Centriole/basal body morphogenesis and migration during ciliogenesis in animal cells

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Accepted 26 September 2006
Journal of Cell Science 120, 7-15 Published by The Company of Biologists 2007
doi:10.1242/jcs.03305

Summary

Cilia, either motile or immotile, exist on most cells in the human body. There are several different mechanisms of ciliogenesis, which enable the production of many kinds of cilia and flagella: motile and immotile, transient and long-lived. These can be linked to the cell cycle or associated with differentiation. A primary cilium is extended from a basal body analogous to the mitotic centrioles, whereas the several hundred centrioles needed to form the cilia of a multi-ciliated cell can be generated by centriolar or acentriolar pathways. Little is known about the molecular control of these pathways and most of our knowledge comes from ultrastructural studies. The increasing number of genetic diseases linked to dysfunctional cilia and basal bodies has renewed interest in this area, and recent proteomic and cell biological studies in model organisms have helped to shed light on the molecular components of these enigmatic organelles.

Key words: Cilia, Centriole, Flagellum, Primary cilium, Epithelia, Cell cycle, Basal body

Introduction

Cilia are found on virtually all cells in the human body; yet despite the importance of these organelles many aspects of their formation and function remain enigmatic. Cell biology textbooks usually pronounce that cilia and flagella are highly conserved structures containing a 9+2 microtubule axoneme made up of nine outer doublet microtubules and two single central pair microtubules (Fig. 1A) surrounded by a ciliary membrane. While it is true that this basic structure is well conserved throughout eukarya, and that 9+2 and 9+0 (lacking the central pair) axonemes are the most common, there are many variations in either the axoneme itself or in the presence of extra-axonemal structures that allow cilia, either motile or non-motile, and flagella to perform specialised functions in diverse organisms and cell types (Afzelius, 2004)[1].

The terms cilia and flagella are sometimes used interchangeably but there are differences: first, only one or two flagella are usually present on cells whereas motile cilia are usually found in higher multiples. This is a generalisation, however, because the sperm of the fern *Marsilea* and the gymnosperm Ginkgo are multiflagellate, displaying up to a thousand flagella (Myles, 1975; Vaughn and Renzaglia, 2006). Second, cilia move with a characteristic ciliary beat that is distinguished by an effective stroke followed by a recovery stroke, whereas flagellar motility usually consists of successive waves originating at either the base or the tip of the flagellum and propagated along its length. Again, there are exceptions to this: the single-celled parasitic protozoan *Trypanosoma brucei* is capable of both flagellar and ciliary beating (our unpublished observations). Third, cilia are often all of one developmental state whereas multiple flagella in the same cell may often be at different stages of development. Fourth, the term cilium is usually used to describe structures shorter than flagella. Fifth, rows of cilia can fuse to form specialised cirri or membranelles.

Flagella often move individual cells, such as mammalian sperm and protists. Cilia can be motile or non-motile and occur singly or in multiples. Multiple cilia are generally motile. They move single-celled protists such as *Tetrahymena* and, in metazoa, material such as mucosal fluids past epithelial cell layers. Single cilia are normally non-motile – the primary cilia on the surface of most mammalian cells are the most common (Wheatley et al., 1996), and much evidence suggests these play pivotal roles in normal tissue homeostasis and disease (reviewed by Davenport and Yoder, 2005), with cilium-generated Hedgehog and Wnt signalling being critical during development (reviewed by Scholey and Anderson, 2006). Certain tissues contain specialised cilia, including motile and non-motile nodal cilia (which are found exclusively in the embryonic node), the transient non-motile kinocilium of the inner ear and the highly modified non-motile connecting cilium of the retina.

As stated above, most axonemes consist of a ring of nine outer doublet microtubules held together by nexin linkages (Fig. 1); this is the basic structure of the primary cilium. Motile

[1]Some exceptions to the canonical axoneme structure. The sperm of certain insects have complex axonemes: A 9+9+2 configuration made up of an outer ring of nine singlet microtubules surrounding nine doublets and a central pair is common [e.g. in honey bees (Lino-Neto et al., 2000), fire ants (Lino-Neto and Dolder, 2002) and stick insects (Afzelius, 1988; Afzelius et al., 1990)]. A 9+7 configuration is found in caddis flies whereas mosquitos of *Culex* spp have a 9+9+1 arrangement (Phillips, 1969). Limpet cilia contain nine outer doublets with five to ten central microtubules (Hackney et al., 1983; Phillips, 1979). The sperm flagellum of the gall midgefly is an extreme case: the axoneme is formed from up to 2500 doublets arranged in a double spiral (Mencarelli et al., 2000). Each doublet has only an outer dynein arm and there is no central pair. Extra-axonemal accessory structures are often found: examples include the outer dense fibres of many sperm (Fouquet and Kann, 1994) and the paraflagellar rod of kinetoplastid protozoa (Maga and LeBowitz, 1999).

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cilia and flagella generally additionally have two singlet central pair microtubules, radial spokes and dynein arms, which are the molecular motors that enable microtubule sliding and consequent ciliary or flagellar beating. The dynein arms are attached to the A-tubule of each doublet such that their motor head domains are in close proximity to the B-tubule of the neighbouring doublet (Gibbons and Gibbons, 1973; Gibbons and Rowe, 1965). With the exception of early ultrastructural observations of cilia and flagella, much of the work on these has been carried out in single-celled organisms, particularly the green alga, *Chlamydomonas reinhardtii* (reviewed by Dutcher, 1995; Silflow and Lefebvre, 2001). Until recently the molecular composition of cilia and flagella was largely obscure. A combination of forward genetic mutational analysis and electrophoresis the *C. reinhardtii* flagella led to predictions that they to contain ~250 polypeptides (reviewed by Dutcher, 1995). More recent studies on a variety of model organisms have used proteomic (Andersen et al., 2003; Broadhead et al., 2006; Keller et al., 2005; Ostrowski et al., 2002; Pazour et al., 2005; Smith et al., 2005), transcriptomic (Blacque et al., 2005; Stolc et al., 2005) and genomic (Avidor-Reiss et al., 2004; Efimenko et al., 2005; Li et al., 2004) approaches and to suggest that the number is much greater. A database of cilia/flagella and basal body components identified in these studies can be found at the Ciliary Proteome Web Server at http://www.ciliaproteome.org/ (Gherman et al., 2006).

Studies primarily in algae have led to a model of ciliary/flagellar motility in which coordinate action of the central pair, radial spokes and dynein arms enable motility. It has been proposed that the central pair signals through the radial spokes to provide an asymmetric stimulus to the dynein motors associated with each of the outer doublet microtubules that enables a flagellar bend to be generated. One hypothesis for how this stimulus is generated relies on the rotation of the central pair microtubules observed in *Chlamydomonas* and *Paramecium* (Mitchell, 2003; Omoto and Kung, 1979; Omoto and Kung, 1980). However, this paradigm does not work for all systems: central pair rotation is not found in all organisms (Gadelha et al., 2006; Sale, 1986; Tamm and Tamm, 1981), which raises the question of how the central pair regulates motility in these systems.

All cilia are extended from a basal body. The Henneguy-Lenhossek theory, first postulated in 1898 and generally accepted today, proposed that these basal bodies are analogous to the mitotic centrioles (Henneguy, 1898; Lenhossek, 1898). Indeed the primary cilium is extended from a single basal body analogous to the pre-existing mature centriole in the cell. By contrast, in multi-ciliated epithelia, ciliogenesis begins with centriole multiplication that yields up to several hundred basal bodies. In both cases migration of the centrioles to the apical cell surface and acquisition of accessory structures follows. Once a centriole has docked with the cell membrane, it is known as a basal body. Finally, an axoneme is extended to form a cilium. Despite the overall similarities there are distinct differences in the mechanisms of formation of single cilia and cilia of multi-ciliated cells in terms of cell cycle regulation and control of centriole number. Our knowledge of the molecular mechanisms of ciliogenesis is largely restricted to intraflagellar transport (IFT), the bidirectional movement of particles along the doublet microtubules, which elongates and maintains the cilium. Many excellent reviews on IFT are available (e.g. Rosenbaum and Witman, 2002; Scholey, 2003). Here, we

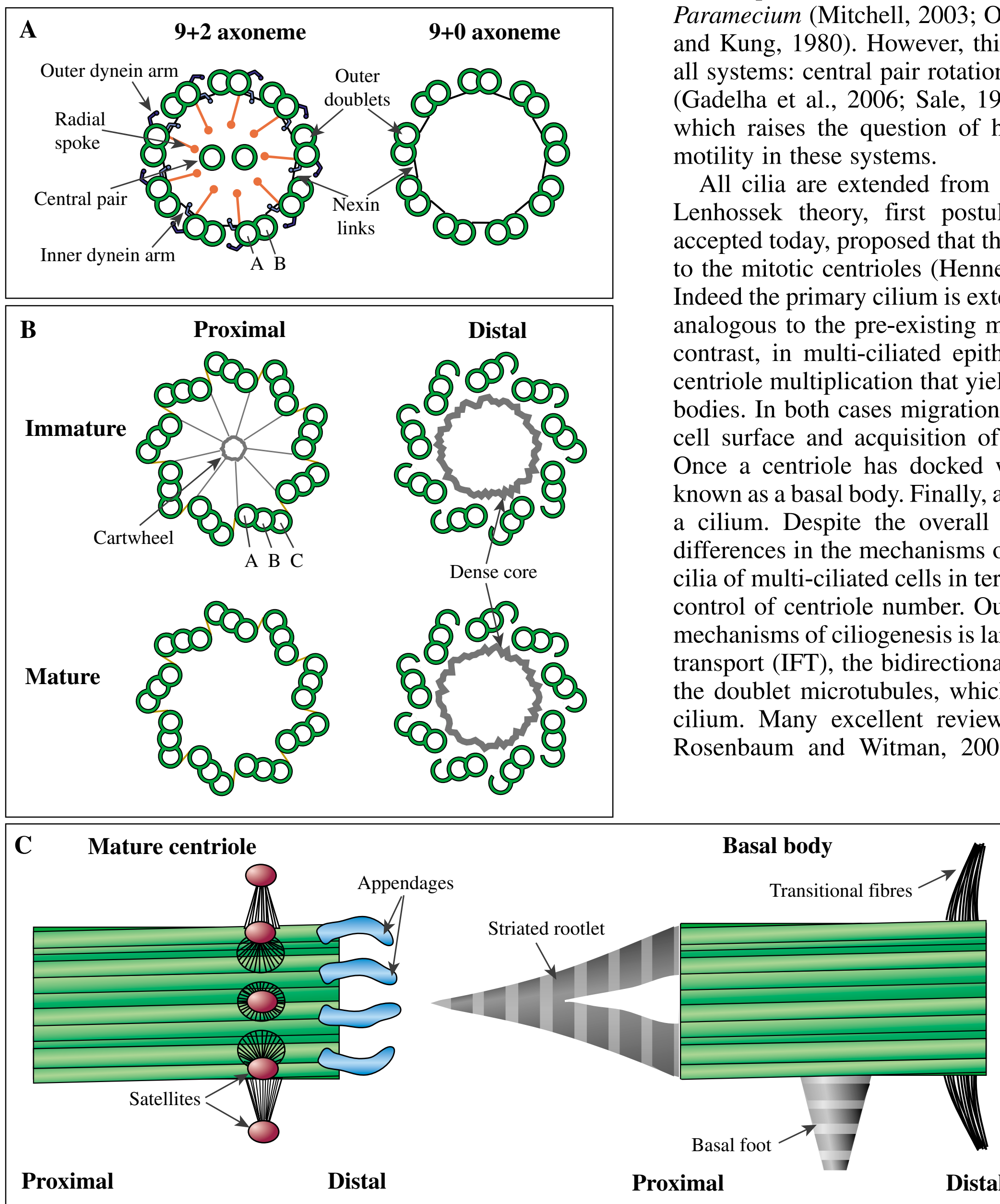

Fig. 1. Structures of centrioles and cilia. (A) The structure of the canonical motile 9+2 axoneme and its most common variation, the immotile 9+0 axoneme. (B) Schematic of a transverse section through the immature and mature centrioles. (C) Schematic of a longitudinal view of the mature centriole, showing the appendages, and a basal body with accessory structures.

instead focus on the earlier stages of ciliogenesis, comparing the ultrastructural details and molecular events that occur during formation of ciliated epithelia and solitary primary cilia.

Multiple pathways of ciliogenesis

At least six mechanisms for generation of cilia and flagella exist and the resulting cilia or flagella can be transient or long-lived (Anderson and Brenner, 1971; Brenner and Anderson, 1973; Flock and Duvall, 1965; Lemullois et al., 1988; Sherwin and Gull, 1989; Sobkowicz et al., 1995; Sorokin, 1968). These enable the production of many kinds of cilia, as well as sperm flagella (Fig. 2). They require various mechanisms for accurate control of centriole number, and there are variations in cell cycle control, in the timing of cilium production in the cell cycle, in the timing of centriole maturation and in whether a probasal body is associated with the cilium or flagellum basal body. Our focus here is on the five major mechanisms for formation of cilia; sperm flagella will not be dwelt upon further.

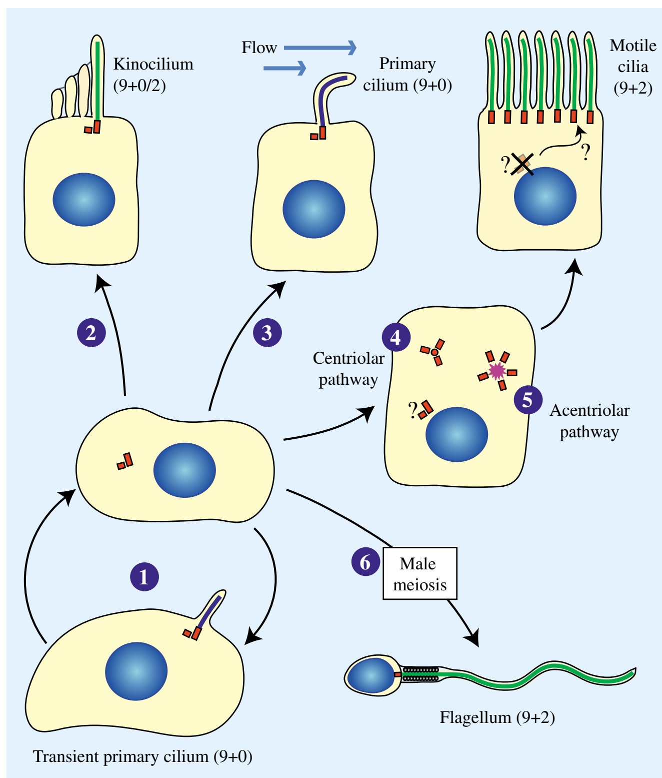

Fig. 2. Multiple pathways of ciliogenesis. Quiescent somatic cells use a single pre-existing mature centriole to subtend a transient primary cilium (Fig. 1 and ❶) lacking central pair microtubules, which is lost as the cell re-enters the cell cycle. In differentiated cells, several different types of single cilia can be produced from a mature centriole, such as the temporary (9+2 or 9+0) kinocilium (❷), which may or may not possess the central pair microtubules (Flock and Duvall, 1965; Sobkowicz et al., 1995) and the primary (9+0) cilium produced on the luminal epithelium of kidney tubules (❸). The pathways that produce several hundred cilia in epithelial cells in the mammalian airway are depicted at ❹ and ❺. Here, hundreds of centrioles are produced, duplicated either using the pre-existing centriole as a template (❹), or formed via a non-templated method (❺). The sperm flagellum produced in male meiosis is depicted at ❻. Green denotes 9+2 axonemes; dark blue denotes 9+0 axonemes; centrioles are shown in red; the deuterosome is shown in purple.

Centriole production

The centriole is a cylindrical structure 0.4 μm long and 0.2 μm in diameter, composed of microtubule triplets that display ninefold radial symmetry. The triplets are positioned around a cylindrical core with a slight twist and are composed of heterodimers of α- and β-tubulin. The immature centriole has a unique feature called the cartwheel (Vorobjev and Nadezhdina, 1987), which is made up of a central rod linked to the inner (A) tubule of each triplet by spokes (Fig. 1B). The mature centriole also has distinct appendages at the distal and subdistal regions (Fig. 1C) (Paintrand et al., 1992). The two centrioles are closely associated, usually in an orthogonal configuration, and surrounded by an amorphous mass of pericentriolar material (PCM) to form the centrosome.

Most differences between ciliogenesis in primary cilia and the cilia of multi-ciliated epithelia are observed in centriologenesis: a primary cilium forms from a pre-existing centriole whereas cilia of multi-ciliated epithelia require de novo production of many centrioles. Multi-ciliated epithelial cells are terminally differentiated. Primary cilia can be formed in quiescent somatic cells or in proliferating populations that make a primary cilium in G1. For cells that will form a primary cilium (Fig. 2, ❶-❸), centriole duplication is cell cycle related. The centriole pair duplicates once per cycle at S phase and maturation (see below) occurs later (Hinchcliffe et al., 1998; Lange and Gull, 1996; Sluder and Hinchcliffe, 1998; Vorobjev and Chentsov Yu, 1982). By contrast, centriole multiplication in cells that will become multi-ciliated (Fig. 2, ❹ and ❺) is differentiation related and often independent of the pre-existing centrioles (Anderson and Brenner, 1971; Dirksen, 1991). In this case, the centrioles that are made mature as they form.

Once formed, centrioles of ciliated cells migrate to the cell surface. For cells that form a primary cilium (Fig. 2, ❶ and ❸), the centriole remains associated with its procentriole daughter throughout ciliogenesis (Alieva and Vorobjev, 2004; Hagiwara et al., 2002; Jensen et al., 2004; Sorokin, 1968); those in multi-ciliated epithelia and the centriole subtending the kinocilium of the inner ear do not have associated procentrioles (Abughrien and Dore, 2000; Anderson and Brenner, 1971; Dirksen, 1971; Hagiwara et al., 1992; Kalnins et al., 1972; Sobkowicz et al., 1995; Sorokin, 1968). During maturation, centrioles acquire additional appendages such as transitional fibres and basal feet that stabilise the centriole/basal body, and IFT extends and maintains the axoneme. This occurs in both primary cilia and cilia of multi-ciliated cells (Rosenbaum and Witman, 2002).

Centriole duplication

During the cell division cycle the centrosome duplicates just before S phase; duplication requires the immature centriole-associated protein centrobin (Zou et al., 2005). Several serine/threonine kinases are implicated in the process, including Polo-like and Aurora kinases (Berdnik and Knoblich, 2002; Hannak et al., 2001; Lane and Nigg, 1996). A procentriole (Andre and Bernhard, 1964; Gall, 1961; Mizukami and Gall, 1966; Renaud and Swift, 1964) forms from the side of each centriole and elongates throughout S phase so that the cell possesses two pairs of centrioles as it

enters G2 phase. Only the mature centriole can produce a cilium. Maturation occurs over 1.5 cell cycles, the maturing centriole acquiring the unique fibrous distal and subdistal appendages mentioned above. Several protein components of these appendages have been identified, including ninein (Mogensen et al., 2000), ϵ-tubulin (Chang et al., 2003), centriolin (Gromley et al., 2003) and CEP110 (Ou et al., 2002). Cenexin is found exclusively at the mature mother centriole and its acquisition at the G2-M transition is a marker for maturation (Lange and Gull, 1995) and essential for appendage production (Ishikawa et al., 2005).

Centrioles are inherited by daughter cells in a semi-conservative manner (Kochanski and Borisy, 1990). Centrosome duplication and DNA regulation are coordinated by a complex pathway involving cyclin-E–Cdk2 (Hinchcliffe et al., 1999; Karsenti, 1999; Meraldi et al., 1999). Centrosome duplication is stimulated by Cdk2. Factors required for entry into S phase, such as Cdk2 and E2F, are required for centrosome duplication, and phosphorylation of the tumour suppressor Rb is also needed (Meraldi et al., 1999). Unduplicated G1 centrosomes are duplication competent, whereas G2 centrosomes placed under identical experimental conditions are not. This strongly suggests the centrosome itself blocks further centrosome duplication (Wong and Stearns, 2003); this might involve a physical connection between the mature and immature centrioles that prevents duplication until after mitosis (Tsou and Stearns, 2006). In most cell lines the centrosome will not re-duplicate during S phase, even in cells treated with hydroxyurea to prolong S phase (Balczon et al., 1995; Meraldi et al., 1999). However, in certain cell lines containing a mutant p53 tumour suppressor, extended cell cycle arrest leads to centrosome accumulation (D'Assoro et al., 2004), which suggests that centrosome duplication is uncoupled from cell division checkpoints in these cells.

Centriole multiplication

Multi-ciliated epithelial cells such as those in the mammalian trachea can each produce 200-300 centrioles during ciliogenesis. Two mechanisms of centriole production have been identified: the centriolar and acentriolar pathways, both of which can occur in a single cell (reviewed by Beisson and Wright, 2003). In contrast to centriole duplication during the cell division cycle, these pathways are linked to differentiation rather than proliferation, and multiple procentrioles are produced simultaneously, making this process distinct from the centriole duplication described above. In the centriolar pathway (Fig. 2, ❹), new centrioles are produced around an existing centriole. Unlike cell-cycle-dependent centriole duplication, however, more than one procentriole may be produced around a single centriole (Anderson and Brenner, 1971; Chang et al., 1979; Hagiwara et al., 1992; Sorokin, 1968; Steinman, 1968). Variations in the number of procentrioles observed could be attributable to the plane of the electron micrograph: among several procentrioles surrounding a centriole, two or three are seen only in glancing section (Sorokin, 1968); serial thin-section electron microscopy will be necessary to resolve the exact number of procentrioles formed in the centriolar pathway. The origin of the core centriole that produces these procentrioles is unclear. Neither the cartwheel, a marker for immature centrioles, nor appendages, present around mature centrioles, appear to be present. Thus the identity of the core centriole is unknown.

In the acentriolar pathway (Fig. 2, ❺), centrioles form around intermediary structures rather than an existing centriole (Anderson and Brenner, 1971; Chang et al., 1979; Dirksen, 1971; Dirksen and Crocker, 1966; Hagiwara et al., 1992; Kalnins et al., 1972; Lemullois et al., 1988; Sorokin, 1968; Sorokin and Adelstein, 1967; Steinman, 1968). The terminology for these intermediary structures is confused in the literature. We use the nomenclature system of Chang et al. (Chang et al. 1979) for the granular structures observed at the first stage of the acentriolar pathway. So-called dense granules (Fig. 3A) form clusters that can be associated with the existing centriole. Some molecular components of these dense granules have been identified: PCM-1 has been detected in them (Kubo et al., 1999) in addition to the striated rootlet protein p195 (Hagiwara et al., 2000). Centrin proteins localise close to granules in ciliogenic epithelial cells, which suggests that they are involved in an early stage of the acentriolar pathway (Laoukili et al., 2000). Dense granule clusters may condense to produce deuterosomes (Fig. 3B, arrowheads), around which

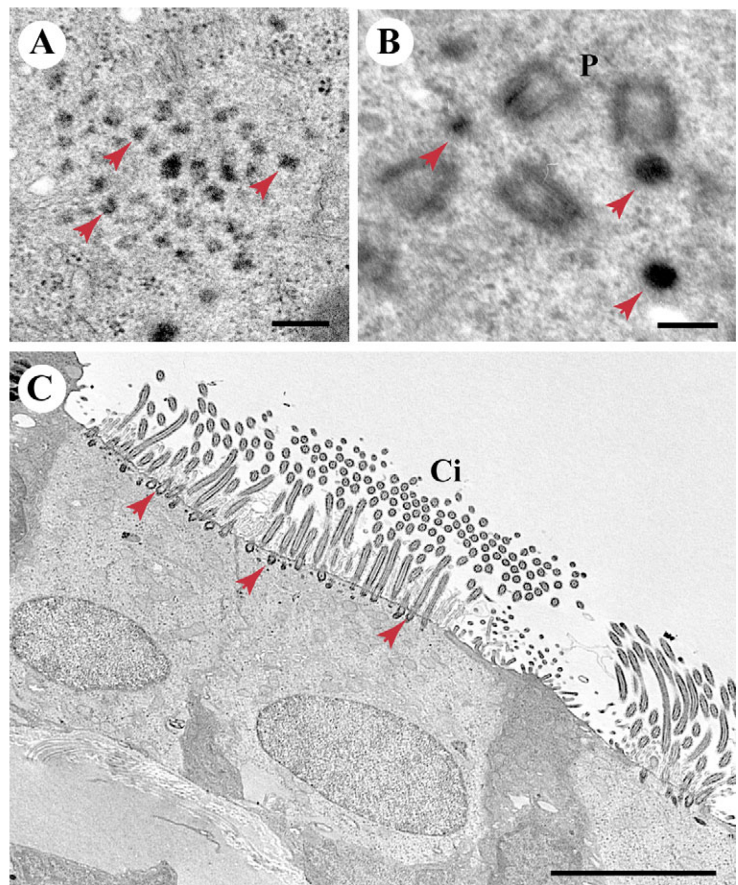

Fig. 3. Ciliogenesis in rat trachea. (A) The first visible stage in the acentriolar pathway is the appearance of dense granules (arrowheads). (B) Procentrioles (P) form around deuterosomes (arrowheads). (C) Mature ciliated cells. The procentrioles have docked with the apical membrane to become basal bodies (arrowheads) and have extended cilia (Ci). Scale bars (A) and (B)=200 nm; (C)=5 μm.

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procentrioles (labelled P in Fig. 3B) develop. Two types of deuterosome exist, classified as solid or hollow on the basis of their ultrastructural appearance; there are organismal and size differences between the two (Dirksen, 1971; Dirksen, 1991; Hagiwara et al., 1992; Sorokin, 1968). The number of procentrioles around a deuterosome correlates with its size (Anderson and Brenner, 1971; Chang et al., 1979; Hagiwara et al., 1992; Kalnins et al., 1972; Sorokin, 1968). Serial thin-section electron microscopy reveals different numbers of procentrioles in each section (Anderson and Brenner, 1971). Thus the centriolar pathway may also produce more procentrioles than are seen in a single section, and there may be less variability in procentriole number in both the centriolar and acentriolar pathways in vivo than previously thought.

What happens to the pair of centrioles initially present in the cell during centriole multiplication? Several possible scenarios exist. First, the centriole pair could migrate to the surface and extend one of the motile cilia. This would imply that all centrioles are identical and that global control of ciliogenesis exists. Second, the two centrioles could migrate to the surface and extend a distinct, primary cilium prior to centriole multiplication and subsequent generation of the multiple motile cilia. This would suggest that an individual cell can form both 9+0 and 9+2 cilia, albeit at slightly different times. This phenomenon has been observed (Anderson and Brenner, 1971; Brenner and Anderson, 1973; Lemullois et al., 1988; Sorokin, 1968), and the presence of developing procentrioles around the mature basal body subtending a primary cilium (Anderson and Brenner, 1971; Sorokin, 1968) makes it probable that the same cell goes on to produce multiple cilia. In this case, the primary cilium is probably only transient, because none has been found in mature ciliated cells (Lemullois et al., 1988); it may function to pattern the apical surface in preparation for formation of motile cilia. The ultimate fate of its basal body – whether it later subtends a motile cilium or returns deeper into the cytoplasm – is unknown, but if it goes on to subtend a 9+2 cilium it is probable that the information needed to template a 9+0 cilium versus a 9+2 cilium does not lie within the centriole. Third, the mature centriole could remain in the cytoplasm. This would imply that existing centrioles are different from those produced by the centriolar and acentriolar pathways and that ciliogenesis is controlled at the level of individual centrioles. Although several studies have failed to observe isolated centrioles in ciliated cells (Anderson and Brenner, 1971; Lemullois et al., 1988; Lenhossek, 1898), one study has observed a centriole deep in the cytoplasm of a ciliated cell (Kalnins et al., 1972). Final resolution of these important questions will require tracking of individual centrioles in live cells during ciliogenesis.

Is cell-cycle-dependent duplication or centriole multiplication the default pathway in cells? Given the deleterious consequences of uncontrolled centriole proliferation, it could be argued that cell-cycle-dependent centriole duplication represents the default pathway. However, whereas most cells lacking centrioles undergo cell cycle arrest (Hinchcliffe et al., 2001), HeLa cells in which the centrosome has been surgically removed can produce multiple centrioles throughout the cytoplasm (La Terra et al., 2005). This implies that an existing centriole prevents production of further centrioles, and that newly synthesised centriole precursors destined for the existing centrosome will, in its absence, form multiple foci throughout the cytoplasm that produce centrioles independently of each other. This suggests that increased centriole production might be the default pathway for cells and that this is suppressed by an existing centriole. However, it is apparent that centriole production in HeLa cells must be uncoupled from the cell cycle and thus these cells do not necessarily represent the in vivo state.

Centriole migration and acquisition of accessory structures

How centrioles migrate during primary cilium formation remains unresolved, although the cytoskeleton is probably involved. Microtubules position the interphase centrosome (Burakov et al., 2003). The Rho-associated protein kinase p160ROCK maintains the position of the mature centriole (Chevrier et al., 2002); however, the upstream and downstream events remain unknown. In multi-ciliated cells, duplicated centrioles migrate towards the apical surface, where they dock to become basal bodies. Much evidence implicates the actin-myosin network in centriole migration in these cells. Actin and myosin are associated with centrioles or centriolar material (Klotz et al., 1986; Lemullois et al., 1988; Lemullois et al., 1987), and treatment of ciliogenic oviducts with agents that inhibit actin polymerisation (Boisvieux-Ulrich et al., 1990) or myosin function (Boisvieux-Ulrich et al., 1987) prevents centriole migration. Conversely, drugs that target the microtubule network do not directly stop migration (Boisvieux-Ulrich et al., 1989). In the ctenophore *Beroe*, centrioles are associated with parallel bundles of actin microfilaments arising in the basal cytoplasm and oriented towards the cell surface (Tamm and Tamm, 1988). They might therefore be pushed to the surface along actin tracks akin to the ‘rocket-like’ motility of intracellular bacteria (see Gouin et al., 2005; Machesky, 1999).

The signalling events regulating these processes also remain obscure. The planar cell polarity effectors inturned (*in*) (Collier and Gubb, 1997) and fuzzy (*fy*) (Park et al., 1996) are implicated in the control of an apical actin array essential for basal body docking (Park et al., 2006). The forkhead/winged helix family of transcription factors is also implicated in centriole migration and/or docking. One member, *Foxj1*, is expressed in ciliated epithelia (Blatt et al., 1999; Hackett et al., 1995; Lim et al., 1997; Pelletier et al., 1998; Tichelaar et al., 1999). *Foxj1*-null mice have situs defects and impaired ciliogenesis (Brody et al., 2000); centriologenesis appears unimpaired but centriole migration fails. The cysteine protease calpain, acting on the cytoskeletal linker protein ezrin, may regulate membrane anchoring (Gomperts et al., 2004), which suggests a need for localised regulation of cytoskeletal proteolysis. By contrast, factors regulating centriole migration during primary cilium development are unknown.

Directional control of centriole migration during formation of single or multiple cilia remains an open question. The Rho family GTPase Cdc42 has a central role in establishment of cell polarity and localisation of the PAR3-PAR6-atypical protein kinase C complex (reviewed by Suzuki and Ohno, 2006) to the membrane in diverse polarity pathways (Etienne-Manneville and Hall, 2001; Joberty et al., 2000). Re-orientation of the centrosome is one step in this process, and

the complex might thus function in centriole/basal body movements. A second complex, comprising the transmembrane protein Crb3 and the apical proteins Pals1 and Patj, is also involved in mammalian epithelial polarity (Roh et al., 2003) (reviewed by Margolis and Borg, 2005). Moreover, the two complexes interact in epithelia (Hurd et al., 2003) and are required for ciliogenesis, interacting with the anterograde IFT motor KIF3 (Fan et al., 2004). Such associations between polarity determinants and the ciliogenesis machinery are intriguing and it is tempting to speculate that polarity complexes provide signals for centriole migration.

After migration, centrioles attach to the membrane and serve as basal bodies for ciliary elongation. Ultrastructural analysis has uncovered a plethora of accessory structures that are associated with centrioles and basal bodies (Fig. 1C), including centriolar satellites, transitional fibres, striated rootlets and basal feet, that are associated with both primary cilia and multi-ciliated epithelia. The timing of acquisition of these structures is unclear: transitional fibres and/or basal feet are found associated with migrating centrioles in some studies (Anderson and Brenner, 1971; Lemullois et al., 1988) but not in others (Frisch and Farbman, 1968; Hagiwara et al., 1992). However, when centriole migration is perturbed (Boisvieux-Ulrich et al., 1990), the centrioles that remain cytoplasmic acquire accessories. Subcellular location is therefore not the determining factor.

Diseases associated with dysfunctional ciliogenesis

An increasing number of genetic diseases are associated with defects in ciliogenesis or ciliary function (reviewed by Afzelius, 2004; Badano et al., 2006; Badano et al., 2005). These comprise a diverse group of pathologies including cystic kidney disease, infertility, retinal degeneration, hydrocephalus, laterality defects and chronic respiratory problems. Recent work has also identified cilia and basal body dysfunction as the underlying cause of various systemic diseases, including Bardet-Biedl, Alstrom, Orofaciodigital and Meckel syndromes (Andersen et al., 2003; Ansley et al., 2003; Kyttala et al., 2006; Romio et al., 2004), and these have expanded the range of ciliopathy phenotypes to include obesity, diabetes, hypertension and cardiac abnormalities (Badano et al., 2006). The affected tissues vary between diseases but all have motile or immotile cilia.

Most of the gene products implicated in the ciliopathies affect ciliary function (e.g. motility or signalling) rather than cilium formation. Others affect ciliogenesis itself. As discussed above, mice lacking the transcription factor FoxJ1 have laterality defects and a loss of cilia (Chen et al., 1998) due to a failure in centriole migration (Brody et al., 2000; Gomperts et al., 2004). A second protein, Seahorse, is implicated in polycystic kidney disease (Sun et al., 2004) and is required to regulate basal body duplication in *Trypanosoma brucei* (Morgan et al., 2005). Seahorse (also known as TbLRTP) localises to the distal part of the trypanosome basal body. Whereas overexpression of the protein suppresses flagellum assembly, RNA-interference experiments result in excess basal bodies; this suggests that Seahorse is a negative regulator of basal body duplication. Polycystic kidney disease and Bardet-Biedl syndrome can be caused by a failure in IFT (Blacque et al., 2004; Li et al., 2004; Ou et al., 2005; Pazour et al., 2000), the final stage of ciliogenesis. Finally, mice lacking the orofaciodigital syndrome gene *OFD1* show defective cilium formation (Ferrante et al., 2001). Based on the phenotypic overlap with Bardet-Biedl syndrome, a role for OFD1 in IFT has been proposed (Ferrante et al., 2001); however, because OFD1 is a centrosomal/basal body protein (Romio et al., 2004), it could also function in earlier stages of ciliogenesis.

Much work on elucidating the biology of cilia disease genes has been carried out in model organisms (e.g. Blacque et al., 2004; Dawe et al., 2005; Pazour et al., 2000; Sun et al., 2004). However, the question of why the defect affects some tissues and not others in mammalian systems is difficult to address in single-celled models. Presumably tissue-specific transcriptional control plays a part. Mice lacking the polycystic kidney disease gene product polycystin 1 have polycystic kidneys but no laterality defects, which correlates with the absence of polycystin 1 in nodal cilia (Karcher et al., 2005). Turnover is also likely to be important. Ciliary tubulin continually turns over (Stephens, 1999); however, the rate varies between tissues. The stability of the cilium and/or the cell probably also varies from tissue to tissue. Comparison of published flagellar proteomes (Broadhead et al., 2006; Ostrowski et al., 2002; Pazour et al., 2005; Smith et al., 2005) from a number of model organisms reveals a surprising diversity despite overall structural conservation (Broadhead et al., 2006). This suggests the canonical axonemal structure is dependent on a central cohort of conserved proteins plus organism, cell and tissue type elaborations. This diversity could contribute to tissue-specific functional specialisation.

Perspectives and conclusions

Despite recent advances, our knowledge of ciliogenesis is still largely confined to ultrastructural observations and scattered information on various proteins. A number of challenges remain. The signals that trigger assembly and disassembly of cilia in tissues are not well understood. It is probable that molecules that control the cell cycle will be involved, at least in cells that form a primary cilium, because the cilium is present in only a limited cell cycle window. Tight control of transcription and translation may be required because many cilia proteins bind or regulate cytoskeletal elements and may have a detrimental effect if released in large quantities into the cytosol.

The paucity of information on the protein constituents of cilia and basal bodies has hampered research. While several recent studies have sought to address this, so that there are now a number of flagellar (Broadhead et al., 2006; Ostrowski et al., 2002; Pazour et al., 2005; Smith et al., 2005) and centrosomal/centriolar (Andersen et al., 2003; Keller et al., 2005) proteomes from evolutionarily diverse organisms, primary cilia have not yet been subjected to proteomic analysis. The composition of the primary cilium is therefore unknown, although it is likely to contain several hundred proteins, including core components shared with motile cilia.

A major challenge is to elucidate the structure and function of the cellular networks that underlie ciliogenesis. Comparative genomics provides one approach to study this. Subtraction of the genomes of organisms that do not build cilia or flagella from those of flagellate organisms yields a dataset that should include regulatory factors as well as structural components (Avidor-Reiss et al., 2004; Li et al., 2004). Importantly, this approach will not identify proteins with cytosolic functions in

addition to roles in ciliogenesis (most obviously tubulin). Work with single-celled model organisms cannot, however, distinguish the mechanisms underlying primary cilium formation from those that organise a multiciliated epithelium.

Finally, altough the list of diseases associated with impaired cilia or basal body function is expanding at a tremendous rate, biology and physiology have not kept pace with the clinical genetics and we do not yet understand why defective cilia or basal bodies lead to such wide-ranging pathologies. Cilia and basal bodies are increasingly implicated in 21st century diseases such as heart disease, obesity and hypertension; yet the underlying mechanisms remain an open question. If we are to treat these diverse pathological conditions, we must focus our efforts on understanding the biology of these iconic but still rather cryptic organelles.

We thank Alison Faragher for electron micrographs of rat trachea. Work in our laboratory is funded by The Wellcome Trust, the BBSRC and the E. P. Abraham Trust. H.D. is a Beit Memorial Research Fellow; K.G. is a Wellcome Trust Principal Research Fellow.

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