interest has therefore been placed on the Ca^{2+} signals that are generated within the primary cilium and their role in coordinating intracellular function.

2. Polymodal nature of TRP channels

TRP channels are generally characterized with polymodal activation properties that implicate them in a broad range of functions (Table 1) [13,14]. This is best demonstrated by TRPV4, which is activated by chemical, mechanical and heat stimuli [15]. Arachidonic acid and its metabolites produced as a result of tissue damage or inflammation activate TRPV4, which in turn mediates Ca^{2+} influx responses that could regulate vaso-relaxation [16]. In mechanically sensitive tissues such as distal nephrons and collecting ducts of kidney as well as blood vessels, TRPV4 plays a principal role in mediating flow-induced intracellular Ca^{2+} influxes [9,17,18]. Consistent

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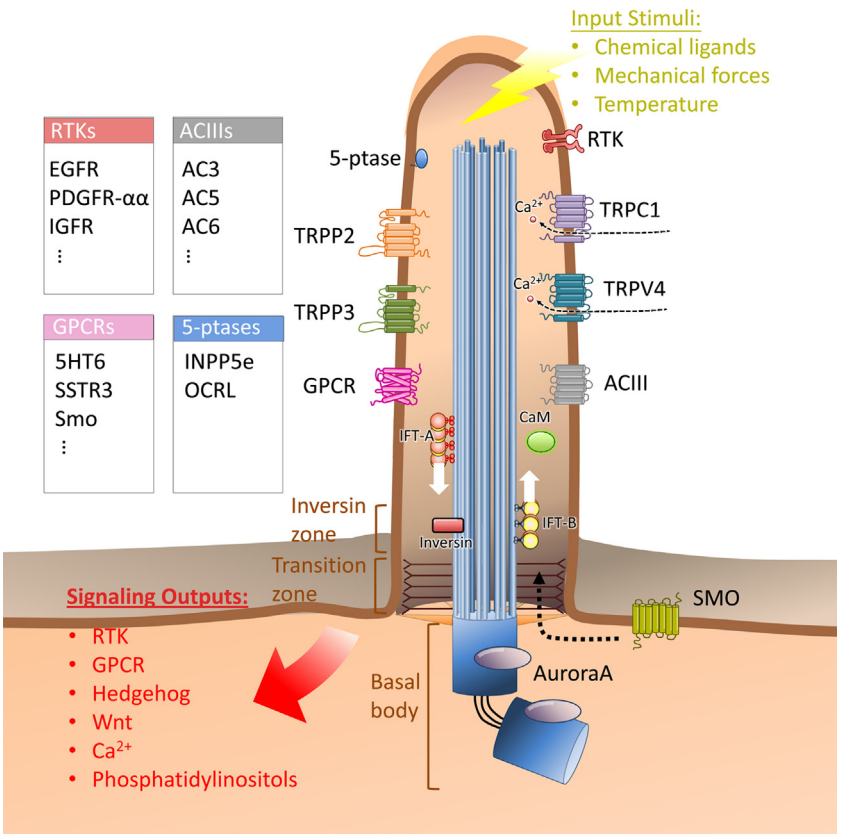

Fig. 1. Primary cilium harbors diverse signaling components to serve its role as a polymodal sensory organelle. The intraflagellar transport (IFT) machinery is a cilium-specific trafficking machinery that regulates anterograde (IFT-B) and retrograde (IFT-A) transport within the cilium. RTK: receptor tyrosine kinase (EGFR: epithelial growth factor receptor; PDGFR-αα: platelet-derived growth factor receptor α homodimer; IGFR: insulin-like growth factor receptor); GPCR: G-protein coupled receptor (5HT6: serotonin receptor isoform 6; SSTR3: somatostatin receptor isoform 3; Smo: smoothened) AC III: adenylate cyclase class III; 5-ptase: phosphoinositide 5-phosphatase (INPP5e: inositol polyphosphate-5-phosphatase; OCRL: oculocerebrorenal syndrome of Lowe); CaM: Calmodulin.

Table 1
A non-exhaustive list of gating stimuli and regulators for each cilium-residing TRP channel. Reported co-assembly partners for respective TRP channels are also listed in the right column together with associated function. Note that these are mostly determined *via* whole cell studies, and their specific effect within primary cilium requires further elucidation.

TRP channels detected in primary cilium	Gating stimuli (direct/indirect)	Channel activity regulators and interacting partners	Heteromeric co-assembly partners and associated function
TRPC1	• Direct membrane stretch [20] • Intracellular Ca^{2+} store depletion [22] • Gq-coupled GPCR/PLCβ [21] • RTK/PLCγ [21] • Nitric oxide [76]	• IP_3R [21,77] • Phosphorylation by PKC [47], PKG [76] • Ca^{2+}-Calmodulin [68] • STIM1 [22] • MARCKS [47] • $PI(4,5)P_2$ [47]	• TRPP2: Gq-coupled GPCR/PLCβ [8]
TRPP2	• Displays some constitutive activity [23] • Direct Ca^{2+} activation [26] • Triptolide [78] • Voltage (positive potential) [28] • EGF [38]	• Microtubule kinesin motors [42] • Actin-binding proteins [42] • IP_3R [79] • EGFR [38] • PLC-γ2 [38] • PACS-1-2 [80] • Phosphorylation by casein kinase 2, glycogen synthase kinase (GSK)-3, protein kinase D [23] • $[Ca^{2+}]$ [26] • $PI(4,5)P_2$ [38]	• PKD1: fluid flow [25] • PKD1L1: fluid flow [12]
TRPP3	• Displays some constitutive activity [24] • Direct Ca^{2+} activation [27] • Hypo-osmotic cell swelling [24] • Voltage (repolarization after depolarization) [24] • Warm temperatures [81]	• $[Ca^{2+}]$ [27] • Actin-binding proteins [41] • $[H^+]$[24] • RACK1 [82]	• PKD1L1: ATP purinergic signaling; warm temperatures; displays constitutive activity? [7,29]
TRPV4	• Hypo-osmotic cell swelling [19] • Fluid flow [9,17] • Warm temperatures (threshold: 27–35 °C) [18] • Phorbol esters [83] • Arachidonic acids and metabolites [16]	• Phosphorylation by PKC, PKA, Src tyrosine kinase [18] • Ca^{2+}-Calmodulin [69] • $PI(4,5)P_2$ [48] • MAP7 [84] • PACSIN3 [48]	• TRPP2: fluid flow; warm temperatures [9] • TRPC1: fluid flow [17]

with a mechanosensitive role, TRPV4 is required by cells to sense changes in cell volume caused by osmolarity changes in the extracellular environment [19], although it remains unclear whether TRPV4 senses mechanical forces directly or indirectly through other transduction pathways. Interestingly, mechanosensitivity of TRPV4 is augmented by warm temperatures [18]. TRPVs are in general thermo-sensitive, and demonstrate sensitivity for different temperature ranges; TRPV4 in particular displays some level of constitutive activity between 27 °C and 35 °C [18]. TRPV4 thus nicely illustrates a polymodal sensor device where one molecular entity detects and responds to multiple different types of inputs.

In contrast, TRPC1 has been proposed as a true mechanosensitive channel that is gated by tension developed in reconstituted lipid bilayer systems [20]. Characteristic of the canonical TRP family, TRPC1 also acts downstream of phospholipase C-β/γ activated by G-protein coupled receptors (GPCRs) or receptor tyrosine kinases (RTKs) [21]. Moreover, TRPC1 has been revealed as a key player in mediating extracellular Ca^{2+} entry in store-operated Ca^{2+} entry (SOCE) through interaction with STIM1 [22]. Gating mechanisms of TRPPs, meanwhile, are less well-defined. When expressed alone, TRPP2 and TRPP3 display low levels of constitutive channel activities that are modulated when these channels co-assemble with other TRP pore-forming monomers or auxiliary non-pore forming subunits [23,24]. For instance, TRPP2 has been proposed to co-assemble with non-pore forming polycystic kidney disease 1 (PKD1) and polycystic kidney disease 1 like 1 (PKD1L1) to form ion channel complexes that mediate cellular sensitivity to mechanical flow stimulation [12,25]. Both TRPP channels are also directly activated by Ca^{2+}, with TRPP2 demonstrating a bell-curve dependence on cytoplasmic [Ca^{2+}] levels [26,27]. Additionally, both TRPP2 and TRPP3 gating display voltage dependence, albeit each orchestrates distinct current responses to changes in membrane potentials [24,28].

Together, these TRP channels may be gated by various stimulatory modes (ligand–receptor, mechanical force, temperature, voltage and direct Ca^{2+} activation), and their co-presence in the primary cilium could equip this organelle with polymodal sensitivity. Indeed, direct measurement of cilium membrane currents has demonstrated sensitivity of the primary cilium to warm temperatures [29], while genetic ablation of primary cilium has also revealed a fundamental role of this organelle in mediating cellular responses to mechanical flow and hydrostatic pressure [12,25,30].

3. Unique structural and functional properties of primary cilium provide distinct local regulation of ciliary TRP channels

The gating and regulatory properties described for each TRP channel above and in Table 1 have been mostly elucidated from plasma membrane or whole cell measurements. This typically involves over-expressing or down-regulating an ion channel of interest in cells, and the resultant effect is assessed either *via* electrophysiological recordings of the cell membrane or monitoring global [Ca^{2+}] changes using cytosolic Ca^{2+} indicator dyes. However, these TRP channels are present in multiple compartments within the cell. This is demonstrated by the concurrent presence of TRPC1, TRPP3, and TRPV4 on the plasma membrane and cilium membrane to execute external Ca^{2+} entry. While TRPP2 is specifically targeted to primary cilia through a highly regulated trafficking mechanism [31,32], the bulk of TRPP2 appears to be present in the endoplasmic reticulum to mediate release of intracellular Ca^{2+} stores [26]. It is imperative to recognize that these compartments possess distinct structural and functional properties. Therefore, the same TRP channel residing in different compartments may be subjected to discrete regulation to perform compartment-specific functions. In the following sub-sections, we will highlight several distinct intrinsic properties of the primary cilium that could provide a basis for cilium-specific regulation of TRP channel activity (Fig. 2).

3.1. Primary cilium may serve as a platform for facilitating heteromeric assembly of TRP channels

TRP monomers generally assemble into homo-tetrameric ion channels, but could also hetero-multimerize with other TRP members from same or different sub-families, or even with auxillary non-pore forming subunits. The primary cilium, which is locally enriched with a unique complement of TRPs from TRPC/P/V sub-families, could house TRP ion channel assemblies of novel molecular stoichiometry that possess gating and regulatory properties distinct from the homomeric channels (Fig. 2A). For instance, TRPV4 has been shown to co-assemble with TRPP2 and TRPC1 [9,17]. Heteromeric TRPV4-TRPP2 channels produce more robust currents and are thus more sensitive than homomeric TRPV4 channels to hypo-osmotic cell-swelling and high extracellular [Ca^{2+}] [9]. As a result, the local enrichment of these novel ion channel complexes could up-regulate the sensitivity of the primary cilium (as compared with plasma membrane) for respective forms of stimuli. Table 1 provides additional examples of heteromeric TRP channel assemblies that could be present in primary cilium.

3.2. Cilium-enriched receptors may locally regulate TRP channels within the compartment

In addition to housing ion channels, the primary cilium is enriched with molecular components from a variety of signaling pathways. These molecules include GPCRs such as serotonin receptor isoform 6 (5HT6) and somatostatin receptor 3 (SSTR3) [33], RTKs such as PDGFR-α and EGFR [34], as well as class III adenylate cyclases (ACs) such as AC 3, 5 and 6 [35–37]. The enrichment of these molecules inside primary cilia suggests that the local signal strength of the downstream pathways could be more robust in the cilium than the surrounding cell membrane. This could also provide a specialized platform for enhanced molecular crosstalk between different ciliary signaling pathways. Importantly, TRP channel activity may be regulated by these signaling pathways through post-translational modifications and transient binding interactions. TRPC1 and TRPP2 act downstream of RTK and Gq-coupled GPCR pathways through phospholipase C-β/γ [21,38], while TRPV4 possesses multiple phosphorylation sites at the amino and carboxyl terminal domains that are regulated by PKA, PKC and Src tyrosine kinases [18]. Thus, local Ca^{2+} signals generated by ciliary TRP channels could serve as effectors of these ligand receptor pathways to modulate specific functions within the primary cilium (Fig. 2B).

3.3. Absence of actin cytoskeleton in the cilium may affect activity and organization of cilia-localized TRP channels

Besides concentrating specific signaling components, the primary cilium also appears to actively exclude certain cellular components that reside in the surrounding cell membrane and underlying cortex. Diverse types of actin regulatory proteins associate with the plasma membrane to control the dynamics of actin cytoskeleton in the cell cortex, and this is critical in a variety of cellular processes including cell migration, endocytosis and exocytosis [39]. Even though the primary cilium membrane is contiguous with the surrounding cell membrane (*i.e.* no membrane barrier), it is surprisingly devoid of actin cytoskeletal structures [40] (Fig. 2C). While it is unclear how cytoskeletal actin may be excluded from the cilium, the sub-micrometer dimensions of the cilium (200 nm in diameter) and the presence of an elaborate microtubule-based axoneme and transport motor system could

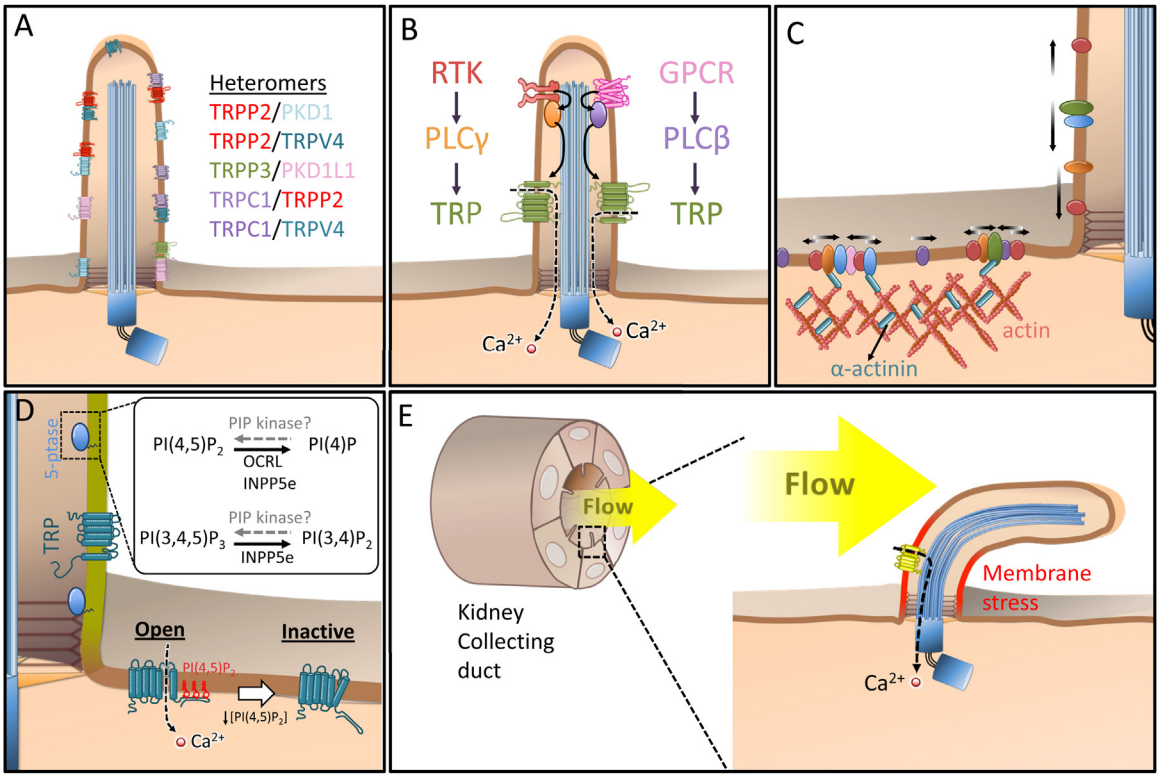

Fig. 2. Proposed cilium-specific regulation of ciliary TRP channels. (A) TRP channels from different sub-families could hetero-multimerize into ion channel assemblies of novel gating and regulatory properties within the primary cilium. (B) Ciliary TRP channels could serve as downstream effectors of receptors selectively concentrated within primary cilium, an example being *via* phospholipase C-β/γ, (C) absence of actin cytoskeleton and actin-associated proteins within primary cilium could result in a dynamic organization of receptors and ion channels that is distinct from contiguous plasma membrane. (D) Enrichment of phosphoinositide 5-phosphatases within primary cilium could alter phosphatidylinositol composition of cilium membrane, modulating phosphatidylinositol-dependent regulation of TRP channel activity. (E) Intrinsic properties of kidney primary cilium could sensitize mechanosensitive ciliary TRP channels to shear stress caused by fluid flow within kidney tubule.

interfere with stable formation of actin structures within the ciliary lumen. This likely affects the function of ciliary TRP channels, as studies have demonstrated that indirect interactions between these TRP channels and actin cytoskeleton through actin binding proteins could potentiate channel activity [41,42]. Indeed, TRPP2 and TRPP3 bind with α-actinin, an actin-crosslinking protein, and this interaction is sufficient to increase the open probability of each channel in reconstituted lipid bilayer systems, without affecting single channel conductance [41,42], suggesting a role of α-actinin in gating TRPP channels. Furthermore, the actin cortex that underlies cell membrane plays a fundamental role in organizing transient nanoscale signaling assemblies on the cell membrane. Several models have been proposed to explain the phenomenon, including tethering between cortical actin filaments and cytosolic domains of transmembrane proteins and regulation of membrane lipid clustering through actin binding proteins [43,44]. This actin-dependent membrane organization is proposed to execute precise control over the strength, kinetics and spatiotemporal localization of cell membrane signals [45]. The lack of actin cortex underlying the primary cilium membrane thereby suggests the receptor and ion channel organization within the cilium membrane should be distinct from that of the surrounding cell membrane (Fig. 2C). Thus, we may expect divergent Ca^{2+} signaling outputs from these two separate compartments, despite utilizing the same ion channel or receptor.

3.4. *Differential phosphatidylinositol composition of primary cilium membrane may alter phosphatidylinositol-dependent regulation of TRP channels*

The functionality of TRP channels is ubiquitously regulated by membrane phosphatidylinositols. Amongst them, the role of $PI(4,5)P_2$ on TRP channel regulation is the most extensively studied, with its action on individual sub-families being distinct [46]. $PI(4,5)P_2$ is a substrate of phospholipase C-β/γ, which breaks it down into IP_3 and DAG. The subsequent activation of protein kinase C by Ca^{2+} (through IP_3-induced release of intracellular Ca^{2+} stores) and DAG in turn phosphorylates and regulates the canonical sub-family of TRP channels including TRPC1 [21,47]. Apart from serving as a substrate for phospholipase C-β/γ, $PI(4,5)P_2$ also regulates TRP channel activity through direct interaction. Indeed, binding with $PI(4,5)P_2$ induces conformational changes in TRPC1 and TRPV4 channels that prime them for channel opening [47,48]. In contrast, Ma et al. [38] reported that TRPP2 is inhibited by high levels of $PI(4,5)P_2$ in the membrane, although the underlying mechanism remains elusive. These data together suggest that the membrane density of $PI(4,5)P_2$ plays a critical role in regulating TRP channel function. However, some TRP channels are less specific to $PI(4,5)P_2$, and may be regulated by other phosphoinositide species such as $PI(3,4,5)P_3$ through similar binding interactions [46]. Interestingly, phosphoinositide 5-phosphatases such as OCRL and INPP5e are concentrated within the primary cilia in a variety of cell types; OCRL is known to metabolize $PI(4,5)P_2$ into PI(4)P, whereas INPP5e converts $PI(3,4,5)P_3$ to $PI(3,4)P_2$ [49–51] (Fig. 2D). While it remains to be determined whether phosphoinositide kinases may be co-enriched within the cilium, a down-regulated local level of $PI(4,5)P_2$ and/or $PI(3,4,5)P_3$ in the cilium membrane (relative to surrounding plasma membrane) could serve as a fundamental mechanism to distinguish ciliary TRP channel activity from that in the plasma membrane. Nevertheless, it is imperative to consider the apparent affinity of TRP channel for each form of phosphoinositide; channels with low apparent affinity may be readily inhibited by mild decrease in phosphoinositide levels, but channels with high

apparent affinity may require a drastic drop in phosphoinositides before inhibition occurs [46]. This is further complicated by the fact that the apparent phosphoinositide affinity of an ion channel is not static and may be regulated by post-translational modifications and binding partners [46]. Thus, it is necessary to compare the relative phosphatidylinositol levels between the cilium membrane and the plasma membrane and assess the resultant effect on individual TRP channel function within the primary cilium.

3.5. *Intrinsic mechanical properties of kidney primary cilium may sensitize ciliary mechanosensitive TRP channels to mechanical flow stimulation*

Within kidney tubules, primary cilia protrude out from the apical face of the cell membrane into the luminal space where fluid motion occurs (Fig. 2E). *In vivo* imaging studies have revealed that fluid flow within the kidney is not continuous, but instead occurs with periodic oscillation in flow rates proposed to be regulated by the tubuloglomerular feedback mechanism [52,53]. As a result, the elongated rod-like structure of the kidney primary cilium could behave as a cantilever beam that gets progressively deflected by elevated luminal flow rates. Indeed, studies have clearly demonstrated the flexural rigidity of the kidney primary cilium to be optimal for sensing changes in flow shear stress (FSS) within a physiological range through correlated changes in its flow-induced bending angle [54–57]. Interestingly, the flexural rigidity of primary cilium was also found to be non-uniform along the axoneme, with the proximal part of the cilium shaft stiffer than the distal segment [55–57]. The higher stiffness of the proximal ciliary shaft may be crucial to keep the kidney primary cilia upright, and sensitize the cantilever-like structure to small variations in the luminal flow rate (Fig. 2E). This is in direct contrast to primary cilia in other cell types such as fibroblasts and retinal epithelial cells, where the ciliary shaft is generally positioned more parallel with the cell surface. Overall, these studies have demonstrated the specialized capacity of the kidney primary cilium to sense mechanical flow, and tremendous efforts have been invested to elucidate the mechanism that translates this mechanical behavior into intracellular signaling events. TRPP2 has been specifically placed in the limelight. It is proposed that TRPP2 forms a complex with mechanosensitive PKD1 within the kidney primary cilium to convert shear forces on the cilium membrane into ciliary Ca^{2+} influxes that could further induce the release of Ca^{2+} from cell body intracellular stores through Ca^{2+}-induced Ca^{2+} response [25,58]. Nevertheless, direct mechanosensitivity of TRPP2 and/or PKD1 has not been proven, and various studies have proposed alternative molecular mechanisms that could endow the primary cilia with mechanotransduction capacity. Notably, the mechanosensitive microtubules within the cilium that compose the axoneme could sense the flow-induced shear stress at the proximal ciliary shaft and transduce this mechanical force onto TRPP2 through conformational changes [59]. Indeed, studies have shown TRPP2 to interact with the microtubule cytoskeleton through microtubule-associated proteins, and altering these interactions could modulate channel activity of TRPP2 [42,59]. Furthermore, TRPC1 and TRPV4 channels also have established roles in flow sensing [17]; TRPC1 in particular has been recognized as a true mechanosensitive channel. Therefore, the mechanosensor within the primary cilia may be expected to consist of a multimeric assembly of proteins that could elicit a ciliary Ca^{2+} influx in response to mechanical deflections of the ciliary structure (Fig. 2E). In addition to kidney cells, primary cilia in other cell types have received recognition as mechanosensitive structures in recent years. The nodal cilia in the embryonic node, in particular, bend under leftward fluid flow generated by surrounding motile cilia [12]. The TRPP2-PKD1L1 complex within the nodal cilium is proposed to transduce this mechanical force into a Ca^{2+} signal that subsequently activates the developmental pathway which determines left-right laterality in the developing embryo [12]. In summary, the ciliary structures of specific cell types are sensitized to sense mechanical flow, which could locally activate cilia-localized TRP channels through direct or indirect mechanotransduction.

4. Illumination of Ca^{2+} signals within primary cilium reveals novel Ca^{2+} regulation within the ciliary compartment

While it is recognized that cilium-localized TRP channels play crucial roles in a variety of cell functions, the nature of ciliary Ca^{2+} signals has been an elusive subject that was revealed only very recently. The volume of a primary cilium is estimated to be in the range of 0.1 femtoliter (fL), merely 1/10,000th of the total cell volume. The disproportionately small volume of the primary cilium in each cell has impeded the use of conventional Ca^{2+} indicator dyes to reveal local Ca^{2+} signals, primarily due to signal saturation from the cell body [60]. This technical hurdle has prompted several groups to develop cilium-targeted genetically encoded Ca^{2+} indicators (GECIs) where GECIs are targeted to the primary cilium through fusion with ciliary proteins [7,60,61]. In particular, Su et al. [60] have constructed and analyzed a library of ciliary targeting sequences fused with varied GECIs and thoroughly assessed these engineered biosensors in terms of targeting efficiency, effects on ciliary structure and dynamic range of GECI responses.

Using these cilium-targeted GECIs, Delling et al. [7] unexpectedly found that the basal ciliary $[Ca^{2+}]$ level (580 nM) in human retinal pigment epithelial (hRPE) cells was approximately five times higher than that in the cell body (107 nM). They further performed patch clamp experiments on hRPE cilium membrane and estimated the channel density to be approximately 29 channels/μm^2, comparable with intracellular compartment membranes *e.g.* mitochondria [29]. Ionic permeability experiments also revealed that ciliary ion channels possessed six times higher permeability to Ca^{2+} than to Na^+. They proposed that a steady basal flow of Ca^{2+} through ciliary TRP channels was able to establish a local elevated $[Ca^{2+}]$ within the narrow ciliary lumen with femtoliter volume; this was likely to be mediated through constitutive activity of a TRPP3-PKD1L1 complex, which they identified within the cilium. Nevertheless, it remains an open question whether loss of function of TRPP3-PKD1L1 complex could directly abolish the elevated basal ciliary $[Ca^{2+}]$ levels. Further work is also required to determine the presence of a Ca^{2+} buffering mechanism (*e.g.* Ca^{2+} binding proteins) or Ca^{2+} efflux mechanism (*e.g.* plasma membrane Ca^{2+} ATPase) that could regulate ciliary $[Ca^{2+}]$ levels.

Some work has also been carried out to understand the exchange of Ca^{2+} between the cilium and cytosol compartments. When caged Ca^{2+} was locally uncaged in the cytosol, Ca^{2+} was observed to freely diffuse into the cilium with no obvious delay. This indicated that Ca^{2+} is normally able to freely diffuse between cilium and cytosol [7]. In contrast, when laser ablation was used to rupture the ciliary tip to induce local Ca^{2+} influx, a strong $[Ca^{2+}]$ rise was detected along the cilium, whereas no significant increase in $[Ca^{2+}]$ was observed in the cytosol. It was hypothesized that the small number of Ca^{2+} ions that diffuse from the ciliary lumen into the cytosol was neither sufficient to result in detectable increase in cytosolic $[Ca^{2+}]$ nor able to induce further Ca^{2+} release in the main cell body.

4.1. *Ciliary Ca^{2+} signals induced by purinergic signaling*

Purinergic signaling is established to induce Ca^{2+} signals in the cytosol through extracellular Ca^{2+} entry (P2X channels) and intracellular store release (G-protein coupled P2Y receptors). Conversely, almost nothing is known about the role of purinergic

signaling in the primary cilium. Su et al. [60] accordingly visualized Ca^{2+} signals induced by purinergic signaling in the cilium and cytosol. By simultaneously monitoring ciliary and cytosolic [Ca^{2+}] changes in fibroblasts under ATP stimulation, they observed elevations in cilium Ca^{2+} signals that accompanied [Ca^{2+}] oscillations in the cytosol, and the propagation of each ciliary Ca^{2+} signal generally occurred from base to tip. While the base initiation of ciliary Ca^{2+} signals likely suggests the diffusion of free Ca^{2+} from the cell body into the ciliary lumen, a contribution from Ca^{2+}-permeable channels in the cilium should not be disregarded. This is supported by patch clamp experiments that detected stronger ionic currents in cilium membrane of multiple cell types upon ATP stimulation [29]. While a recent study has revealed the presence of cyclic AMP signal-related $P2Y_{12}$ purinergic receptors within the cholangiocyte primary cilium [62], further work is required to determine the presence of Ca^{2+} signal-related purinergic receptors in the primary cilium and their precise role in gating cilia-localized Ca^{2+}-permeable channels.

4.2. Ciliary Ca^{2+} signals induced by mechanosignaling

Ca^{2+} signals within primary cilium mechanically deflected by fluid shearing forces have also been revealed by combining biosensor technology with mechanical flow systems. The kinetics of flow-induced ciliary Ca^{2+} signals is interestingly dependent on the type of mechanical flow system used. Jin et al. [61] developed a novel cilia imaging technique to facilitate side-view imaging of the cilium shaft in the XY imaging plane; kidney cells were seeded onto precision microwire and assembled into an open fluid perfusion chamber where fluid flow may be supplied. Using this imaging platform, ciliary Ca^{2+} signals induced by 0.7 dyne/cm^2 of FSS were mild and lasted on a timescale of seconds. In contrast, Su et al. [60] used a closed flow perfusion system that could better resemble the closed environment of kidney tubules *in vivo*. Ciliary Ca^{2+} signals induced by 1 dyne/cm^2 of unidirectional laminar FSS were more robust and sustained on the scale of minutes. Clearly, the types of fluid flow that were delivered to cells in these studies are rather simplified and do not accurately reflect the complex nature of fluid flow that occurs along different segments of kidney nephrons *in vivo* [52,53]. Therefore, while the findings of these studies deliver a first glimpse of Ca^{2+} signals in mechanically deflected primary cilia, much work is still required to determine ciliary Ca^{2+} signals under physiological states of fluid flow, and the correlation with Ca^{2+} signals in the cell body.

5. Functional significance of ciliary Ca^{2+} signals

While genetic studies have demonstrated a central role of ciliary TRP channels in regulating embryonic patterning and epithelial tissue morphogenesis [7,11,12], surprisingly little is known about the exact molecular function of ciliary Ca^{2+} signals within the cilium or in the cell body. This is likely attributed to the limited existing knowledge of ciliary protein function. Nevertheless, several proteins within the primary cilia are known to interact with Ca^{2+} or Ca^{2+}-binding proteins, and could serve as a stepping-stone to elucidate the functional role of ciliary Ca^{2+} signals.

The proximal segment of primary cilium is organized by a highly conserved protein known as inversin [63] (Fig. 1). Loss of function of inversin is known to cause embryonic left–right determination defects and polycystic kidneys [64]. Interestingly, inversin contains two IQ domains (IQ1 and IQ2). *In vitro* studies have not only demonstrated direct Calmodulin (CaM) binding by these IQ domains, but also revealed that CaM-binding with IQ2 domain is negatively regulated by Ca^{2+} [65]. Since CaM has been reported in primary cilia of several cell types [63,66,67], one could speculate that local change in ciliary Ca^{2+} signals could regulate inversin–CaM interaction within the primary cilia. Moreover, several cilium-localized TRP channels possess CaM interaction sites which modulate channel activity [68,69]. Dissecting the functional interaction between inversin, CaM and ciliary TRP channels could therefore reveal their role in mediating embryonic patterning and kidney morphogenesis.

In addition to the inversin compartment, components in other parts of the ciliary structure could also be regulated by changes in ciliary [Ca^{2+}]. An example is IFT 25, a component of the anterograde intraflagellar transport machinery which plays an essential role in trafficking sonic hedgehog signaling components into the primary cilium [70] (Fig. 1). A recent crystallographic study has detected a Ca^{2+} binding site in IFT 25, which could regulate the dynamics of IFT25 transport along the ciliary axoneme [71]. Interestingly, Delling et al. [7] have also proposed Ca^{2+} entry through ciliary TRP channels to locally deliver free Ca^{2+} to the periciliary region, where Ca^{2+}-regulated kinases such as aurora A kinase reside at the centrosome [72] (Fig. 1). Understanding the molecular role of Ca^{2+} in regulating ciliary proteins would reveal the functional role of Ca^{2+} signaling within the primary cilium.

6. Conclusion and outlook

To accomplish its role as a multi-sensory antenna, the mammalian primary cilium has evolved mechanisms to direct polymodal TRP channels from distinct sub-families to reside in the cilium membrane. While rigorous studies on TRPP2 have determined a trafficking mechanism that regulates transport between the endoplasmic reticulum, plasma membrane and cilium membrane [31,32], much work is still required to understand how TRPC1, TRPP3 and TRPV4 are transported into the cilium. In this review, we have highlighted several intrinsic properties of the cilium that differentiate it from the surrounding plasma membrane, and further evaluated the potential of these factors in modulating the activity of residing TRP channels to implement cilium-specific function. Dissecting cilium-specific activity from the rest of the cell body requires obtaining adequate ciliary signal-to-background ratio, but this is impeded by the disparate volume ratio between the solitary primary cilium and the cell body. There is hence growing interest to develop tools that can specifically visualize and manipulate activities within this sub-micrometer sized compartment on the cell membrane [7,60,61,73,74].

Ciliary [Ca^{2+}] is regulated both by cilium-specific machinery and cytosolic Ca^{2+} changes [7]. As a result, it is relevant to dissect and resolve ciliary [Ca^{2+}] changes that result from global Ca^{2+} signals spread from the main cell body and local Ca^{2+} entry from ciliary ion channels, and determine ciliary effectors that could possess sensitivity to distinguish between Ca^{2+} signals originating from these distinct sources. Moreover, it is currently unclear why the primary cilium needs to be equipped with polymodal sensitivity. A recent study has suggested the primary cilium receives extracellular signals and specifically transduces them to the underlying centrosome to regulate cell cycle progression [75]. Overall, defining the functional significance of ciliary Ca^{2+} signals in the primary cilium/centrosome region promises valuable insight into the complex roles of cilium-localized TRP channels in disease and development.

Conflict of interest

The authors declare no competing financial interests.

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