

Cilium Assembly



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Introduction

Reactome is open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.

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This document contains 4 pathways and 2 reactions (see Table of Contents)

Cilium Assembly 7

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Cilia are membrane covered organelles that extend from the surface of eukaryotic cells. Cilia may be motile, such as respiratory cilia) or non-motile (such as the primary cilium) and are distinguished by the structure of their microtubule-based axonemes. The axoneme consists of nine peripheral doublet microtubules, and in the case of many motile cilia, may also contain a pair of central single microtubules. These are referred to as 9+0 or 9+2 axonemes, respectively. Relative to their non-motile counterparts, motile cilia also contain additional structures that contribute to motion, including inner and outer dynein arms, radial spokes and nexin links. Four main types of cilia have been identified in humans: 9+2 motile (such as respiratory cilia), 9+0 motile (nodal cilia), 9+2 non-motile (kinocilium of hair cells) and 9+0 non-motile (primary cilium and photoreceptor cells) (reviewed in Fliegauf et al, 2007). This pathway describes cilia formation, with an emphasis on the primary cilium. The primary cilium is a sensory organelle that is required for the transduction of numerous external signals such as growth factors, hormones and morphogens, and an intact primary cilium is needed for signaling pathways mediated by Hh, WNT, calcium, Gprotein coupled receptors and receptor tyrosine kinases, among others (reviewed in Goetz and Anderson, 2010; Berbari et al, 2009; Nachury, 2014). Unlike the motile cilia, which are generally present in large numbers on epithelial cells and are responsible for sensory function as well as wave-like beating motions, the primary cilium is a non-motile sensory organelle that is present in a single copy at the apical surface of most quiescent cells (reviewed in Hsiao et al, 2012). Cilium biogenesis involves the anchoring of the basal body, a centriole-derived organelle, near the plasma membrane and the subsequent polymerization of the microtubule-based axoneme and extension of the plasma membrane (reviewed in Ishikawa and Marshall, 2011; Reiter et al, 2012). Although the ciliary membrane is continuous with the plasma membrane, the protein and lipid content of the cilium and the ciliary membrane are distinct from those of the bulk cytoplasm and plasma membrane (reviewed in Emmer et al, 2010; Rohatgi and Snell, 2010). This specialized compartment is established and maintained during cilium biogenesis by the formation of a ciliary transition zone, a proteinaceous structure that, with the transition fibres, anchors the basal body to the plasma membrane and acts as a ciliary pore to limit free diffusion from the cytosol to the cilium (reviewed in Nachury et al, 2010; Reiter et al, 2012). Ciliary components are targeted from the secretory system to the ciliary base and subsequently transported to the ciliary tip, where extension of the axoneme occurs, by a motor-driven process called intraflagellar transport (IFT). Anterograde transport of cargo from the ciliary base to the tip of the cilium requires kinesin-2 type motors, while the dynein-2 motor is required for retrograde transport back to the ciliary base. In addition, both anterograde and retrograde transport depend on the IFT complex, a multiprotein assembly consisting of two subcomplexes, IFT A and IFT B. The primary cilium is a dynamic structure that undergoes continuous steady-state turnover of tubulin at the tip; as a consequence, the IFT machinery is required for cilium maintenance as well as biogenesis (reviewed in Bhogaraju et al, 2013; Hsiao et al, 2012; Li et al, 2012; Taschner et al, 2012; Sung and Leroux, 2013). The importance of the cilium in signaling and cell biology is highlighted by the wide range of defects and disorders, collectively known as ciliopathies, that arise as the result of mutations in genes encoding components of the ciliary machinery (reviewed in Goetz and Anderson, 2010; Madhivanan and Aguilar, 2014).

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Anchoring of the basal body to the plasma membrane 7

Location: Cilium Assembly

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Cilium biogenesis is initiated by the docking of basal bodies, a centriole-derived organelle, to the plasma membrane (reviewed in Reiter et al, 2012). The centriole consists of a multiprotein core surrounded by a ring of nine microtubule triplets; the mother centriole additionally has 'distal' and 'subdistal appendages' that are critical for ciliogenesis (reviewed in Kim and Dynlacht, 2013; Firat-Karalar and Stearns, 2014; Bettencourt-Dias et al, 2011). Basal bodies initiate and anchor the extension of the axonemal microtubules and also associate with secretory vesicles which are thought to provide membrane components for the extension of the ciliary membrane (Sorokin, 1962; Sorokin, 1968; Bachmann-Gagescu et al, 2011; Tanos et al, 2013; reviewed in Ishikawa et al, 2011; Reiter et al, 2012). Basal bodies are attached to the plasma membrane through a proteinaceous network of transition fibers that form part of the 'transition zone' at the ciliary base. The transition zone acts as a selective barrier or ciliary pore, excluding vesicles and limiting the diffusion of proteins and lipids from the cytosol or plasma membrane (Deane et al, 2001; Craige et al, 2012; Reiter et al, 2012). In addition to the transition fibres, the transition zone also consists of the ciliary necklace (a row of protein particles at the ciliary membrane at the base of the cilium) and the Y-links (that connect the axonemal microtubules to the membrane at the ciliary necklace) (Williams et al, 2011; reviewed in Hsiao et al, 2012; Reiter et al, 2012).

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Cargo trafficking to the periciliary membrane ↗

Location: Cilium Assembly

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Proteomic studies suggest that the cilium is home to approximately a thousand proteins, and has a unique protein and lipid make up relative to the bulk cytoplasm and plasma membrane (Pazour et al, 2005; Ishikawa et al, 2012; Ostrowoski et al, 2002; reviewed in Emmer et al, 2010; Rohatgi and Snell, 2010). In addition, the cilium is a dynamic structure, and the axoneme is continually being remodeled by addition and removal of tubulin at the distal tip (Marshall and Rosenbaum, 2001; Stephens, 1997; Song et al, 2001). As a result, the function and structure of this organelle relies on the directed trafficking of protein and vesicles to the cilium. Small GTPases of the RAS, RAB, ARF and ARL families are involved in cytoskeletal organization and membrane traffic and are required to regulate the traffic from the Golgi and the trans-Golgi network to the cilium (reviewed in Deretic, 2013; Li et al, 2012). ARF4 is a Golgi-resident GTPase that acts in conjunction with a ciliary-targeting complex consisting of the ARF-GAP ASAP1, RAB11A, the RAB11 effector FIP3 and the RAB8A guanine nucleotide exchange factor RAB3IP/RABIN8 to target cargo bearing a putative C-terminal VxPx targeting motif to the cilium. A well-studied example of this system involves the trafficking of rhodopsin to the retinal rod photoreceptors, a specialized form of the cilium (reviewed in Deretic, 2013). ARL3, ARL13B and ARL6 are all small ARF-like GTPases with assorted roles in ciliary trafficking and maintenance. Studies in C. elegans suggest that ARL3 and ARL13B have opposing roles in maintaining the stability of the anterograde IFT trains in the cilium (Li et al, 2010). In addition, both ARL3 and ARL13B have roles in facilitating the traffic of subsets of ciliary cargo to the cilium. Myristoylated cargo such as peripheral membrane protein Nephrocystin-3 (NPHP3) is targeted to the cilium in a UNC119- and ARL3dependent manner, while ARL13B is required for the PDE6-dependent ciliary localization of INPP5E (Wright et al, 2011; Humbert et al, 2012; reviewed in Li et al, 2012). ARL6 was also identified as BBS3, a gene that when mutated gives rise to the ciliopathy Bardet-Biedl syndrome (BBS). ARL6 acts upstream of a complex of 8 other BBSassociated proteins known as the BBSome. ARL6 and the BBSome are required for the ciliary targeting of proteins including the melanin concentrating hormone receptor (MCHR) and the somatostatin receptor (SSTR3), among others (Nachury et al, 2007; Loktev et al, 2008; Jin et al, 2010; Zhang et al, 2011). Both the BBSome and ARL6 may continue to be associated with cargo inside the cilium, as they are observed to undergo typical IFT movements along the axoneme (Fan et al, 2004; Lechtreck et al, 2009; reviewed in Li et al, 2012).

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Intraflagellar transport 7

Location: Cilium Assembly

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Intraflagellar transport (IFT) is a motor-based process that controls the anterograde and retrograde transport of large protein complexes, ciliary cargo and structural components along the ciliary axoneme (reviewed in Cole and Snell, 2009). IFT particles contain two multiprotein IFT subcomplexes, IFT A and IFT B, with ~6 and ~15 subunits, respectively. Linear arrays of IFT A and IFT B 'trains' assemble at the ciliary base along with the active plus-end directed kinesin-2 motors and the inactive dynein motors and traffic along the microtubules at a rate of ~ 2 micrometers per second. At the ciliary tip, the IFT trains disassemble, releasing cargo and motors, and smaller IFT trains are subsequently reassembled for retrograde traffic driven by the now active minus-end directed dynein-2 motors. Retrograde trains travel down the length of the axoneme at a rate of ~3 micrometers per second and are disassembled and recycled for further rounds of transport at the ciliary base (reviewed in Taschner et al, 2012; Bhogaraju et al, 2013; Ishikawa et al, 2011). Mutations in kinesin-2 motors or IFT B complex members tend to abrogate cilium formation, while mutations in dynein-2 motor or in IFT A complex members generally result in short, bulging cilia that abnormally accumulate IFT particles. These observations are consistent with a primary role for IFT B and IFT A complexes in anterograde and retrograde transport, respectively (see for instance, Huangfu et al, 2005; Follit et al, 2006; May et al, 2005; Tran et al, 2008; reviwed in Ishikawa et al, 2011). In addition to the IFT A and B complexes, the IFT particles may also contain the multi-protein BBSome complex, which displays typical IFT-like movement along the ciliary axoneme and which is required for cilium biogenesis and delivery and transport of some ciliary cargo (Blaque et al, 2004; Nachury et al, 2007; Ou et al, 2005; Ou et al, 2007; reviewed in Sung and Leroux, 2013; Bhorgaraju et al, 2013).

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ATAT acetylates microtubules *▼*

Location: Cilium Assembly

Stable identifier: R-HSA-5618328

Type: transition

Compartments: cytosol



Cilia are enriched in acetylated tubulin, a marker that is associated with stability, and the kinesin motor preferentially travels on acetylated microtubules (Johnson et al, 1998; Reed et al, 2006; Cai et al, 2009). Alpha tubulin acetyltransferase (ATAT), also known as MEC17, has been shown to catalyze the acetylation of alpha tubulin at K40 (Akella et al, 2010; Shida et al, 2010; Montagnac et al, 2013; Taschner et al, 2012). ATAT preferentially acetylates polymerized alpha tubulin and may access the luminal K40 residue through 'breathing' of the microtubules protofilaments (Shida et al, 2010). ATAT was identified as an interacting protein with components of the BBSome, and siRNA knockdown of ATAT delays assembly of the primary cilium (Jin et al, 2010; Shida et al, 2010). BBIP1 is another component of the BBSome that has been shown to affect tubulin acetylation and stability, potentially through its interaction with HDAC6. BBIP1 exerts its effect on microtubule acetylation independently of its role as a component of the BBSome; it may exert its indirect effect by promoting ATAT-mediated acceleration, by counteracting HDAC6-mediated deacetylation, or by another mechanism (Loktev et al, 2008)

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HDAC6 deacetylates microtubules 7

Location: Cilium Assembly

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Type: transition

Compartments: cytosol



HDAC6 is a microtubule-associated deacetylase that targets the K40 acetyl groups of alpha tubulin (Hubbert et al, 2002; Loktev et al, 2008; Zhang et al, 2008). HDAC6 also interacts with BBIP1, a component of the BBSome that is required for BBSome assembly, and additionally (and independently of its role in the BBSome) plays a role in microtubule polymerization and acetylation (Loktev et al, 2008). Depletion of BBIP1 causes a marked reduction in cytoplasmic microtubule acetylation, and this defect is partially overcome by inhibition of HDAC6. These data suggest that BBIP1 may exert its effect on microtubule acetylation by negatively regulating HDAC6, although other mechanisms are also possible (Loktev et al, 2008).

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