Cilia, centrioles and ciliogenesis

Cilia and flagella:

Cilia and flagella are essentially the same organelle in two different contexts. These organelles are 'hair-like' projections from cell surface. We will broadly refer to them as cilia, particularly to avoid confusion with bacterial flagella.

There are two major types of cilia in eukaryotes- motile cilia that provide motility and non-motile primary cilia that function as sensory organ or cells 'antenna'.

Cilia are evolutionary conserved organelles present in various unicellular eukaryotes to most cell types in human. However, organisms that don't have cilia include fungi, red algae, amoeba and higher plants. Examples of unicellular organisms that contain one to two motile cilia (commonly referred to as flagella in these organisms) are Naegleria, Trypanosoma, Toxoplasma, Plasmodium, Leishmania, Chlamydomonas etc. However, some unicellular ciliates contain multiple cilia, for example Paramecium and Tetrahymena.

The long flagella in mammalian sperms are also motile cilia. Motile function (generally 'wave' like motion) of these cilia helps these cells to swim or move.

On the other hand, hundreds of motile cilia are found in well-differentiated epithelial cells in different parts of multi-cellular organisms. A coordinated ciliary motility ('beat' like motion) of these cilia generates directional fluid flow. Such multiciliated cells are found in epithelia of respiratory tract, brain ventricle and female oviduct.

In contrast, a non-motile cilium (often called as primary cilium) is found in most vertebrate cells at some point during their life cycle. Primary cilia transduce physiological (chemical, mechanical and developmental) signals. One of the important roles of primary cilia is to transduce Hedgehog signaling (Hh) that plays a key role in tissue pattering during mammalian development.

The Hh signaling pathway initiates a signal transduction cascade upon Hh ligand binding to its receptor Patched (Ptch1; on ciliary membrane), relieving Ptch1 inhibition on another membrane protein Smoothened (Smo). When relieved from inhibition, Smo accumulates on the primary cilium, and this increased ciliary Smo activates Gli transcription factors inside cilia. Activated Gli transcription factors are transported into cytoplasm by cytoplasmic dynein and Intra Flagellar Transport (IFT) particles and initiate expression of nuclear Hh target genes.

Bacterial flagella:

Most bacteria swim toward nutrients using more than one flagella. Although bacterial flagella provide motility similar to eukaryotic cilia or flagella, they are structurally entirely different. Bacterial flagella don't have microtubules or dynein. They generate movement through propeller-like rotation, instead of the 'wave' or 'beating' motion of eukaryotic cilia. Regulation of bacterial flagella assembly is also distinct from ciliogenesis in eukaryotes. One of the major components of the bacterial flagella is the filamentous protein Flagellin.

Ultrastructure of cilia:

The structure of a cilium is very complex as a cilium contains more than hundred proteins that are recruited and assembled in a highly ordered fashion. A cilium is composed of a microtubular axoneme that is assembled on a basal body and ensheathed within a ciliary membrane. Although the ciliary membrane looks like an extension of cell membrane, it significantly differs from plasma membrane in composition, and contains specific signal receptors.

The wall of the ciliary axoneme is made of nine doublet microtubules (the distal ciliary tip is composed of singlet microtubules) arranged in radial symmetry. The doublet structure consists of the A tubule (one complete microtubule, 13 protofilaments) connected to an incomplete second microtubule or the B tubule. Tubulin of ciliary axoneme are stabilized by numerous post-translational modifications including acetylation and glutamylation. Generally, axoneme of a motile cilium consists of nine peripheral doublet microtubules associated with inner and outer dynein arms and nexin connections, radial spokes and a central pair of singlet microtubules ('9 + 2' configuration). In contrast, a primary cilium shows a '9 + 0' configuration (without central microtubules) and also lacks motility-associated proteins such as dynein. Ciliary dynein is a large protein complex that appears as two globular head domains connected to the base through stems. The base is strongly attached to the A tubule, while the heads are loosely attached to the B tubule of another doublet microtubule (MT). One head domain attempts to move with respect to another head domain (from '+' to '-' end) utilizing ATP, thus generates a 'sliding' force to the B tubule. Since the doublet MTs are connected to each other, the sliding force causes a bending motion of the axoneme that generates the ciliary motility.

The axoneme is assembled on a basal body that is a modified centriole. Therefore, it is very important to know about the structure and formation of centrioles in order to understand the ciliary assembly, also called ciliogenesis.

Centrioles and centrosomes:

Centrioles are tiny microtubule based barrel-shaped structures present in all cells that are able to build cilia. The wall of a centriole is made of nine triplet microtubules arranged in a radial symmetry. The triplet microtubule array is made of A, B and C tubule arranged in an inside to outside fashion. In animal cells, a pair of centrioles forms a centrosome that serves as the major microtubule organizing center (MTOC). The centrioles of a centrosome are surrounded by a proteinaceous matrix called pericentriolar material (PCM). The two centrioles of a centrosome are not identical. One, known as the mother (or maternal), is formed at least one centriole duplication cycle earlier than the other, the daughter, and contains distal and subdistal appendages. During ciliogenesis, the mother centriole is attached to the plasma membrane and is converted to a basal body.

A major component of PCM is γ-tubulin. In the centrosome matrix, γ-tubulin forms the γ-tubulin ring complex (γ-TuRC), a ring-like macromolecular assembly that helps in microtubule nucleation. Centrosomes regulate the cytoskeletal dynamics and cell polarity in interphase cells. During mitosis, centrosomes serve as the poles of bipolar spindle assembly that mediates accurate chromosome segregation between daughter cells.

Centriole duplication:

The canonical centriole duplication occurs during S-phase (a cell cycle phase when cells replicate their chromosomes). During G1 phase, a cell has one centrosome that contains a mother and a daughter centriole, positioned orthogonally to each other and linked by flexible connecting fibers. The initial step of centriole duplication is centriole disengagement that leads to dissolution of the connecting fibers between the two centrioles and subsequent loss of the orthogonality. During S-phase, a cartwheel is formed at the proximal ends of each centriole. The cartwheel provides the platform for stepwise assembly of centriolar proteins and also determines the nine-fold symmetry that is strictly maintained throughout the length of the centriole length as well as in the ciliary axoneme. Each procentriole is formed upon stepwise recruitment of various centriolar proteins from bottom to top fashion onto the cartwheel and the formation of the microtubule-based wall. During G2 phase of cell cycle, procentrioles elongate and mature. Subsequently, γ-tubulin and other PCM proteins are also assembled to form the second centrosome that has one pre-existing and one newly formed centriole. Centrosomes separate and move to the opposite ends of a cell during G2-M transition, which is followed by formation of a mitotic spindle.

During cellular quiescence or G0 phase, the mother centriole is converted to the basal body that, with the help of intraflagellar transport (IFT) machinery assembles a primary cilium. A primary cilium must resorb when the quiescent cells re-enter the S-phase to liberate the centrioles for new centriole assembly. Thus, the dynamic ciliary assembly and disassembly process is strictly coordinated with centriole duplication cycle to maintain the centriole homeostasis. In cell culture laboratories, cellular quiescence is commonly achieved by starving the cells for serum that provides the growth factors required for proliferation of cells in culture medium. Almost 85-90% of serum starved cells form primary cilia. Upon serum re-addition, most of these cells resorb their cilia and synchronously enter cell cycle. Recent studies have shown that ciliary disassembly are induced by many cell cycle regulating kinases such as Aurora kinase A, Nek kinases, Plk1, and Mps1 kinase. The two suggested mechanisms for ciliary disassembly are - a) destabilization of axonemal microtubules by the tubulin deacetylation activity of HDAC6 and b) increasing the rate of depolymerization at the ciliary tip by members of depolymerizing kinesin family.

Transition fibers, transition zone and ciliary necklace:

When the centriole is converted to a basal body, the distal appendages transform into transition fibers that binds to membrane. The Transition Zone (TZ) is a specialized compartment between the distal edge of the centriole (triplet MT-based wall) and the proximal-most end of axoneme (doublet MT-based wall). The doublets MTs in this region are linked to the surrounding ciliary membrane by Y-shaped fibers (Y-link). These linkers coincide with a characteristic circumferential arrangement referred to as the ciliary necklace that can be observed by freeze fracture electron microscopy.

The transition zone, which is formed during the early stages of ciliogenesis, stabilizes basal body–membrane connections and also serves as a selective membrane diffusion barrier, contributing to compartmentalization (cilioplasm vs cytoplasm).

Another distinctive sub-domain of some types of mammalian cilia is the ciliary pocket, an invagination of the plasma membrane at the root of a cilium.

Ciliary assembly:

Ciliogenesis initiates when vesicles derived from golgi (sometime referred to as ciliary vesicles, CV) are attached to the mother centriole through distal appendages. An immature TZ region begins to emerge and invaginate the CV. The membrane surface grows by the fusion of additional vesicles trafficked from golgi. Then, the centriole-vesicle migrates to the cell surface and fuses with it. The dynamic elongation and maintenance of the ciliary axoneme is mediated by the intraflagellar transport (IFT).

Intraflagellar transport (IFT):

IFT is the bidirectional movement of multiprotein complexes (IFT particles) along the axonemal MTs. These IFT parcels carries building blocks of cilia from the base of cilia to the newly forming ciliary tip during ciliogenesis.

The anterograde movement of IFT particles (base to tip) is driven by the function of motor activity of plus-end directed kinesins (members of the Kinesin-2 family). The activity of cytoplasmic dynein complex (cytoplasmic dynein 2) returns the IFT train to the cell body by the retrograde movement. Although the two IFT complexes, complex A and B move together as the IFT train, they bind to specific cargo at different time. IFT complex B carries the cargo during anterograde movement, while IFT complex A brings back cargos during retrograde movement. It is thought that at the ciliary tip, IFT train unloads cargo (building blocks), turns around, switches from Kinesin to cytoplasmic dynein powered locomotion, reloads cargo (recyclable components) and moves back to the basal body.

The canonical anterograde IFT motor heterotrimeric Kinesin-2 is indispensable for the assembly and maintenance of cilia. On the other hand, depletion of the function of cytoplasmic dynein 2, another multiprotein complex, leads to stumpy cilia swollen with accumulated IFT particles at the tip. Most of the IFT proteins, particularly the components of IFT complex B (such as IFT88, IFT20 etc) are also crucial for cilia formation, maintenance and function.

Ciliopathies:

Since motile and sensory cilia play role in so many different physiological processes at different times and parts of human body, it is expected that abnormal cilia structure and/or function may lead to many diseases in human. The disorders that are associated with ciliry dysfunction mostly due to genetic mutations in various genes that regulate cilia assembly, motility or sensory functions are broadly grouped as ciliopathies. Kartagener's syndrome is an example of a complex ciliopathy with the symptoms of bronchiectasis, situs inversus, chronic sinusitis and infertility due to immotile sperms.

Legends:

Slide-3: Human Retinal Pigment Epithelial cells (RPE1) containing primary cilia. Hair like primary cilia are marked by antibody against acetylated tubulin (green), while centrosomes are stained by γ-tubulin (red). Nuclear DNA is stained as blue.

Slide-11: Representative image of a RPE1 cell with a primary cilium. The cell is stained for acetylated tubulin (green; ciliary axoneme), and Arl13B (red), a specific component of ciliary membrane. Nuclear DNA is stained as blue.

References:

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