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Review

Autophagy and regulation of cilia function and assembly

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Motile and primary cilia (PC) are microtubule-based structures located at the cell surface of many cell types. Cilia govern cellular functions ranging from motility to integration of mechanical and chemical signaling from the environment. Recent studies highlight the interplay between cilia and autophagy, a conserved cellular process responsible for intracellular degradation. Signaling from the PC recruits the autophagic machinery to trigger autophagosome formation. Conversely, autophagy regulates ciliogenesis by controlling the levels of ciliary proteins. The cross talk between autophagy and ciliated structures is a novel aspect of cell biology with major implications in development, physiology and human pathologies related to defects in cilium function.
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Facts

- Autophagy is a degradative and recycling mechanism that allows cells to adapt to stress situations including nutrient deprivation.
- Primary cilia (PC) and motile cilia (MC) are sensory structures at the cell surface. PC sense nutrient, growth factor and calcium changes in the extracellular environment and also respond to mechanical stress. MC are beating structures that contribute to innate defense in the lung, for example.
- The PC is a site for the recruitment of autophagy-related (ATG) proteins that mediate autophagosome formation in response to cilium-dependent signaling. In turn, autophagy regulates the biogenesis of cilia by degrading certain proteins involved in cilia formation.
- Modulation of autophagy represents a new therapeutic opportunity in diseases related to defective cilia function.

Open Questions

- How are ATG proteins transported to the PC?
- How do ATG proteins recruited to the PC contribute to the biogenesis of phagophores and autophagosomes?
- Ciliary proteins are degraded by autophagy. How selective is this degradative pathway? Do some of these proteins contain motifs that result in selective engulfment by autophagosomes?
- What are the determinants that switch the degradation of ciliary proteins between basal and induced autophagy?
- Is there any specific function for cilium-dependent autophagy in ciliated cells?
- Do signals that trigger cilium-dependent autophagy depend on the stimuli (chemical, mechanical) or do all stimuli converge to a single signaling pathway upstream of the autophagy machinery?
- Can modulation of autophagy contribute to the restoration of cilium functions?

Receptors, transporters and specialized plasma membrane structures such as cilia constitute the interfaces between the extracellular and intracellular milieu and allow the cells to trigger specific responses to adapt to changes imposed by the environment. Among these adaptive responses, macroautophagy (hereafter referred to as 'autophagy') is a catabolic process conserved in eukaryotic cells by which the cell recycles its own constituents.[1] Autophagy is involved in the adaptation to starvation, cell differentiation and development, the degradation of aberrant structures, organelle turnover, and in tumor suppression, innate and adaptive immunity, lifespan extension, and cell death. The process of autophagy has been the subject of tremendous scientific interest in recent years because of the importance of autophagy in physiology and development and its dysregulation in various human pathologies.[2,3]

Cilia are microtubule-based structures present at the surface of many cells.[4,5] Depending on the microtubule composition and function, cilia fall into two classes: motile

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Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; AMPK, AMP-activated protein kinase; APC, adenomatosis polyposis complex; ATG, autophagy-related; COPD, chronic obstructive pulmonary disease; Gli, glioma-associated oncogene family; HDAC6, histone deacetylase-6; Hh, hedgehog; IFT, intraflagellar transport; MC, motile cilium; OFD1, oral-facial-digital syndrome type 1; PC1, polycystin 1; PC2, polycystin 2; PC, primary cilium; PCP, planar cell polarity; PDGF, platelet-derived growth factor; PDGFRα, platelet-derived growth factor receptor alpha; Ptc1, patched 1; PKD, polycystic kidney disease; Shh, sonic hedgehog; Smo, smoothened; Sufu, suppressor of fused protein; Vangl2, Van Gogh-like 2 protein

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cilia (MC) and primary cilia (PC).[6] MC are beating organelles that are present at the surface of epithelial cells in the airway, in the reproductive tract and in the brain. The PC is generally considered as a non-motile organelle that grows from the centriole and protrudes from the plasma membrane of most cell types.[4,5] It functions as a transducer of inputs from the extracellular environment into different cellular signaling pathways, acting as a sensory antenna.[5]

Recent studies have shown that signals emanating from ciliated structures control the formation of autophagosomes.[7] A role for autophagy in ciliogenesis and control of cilia length has also been demonstrated.[7–9] The aim of this review is to present the recent findings that uncover the interplay between cilia and autophagy and to discuss their potential impact on cell homeostasis and oncilia-related disease.

Cilium: Structure and Signaling

Structure. Conventionally, cilia are classified as motile and non-motile according to their microtubule pattern.[4,10] MC is formed by a microtubule-based axoneme of nine outer microtubule doublets and two single central microtubules (Figure 1). Two dynein motors and a radial spoke are attached to the doublets along the axoneme, which generate fluid and beat-like movements. MC are found on the surfaces of the epithelial cells lining the airways and the reproductive tracts, as well as on epithelial cells of the ependyma and in the brain. Motility of the cilium in these epithelial cells is responsible for mucociliary clearance and ependymal flow.

PC are solitary and non-motile, and consist of an axoneme of nine outer doublet microtubules extending from a basal body that is derived from the older (mother) centriole of the centrosome.[4] Two main ciliogenesis models have been proposed.[11] In quiescent polarized cells, the centrioles are sited close to the plasma membrane where the mother centriole serve as a docking center for the growth of the axoneme. PC project from the apical surfaces of the plasma membrane of a wide range of polarized cells including stem, epithelial, endothelial, connective-tissue muscle cells and neurons. In non-polarized cells, for instance, an intracellular ciliary vesicle grows around the mother centriole together with the axoneme (Figure 1). This vesicle will later fuse with the plasma membrane, resulting in the protrusion of the PC into the extracellular space and the generation of the ciliary pocket.[11] Continuous with the plasma membrane, but functionally different, the ciliary pocket is a discrete structure that contributes to the function of the PC. It is characterized by the presence of clathrin-coated vesicles resulting from the vesicular trafficking that originates and ends in this region.[12–14] Although it is not isolated from the cytoplasm, the PC is a highly compartmentalized organelle (Figure 1), surrounded by a specialized region of the plasma membrane that holds a different concentration of channels and receptors. The ciliary axoneme indeed is separated from the rest of the intracellular compartments by the 'ciliary necklace', which is located at the base of the cilium and connects the transition zone fibers of the basal body to the 'ciliary pore complex'.[15] This complex is a highly organized structure molecularly similar to the nuclear pore, where different precursors and intraflagellar transport (IFT) proteins, along with proteins of different signaling pathways, are selected and concentrated before entering the axoneme.[16]

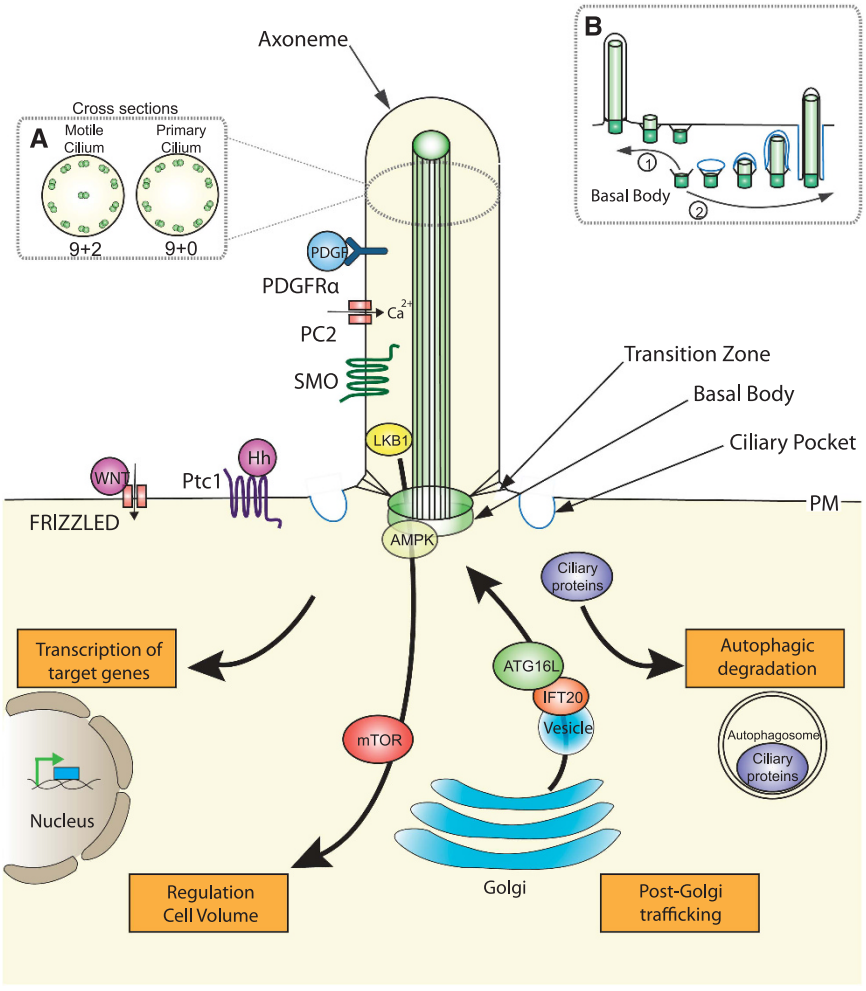
Continuous trafficking of cargo molecules along the ciliary axoneme is controlled by the evolutionarily conserved IFT mechanism.[17] Trafficking along the axoneme is maintained through the action of two motor protein families: kinesins, responsible for anterograde IFT, and dyneins, responsible for retrograde IFT. In addition, two large complexes of >20 proteins called IFT A and IFT B are also essential for IFT, exemplified by mutations in IFT B, which lead to the absence of cilia or to shortened cilia, whereas defects in IFT A proteins lead to a bulged cilia.[18] Extraciliary functions of some of these subunits have been also described,[19–21] as for example, continuous shuttling of the protein IFT20 between the Golgi and the basal body, which facilitates the mobilization of specific cargo.

Ciliary signaling. The major function of PC is to sense extracellular stimuli (Figure 1). Thus, PC responds to different sensory modalities such as mechanical stimuli (e.g., a shear stress that results in bending of the cilia) or chemosensation (e.g., specific ligand, growth factor, hormone or morphogen recognition). A variety of signaling pathways are coordinated through this organelle during development, tissue homeostasis, cell migration, cellular differentiation, cell cycle and apoptosis. Examples are the Hedgehog (Hh) pathway, platelet-derived growth factor (PDGF) pathway, the Wnt pathway and the Ca^{2+}signaling cascade (see Figure 1 for an overview of ciliary structure and signaling).[5,22]

Hh signaling. The Hh pathway is involved in regulation of cell proliferation, cell fate determination, the epithelial-to-mesenchymal transition and in homeostasis maintenance in adult cells.[23,24]Mammalian Hh signaling pathway requires the PC as its major signaling components are localized to the cilium, and IFT proteins are essential for trafficking of Hh molecules.[18]

There are three secreted Hh ligand family member proteins: Sonic Hh (Shh), Indian Hh and desert Hh.[25,26] Hh signaling depends on the balance between activator and repressor forms of the glioma-associated oncogene family (Gli) of zinc-finger transcription factors. The basic signaling pathway involves a 12-transmembrane domain receptor protein Patched 1 (Ptc1), the 7-transmembrane domain protein Smoothened (Smo), the suppressor of fused protein (Sufu) and the Gli transcription factors. Hh signaling is suppressed in the absence of Hh ligand through the physiological inhibitory effect of Ptc1 over Smo. When the pathway is activated by Hh ligand binding to Ptc1, Smo is released from Ptc1 and re-localizes to the PC axoneme where it inhibits Sufu activity. Sufu controls the processing and translocation of Glis from the cilium to the nucleus.

The Gli protein family includes Gli1, Gli2 and Gli3, which balances between activator (Gli^A) and repressor forms (Gli^R). Activator forms of Gli (Gli^A) directly induce transcription of Hh target genes, including Ptc1 and Gli gene family members.[27–29] The activity of Gli1 depends on Gli2, and Gli1 and Gli2 act primarily as transcriptional activators. Gli3 exists



	ULK1		BECLIN 1		VPS15		VPS34		ATG14L		ATG7		ATG5		ATG16L		LC3	
serum	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
basal body	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+
axoneme	-	-	-	-	ND	+	-	-	-	-	-	-	-	-	ND	+	+	+

Figure 1 Structure and signaling from the cilium and relationship with the autophagic pathway. Cilia are microtubule-based structures that project from the plasma membrane (PM) to the extracellular environment and act as sensory antennas. Cilia are formed of a basal body, a transition zone (transition fibers and ciliary necklace) and an axoneme. Inset A schematically shows the PC structure of nine microtubule doublets and the MC structure of nine microtubules doublets and two single central doublets. Inset B presents two ciliogenesis models for the PC: (1) Representation of the ciliogenesis in polarized cells, where the basal body docks directly to the PM. (2) Representation of the intracellular pathway during which a ciliary vesicle grows around the mother centriole and the axoneme. This vesicle later fuses with the PM and forms the ciliary pocket (in blue). The PC function as sensors signaling pathway ligands, which dock withreceptors (such as Ptc1 and PDGFRα) located on the axoneme, orions (e.g., Ca^{2+}) to mediate transcription of target genes (see the text for details). PC also respond to mechanical stimuli (e.g., fluid flow/shear stress) via, for example, the recruitment of Lkb1 and AMPK at the axoneme and basal body, respectively, in order to regulate the cell volume through mTOR. Ciliogenesis and the autophagic pathway are interconnected. Several ciliary proteins are degraded by autophagy (see Table 1). IFT20, which interacts with ATG16L, regulates the trafficking of ciliary components from the Golgi to the cilium. The table shows the localization of the ATG machinery induction in the basal body and/or in the axoneme in complete medium or upon serum starvation. 'ND' means 'not determined' (from results Pampliega *et al.*[7])

in two forms: a non-processed, full-length form that can function as transcriptional activator, and a truncated amino-terminal fragment that acts as transcriptional repressor.[27–29] Also, it has been recently described that the activity of Kif7 at the cilium creates a specialized compartment at the top of the cilia where the activity of the Gli proteins is regulated.[30] The bifunctional Gli proteins have roles in embryogenesis and adult homeostasis as their target genes regulate cell proliferation (*cyclin* and *Myc* genes), cell death (*Bcl-2*), differentiation (genes encoding Forkhead family transcription factors) and stem cell renewal (*Bmi-1*). Target genes regulated by the Hh pathway depend on tissue and cell types.[23,31]

PDGF pathway. PDGF receptor alpha (PDGFRα), a widely expressed protein, localizes specifically to the PC in quiescent fibroblasts in a ligand-dependent manner, and induces activation of Akt, Mek1/2, Erk1/2 and Rsk pathways at the axoneme and in the basal body.[4,5] PC-dependent PDGFRα pathway induces MAPK activation, as well as regulates the ubiquitous plasma membrane Na^+/H^+ exchanger NHE1. Both MAPK and NHE1 ultimately regulate cell proliferation, survival and migration during embryogenesis.[32,33] Defects in PDGFRα pathway can lead to different human pathologies including gastrointestinal stromal tumors, lung tumors and ovarian carcinoma.[34]

Wnt pathway. Canonical and non-canonical Wnt pathways are crucial during development.[35,36] The non-canonical Wnt pathway is controlled by a membrane protein called Van Gogh-like 2 (Vangl2) and regulates cytoskeletal changes, cell adhesion, migration and polarity.[37] Vangl2 functions in asymmetric positioning of MC and in centrosome positioning, which is important for cytoskeletal rearrangement. Mutations of different ciliary proteins, such as Kif3a, Ift88, Ofd1 (the protein mutated in oral-facial-digital syndrome type 1), polycystin 1 (PC1) and polycystin 2 (PC2), result in dysregulation of planar cell polarity (PCP) and are associated with hyper- or de-activation of the Wnt pathway depending of the tissue, timing, and localization of these proteins. Components of the PCP pathway, such as Vangl2 and inversin (also called nephrocystin 2), localize at the PC, specifically at the basal body, during development. Inversin serves as a molecular switch between canonical and non-canonical Wnt pathways by regulating Disheveled protein degradation at the basal body by the adenomatosis polyposis complex (APC).[37]

The canonical Wnt pathway is regulated by β-catenin and controls proliferation, apoptosis and cell fate determination via activation of target genes. The connection between the canonical Wnt pathway and PC seems to be linear. Activation of Wnt target genes depends on ligand binding to the Frizzled receptor at the plasma membrane and subsequent β-catenin translocation into the nucleus. The stability of β-catenin depends on its degradation by the APC complex at the basal body. Recent studies have established the association of Wnt pathways with ciliary components such as kinesins. These findings shed light on the contribution of the interaction of Wnt pathway with the PC in the regulation of cytoskeletal architecture.[37]

Calcium signaling. The Ca^{2+}-specific cation channel PC2, which forms an ion channel complex with the G-protein coupled receptor PC1 localized in the axoneme, regulates calcium signaling.[38,39] PC1 and PC2 are necessary for the calcium response and nitric oxide release in endothelial cells in response to blood flow.[40] Extracellular flow-induced calcium influx leads to Ca^{2+} release from the endoplasmic reticulum via ryanodine and inositol triphosphate receptors, resulting in AMP release.[41] Kidney epithelial cells respond to urinary flow in the lumen of the renal tubules by bending of the PC, which acts as a mechanosensory antenna, increasing membrane permeability to Ca^{2+} associated with the generated shear stress on the tubule lining cells, and, therefore, regulating the intracellular calcium response. It has also been shown that different renal cells have additional cilia-independent mechanosensitive Ca^{2+} responses.[42,43] Recently, Delling *et al.*[44] showed by using a ratiometric Ca^{2+} sensor for individual cilia and patch-clamp methods that the PC is a specialized organelle regarding intracellular ions. The study demonstrated that ciliary calcium channels, specifically the heteromeric PKD1L1–PKD2L1 channel, maintain a high Ca^{2+} concentration in the cilium with respect to cytoplasm in resting state, thus regulating a steady diffusion of this signaling molecule into the cytoplasm.

Autophagy and Cilium

The autophagic pathway

Role of autophagy: Autophagy is a constitutive degradative process that eliminates non-functional or redundant organelles and protein aggregates in the cytoplasm.[1,45–47] Levels of autophagy are upregulated in response to sublethal stress, enabling an organism to adapt to stress conditions, to generate intracellular nutrients and energy, and to eliminate cellular damage. In most cases, stimulation of autophagy protects cells from insults and contributes to cell survival.[46] During nutrient deprivation, autophagy is essential to generate metabolites and to maintain ATP levels in various tissues such as the liver.[48,49] At birth, soon after the interruption of the maternal nutrient supply via the placenta, autophagy allows neonates to adapt this abrupt nutritional modification.[50]

Although in general autophagy is an in bulk process, it can also be highly selective toward cellular structures and microorganisms invading the cytoplasm. The paradigm for selective autophagy is the recognition of the cargo by autophagy receptors (p62/SQSTM1, NBR1, NDP52, optineurin and BNIP3L), which connect the cargo to the autophagy machinery (reviewed in references Birgisdottir *et al.*[51]; Okamoto[52]; Rogov *et al.*[53]; and Stolz *et al.*[54]).

Autophagy has an essential role in multitude of physiological processes including cell differentiation and development, cellular quality control, tumor suppression, innate and adaptive immunity, lifespan extension, and cell death.[47,55] Its role in disease and its potential targeting with therapeutic agents have been extensively described in recent reviews.[2,3,46,47,56]

Regulation of autophagy: The core molecular machinery engaged in autophagosome formation, as well as the signaling pathways that stimulate autophagy have been reviewed in detail elsewhere.[57–61] Fifteen autophagy-related (ATG) proteins constitute the core machinery of autophagosome formation.[59] In mammalian cells, these ATG proteins are recruited to form a phagophore, or isolation membrane, which subsequently elongates to form the autophagosome (Figure 2). Recent studies have shown that phagophore elongation takes place at the endoplasmic reticulum in a structure called the omegasome characterized by the presence of the phosphatidylinositol 3-phosphate binding protein DFCP1.[58,61,62] In addition, other membrane sources also contribute to the biogenesis of the autophagosome, including those of the endosomes and the Golgi apparatus, the contact site between the endoplasmic reticulum and mitochondria, endoplasmic reticulum transition elements, and the plasma membrane (for recent reviews, Lamb *et al.*[58]; Shibutani and Yoshimori[61]; and Puri *et al.*[63]). Each functional module from the initiation of the autophagosome formation to the elongation/closure step involves different ATG proteins. The ULK1 complex (mammalian ortholog of yeast Atg1) and the phosphatidylinositol 3 kinase (PIK3) complex I (which contains PIK3C3/VPS34 and its adapter VPS15, ATG14L and Beclin 1, yeast Atg6) initiate autophagosome formation. A variety of signaling pathways that sense nutrient availability, ATP levels, growth factors and reactive oxygen species control autophagy by regulating the activity of the ULK1 complex and/or the activity of the PIK3 complex 1.[58]

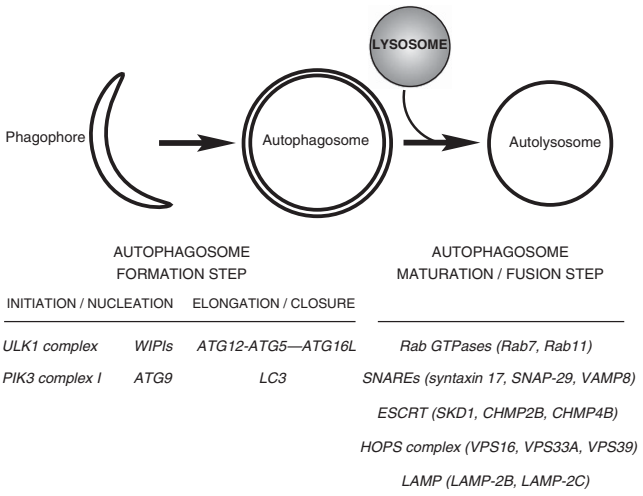


Figure 2 The autophagic pathway. There are two important stages in autophagy pathway: (i) autophagosome formation, which includes the initiation/nucleation (formation of the phagophore) and elongation/closure, and (ii) autophagosome fusion with the lysosome (The autophagosomes can merge with endocytic compartments before the fusion with the lysosome). ATG proteins involved in the formation of autophagosome and a non-exhaustive list of the protein families involved in the maturation/fusion step are shown in italics

Phosphatidylinositol 3-phosphate, which is produced by the enzymatic activity of PIK3 class III (PIK3C3/VPS34), recruits WIPI1/2 (homologs of yeast Atg18) at the phagophore. One of the functions of WIPI2 is to control the transport of the multi-membrane spanning ATG9 between the phagophore and a peripheral endosome/Golgi localization.[64] The trafficking of ATG9 to the phagophore is an early event that occurs soon after autophagy induction.[58,65] Another function of WIPI2 is to recruit ATG16L to contribute to the elongation of the phagophore.[66] The last functional module consists of the two ubiquitin-like conjugation systems: ATG12–ATG5 (this conjugate interacts with ATG16L) and the LC3–phosphatidylethanolamine (yeast Atg8), which are involved in the elongation and closure of the autophagosomal membrane.

Once formed, most autophagosomes mature into autolysosomes after merging with endocytic/lysosomal compartments with the help of Rab GTPases (Rab7, Rab11), SNAREs, ESCRT proteins,[67–70] the HOPS complex[71–73] and some of the lysosomal membrane glycoproteins LAMP-2 variants (Figure 2).[74] The very last stage of autophagy is the degradation and recycling of components from the lysosomal lumen to the cytosol, as well as the mammalian target of rapamycin (mTOR)-regulated restoration of lysosomes.[75] In addition to the maturation of autophagosomes, it is worth noting that SNAREs and elements of the endocytic machinery also contribute to the formation of autophagosomes.[61,71,76]

Autophagy and PC. Until recently, the functional interaction between autophagy and PC had not been characterized. Pampliega *et al.*[7] demonstrated that induction of autophagosome formation during starvation depends on functional cilia and the Hh pathway, and observed that components of the autophagic machinery are located at the axoneme and basal body of the cilium (see Figure 1 for localization of different autophagic machinery components to the cilia). A functional PC and Hh activation are required for the recruitment of ATG16L to the basal body upon serum removal, which is delivered in IFT20-containing vesicles. This serum-dependent relocation of specific ATG proteins to pre-existing basal bodies leads to maximal activation of autophagy. Moreover, the contribution of the plasma membrane to autophagosome formation[76,77] together with the presence of a subset of ATG proteins along the ciliary membrane, suggest a direct regulatory effect of the PC on the biogenesis of autophagosomes from the plasma membrane.

However, the relationship between autophagy and Hh signaling appears to be complex. The study of Jiménez-Sánchez *et al.*[78] showed that Hh signaling represses autophagy in fibroblasts, HeLa cells and *Drosophila melanogaster*. Interestingly, HeLa cells are poorly ciliated cells, and PC is not required for Hh signaling in *Drosophila melanogaster*.[18] In support of the hypothesis that autophagy is stimulated by a cilia-dependent Hh pathway, Shh treatment induced autophagy in ciliated vascular smooth muscle cells[79] and in ciliated hippocampal neurons.[80] The role of Hh signaling in autophagy is probably dependent on multiple parameters including cell growth and the presence of PC.

In addition to the modulatory effect of PC on autophagy activation, ciliogenesis is also regulated by autophagy. Autophagy has been shown to degrade components essential for ciliogenesis, such as IFT20, and also negative regulators of ciliogenesis such as the centriolar satellite protein OFD1 (see Figure 1).[9] This allows the autophagic process to control the length of the PC in response to the environmental changes. The degradation of IFT20 occurs predominantly via basal autophagy,[7] and that of OFD1 is observed early during serum starvation-induced autophagy.[9] OFD1 is encoded by the gene involved in the X-linked ciliopathy, the OFD1. OFD1 functions as a suppressor of ciliogenesis at the centriolar satellites. Consequently, the degradation of the centriolar satellite pool of OFD1 by autophagy induces ciliogenesis (Figure 3).

Interestingly, as starvation persists, IFT20 becomes again a preferred cargo for autophagy, and degradation of the ciliary vesicular components imposes a negative control on ciliary growth (Figure 3). This cargo switch between basal and inducible autophagy determines that in basal conditions, IFT20, a positive regulator of ciliogenesis, is degraded by autophagy, whereas OFD1 is located at the centriolar satellites inhibiting ciliogenesis via sequestration of BBS4 (Table 1). Upon serum starvation, ciliogenesis is induced, and OFD1 is degraded by autophagy, whereas IFT20 protein contributes to ciliary trafficking (Figure 3). This switch between basal and inducible ciliophagy highlights the importance of the interplay between autophagy and ciliogenesis for cell homeostasis.

An interesting aspect that requires further clarification is the differences of ciliary length in autophagy-deficient MEFs between the two studies under basal conditions.[7,9] Differences in the state of confluence of the cells – known to influence ciliogenesis – could be behind the discrepant findings. More broadly, additional studies are needed to determine the influence of the physiological context on the interplay between PC-related signaling and the autophagic pathway.

Autophagy and ciliopathies. Cilia defects are associated with a wide range of pathologies called 'ciliopathies' and can affect many organs and systems.[18] Polycystic kidney disease (PKD) is a genetic disorder that causes formation of fluid-filled cysts in the renal tubules.[81]Autosomal dominant PKD (ADPKD), the most common form of PKD, is due to mutations in two genes: *PKD1* (around 85% of cases) and *PKD2* (around 15% of cases) that encode PC1 and PC2 ciliary proteins, respectively.[82] PC1 and PC2 are critical for cellular repair and controlled growth, as well as for division of tubule cells after kidney injury, thus explaining the injury sensitivity of ADPKD kidneys.[83,84] ADPKD leads to kidney enlargement with increasing numbers and size of cysts over time because of abnormal renal epithelial cell growth, together with a disturbed fluid transport resulting in end-stage renal disease in 50% of patients.

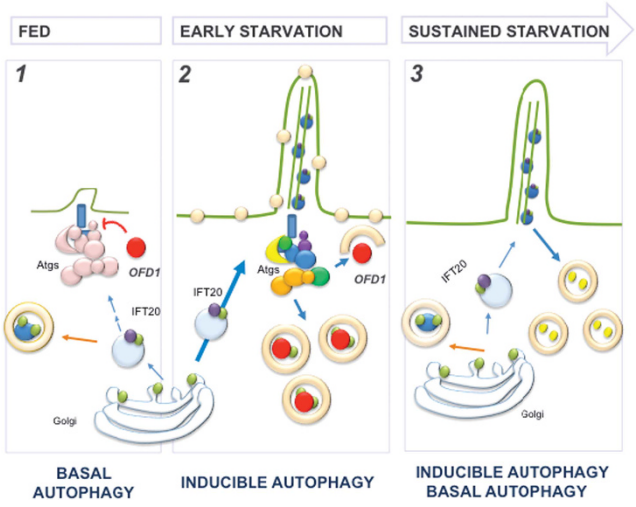


Figure 3 Autophagy as a novel modulator of ciliogenesis. (1) Basal autophagy prevents ciliary growth under basal conditions through degradation of proteins involved in intraflagellar trafficking such as IFT20, a protein that shuttles from Golgi to cilia to contribute to ciliogenesis. (2) Early upon nutrient removal, induction of autophagy favors degradation of the endogenous inhibitor of ciliogenesis OFD1. This switch in autophagic cargo allows an increase in the delivery of IFT20 to the base of the cilia and promotes ciliogenesis. Ciliary growth leads to activation of signaling pathways that promote recruitment of specific ATG proteins to complexes already present at the ciliary base to promote maximal activation of inducible autophagy. (3) If starvation is sustained, there is a switch toward IFT20 degradation to prevent unlimited growth of the cilia and excessive activation of the autophagic process

The role of PC is complex in PKD. It has been shown that mutations in various ciliary proteins, including IFT88 (also known as Tg737, Polaris, or Orpk), IFT20, IFT140, and a subunit of kinesin-II Kif3-*a*, interfere with cilia structure and can result in cyst formation. Mutations of PC1 and PC2, however, result in functional defects in cilium-dependent signaling, like defective mechanosensation that decreases intracellular Ca^{2+} and reduces intracellular cAMP clearance.[85] The role of IFT88 (Tg737) has been extensively studied in PKD. Loss of the ciliary protein IFT88 induces embryonic lethal PKD, and reintroduction of the protein ameliorates the phenotype.[86,87] Mice with a hypomorphic mutation in the gene encoding IFT88 (Tg737) show a similar phenotype to autosomal recessive PKD, with cystic kidneys, hepatic biliary disease, large cysts in the collecting duct and inability to concentrate urine.[85,86] In ADPKD models, loss of cilia reduce cystogenesis; however, this reduction is independent of mTOR or cAMP signaling.

The role of autophagy in PKD is under discussion because of the diverse effects of the pharmacological agents used in PKD models, as well as in the trials with the mTOR complex 1 inhibitor rapamycin and other autophagy inducers.[88,89] It was previously reported that mTOR signaling is activated in PKD, and rapamycin was identified as an effective therapeutic agent against cystogenesis in rat and mice PKD models. However, rapamycin (everolimus) was not a successful treatment in clinical trials with PKD patients.[88–90] The regulation of the mTOR pathway is crucial during cystic development. Epithelial cells lining the cysts expressing mutated PC1 protein have increased levels of cell proliferation and abnormally activated mTOR signaling because of a disrupted interaction between PC1 and tuberin, a product of *TSC2* gene, which controls mTOR activity.[91] Boehlke *et al.*[92] showed that in kidney epithelial cells, the ciliary axoneme-dependent Lkb1/AMP-activated protein kinase (AMPK) pathway controls cell volume upstream of the inhibition of the mTOR pathway. In non-ciliated cystic renal epithelial cells, or cell lines with mutations in ciliogenesis-related proteins, this regulation of cell volume is

Table 1 Examples of ciliary proteins degraded by autophagy

Autophagy substrate	Cellular localization	Ciliary role	Associated ciliopathy	Ref
IFT20[a]	Golgi, basal body and axoneme	IFT B anterograde IFT complex and ciliary assembly via vesicular transport of proteins to the basal body	PKD	Pampliega *et al.*[7]
OFD1[b]	Distal centriole	Cilia length and centriolar recruitment of IFT88	Oral-facial-digital syndrome	Tang *et al.*[9]
PC2	Axoneme and endoplasmic reticulum	Ca^{2+}-specific cation channel	PKD	Hessel *et al.*[100]
IFT88	Axoneme	Ciliary assembly and IFT B anterograde IFT complex	COPD	Lam *et al.*[8]
Centrin1	Basal body	Ca^{2+} signaling in the connecting cilium	COPD	Lam *et al.*[8]
ARL13	Axoneme	IFT A assembly stability	COPD	Lam *et al.*[8]
Pericentrin	Basal body	Ciliary assembly	COPD	Lam *et al.*[8]

Abbreviations: COPD, chronic obstructive pulmonary disease; PKD, polycystic kidney disease
[a]IFT20 is an autophagy substrate under basal condition
[b]OFD1 is an autophagy substrate under autophagy stimulation

disrupted. Whether autophagy has a role in the cilium-dependent regulation of kidney epithelial cell volume remains to be investigated.

In addition, Belibi *et al*.[93] showed that the presence of autophagosomes in cystic kidneys in response to hypoxia is due to a blockage of autophagic flux. In addition, mouse kidney cells that express a mutated *PKD1* failed to induce autophagy in response to glucose starvation.[94] These studies suggest that autophagy is altered in PKD patients either because of a reduction in the biogenesis of autophagosomes, or because of limitations on the autophagic flux. A recent study reports that autophagy has a role in PKD by altering ciliary protein turnover.[95] In support of this hypothesis, the expression level of PC1 is critical for regulation of normal tubule formation in cultured cells and mouse models.[81] PC1 overexpression is observed in cystic epithelia of ADPKD patients,[96] and its overexpression disrupts tubule formation in renal epithelial cells in mice.[97] In contrast, the downregulation of PC1 induces cystogenesis in mouse models.[98] A major conclusion of these studies is that the dosage of PC1 is important for cystogenesis.

Recently Cebotaru *et al*.[95] showed that PC1 controls the degradation of PC2 by autophagy through a mechanism dependent on histone deacetylase-6 (HDAC6). A pathogenic mutant of PC1 failed to induce autophagy-dependent PC2 degradation.[95] Together these findings suggest that autophagy must be considered as a factor in the development of renal ciliopathies.

Autophagy and MC. Epithelial cells in the airway are ciliated with multiple MC nucleated by a basal body. The presence of cilia in the respiratory track is critical for normal lung function, and alterations in these MC contribute to the in development of pathologies such as chronic obstructive pulmonary disease (COPD), emphysema and chronic bronchitis.[99] In COPD, patients who were chronic cigarette smokers, a significant cilia shortening is observed that leads to impaired mucociliary clearance.[100,101] Cilium shortening leads to excess mucus production in the lung epithelial cells and interferes with the protection of airways from infections. The lung tissues of COPD patients have been previously shown to accumulate autophagosomes.[102] Lam *et al*.[8] demonstrated that cilia shortening in chronic cigarette smoker correlates with a HDAC6-dependent autophagy degradation of ciliary proteins. In support of the proposed role of autophagy in the negative regulation of ciliary growth, autophagy-impaired cells, *Becn1*$^{+/-}$, *Maplc3b*$^{-/-}$ and *Hdac6-/Y* cells, were protected from cilia shortening. In addition, ciliary proteins, such as IFT88, ARL13, centrin1 and pericentrin, are sequestered in autophagosomes (Table 1). Lam *et al*.[8] propose a new role for selective autophagy in degradation of ciliary proteins and in the regulation of MC length in response to chronic cigarette smoking stress in lung epithelial cells. This hypothesis is supported by previous observations of hyper-responsiveness in lung-specific Atg7 knock-out mice with abnormal ciliogenesis and long cilia formation in mTOR-activated cells.[103,104] Basal autophagy regulates the turnover of proteins involved in cilia function, thus controlling sensitivity of the cell to different stressors, such as cigarette smoking. Therefore, inhibition of autophagy in lung epithelial cells may have therapeutic relevance in COPD, chronic bronchitis or emphysema patients.

Conclusions and Future Directions

Cilia-mediated autophagy can be induced by different stimuli because of the fact that the cilium can sense various extracellular changes, such as nutrient deprivation, calcium influx, presence of morphogens or flow-induced shear stress. The autophagic machinery is recruited to the ciliary structures *in vitro* in response to serum starvation, suggesting a novel cellular localization for autophagosome biogenesis regulated by active recruitment of ATG16L1 via IFT20 vesicles (Figure 1). The nature and mechanism of this vesicular trafficking should be further investigated in order to complete our understanding of the reciprocal relationship between PC and autophagosome biogenesis. A growing number of ciliary structures (the axoneme, basal body and the ciliary pocket) have been identified as platforms for multiple signaling pathways (see Figure 1). Thus, it would be interesting to examine the interplay between autophagy and the transduction of different pathways in a cilia-dependent manner. Several studies cited in this review examined the turnover of ciliary proteins as autophagy substrates in different cellular contexts. The degradation of ciliary components can suggest that a selective form of autophagy, defined by the term 'ciliophagy', acts on certainciliary structures (see Table 1 for examples of ciliary proteins degraded by autophagy).[105] The study by Lams *et al*.[8] demonstrated that cross-talk between autophagy and cilia is not limited to the PC but also extends to the MC. Cilia/flagella have a role in many processes including development and sensory reception in invertebrate organisms (*Caenorhabditis elegans*, *Drosophila melanogaster*).[21,106] These models have contributed to our understanding of the function of autophagy,[107–110] and it would be useful to investigate the interplay between autophagy and ciliated structures in these organisms to better understand its physiological and developmental impact.

The coordinated action of autophagy and ciliogenesis during nutritional stress is likely just the tip of the iceberg of the process now known as ciliophagy that has been implicated in normal functioning of various ciliated organs such as brain, kidney, skin, bone, colon and blood vessels and in the molecular basis of pathologies related to defects in ciliary functions.

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