Evolution

Tracing the origins of centrioles, cilia, and flagella

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Centrioles/basal bodies (CBBs) are microtubule-based cylindrical organelles that nucleate the formation of centrosomes, cilia, and flagella. CBBs, cilia, and flagella are ancestral structures; they are present in all major eukaryotic groups. Despite the conservation of their core structure, there is variability in their architecture, function, and biogenesis. Recent genomic and functional studies have provided insight into the evolution of the structure and function of these organelles.

Structure and assembly of CBBs and cilia/flagella

The microtubule (MT) cytoskeleton is the main component of macromolecular machineries, such as the mitotic spindle and centrioles/basal bodies (CBBs). CBBs nucleate formation of the centrosome—the primary microtubule-organizing center (MTOC) in animal cells that is involved in cell proliferation, migration, and polarity—and of cilia/flagella, which are essential for motility and sensing/responding to environmental cues (Fig. 1; Bettencourt-Dias and Glover, 2007; Nigg and Raff, 2009). CBBs are cylinders generally constructed from nine microtubule triplets organized in a ninefold symmetrical configuration reinforced by a cartwheel-like structure (Fig. 1, A and B). The canonical animal centrosome is usually composed of a mature mother and a daughter centriole (Fig. 1, A and B). Mature centrioles may acquire additional specialized structures, such as subdistal and distal appendages, involved in cytoplasmic MT anchoring and positioning the CBB within the cell (Fig. 1 A; Bornens, 2002; Azimzadeh and Marshall, 2010). Centrioles, then called basal bodies, anchor at the cell membrane to initiate the growth of the axoneme, the MT core of cilia and flagella (Fig. 1 C). Basal body differentiation may include the addition of structures such as striated rootlets, basal feet, and transition fibers, linking the basal body to the cytoskeleton and orienting it in relation to other organelles and other cells (Fig. 1, A and C). The axoneme assembles through the extension of the two most inner MTs of the basal body and is usually encased in a specialized membrane. Cilia may also contain a pair of central MTs, inner and outer dynein arms, and radial spokes that drive and regulate ciliary motility (Fig. 1 C; Satir and Christensen, 2007; Gerdes et al., 2009).

CBBs can assemble adjacent to other centrioles or de novo in the absence of preexisting structures (Fig. 1 B). In most eukaryotic cell types, cilia biogenesis, integrity, and function rely on a bi-directional transport mechanism along axonemal MTs, called intraflagellar transport (IFT; Fig. 1 C; Kozminski et al., 1993; Marshall and Rosenbaum, 2001; Engel et al., 2009). In most organisms, anterograde (base to tip) IFT is driven by heterotrimeric kinesin-II, whereas the retrograde (tip to base) movement is mediated by dynein heavy chain (Fig. 1 C; Davis et al., 2006).

Methods to infer how CBBs and derived structures evolved

Eukaryotes evolved more than two billion years ago, but we lack a fossil record from which we can track the evolution of the structure and function of subcellular organelles (Cavalier-Smith, 2010; El Albani et al., 2010). We can only infer details of early eukaryotic history using features of extant organisms. We use the general parsimony principle that the most likely evolutionary scenario is the one that requires the least amount of events (see for example Ouzounis (2005) for a discussion of ancestral genome reconstruction). So, if we observe that a given structure or gene exists in several eukaryotic groups, we assume that it emerged before their divergence, in the last eukaryotic common ancestor (LECA), and reject a scenario of convergent evolution.

The recent sequencing and annotation of eukaryotic genomes, combined with advancements in RNAi and mass spectrometry, has enabled the characterization of the composition and function of CBBs and associated structures in several eukaryotic groups (Figs. 2 and 3; Wigge et al., 1998; Ostrowski et al., 2002; Andersen et al., 2003; Keller et al., 2005; Pazour et al., 2005; Smith et al., 2005; Broadhead et al., 2006; Cao et al.,

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Abbreviations used in this paper: CBB, centriole/basal body; IFT, intraflagellar transport; LECA, last eukaryotic common ancestor; MT, microtubule; MTOC, microtubule-organizing center.

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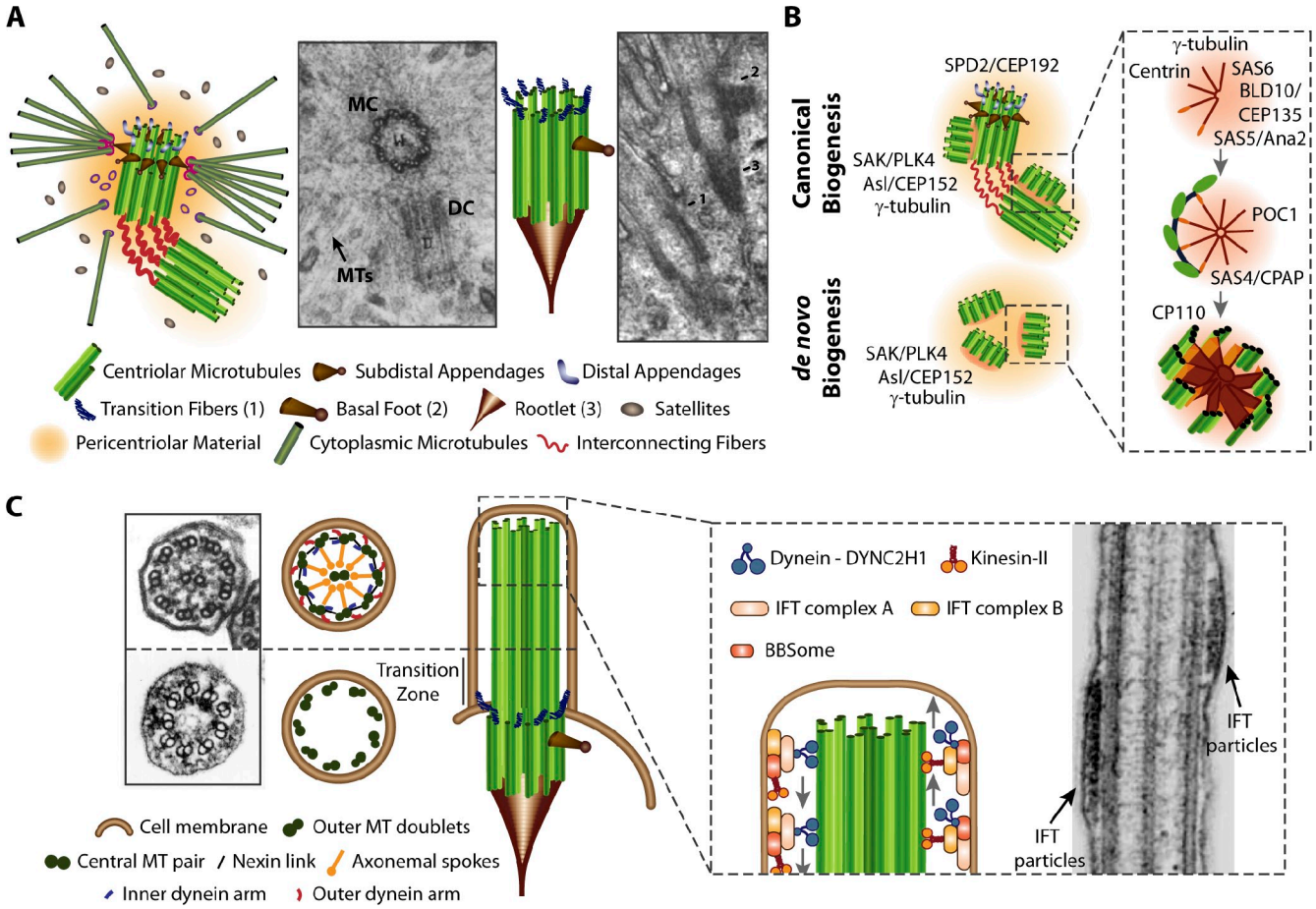

Figure 1. **Structure and biogenesis of centrosomes and cilia.** (A) On the left, a schematic and EM micrograph (reproduced from Vorobjev and Chentsov, 1982) of an animal prometaphase centrosome composed of mother (MC) and daughter (DC) centriole arranged in an orthogonal fashion. The mother centriole harbors subdistal and distal appendages. On the right, a schematic and EM longitudinal section (reproduced with permission from the *Journal of Cell Science;* Sorokin, 1968) of a basal body from rat lung multiciliated cells bearing rootlets and lateral/distal appendages. (B) Key regulatory and structural components in CBB biogenesis (canonical [top] and de novo [bottom]; Azimzadeh and Marshall, 2010). (C) Schematic of the basal body, when docked at the cell membrane and growing the axoneme of cilia/flagella. EM cross section of tracheal motile cilia (top: reproduced from Satir and Dirksen (1985) in *Handbook of Physiology* with permission from the American Physiology Association) and renal nonmotile primary cilia (bottom: image courtesy of H. Zentgraf, German Cancer Research Center, Heidelberg, Germany). Cilia/flagella are assembled via the intraflagellar transport (IFT) system. EM longitudinal section of the *Chlamydomonas* flagellum adapted from Pedersen et al. (2006) with permission from Elsevier.

2006; Reinders et al., 2006; Kilburn et al., 2007; Liu et al., 2007; Baker et al., 2008a,b; Arnaiz et al., 2009; Boesger et al., 2009; Mayer et al., 2009). These data have allowed the use of comparative genomics in the study of CBBs and derived structures (Avidor-Reiss et al., 2004; Li et al., 2004; Branche et al., 2006). A strong correlation between the presence of a given set of genes and a structure in a wide number of species points to an involvement of those genes in the biogenesis/function of those structures (Figs. 2 and 3; Carvalho-Santos et al., 2010). This also includes organisms for which no functional studies have been performed. Moreover, these phylogenetic profiles can suggest paths for the evolution of CBBs and cilia.

Eukaryotic origin of CBB and cilia/flagella

The structure and origin of the first CBB. Both the CBB and axoneme are remarkably conserved structures found in all major eukaryotic groups, which suggests their presence in the LECA (Fig. 2; Table S1; Cavalier-Smith, 2002). Organisms in most taxonomic groups show a ninefold symmetric CBB with cartwheel and MT triplets and an axoneme composed of nine MT doublets bound to outer and inner dynein arms and radial spokes, surrounding a central MT pair (Fig. 2). A similar structure is thus likely to have been present in the LECA (Fig. 4).

Other structures associated with the CBB, such as appendages (transition fibers/distal appendages, subdistal or other lateral appendages, basal feet or connecting fibers, and non-MT–based rootlets), may have also been present in the LECA (Figs. 2 and 4). It has been suggested that the association of CBBs with other cytoplasmic cytoskeleton elements, mediated by those appendages, is an ancestral feature ensuring cell polarization relative to its motile/sensory apparatus and the propagation of cell geometry through cell division (Azimzadeh and Marshall, 2010). For instance, in *Chlamydomonas*, the basal bodies anchor a set of stable MT bundles that are involved in division plane determination and thus impact the cell geometry of daughter cells. Basal bodies from *Paramecium*, *Trichomonas*, Chitrids, and choanoflagellates also show an association with cytoplasmic microtubules either directly or through an MTOC.

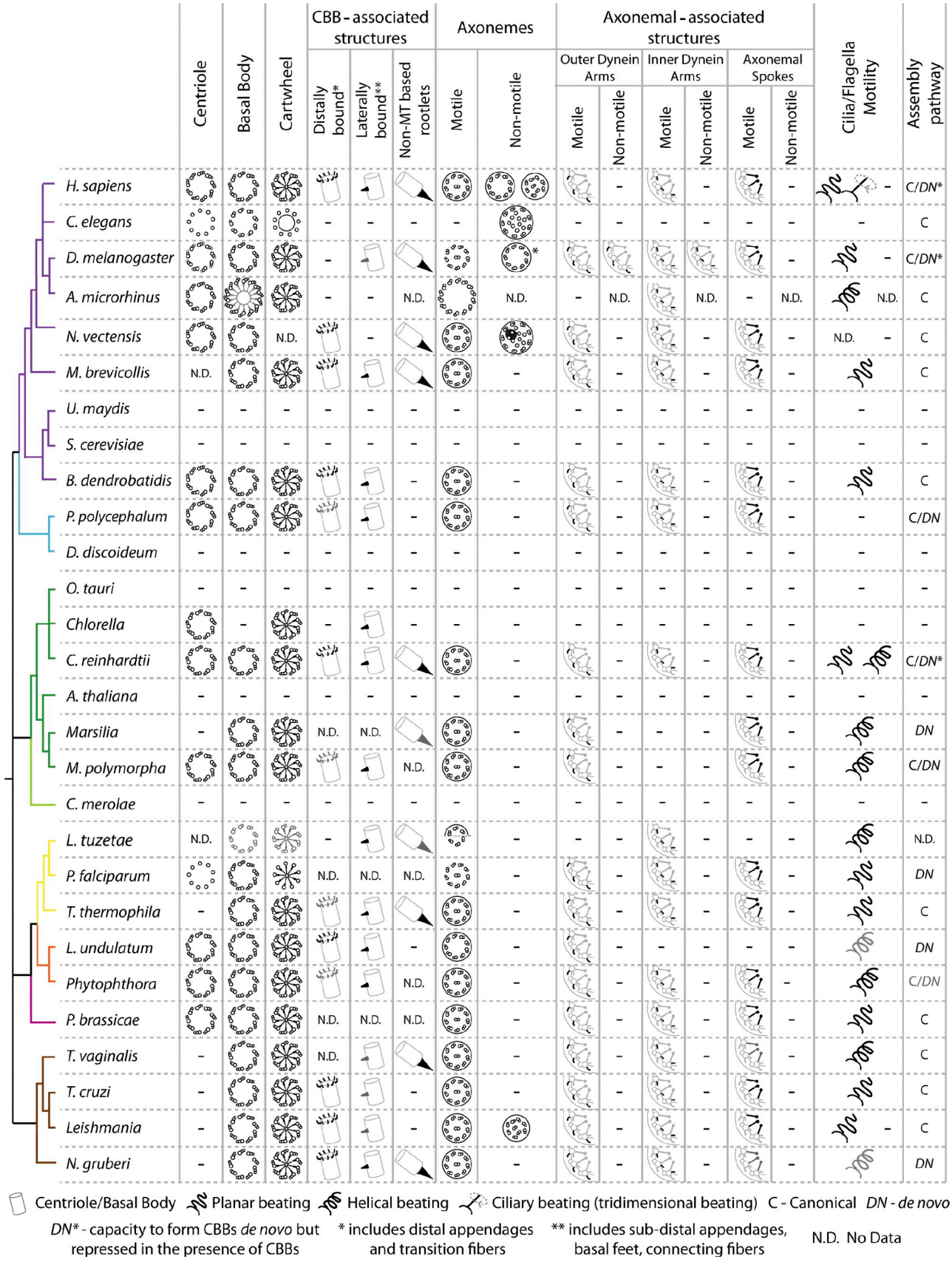

Figure 2. **Structure and distribution of CBB, cilia/flagella, and associated structures in eukaryotes.** Simplified taxonomic tree representing major eukaryotic groups in different colors (these groups contain a common ancestor and all its descendants; adapted from Hedges (2002) and Baldauf (2003)). Unikonts include eukaryotic cells that, for the most part, have a single emergent flagellum and are divided into Opisthokonts (propel themselves with a single posterior flagellum; Metazoans, Fungi, and Choanoflagellates) and Amoebozoa (Cavalier-Smith, 2002). Bikonts include eukaryotic organisms with

Unfortunately, the little information available about the morphology and composition of these associated structures does not allow us, at the time, to discriminate between scenarios of common ancestry or convergent evolution.

Finally, it is likely that early flagella performed both motile and sensory functions, as these features are observed in multiple branches of the eukaryotic tree of life (Fig. 2; Satir et al., 2008; Bloodgood, 2010).

Three different hypotheses have been proposed for the presence of the CBBs and cilia/flagella in the LECA: endosymbiotic, viral, and autogenous origin. The first proposes that the cilium originated from a permanent symbiosis between an ancient Spirochete bacterium and an Archaebacterium (Sagan, 1967). Satir et al. (2007) proposed that the CBB evolved from an infection with a virus bearing a ninefold symmetry, whose capsid gave rise to a cartwheel with the ability to elongate MTs. To date, these hypotheses have had little morphological and molecular support (Satir et al., 2008).

The third hypothesis, first described by Pickett-Heaps (1974), proposes an autogenous origin of the CBB/cilium from an MTOC that organized cytoplasmic and mitotic MTs. The ninefold symmetry of these structures would have been the result of an evolutionary optimization of cilium motility (Cavalier-Smith, 1978; Mitchell, 2004; Satir et al., 2008). Although remote homologues of tubulin exist in Prokaryotes (Löwe and Amos, 2009), recent data suggest that most CBB components originated in eukaryotes: (a) no bacterial, archaeal, or viral counterparts of CBB components were found using defined conserved regions from proteins of the CBB assembly pathway (Carvalho-Santos et al., 2010; unpublished data); and (b) many CBB/cilium components such as tubulins, microtubule motors, and the IFT machinery are eukaryotic duplicates of cytoplasmic proteins (Fig. 3; Dutcher, 2003; Azimzadeh and Bornens, 2004; Jékely and Arendt, 2006; Wickstead and Gull, 2007). These results support the scenario of a eukaryotic/autogenous origin of the CBB, which we discuss in the next section.

Minimal requirements for the assembly and function of CBBs and derived structures. Recent studies on the molecular mechanisms involved in the assembly and function of cilia and flagella reveal that the ninefold symmetrical CBB and axoneme is universally dependent on molecules such as SAS6, SAS4/CPAP, and BLD10/CEP135 (Fig. 1 B; Carvalho-Santos et al., 2010; Hodges et al., 2010). On the other hand, although it is not clear whether there is an ancestral mechanism for association of CBBs with the cytoplasmic cytoskeleton as discussed above, at least one component required for CBB migration and attachment to the actin cytoskeleton, MKS1, is ancestral (Fig. 3; Keller et al., 2005; Dawe et al., 2007, 2009).

The assembly of the axoneme and localization of receptors to the ciliary membrane was shown to be dependent on polarized vesicular trafficking and on the IFT machinery (Fig. 1 C and Fig. 4, B, B′, C, C′, and E; Nachury et al., 2010). Cargo destined for the cilium is assembled and sorted at the Golgi and delivered at the flagellar base. This process involves essential regulators of polarized transport, such as Rab8 and the exocyst and sorting complexes such as the BBSome (Figs. 3 and 4; Peränen et al., 1996; Moritz et al., 2001; Koumandou et al., 2007; Nachury et al., 2007; Yoshimura et al., 2007; Pereira-Leal, 2008; Jin et al., 2010). Cilia compartmentalization may be dependent on different mechanisms, as large proteins do not freely enter the ciliary lumen (Nachury et al., 2010). Recently, septins, GTP-binding proteins involved in compartmentalization, were implicated in the definition of a ciliary-specialized membrane compartment at the base of the cilium (Fig. 3; Fig. 4, B and E; Hu et al., 2010; Kim et al., 2010). Also, a Ran-GTP gradient may selectively control which molecules enter the cilium, similar to the mechanism that regulates transport through the nuclear pore (Dishinger et al., 2010). Although still poorly understood, this system may be crucial for transport into the cilium and for shuttling components between the cilium and the nucleus, hence regulating transcription in cilia-mediated signaling events.

Finally, the motility of the axoneme is dependent on a variety of protein complexes, such as dynein arms (Fig. 1 C and Fig. 3; Satir and Christensen, 2007).

Evolutionary scenarios for the origin of CBB/cilium apparatus in eukaryotes. A tubulin-like cytoskeleton, membrane compartmentalization with coat-like molecules, and an internal genome-containing compartment are all found in bacteria, and in particular in a recently characterized superphylum of bacteria suggested to be ancestors of eukaryotes, which includes the Planctomycetes, Verrucomicrobia, and Chlamydiae (Devos and Reynaud, 2010). Ran GTPase, which controls nucleo-cytoplasmic shuttling, appears to have emerged before the radiation of the Rab family (Colicelli, 2004). Thus, many of the essential components required to assemble a cilium, either sensory or motile, can be traced back to the proto-eukaryote.

The origin of the motile cilium Several unicellular organisms use their flagellum to attach to surfaces and pull the cell body in a process called gliding, which allows cells to move in an almost amoeboid fashion. A simple form of motility such

two emergent flagella (Cavalier-Smith, 2002). Branch color code: purple, Opisthokonts; blue, Amoebozoa; green, Plants; yellow, Alveolates; orange, Stramenopiles; rose, Rhizaria; brown, Excavates and Discicristates. We represent the symmetry and number of CBB MTs, either when nucleating an axoneme (basal body) or not (centriole), and of axonemes as well as the presence/absence of central MT pair (−, not present). In organisms for which data were available, we also included the structure of the cartwheel and of several associated structures (transition fibers/distal appendages involved in CBB anchoring at the cell membrane; lateral appendages including subdistal appendages, basal foot or connecting fibers linking CBBs to each other or to cytoskeleton components; and non-MT based rootlets that link the proximal part of the CBB to other organelles). We also included information on the presence/absence of axonemal-associated structures (dynein arms and radial spokes) as well as their beating patterns. Finally, we represent the pathways used for CBB assembly, canonical, de novo, or both. In *Drosophila melanogaster*, the gray centriolar MTs represent the fact that certain tissues present centrioles with doublets whereas others show triplets. The asterisk in *D. melanogaster* 9+0 sensory axoneme reflects the possibility that this structure is motile (Göpfert and Robert, 2003). In *Acerentomon microrhinus*, the CBBs formed during spermatogenesis have a different symmetry and the cartwheel is also represented in these structures. In the remaining cases, gray structures are used when evidence pointing to their presence is not robust (poor EM data or data from a related species). For references please check Table S1.

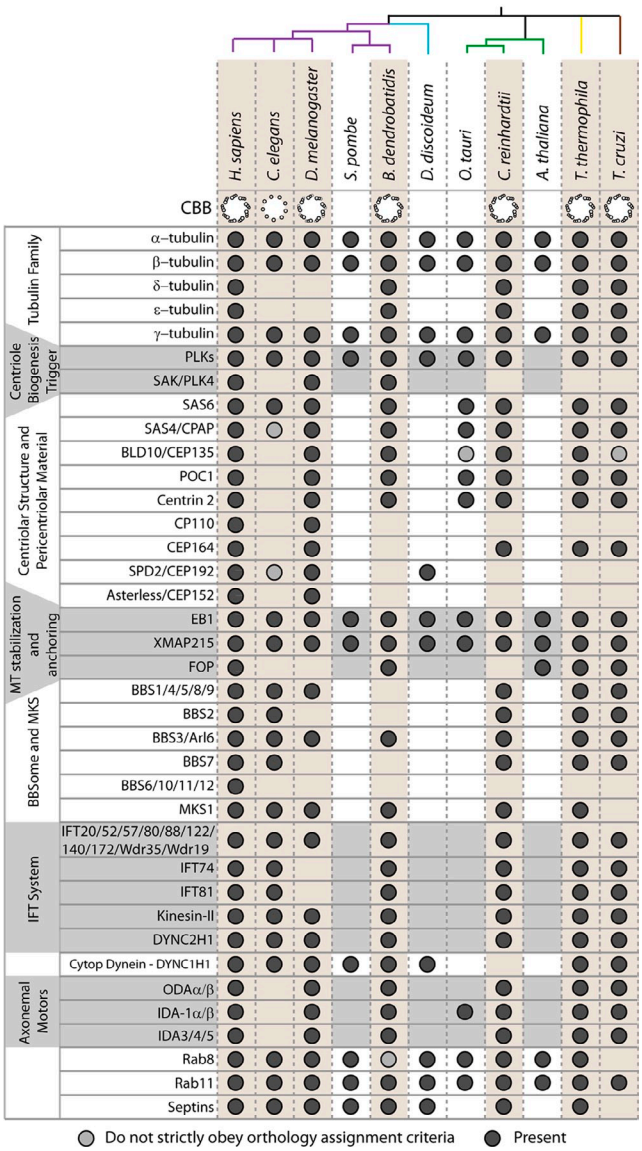

Figure 3. **Phylogenetic profile of CBB/axoneme structure and components.** Simplified taxonomic tree representing major eukaryotic groups in different colors using the same color code as in Fig. 2 (adapted from Hedges (2002) and Baldauf (2003)). Phylogenetic profile of proteins involved in CBB and cilia/flagella assembly and function. Data adapted from Jékely and Arendt (2006), Wickstead and Gull (2007), Carvalho-Santos et al. (2010), Hodges et al. (2010), Wickstead et al. (2010) and our unpublished data.

as this could have been the first proto-cilium–based motility (Satir et al., 2008). This is supported by the fact that this type of movement is found in at least three different eukaryotic groups (Plants, Discicristates, and Cercozoa; Bloodgood, 1981; Kozminski et al., 1993; Saito et al., 2003; Chantangsi et al., 2008; Satir et al., 2008; Cavalier-Smith et al., 2009).

Several proteins had to emerge in early eukaryotes to allow the evolution of a motile structure with ninefold symmetry, formed by triplets and doublets of closed and opened MTs. These probably include ε- and δ-tubulins, allowing for the construction of the unique opened MTs of the CBB and cilium, and other proteins such as SAS6, SAS4/CPAP, BLD10/CEP135, POC1, and CEP164 involved in cartwheel, centriolar MTs, and appendage assembly (Figs. 1–4; Carvalho-Santos et al., 2010; Hodges et al., 2010; Azimzadeh and Marshall, 2010). In addition, multiple rounds of dynein duplication with subfunctionalization originated the outer and inner dynein arms, which produce the flagellar beating force (Wickstead and Gull, 2007). The emergence of a variety of molecules needed for biogenesis and orientation of the central MT pair would have allowed the evolution of more complex beat waveforms (Fig. 4, D and E). The ninefold symmetry of the axoneme appears to be the most efficient conformation to enclose the central pair (Mitchell, 2004, 2007; Satir et al., 2008). Whether the CBB appeared first, at the same time, or after the axoneme is not clear, but morphological distinctions between the two structures (doublet vs. triplet MTs, central MT pair, etc.) were likely present in the LECA (Figs. 2 and 4).

Evolving a sensory organelle. The evolution of a sensory cilium required the targeted trafficking of receptors and signaling molecules to a specialized membrane domain. Jékely and Arendt (2006) recently proposed an evolutionary scenario in which the initial structure to appear during CBB/cilia evolution was an individualized sensory patch resulting from polarized vesicle transport from the Golgi to the plasma membrane using specific coats (Fig. 4, B, B′, and E). This polarized transport relied on a system of motors and adaptors that would later evolve into the IFT system (Fig. 4, C and C′). The combination between the sensory and motility functions on the proto-cilium would culminate in the evolution of an organelle similar to those found in extant organisms (Mitchell, 2007; Satir et al., 2008).

Ample evidence supports the idea that sensory functions of the cilium evolved from polarized trafficking. The IFT system is likely to be a specialization of a more general transport system already present in the LECA: (a) coat proteins and small GTPases, typically found on the cytosolic face of organelles, have counterparts in the IFT machinery; (b) kinesins and dyneins involved in IFT have cytoplasmic counterparts involved in polarized transport (Wickstead and Gull, 2006, 2007; Wickstead et al., 2010); (c) some IFT components have nonciliary functions such as polarized secretion in nonciliated lymphoid cells and in exocytosis at the synapse of nonciliated secondary neurons in the retina (Finetti et al., 2009; Sedmak and Wolfrum, 2010); (d) the kinesin-II family of IFT motors also functions in polarized cells including intracellular melanosome transport and mRNA and MT stabilization (Wickstead and Gull, 2006; Jaulin and Kreitzer, 2010; Wickstead et al., 2010); and (e) several IFT proteins localize to spindle poles in mitosis, suggesting cytoplasmic roles in microtubule organization and spindle positioning (Fig. 3; Fig. 4, B, B′, C, C′, and E; Wickstead and Gull, 2006; Jaulin and Kreitzer, 2010; Wickstead et al., 2010; Delaval et al., 2011). Finally, cilia are specialized compartments, with different protein and lipid composition from those of cytoplasm and nonciliary membrane (Nachury et al., 2010). Diffusion barriers are known to play important roles in the definition of cellular compartments. It is plausible that the early creation of a diffusion barrier, perhaps organized by septins, which our unpublished data suggest are ancestral molecules, led to the individualization of a ciliary membrane (Fig. 4, B, C, C′, and E).

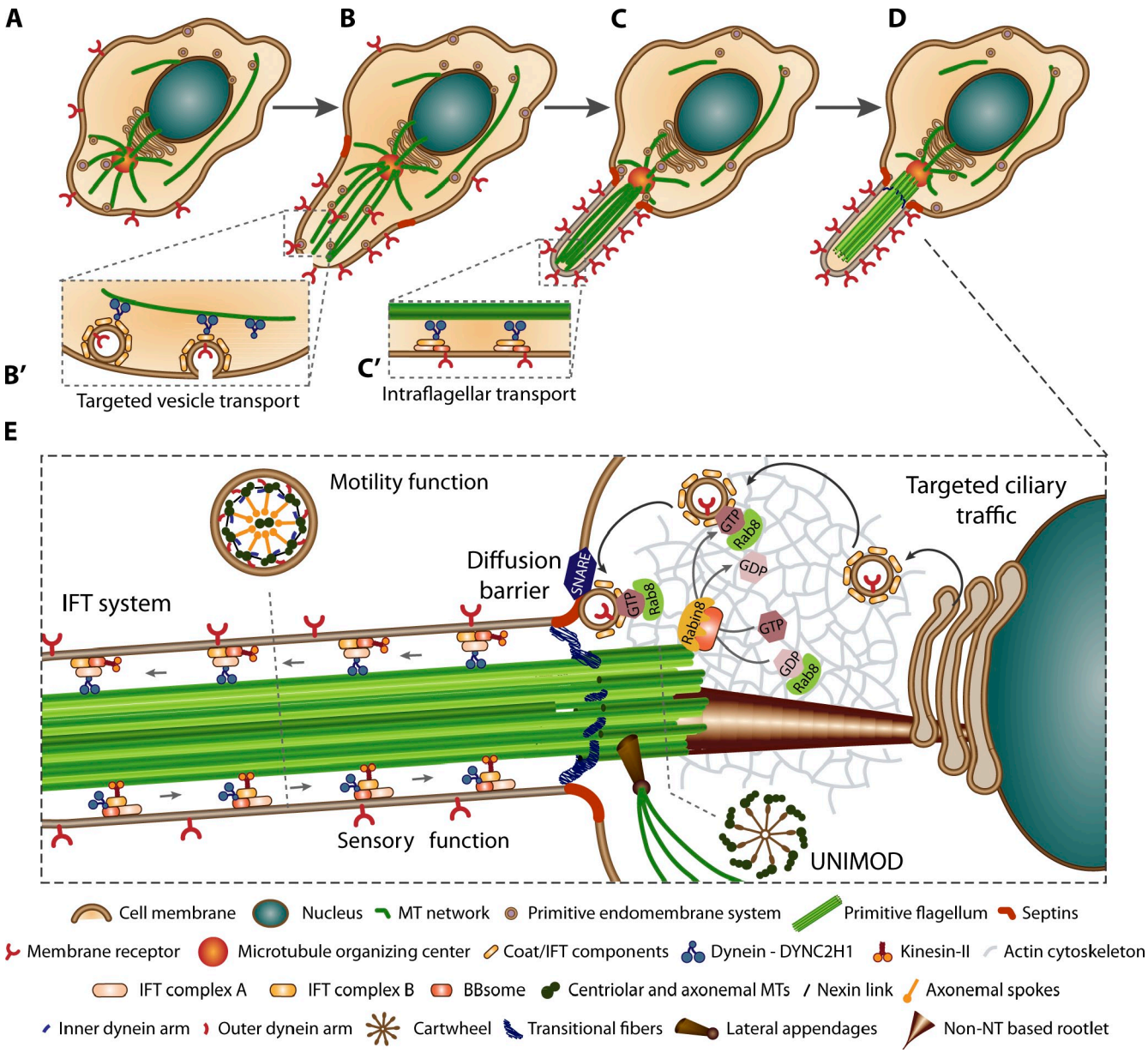

Figure 4. **Autogenous theory for the origin of the sensory/motile flagellum.** (A) The eukaryotic cell where the proto-cilium evolved likely had a cytoskeleton composed of actin and MTs that converged in the MTOC, a nucleus, and an endomembrane system. (B and B′) Targeted traffic of cell membrane components to a cell membrane patch that started protruding through the directed force produced by the MTs anchored at the MTOC. This protrusion would evolve to become a specialized structure with specific membrane composition maintained by diffusion barriers. (C) The evolving proto-cilium was likely capable of environmental sensing and gliding, which might have driven the implementation of this organelle. (C′) An IFT system was recruited to assemble these structures. (D) Further on, the bundle of MTs would evolve in order to create a specialized arrangement of closed and open MTs forming a ninefold symmetric structure capable of bending due to the presence of molecular motors. The basal body gave support to the motile axoneme at the cell membrane. (E) In conclusion: the ancestral CBB/cilium apparatus would have been characterized by the following characteristics: (a) a ninefold symmetric CBB composed of MT triplets, with a cartwheel, lateral and distal appendages, and rootlets being defined by a set of proteins, the UNIMOD (UNIversal MODule); (b) an axoneme with both motile and sensorial functions presenting ninefold symmetry composed of nine doublets, central pair, outer and inner dynein arms, and maintained by the IFT system; (c) a specialized membrane created by a diffusion barrier both at the level of the membrane and of diffusion of components at the transition zone; and (d) targeted transport of membrane and other components from the Golgi to the ciliary base. Adapted from Satir et al. (2008).

Less clear is whether the sensory patch predates the polarization of microtubules into a proto-cilium, whether the targeting of sensory and signaling molecules was a later adaptation, or even if they formed concomitantly. As the sensory and motility functions of the cilium appear to be widespread, it is not possible at this point to order their emergence in evolution.

Diversification of CBB/cilium structure and their molecular components

The CBB/cilium as a functional unit. Variations on the ancestral 9+2 axoneme configuration are observed among eukaryotes. Interestingly, these alterations correlate with changes in beat waveforms or loss of motility (Fig. 2). For instance, certain plants, diatoms, insects, and gregarines possess motile axonemes

that lack a central pair, outer or inner dynein arms, or show deviations from the ninefold symmetry (Fig. 2; Manton et al., 1970; Heath and Darley, 1972; Baccetti et al., 1973; Schrevel and Besse, 1975; Prensier et al., 1980; Woolley, 1997; Okada et al., 2005; Riparbelli et al., 2009; Dallai et al., 2010). Although 9+2 axonemes use planar or three-dimensional beat waveforms, axonemes lacking the central pair typically have a simpler helical beat (Fig. 2; Holwill, 1966; Leadbeater and Dodge, 1967; Prensier et al., 1980; Goldstein and Schrével, 1982; Gibbons et al., 1983; Werner and Simmons, 2008). Nonmotile sensory cilia can show a variety of axoneme structures, as it is in the extreme case of nonmotile *Caenorhabditis elegans* cilia (Inglis et al., 2007). This observation suggests that the main constraint limiting the variation in axoneme architecture is their motility (Mitchell, 2007; Satir et al., 2008).

Evolutionary constraints applied to the axoneme structure are likely to be extended to the CBBs. For example, *C. elegans*, which does not have motile cilia, contains a small MT doublet "basal body" at the base of sensory cilia, whereas its embryonic centrioles, which are not associated with cilia, only have singlet MTs (Fig. 2). Similarly, *Drosophila* embryonic centrioles, which are not associated with cilia, are composed of MT doublets, whereas during motile flagella formation in spermatogenesis, CBBs that nucleate motile cilia are composed of MT triplets (Fig. 2; Callaini et al., 1997). In the extreme case, many species have completely lost their CBBs and cilia, such as Angiosperms, some fungi, and certain amoebas (Fig. 2).

CBB and cilia assembly in different contexts. The pathway of CBB biogenesis varies among eukaryotes being performed either in proximity to an already existing "parental" structure or de novo (Fig. 1 B). Canonical and de novo CBB assembly can also coexist in the same organism, such as in parthenogenic insects (Riparbelli and Callaini, 2003; Ferree et al., 2006). Because in animals and *Chlamydomonas* experimentally induced de novo biogenesis lacks control on the place, time, and number of CBBs assembled, it has been suggested that the presence of a parental structure provides a scaffold for the regulation of new centriole assembly (Marshall et al., 2001; Rodrigues-Martins et al., 2007b). It is thus striking how organisms such as the amoeboflagellate *Naegleria* and sperm cells of *Plasmodium*, ferns, or diatoms that form CBBs de novo show impressive number, spatial, and time control (Fig. 2; Dingle and Fulton, 1966; Mizukami and Gall, 1966; Manton et al., 1970; Heath and Darley, 1972; Fritz-Laylin et al., 2010a). Perhaps in all organisms regulation of the location and numbers is achieved by controlled availability and localization of assembly components, which is often but not always specified by a parental CBB.

Several unicellular organisms assemble new cilia/flagella apparatuses during cell division without disassembling the old cilia/flagella and with no disruption of the ability to move. This is the case for ciliates, *Trypanosoma*, and *Giardia*, among others (Allen, 1969; Dute and Kung, 1978; Iftode and Fleury-Aubusson, 2003; Nohynková et al., 2006; Elias et al., 2007; Lacomble et al., 2010). Disassembly of cilia/flagella in mitosis, followed by reassembly in the next cycle is seen in unicellular and multicellular organisms, such as *Chlamydomonas* and human cells (Johnson and Porter, 1968; Cavalier-Smith, 1974; Rieder et al., 1979). It has been suggested that *Chlamydomonas* reabsorb their flagella due to the presence of a cell wall, which inhibits the migration of the basal bodies into opposite poles (Parker et al., 2010). In human cycling ciliated cells, where cilia disassembly is also observed, it seems plausible that this mechanism requires regulatory coordination with the cell cycle, though currently little is known about the mechanisms regulating this process.

Ancestral and acquired functions of the CBB. The animal CBB has a dual function as an axonemal nucleator and as part of the cytoplasmic MTOC. In animals, the centriole, as part of the centrosome, has important functions in embryonic development, asymmetric divisions, and male meiosis (Rodrigues-Martins et al., 2008; Debec et al., 2010). A centrosome composed of centrioles and PCM is primarily found in animals and Fungi (Opisthokonts); however, other examples are found outside of this taxon, such as in brown algae and *Plasmodiophorids* (plant parasites belonging to the Cercozoa group; Fig. 2 and Table S1). Although all species that present CBBs also present cilia, a weaker correlation is seen between the presence of the CBB and CBB-containing centrosomes. This observation suggests that the ancestral CBB function would be mainly to template/support the axoneme.

During cell division, the spindle ensures equal separation of the sister chromatids into the two daughter cells. The MTOCs that contribute to spindle MT nucleation and organization are highly variable among eukaryotes. The localization of CBBs at or close to the poles of the spindle is often achieved through the interaction of MTs nucleated by the CBB and the spindle itself. It has been suggested that this localization is a strategy to ensure equal inheritance of these structures so that both daughter cells can form cilia (Pickett-Heaps, 1971, 1974). However, even if this was originally the case, the CBB has important functions in cell division, suggesting possible cooption of the structure to actively participate in the process and coordinate it with other cell functions.

Molecular mechanisms in CBB evolution. There is a core mechanism of CBB assembly conserved among eukaryotes determined by an evolutionarily cohesive and ancestral gene module (UNIMOD; Fig. 1 B and Fig. 3). The use of that module extends to different tissues within the same organism, as we and others have shown that canonical, de novo and assembly in multiciliated cells use the same molecular pathway to assemble the centriole structure (Rodrigues-Martins et al., 2007a; Vladar and Stearns, 2007; Kuriyama, 2009). Perhaps one of the most striking features of the evolution of CBBs is the almost perfect correlation between the absence of the structure and absence of that ancestral gene module that is strictly required to assemble the structure (Figs. 2 and 3). Exceptions to this rule may provide interesting examples of eukaryotes that are in the process of losing the structure. That is the case of the smallest eukaryotic cell, the algae *Ostreococcus*, which does not seem to have a CBB but encodes some of its components in the genome (Figs. 2 and 3; Henderson et al., 2007; Merchant et al., 2007; Wickstead and Gull, 2007; Keller et al., 2009; Carvalho-Santos et al., 2010; Hodges et al., 2010).

Despite the conservation of structural components, proteins that contribute additional regulatory steps, upstream or downstream of CBB assembly, appear to have been added in a stepwise, taxon-specific manner throughout evolution (Carvalho-Santos et al., 2010; Hodges et al., 2010). For example, SPD2/CEP192 is a centrosomal protein involved in PCM recruitment, which is only present in Amoebozoa and Animals (Kemp et al., 2004; Pelletier et al., 2004; Dix and Raff, 2007; Giansanti et al., 2008; Zhu et al., 2008; Carvalho-Santos et al., 2010; Hodges et al., 2010). SPD2/CEP192 is required for the duplication of the naked PCM-less sperm centriole in animals, suggesting a specific role at this developmental stage (Kemp et al., 2004; Pelletier et al., 2004; Dix and Raff, 2007). CP110 and CEP97, two centriolar proteins that have been proposed to coordinate ciliogenesis with the cell cycle, are confined to the Metazoans (Fig. 3; Carvalho-Santos et al., 2010; Hodges et al., 2010). Thus, these proteins might have been added to the pathway of CBB/cilium biogenesis as additional mechanisms to regulate this process through the cell cycle.

Additionally, gene duplication plays an important role in the evolution of CBB components and, in the case of BLD10/CEP135, this is a source of tissue specificity in CBB and flagella biogenesis (Carvalho-Santos et al., 2010). Protein divergence among orthologues may also contribute to species-specific adaptations in CBB assembly (Carvalho-Santos et al., 2010).

Conclusions and perspectives

Comparative genomics, powered by the rapid increase in the availability of complete genome sequences from all branches of the eukaryotic tree, is revealing the nature of our eukaryotic ancestor and its evolution. As we sequence the genomes of more diverse eukaryotes, we also predict an increasingly complex LECA (Figs. 3 and 4; Fritz-Laylin et al., 2010b; Koonin, 2010). Because we have not yet found intermediate structures, we can only speculate how CBBs and cilia could have emerged from simpler, preexisting components. We hypothesize that the creation of diffusion barriers coupled with gene duplication and divergence may have provided a mechanistic driving force for the emergence of eukaryotic subcellular structures. Diversification of these structures and their function in different eukaryotes and different tissues likely proceeded through stepwise addition, duplication, and divergence of molecular components.

CBBs and derived structures are unequivocally identified by electron microscopy, in particular their characteristic ninefold symmetry, which has permitted their identification in different branches of the eukaryotic tree. These structures were lost in some Fungi, Amoebozoa, Alveolates, and Angiosperms, concomitant with loss of genes that are exclusively needed for CBB architecture such as SAS6, SAS4/CPAP, and BLD10/CEP135 (Figs. 2 and 3). This coarse correlation has been widely used for predicting new components of these structures (Avidor-Reiss et al., 2004; Li et al., 2004). As phylogenetic patterns for structural parts of CBBs and derived structures emerge, it will be important to investigate whether they correlate with certain molecular and morphological signatures (Fig. 2). For example, it is of key importance to piece together the evolution of the transition zone, the axonemal region where the transformation from basal body to axoneme occurs, because its components are often involved in many human diseases. This type of analysis should help us to identify new molecular components of this structure as well as having a better understanding of their mechanism of action.

The identification of pairs of closely related species where these structures were maintained in one organism and lost from the other (e.g., *Thalassiosira* vs. *Phaeodactylum* [flagellated vs. nonflagellated diatoms], *Chlamydomonas* vs. *Ostreococcus* [flagellated vs. nonflagellated green algae], and *Plasmodium* vs. *Theileria* [flagellated vs. nonflagellated apicomplexa]) may provide additional insights into how cellular structures and genes are lost during evolution. Finally, in the future it will also be important to understand how different cellular mechanisms have coevolved, in particular complexes determining cell polarity, protein trafficking, and the MT cytoskeleton. The centriole and its derived structures are thus an ideal paradigm to study the mechanisms involved in the evolution of the eukaryotic cell.

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