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Review

Golgi bypass of ciliary proteins

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ABSTRACT

Primary cilia represent small, yet distinct compartments of the plasma membrane. They are speculated to exercise chemo- and mechanosensory functions and to serve as signaling hubs for crucial pathways such as the Wnt and hedgehog cascades. It is therefore necessary that specific integral membrane proteins, in particular sensors and receptors, are sorted to the cilium and not to the surrounding somatic plasma membrane upon being synthesized at the rough endoplasmic reticulum. Apparently no singular zip code for the primary cilium exists but rather several ciliary targeting signals whose biochemical and cell biological implications are just about being unravelled. Among the better understood proteins residing in the primary cilium is polycystin-2 which is mutated in patients suffering from autosomal-dominant polycystic kidney disease. A special case in the context of this review concerns the connecting cilium which serves as the trafficking pathway for proteins involved in visual sensation of retinal photoreceptor cells. In order to efficiently capture photons, the photopigments are organized in discs or membrane invaginations. Mutations in certain proteins involved in these processes lead to retinal degeneration and ultimately to blindness. One example is peripherin/rds which is mutated in the *rds* (retinal degeneration slow) mouse. The trafficking of peripherin/rds from the inner to the outer segment of photoreceptor cells by way of the connecting cilium also seems to diverge at the Golgi apparatus, and the routes of polycystin-2 and peripherin/rds may represent paradigms of ciliary proteins for the type IV pathway of unconventional protein secretion.

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Contents

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1. Introduction

After having been neglected for decades primary cilia have become one of the most intensely investigated organelles in the field of cellular biology. They are singular, typically ~3–5 μm long extensions of most mammalian cell types, and due to the presence of only 9 peripheral microtubular doublets and no central pair of microtubules are believed to be immotile in most cases although there seem to be exceptions [1]. The renaissance of primary cilia started with the discovery that proteins mutated in patients suffering from polycystic kidney disease are located in primary cilia [2,3]. Despite intense research efforts their function is still not understood. They may play a role in chemosensation [4] and mechanosensation [5] although the latter has been disputed recently [6]. Furthermore primary cilia are thought to be involved in several signal transduction cascades and a bewildering variety of genetic diseases such as autosomal-dominant and autosomal-recessive polycystic kidney disease, nephronophthisis, Bardet-Biedl syndrome, Joubert syndrome, Meckel syndrome or retinal degeneration [7,8]. In light of those facts a new term has been coined to name the diseases originating from a malfunctioning primary cilium, they are now called ciliopathies.

1.1. The formation of primary cilia

More than 5 decades ago Sergei Sorokin published a careful ultrastructural characterization of what has been categorized as the intracellular pathway of cilia formation [9], the results of which were confirmed in 1971 [10]. He concentrated his studies on fibroblasts of the developing duodenum where primary cilia can be regularly observed (but which at the same time are among those cells where the function of cilia is the least understood). Since the basal body of primary cilia originates from the mother centriole of the centrosome, Sorokin focused on this particular organelle. In what he considered the first stage of cilium formation he described the association of a round vesicular structure, the primary ciliary vesicle, with the distal end of the mother centriole. As the primary ciliary vesicle attaches to the mother centriole, an amorphous material emerges between the vesicle and the centriole which is pushed forward by the outgrowing axoneme so that the vesicle flattens and assumes a cap- and bell-shaped structure (Fig. 1a, b). Finally the centrosome with the elongated mother centriole together with its membranous sheath moves towards the plasma membrane where the outer membrane of the sheath, the prospective pocket membrane, fuses with the plasma membrane. In consequence, the inner membrane gains access to the extracellular space and becomes the ciliary membrane compartment, and the mother centriole becomes the basal body of the newly formed cilium (Fig. 1c).

Due to the fact that the centrosome typically lies in a perinuclear position close to the Golgi apparatus Sorokin speculated that the primary vesicle and also the incoming additional vesicles which provide the necessary membrane material for the elongation of the primary vesicle are derived from the Golgi apparatus. No firm experimental evidence was provided for this hypothesis but it nevertheless found wide acceptance in the subsequent literature. In 2015, an article appeared in which an even earlier stage of ciliogenesis was identified. The mother centriole can be discerned from the daughter centriole through the presence of filamentous, electron-dense extensions, the distal appendages, at the *plus* end of its microtubular doublets and triangular extensions, the subdistal appendages, a short distance further down towards the *minus* end of the microtubules. Vesicles of 40–60 nm in diameter were seen to attach to the far end of the distal appendages (Fig. 1a, b1), henceforth called distal-appendage vesicles, and believed to fuse with each other in a second step to form the ṗrimaryc̈iliary vesicle [11].

1.2. Theoretical considerations concerning the transport of integral membrane proteins to the primary cilium

Over the years a code has emerged which explains the specific targeting of integral membrane and other proteins towards distinct organelles, such as the hydrophobic signal peptide at the NH_2-terminus of plasma membrane and secreted proteins or a stretch of basic amino acids in proteins destined for transport into the nucleus. At least for some membrane proteins of primary cilia and of the outer segments of retinal photoreceptor cells, which are connected with the cell body by the connecting cilium (a structure analogous to the primary cilium) (Fig. 1c), critical residues have been identified as putative ciliary targeting signals, such as the VxP motif in rhodopsin [12] and polycystin-2 [13]. It is not understood, however, how these proteins reach the ciliary compartment. Since polycystin-2, for example, can only be found in primary cilia and not in the surrounding somatic plasma membrane something like a barrier has to exist at the base of the cilium which prevents the escape of polycystin-2 from the cilium. Therefore transport vesicles carrying polycystin-2 should tether at the barrier or on the ciliary side of it. Alternatively, transport vesicles with ciliary membrane proteins might also fuse with the somatic plasma membrane and then the ciliary proteins move into the primary cilium via lateral diffusion, as is the case for smoothened [14]. In such a scenario the putative barrier at the base of primary cilia would act in a one-way fashion. And finally, ciliary integral membrane proteins might first be incorporated into the somatic plasma membrane, then endocytosed and redirected from an endosomal compartment to the cilium. At the current state of knowledge it is not clear what the predominant route to the cilium is.

2. Trafficking of peripherin/rds to the connecting cilia of photoreceptor cells

Since the outer segments of photoreceptor cells in the retina do not contain the necessary components of the protein synthetic machinery, newly synthesized proteins have to be transported from the inner to the outer segment via the connecting cilium, an organelle with structural and functional features related to those of the primary cilium (Fig. 1c). It therefore comes as no surprise that mutations in certain genes not only lead to polycystic kidney disease but also to the degeneration of photoreceptor cells. Murine peripherin/rds is a 346-amino acid long protein [15] with 4 membrane-spanning domains [16,17] which has been localized to the discs in the outer segments of photoreceptor cells [16,17]. Its importance for the formation of these discs can not only be appreciated by the fact that it is mutated in the *rds* mouse model of retinal degeneration [15] but also from mutations of the human orthologue in a number of retinal diseases [18].

Integral membrane and secretory proteins are subject to glycosylation at asparagine residues in the endoplasmic reticulum. These *N*-linked sugar residues are processed upon transit of these proteins through the Golgi apparatus by the enzyme mannosidase II, which resides predominantly in the mid- and trans-Golgi cisternae [19], such that the sugar residues become resistant to the action of endoglycosidase H although the *N*-linked sugar residues can still be removed by treatment with the enzyme peptide:*N*-glycosidase F (PNGase F) [20]. Already early on it was demonstrated that peripherin/rds isolated from rod outer segments is *N*-glycosylated and still sensitive to treatment with endoglycosidase H [16,17], and this finding was confirmed in a more recent study [21]. Due to the fact that no suitable cell line model exists which elaborates

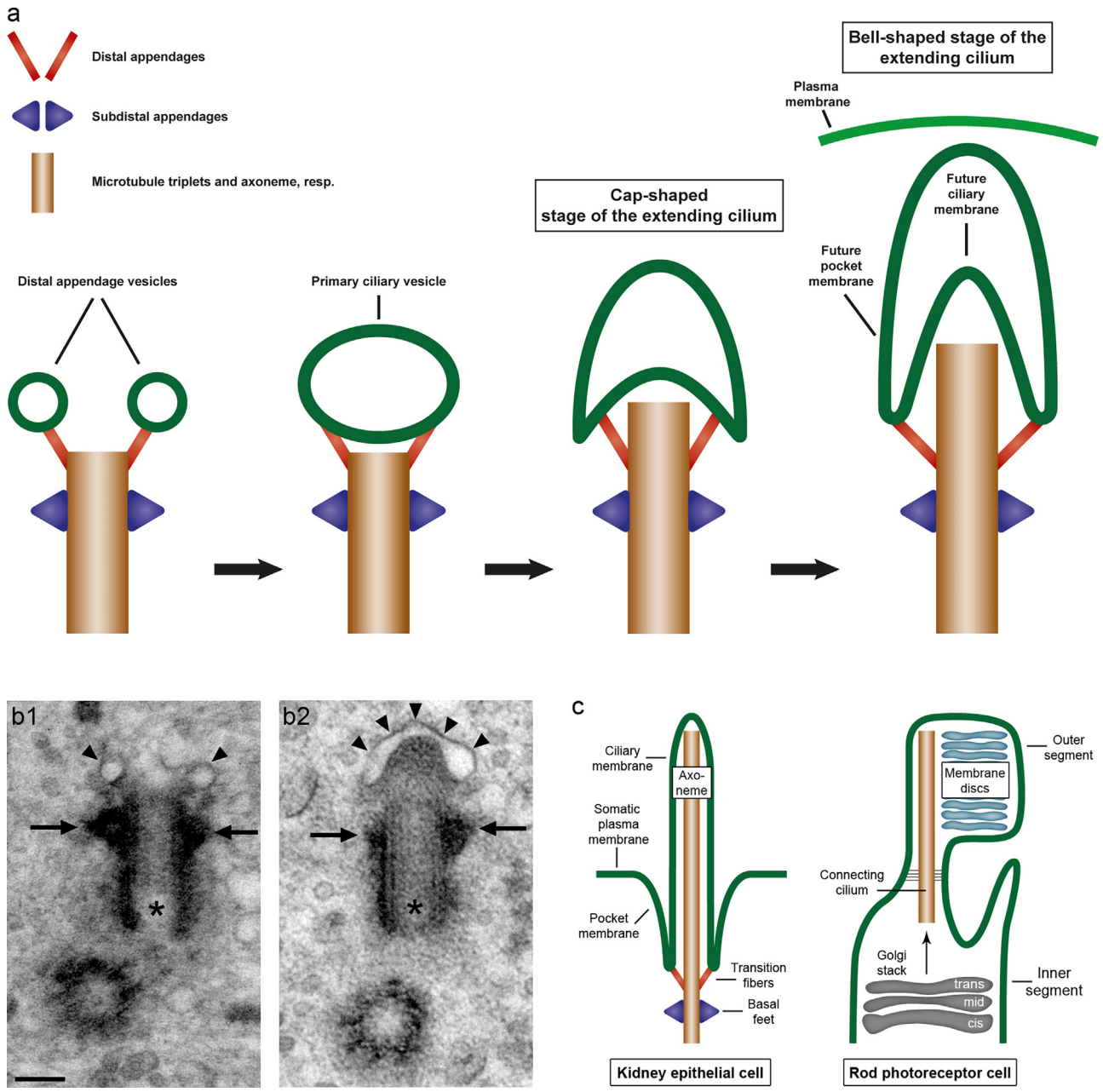

Fig. 1. Development and structure of primary cilia and connecting cilia. (a) Schematic view of ciliogenesis. The first known step involves the association of vesicles to the distal appendages of the mother centriole which subsequently fuse to form a primary ciliary vesicle. Through the extension of the axoneme the primary ciliary vesicle becomes indented at its proximal end and elongated at its distal end. Finally the ciliary vesicle fuses with the overlying plasma membrane. (b1, b2) Transmission electron micrographs of ciliogenesis in immortalized retinal pigment epithelial cells. Both pictures show a longitudinal section of the mother centriole (asterisk) and a cross section of the daughter centriole. In contrast to the daughter centriole, the mother centriole elaborates subdistal (arrows) and distal appendages. At the earliest stage of ciliogenesis, small vesicles (arrow heads) associate with the distal appendages to form distal appendage vesicles (b1). After fusion of the distal appendage vesicles to a primary ciliary vesicle, the axoneme extends at its distal end so that the primary ciliary vesicle (arrow heads) flattens and assumes a cap-shaped structure (b2). Bar, 200 nm. Pictures taken by Karin Schadendorf. (c) Schematic view of a mature primary cilium, e.g. in a kidney epithelial cell, and of a connecting cilium in a rod photoreceptor cell. In the photoreceptor cell the cilium connects the inner segment of the cell, where the protein synthetic machinery is located, with the outer segment, where the stack of membrane discs containing the photoreceptors are located.

the intricate organization of photoreceptor cells, the trafficking of peripherin/rds to the primary cilium was investigated. Treatment with the Golgi-disrupting agent brefeldin A, a fungal metabolite inhibiting the monomeric GTPase Arf1 which thereby leads to the collapse of the Golgi apparatus [22,23], only marginally affected the trafficking of peripherin/rds to the primary cilium [21]. In addition, treatment with 30N12, a drug inhibiting transport from the trans-Golgi compartment to the plasma membrane, had no effect on the number of peripherin/rds-positive primary cilia, and neither had monensin, an antibiotic also acting at the trans-side of the Golgi apparatus [21]. Taken together, peripherin/rds appears to reach the cilium using a transport route that bypasses the Golgi apparatus.

Certain mutated integral membrane proteins such as CFTR also bypass the Golgi apparatus, and they do not appear to rely on the COPII coat complex when leaving the endoplasmic reticulum [24]. In contrast (and similar to the data described below for polycystin-2), the transport of peripherin/rds begins in a COPII-dependent fashion at the endoplasmic reticulum [21]. Furthermore at least in some cases, bypass of the Golgi apparatus has been shown to depend on the action of GRASP55, a protein located at the cis-side

of the Golgi apparatus [25]. However, the knock-down of GRASP55 had no effect on the transport of peripherin/rds to the primary cilium [21].

IFT (intraflagellar transport) complexes are important for the antero- and retrograde movement of proteins in primary cilia, but some IFT proteins have been found at other intracellular sites in addition to primary cilia. IFT88 interacts with DGKδ [26], a member of the family of diacylglycerol kinases (DGK) which catalyze the transfer of a phosphate residue onto diacylglycerol to generate phosphatidic acid. DGKδ and IFT88 are both present at the endoplasmic reticulum, specifically endoplasmic reticulum exit sites, where DGKδ also associates with SEC13, a known component of COPII vesicles. Furthermore there is evidence that the deletion of DGKδ slows down the budding of COPII vesicles from the endoplasmic reticulum. Remarkably mouse embryonic fibroblasts deficient for DGKδ also elaborate shorter primary cilia, and their primary cilia contained less of a GFP-peripherin/rds fusion protein synthesized in those cells [26].

3. The case of polycystin-2

3.1. General features of polycystin-2

Among the better understood proteins residing in the primary cilium is polycystin-2 which is mutated in patients suffering from autosomal-dominant polycystic kidney disease. Autosomal-dominant polycystic kidney disease represents one of the most common monogenetic diseases affecting patients. It is characterized by the lifelong formation of fluid-filled cysts originating from most, if not all, parts of the nephron and of collecting ducts thus ultimately leading to end-stage renal failure. Eighty-five percent of the patients suffer from mutations in the *PKD1* gene [27] and 15% from mutations in the *PKD2* gene [28]. The latter encodes polycystin-2, a 968-amino acid protein with 6 membrane-spanning domains and both its NH_2- and the COOH-terminus extending into the cytoplasm [29]. Between the 5th and the 6th membrane-spanning segment lies a pore-forming domain which conducts cations, and the similarity of polycystin-2 with other proteins of the TRP (transient receptor potential) channel family on the sequence level has been subsequently confirmed structurally [30–32]. After having been hotly debated in the past [33] it is now more or less generally acknowledged that polycystin-2 resides in the endoplasmic reticulum and in the primary cilium. A motif in its COOH-terminus has been made responsible for the location of polycystin-2 in the endoplasmic reticulum [34] but it is not clear whether this motif serves as a retention or retrieval signal, i.e. whether (except for that pool of polycystin-2 which reaches the primary cilium) polycystin-2 never leaves the endoplasmic reticulum or whether polycystin-2 is able to escape from the endoplasmic reticulum and is immediately returned to this organelle.

3.2. The path of polycystin-2 to the primary cilium

3.2.1. Polycystin-2 appears to take an unconventional route to the primary cilium

Due to the presence of 6 hydrophobic domains it was immediately hypothesized that polycystin-2 represents an integral membrane protein, and this was confirmed experimentally [34]. Using immunofluorescence [34–36], density gradient centrifugation [36,37], surface biotinylation [34] and - most relevant in the context of this review - sensitivity to treatment with endoglycosidase H [34,38] it was shown that the major portion of polycystin-2 resides intracellularly, i.e. in the endoplasmic reticulum, but by immunofluorescence polycystin-2 also reaches the primary cilium (Fig. 2a). A shortened polycystin-2 protein lacking almost the complete COOH-terminus and therefore also the retention/retrieval signal is again incorporated into the primary cilium (Fig. 2b) but also into the somatic (i.e. non-ciliary) plasma membrane.

The treatment of lysates from cells producing full-length polycystin-2 with PNGase F revealed that polycystin-2 is *N*-glycosylated, and since all sugars could be removed with endoglycosidase H as well this argued that polycystin-2 did not reach the mid-Golgi compartment [34,38]. At first glance such a result suggests that polycystin-2 does not traverse through the Golgi apparatus on its way to the primary cilium. It has to be kept in mind, however, that the primary cilium constitutes only a very

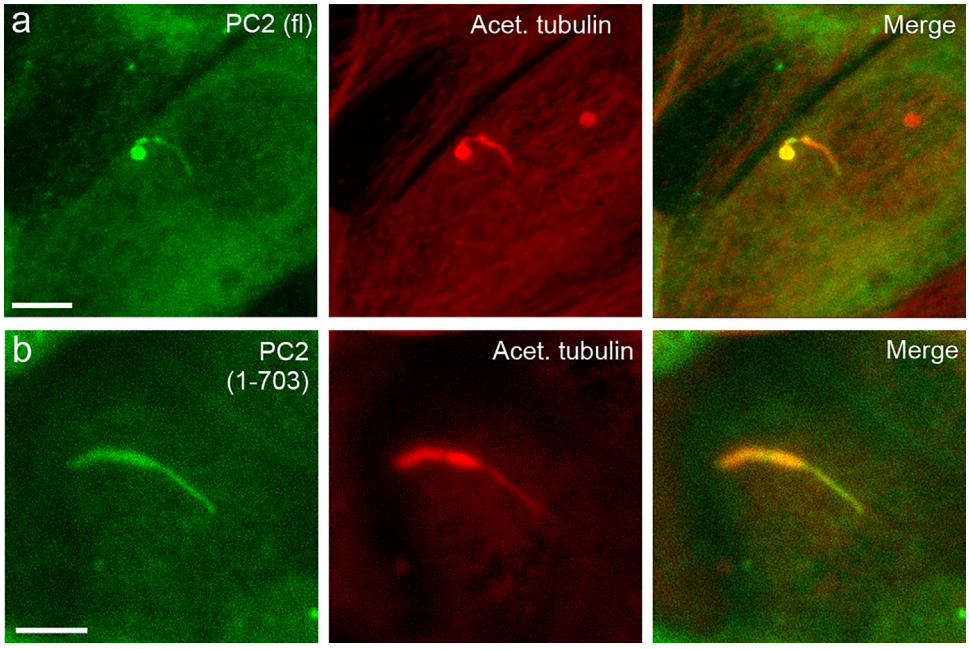

Fig. 2. Ciliary location of polycystin-2 in LLC-PK_1 cells. Immunofluorescence staining for human full-length [PC2 (fl)] and a truncated polycystin-2 protein lacking the COOH-terminal 265 amino acids [PC2 (1-703)] shows that both proteins are located in primary cilia. Acetylated tubulin (Acet. tubulin) was used as a marker to demonstrate the presence of cilia. Taken from the J. Cell Biol. 192, 631-645 (2011).

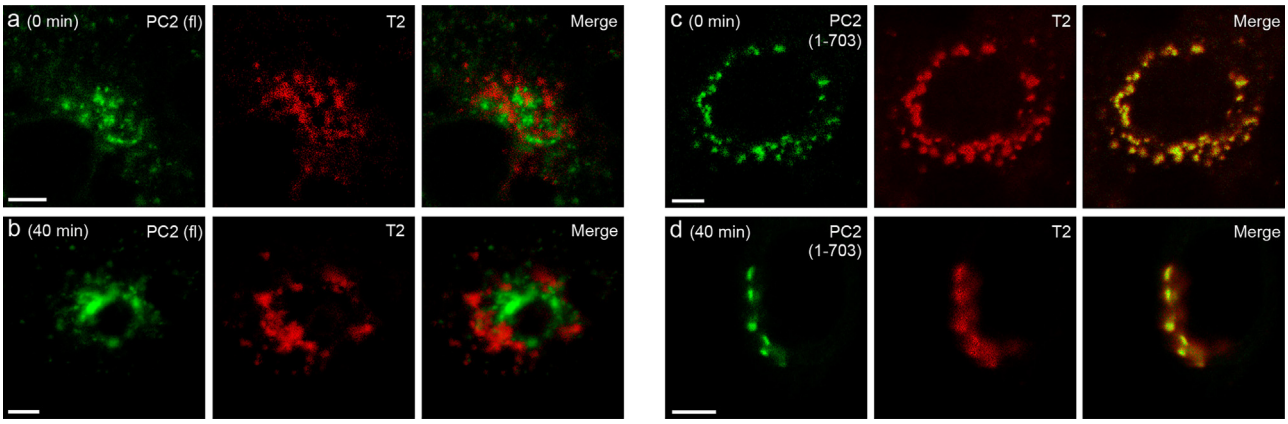

Fig. 3. Live-cell imaging of the intracellular trafficking of human polycystin-2 in LLC-PK$_1$ cells. Cells were incubated for 5 h at 15 °C in order to arrest trafficking between the endoplasmic reticulum and the Golgi apparatus. When the cells were transferred back to 37 °C (0 min), intracellular transport proceeded. It can be seen that 40 minutes after release from the temperature block, the full-length polycystin-2 protein [PC2 (fl)], which only reaches the primary cilium and not the somatic plasma membrane, does not co-localize with *N*-acetylgalactosaminyltransferase-2 (T2), a marker of the Golgi apparatus. In contrast, a truncated polycystin-2 protein encompassing only the NH_2-terminal 703 amino acids, which is incorporated both into the ciliary and the somatic plasma membrane compartments, traverses the Golgi apparatus. Taken from the J. Cell Biol. 192, 631–645 (2011).

small portion of the entire cell and that endoglycosidase H-resistant polycystin-2 in the primary cilium may escape undetected. We therefore resorted to metabolic labeling (pulse-chase) experiments in order to track newly synthesized polycystin-2. When polycystin-2 was radioactively labeled with ^{35}S-methionine/cysteine for a short period of 5 min (pulse) and the fate of the labeled protein followed for up to 24 hours (chase), it was always sensitive to a treatment with endoglycosidase H [39].

The monomeric GTPase Sar1 is essential for the budding of transport vesicles at the endoplasmic reticulum before they move to the Golgi apparatus. Upon the transient expression of GDP- and GTP-locked mutant forms of Sar1 a markedly decreased number of primary cilia positive for polycystin-2 was observed [39] which demonstrated that (similar to peripherin/rds) the transport of polycystin-2 to the primary cilium originates in a classic, Sar1 (and therefore also COPII)-dependent fashion at the endoplasmic reticulum. Further transport of COPII vesicles to the Golgi apparatus can be stalled by incubating cells at 15 °C, thus synchronizing intracellular transport; by returning the cells to 37 °C the transport vesicles rapidly move to the Golgi apparatus. Remarkably the full-length polycystin-2 protein, which exclusively travels to the primary cilium, was not observed in the Golgi apparatus although a truncated polycystin-2 protein lacking the retention/retrieval signal, which is incorporated both into the ciliary and into the somatic plasma membrane, was detected in the Golgi apparatus [39] (Fig. 3). The interpretation that polycystin-2 does not traverse the Golgi apparatus on its way to the primary cilium was corroborated by the ultrastructural localization of polycystin-2 using the tetracysteine technique [39]. Remarkably, however, when the cells were treated with brefeldin A both the full-length and the truncated polycystin-2 protein no longer reached the primary cilium [39]. Therefore an intact Golgi apparatus is required for the trafficking of polycystin-2 to the primary cilium and it appears that polycystin-2 is transported from the endoplasmic reticulum to the Golgi apparatus but does not migrate through it.

3.2.2. Additional issues to be considered and counter-arguments against the bypass of the Golgi apparatus by polycystin-2

According to the data presented above a minor portion of the full-length polycystin-2 protein escapes from the endoplasmic reticulum, reaches the cis-side of the Golgi apparatus and from there moves [through the recycling endosome? (see below)] to the primary cilium (Fig. 4). The picture, however, may not be quite so clear-cut.

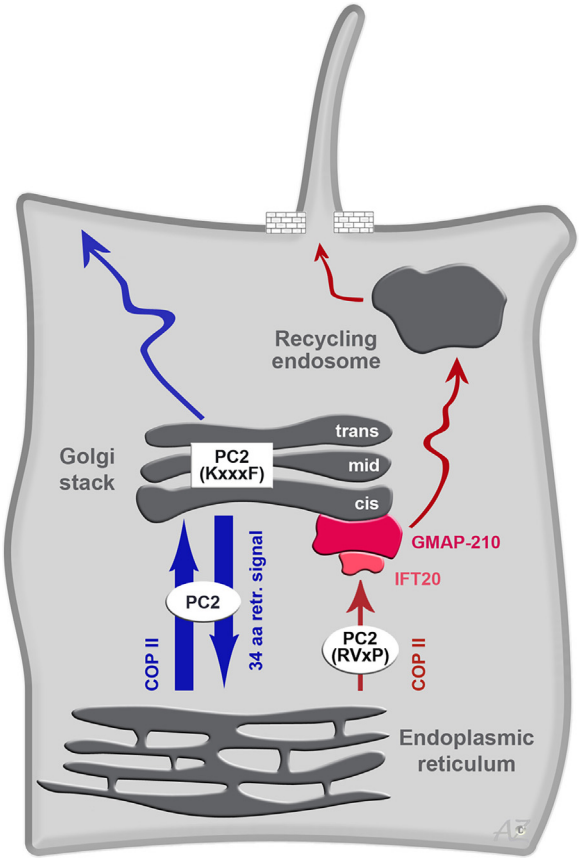

Fig. 4. Hypothetical model depicting the intracellular trafficking of polycystin-2. Polycystin-2 is synthesized at the rough endoplasmic reticulum. Most of it may leave the endoplasmic reticulum but is immediately retrieved by virtue of a 34-amino acid long domain in its COOH-terminus [34] (blue arrows between endoplasmic reticulum and Golgi stack). Polycystin-2 mutant proteins lacking the retrieval signal move through the Golgi stack, for which the K̆xxxF̈motif is necessary [39], and further towards the somatic plasma membrane. Some of polycystin-2 (red arrow) presumably escapes to the cis-side of the Golgi apparatus (via the ciliary targeting signal R̈VxP? [13]) and interacts with a complex containing GMAP-210 and IFT20, from there polycystin-2 may traffic further to the recycling endosome and finally to the base of the primary cilium.

For one, a truncated polycystin-2 protein lacking the retrieval/retention signal for the endoplasmic reticulum which reaches both the somatic and ciliary plasma membrane compartments (Fig. 2) gains resistance to endoglycosidase H already 2 h after its synthesis [39]. Therefore a variant of polycystin-2 which is able to traffic to the plasma membrane moves through the Golgi apparatus. The further characterization of the intracellular trafficking pathways of polycystin-2 was aided by the identification of 2 amino acid residues, lysine at position 572 and phenylalanine at position 576, which are essential for the transport of polycystin-2 to the somatic plasma membrane. Surprisingly a truncated polycystin-2 mutant protein, in which these 2 amino acids were changed to an alanine, still reached the primary cilium although it no longer reached the somatic plasma membrane, and it was still sensitive to a treatment with endoglycosidase H [39].

Secondly, another study investigated the combined intracellular trafficking of polycystin-1 and polycystin-2. The authors argued that a complex consisting of polycystin-1 and polycystin-2 migrates through the Golgi apparatus, and that the trafficking of polycystin-2 to the primary cilium depends on the presence of a wild-type polycystin-1 protein [40]. When primary cilia were isolated from cells producing both proteins, polycystin-2 was resistant to the action of endoglycosidase H [40] arguing that it moves through the Golgi apparatus. This contrasts with the notion that other studies [13,39] have found no evidence that polycystin-2 depends on polycystin-1 in order to reach the primary cilium.

The fungal metabolite brefeldin A is routinely used to interfere with the structure and function of the Golgi apparatus. It has been shown to exert its action through inhibiting the GTP loading of the monomeric GTPase ARF1 [22,23]. However, brefeldin A also affects certain endocytotic processes [41,42] and it therefore cannot be ruled out that the effect we have observed on the trafficking of polycystin-2 upon treatment with brefeldin A results from the inhibition of an endocytotic pathway (see below).

3.2.3. The role of IFT20 in the trafficking of polycystin-2 to the primary cilium

IFT20, another IFT protein, not only is present in primary cilia but it also associates with the Golgi apparatus, in particular the cis- and mid-Golgi compartment [43]. When the intracellular levels of IFT20 are reduced by RNA interference, this also leads to reduced levels of polycystin-2 in primary cilia [43]. Proteins interacting with IFT20 have been identified by immunoprecipitation and subsequent mass spectrometry. One of them is GMAP-210 (Golgi microtubule-associated protein 210) which is important for the association of IFT20 with the Golgi apparatus [44]. In cells isolated from mice lacking Gmap-210 the ciliary levels of polycystin-2 are diminished [44] which corroborates the functional significance of a complex between IFT20 and GMAP-210. *Ift20* knock-out mice develop polycystic kidney disease 5 to 10 days after birth [45] thus emphasizing the importance of the IFT20 protein in the context of this disease. Since the mice lacking Gmap-210 die on the day of the birth [44] it is not clear whether the absence of this protein also leads to the development of cystic kidneys.

The above results for IFT20 and GMAP-210 were corroborated in a subsequent study using a dynamic assay [46]. In this publication additional IFT20-interacting proteins were isolated, among them the exocyst subunits Exo70 and Sec8, and the BLOC-1 (biogenesis of lysosome-related organelles complex-1) component pallidin. BLOC-1 seems to be required for the vesicular transport from the sorting endosome, the vacuolar compartment of the early endosome [47], to the recycling endosome and lysosome. The blockade of this pathway through a knock-down of pallidin and dysbindin, another BLOC-1 subunit, or through the expression of a dominant-negative myosin Vb protein leads to reduced ciliary polycystin-2 levels and to an accumulation of polycystin-2 in the recycling endosome [46]. Similar results were obtained when the function of the recycling endosome was disrupted through expression of a dominant-negative form of the monomeric GTPase Rab11a [46]. An inactivation of the genes encoding pallidin and dysbindin results in distended renal collecting ducts but no drastic cystic kidney phenotype was observed [46]. It is remarkable that the exocyst components Sec8 and Sec10 [48] and the BLOC-1 subunit pallidin [46] also interact with polycystin-2. And analogous to the results obtained after the knock-down of the BLOC-1 subunits pallidin and dysbindin, a knock-down of the exocyst subunits Sec10 [48], Exo70 and Sec8 [46] leads to reduced levels of polycystin-2 in the primary cilium.

Taken together, these results can be interpreted such that polycystin-2 is able to leave the endoplasmic reticulum, then reaches the ER-Golgi intermediate compartment (ERGIC) and from there moves to the recycling endosome and finally to the primary cilium. This model incorporates the brefeldin A experiments and many of the other data discussed above.

4. Trafficking of other integral membrane proteins to the primary cilium

Of course primary cilia do not only contain polycystin-2 but a number of additional integral membrane proteins with diverse functions. Among the best known is smoothened which usually resides in the somatic plasma membrane and moves to the primary cilium upon the binding of sonic hedgehog to its receptor patched. Furthermore a variety of G protein-coupled receptors has been localized to primary cilia whose transport to the cilium, in particular regarding the earliest steps, has been poorly characterized so far. The tubby-like protein 3 (TULP3) is not only essential for the transport of these G protein-coupled receptors to the primary cilium [49,50] but also for the transport of polycystin-2 and fibrocystin [49], a protein mutated in patients suffering from autosomal-recessive polycystic kidney disease. Since TULP3 has been localized to the primary cilium [50], it is unlikely to play a role at earlier stages, in particular not at the endoplasmic reticulum and the Golgi apparatus. On the other hand, the trafficking of wild-type smoothened does not depend on TULP3 [50]. In contrast to the wild-type smoothened protein, the constitutively active M2 mutant form of smoothened is constantly present in the primary cilium [51]. We have found out that while the wild-type smoothened protein moved through the Golgi apparatus, this mutated smoothened protein did not [39] (Fig. 5).

The distinct trafficking pathways of polycystin-2 and fibrocystin on the one hand, and of smoothened on the other hand also become clear from the fact that the knock-down of IFT20 and of GMAP210 had a marked effect on the transport of the former two proteins to the primary cilium but not the latter [46]. But even the trafficking pathways of polycystin-2 and of fibrocystin may differ because dominant-negative myosin Vb prevents the transport of polycystin-2 to the cilium whereas that of fibrocystin (and of smoothened) is not affected [46].

5. Final remarks

Although there is good evidence that some integral membrane proteins utilize an unconventional intracellular trafficking pathway on their way to the primary cilium, it is still not clear where such a pathway diverges from the conventional route and what signals govern where the two pathways branch. Ciliary targeting signals have been identified in several proteins, such as the VxP motif in polycystin-2 [13], rhodopsin [12] and the cyclic nucleotide-gated channel CNGB1 [52], or the (V/I)KARK and the Ax(S/A)xQ motifs in a variety of G protein-coupled receptors [49,53,54]. For the VxP motif

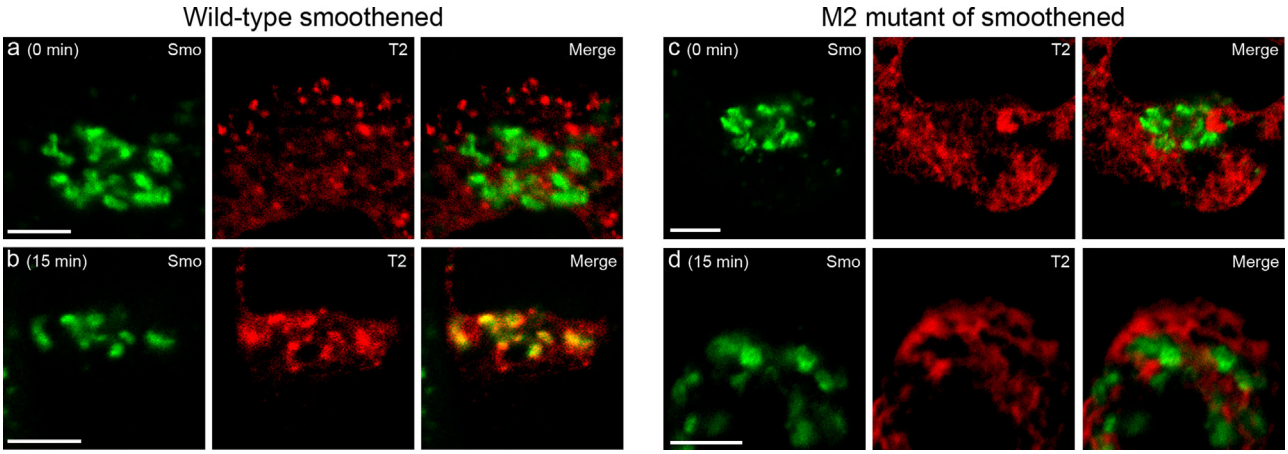

Fig. 5. Live-cell imaging of the intracellular trafficking of the wild-type form and the M2 mutant of smoothened in LLC-PK$_1$ cells. Cells were treated as described in the legend to Fig. 3. It can be seen that wild-type smoothened (Smo) is transported through the Golgi apparatus as shown by co-localization with *N*-acetylgalactosaminyltransferase-2 (T2), a marker of the Golgi apparatus, but the M2 mutant is not. Taken from the J. Cell Biol. 192, 631–645 (2011).

a molecular network has been identified already [55,56] but in light of the fact that the trafficking pathways, at least at their early stages, probably diverge for the different proteins the VxP motif may exert its influence later. One also has to wonder whether there is a distinct coat complex covering the vesicles destined for the primary cilium. At least for one G protein-coupled receptor, SSTR3, its trafficking to the primary cilium depends on the presence of the BBSome, a novel coat complex consisting of proteins which are mutated in patients suffering from the ciliopathy Bardet-Biedl syndrome [57]

Even for polycystin-2 the picture probably is more complicated than described above. There is evidence that the intracellular transport of polycystin-2 is regulated by the endosomal network. The knock-down of SDCCAG3, a protein located at early and recycling endosomes, reduced the ciliary presence of polycystin-2 [58], as did the knock-down of phosphoinositide 3-kinase-C2α [59], an enzyme acting at various stages of the endocytotic network. Furthermore the sorting nexin SNX3, a protein capable of binding to phosphatidylinositol (3)-phosphate and to early endosomes, has been shown to interact with the COOH-terminus of polycystin-2, and the retromer subunit VPS35 was demonstrated to interact with the NH_2-terminus of polycystin-2 [60]. It was not reported, however, whether those two proteins regulate the trafficking of polycystin-2 to the primary cilium.

There are several open questions which remain to be answered before the trafficking of some integral membrane proteins to the primary cilium can be considered as a special case of unconventional protein secretion. What is the role of GRASP55 and GRASP65? Do vesicles originating at the endoplasmic reticulum carry specific modifications in their coat? Do those vesicles fuse with the Golgi apparatus or do they only briefly touch its cis-side and then become immediately diverted to their next destination? How are cilium-specific integral membrane proteins sorted into distinct vesicles? Answers to those questions should provide valuable insight into the molecular and cell biological mechanisms underlying the trafficking of ciliary membrane proteins.

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