Assembly and Motility of Eukaryotic Cilia and Flagella. Lessons from *Chlamydomonas reinhardtii*[1]

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Cilia and flagella are among the most ancient cellular organelles, providing motility for primitive eukaryotic cells living in an aqueous environment. During adaptation to life on land, some groups of organisms, including advanced fungi, red algae, cellular slime molds, conifers, and angiosperms, lost the ability to assemble flagella (Raven et al., 1999). The centriole or basal body, which organizes the assembly of flagella, also is absent in these groups. In other lineages, flagella were retained only on gametic cells. Land plants are believed to have arisen from one group of green algae, the charophytes (for review, see Bhattacharya and Medlin, 1998; Qiu and Palmer, 1999), in which the only flagellated cells are motile sperm. The first land plants, bryophytes, which are thought to be the ancestors of higher plants, also produce flagellated sperm cells that require water to swim to the egg. Ultrastructural features of the basal body apparatus in the flagellated cells have provided important morphological data for phylogenetic studies of algae and bryophytes.

The absence of centrioles and flagella in all but sperm cells also characterizes seedless vascular plants (pteridophytes) including ferns and the genus *Equisetum* (Raven et al., 1999). Water is required for these sperm to swim to the archegonium containing the egg. A further adaptation for colonization on land developed in gymnosperm phyla represented by cycads and gingko. These plants produce pollen grains that are transferred to the vicinity of the female gametophyte. A pollen tube extends toward the archegonium and bursts to release flagellated sperm that swim through the released fluid to fertilize the egg. Sperm in seedless vascular plants, cycads, and gingko are large (up to 300 μm in diameter), spectacularly complex cells that swim with hundreds to thousands of flagella. The de novo synthesis of the centrioles during the formation and differentiation of these sperm cells was documented a century ago, but fascinating questions remain about the developmental mechanisms for these events (see, for example, Hart and Wolniak, 1998).

How do flagella of plants and animals differ? The main evolutionary difference is that in animals, flagella acquired new functions as multicellular forms evolved. For example, in mammals, epithelial cells in the respiratory system, the female reproductive system, and in the ventricles of the brain differentiate to produce multiple cilia that beat coordinately to propel fluids over the tissues. Most types of mammalian cells express a primary cilium whose growth is nucleated by the older of the two centrioles in a cell. These organelles have been shown to play crucial roles in embryo development (for review, see Schneider and Brueckner, 2000). Modified primary cilia play important roles in the function of sensory cells such as photoreceptor cells (for review, see Rosenbaum et al., 1999).

Chlamydomonas reinhardtii, a unicellular, biflagellate green alga in the order Volvocales, offers unique advantages for studying eukaryotic flagella and basal bodies (Fig. 1). These cells use flagella for motility and for cell-cell recognition during mating. Located on the surface of the cell, flagella may be isolated easily for biochemical analysis. More than 200 different proteins have been identified in the axoneme; at least an equal number of proteins comprise the basal body apparatus that regulates the assembly and positioning of the flagella. Although *C. reinhardtii* and mammals are separated by more than 10^9 years of evolution, *C. reinhardtii* flagella are amazingly similar in structure and function to mammalian cilia and flagella. For example, some of the flagellar proteins in *C. reinhardtii* show more than 75% identity and similarity to proteins with similar function in human sperm.

FLAGELLAR FUNCTION IN *C. REINHARDTII*

All plants need to optimize their exposure to light by growing toward it, as land plants do, or by swimming toward it, as flagellated organisms do. *C. reinhardtii* cells swim to optimal light levels by regulating flagellar beating in the process of phototaxis. The two anterior flagella, 12 μm in length, propel the cell forward by beating in opposite directions with an

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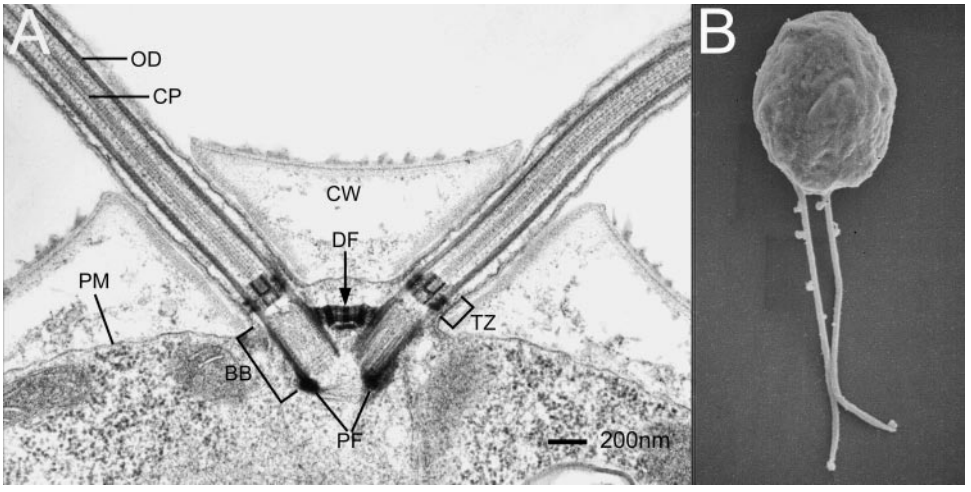

Figure 1. *C. reinhardtii* flagella. A, A longitudinal section through the basal bodies and flagella of *C. reinhardtii*. CW, Cell wall; PM, plasma membrane; OD, outer doublet microtubule of the axoneme; CP, central pair microtubules; BB, basal body; DF, distal striated fiber; TZ, transition zone; PF, proximal fiber connecting the two basal bodies. Electron micrograph by Dr. William Dentler (adapted from Trends in Cell Biology, Vol 1, No. 6, 1991, with copyright permission from Elsevier Science). B, Scanning electron micrograph of a *C. reinhardtii* cell. Image provided by Dr. William Dentler.

asymmetric ciliary waveform (something like a breast stroke) with a frequency of approximately 60 beats s^{-1}. Light is sensed by a rhodopsin-like photoreceptor located in the eyespot on the side of the cell (for review, see Hegemann, 1997; Sineshchekov and Govorunova, 1999). During phototactic behavior, light intensity in the environment is surveyed as the cells swim in a helical path, rotating around their longitudinal axis. A photoreceptor current is generated across the plasma membrane at the eyespot; signal transduction involves changes in the intraflagellar free Ca^{2+} concentration. The two flagella, denoted by their positions cis or trans to the eyespot, show a differential response in beating to changes in submicromolar calcium levels, allowing the cell to change swimming direction (for review, see Mitchell, 2000). Mutations that affect phototactic behavior are being analyzed to define the molecular mechanisms in the signal transduction pathways (Hegemann, 1997).

Although *C. reinhardtii* cells need light for photosynthesis, too much light can damage the chloroplast. When cells are exposed to a large increase in light intensity, they undergo a photoshock response characterized by a remarkable change in flagellar waveform (Sineshchekov and Govorunova, 1999). The flagella stop beating and then beat with a symmetric flagellar waveform (resembling a sinusoidal pattern) to move the cell briefly backward. The shift from ciliary "breast stroke" to flagellar "flutter kick" beating is thought to be controlled by activation of voltage-gated Ca^{2+} channels in the flagellar membrane. The resulting transient burst of intraflagellar free Ca^{2+} signals the change in flagellar beating mode.

C. reinhardtii flagella also play an important role during sexual reproduction. When starved for nitrogen, vegetative cells differentiate into gametes of mating type plus or mating type minus, a process controlled by the mating type locus (for review, see Goodenough et al., 1995). This process includes expression of Hyp-rich glycoproteins known as flagellar agglutinins on the flagellar surfaces, allowing gametes of opposite mating type to recognize and adhere to one another (for review, see Adair, 1985). Simple flagellar contact is enough to trigger an increase in cAMP levels, inducing downstream signaling pathways that lead to cell fusion (for review, see Pan and Snell, 2000).

WHY USE *C. REINHARDTII* TO STUDY FLAGELLA?

The *C. reinhardtii* system has many advantages for genetic approaches to understanding the assembly and function of flagella and the basal body apparatus. Genetic techniques that have been used to great success in the budding yeast *Saccharomyces cerevisiae* such as tetrad analysis and stable diploid construction are routine for *C. reinhardtii*. The genetic map consists of more than 200 mutations on 17 linkage groups (for review, see Harris, 2001). Most importantly, flagella are nonessential organelles in *C. reinhardtii*. Mutants with paralyzed flagella, even mutants totally lacking flagella, are fully viable. Although flagella are normally used in the mating process, it is experimentally possible to induce mating between cells lacking flagella. Various screening and selection strategies have been employed to identify over 125 different mutant loci that affect the assembly and function of the flagellar apparatus (Fig. 2; for review, see Dutcher, 2000).

C. reinhardtii is the only system in which transformation of nuclear, chloroplast, and mitochondrial genomes has been achieved (Harris, 2001). The nu-

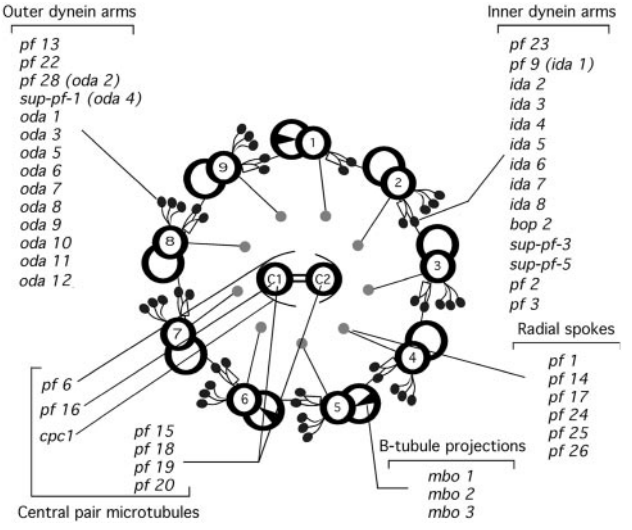

Figure 2. Schematic diagram of the flagellar axoneme in cross-section. Mutations in *C. reinhardtii* that affect the assembly or function of specific structures are indicated. (Reproduced from Porter and Sale [2000] by copyright permission of the Rockefeller University Press).

clear genome may be transformed using various methods, including particle bombardment, agitation with glass beads, or electroporation. Transformation of plasmid DNA into the nucleus results in random, nonhomologous integration events, whereas transformation of the chloroplast genome occurs exclusively by homologous recombination. Tagging of genes by insertional mutagenesis and subsequent analysis of genomic DNA flanking the inserted plasmid has been a fruitful approach for identifying and cloning genes encoding components of flagella and the basal body apparatus. These mutations are generally null mutations due to the deletions that accompany plasmid insertion. Wild-type genes or altered genes can be transformed into these null mutants for phenotype testing. In a reverse genetic approach, banks of insertional mutant strains with motility defects have been screened for mutations in specific genes by hybridizing blots of genomic DNA to sequences of interest. A hybridizing restriction fragment present in DNA from wild-type cells but absent or altered in size in DNA from a mutant strain indicates that the mutant strain may carry an insertional mutation in the gene of interest (for review, see Pazour and Witman, 2000).

Despite the importance of the insertional mutagenesis approach, the phenotype of a null mutation is not usually as informative as the phenotypes of more subtle mutations in the same gene. Further, null mutations in some genes of interest may have a lethal phenotype. In addition, because hundreds of mutants with interesting phenotypes are already on the genetic map, map-based cloning of genes identified by mutation will be important for future progress. To this end, a molecular map coaligned with the genetic map of the *C. reinhardtii* genome has been developed (Silflow, 1998). This map contains nearly 300 molecular markers, including RFLP and single nucleotide polymorphism markers, with an average spacing of four genetic map units. As new sequences are placed on the molecular map, their possible linkage to specific mutations on the genetic map can be assessed. Molecular markers on each linkage group also facilitate the genetic mapping of new mutations. The molecular map has served as the basis for developing a physical map of the genome based on overlapping contigs of bacterial artificial chromosome clones aligned with the genetic and molecular map. The physical map has recently been used for map-based cloning of genes involved in flagellar function in our laboratories.

C. REINHARDTII FLAGELLA: STRUCTURE AND MOTILITY

The structure and mechanism of motility of *C. reinhardtii* flagella are representative of "9 + 2" motile cilia and flagella in diverse eukaryotic systems (Figs. 1A and 2). Nine doublet microtubules, each composed of a complete A tubule (13 protofilaments) fused to a partial (11 protofilaments) B tubule, form an elongated cylinder growing from the A and B tubules of the triplet microtubules that form the wall of the basal body. A specialized region of the plasma membrane extends to cover the bundle of microtubules and associated proteins, termed the axoneme. At the base of the axoneme is a specialized structure called the transition zone that is involved in flagellar excision, among other possible functions. When flagella are excised in response to lowered pH or other stimuli, doublet microtubules are cleaved just distal to the transition zone, and the plasma membrane seals the resulting gap. Katanin, a microtubule-severing protein, localizes to the region of the transition zone (for review, see Quarmby and Lohret, 1999). Two central pair microtubules begin just above the transition zone, and extend the length of the axoneme in the center of the ring of doublet microtubules.

A combination of genetic and biochemical approaches has unraveled the protein composition and functions of structures within the axoneme. Ultrastructural analysis of mutant strains has revealed missing or altered structures that result in specific motility defects. Comparison of axonemal proteins from the mutant cells with those from wild-type cells has identified proteins missing in the mutant that may be components of the missing structure (for review, see Dutcher, 2000). Gamete fusion results in quadriflagellate, temporary dikaryons that are useful for in situ complementation tests. In dikaryons derived from mating mutant cells with wild-type cells, defective proteins in flagella of mutant cells may be rescued by proteins from the wild-type cells, result-

ing in four flagella with wild-type motility. Another powerful approach for studying the mechanisms of flagellar beating is the in vitro reactivation of axoneme beating or outer doublet sliding in the presence of MgATP. These in vitro assays using axonemes from mutant and wild-type strains and using drugs to inhibit the activity of specific enzymes have provided insight into the regulation of flagellar dynein activity (for review, see Porter and Sale, 2000).

How do flagella move? Flagellar beating is driven by flagellar dyneins, molecular motor proteins that bind the A tubule of each outer doublet as cargo. In the presence of ATP, the dyneins bind the B tubule of the adjacent outer doublet and move along the B tubule toward its minus end, resulting in sliding between pairs of outer doublets. Interdoublet sliding is constrained by doublet attachment to the basal body and by structural links between the doublets, resulting in flagellar bending.

The dyneins are arranged in two macromolecular complexes called arms that are different in function and composition (for review, see King, 2000; Mitchell, 2000; Porter and Sale, 2000). The inner arm dyneins, located closest to the central pair microtubules, are remarkably complex. Seven distinct isoforms have been resolved biochemically. These contain 11 heavy chains of >400 kD, together with numerous intermediate and light chains. The inner arm components are arranged along the A tubule with a 96-nm longitudinal repeat pattern that has been analyzed using computer averaging of electron micrographs from sections of axonemes (for review, see Porter and Sale, 2000). Among the protein components of the inner arm dynein are actin and the calcium-binding protein, centrin. Mutations in genes encoding inner arm dynein components result in changes in the waveform rather than beat frequency. The *ida5* mutation contains a defect in the unique gene encoding conventional actin. The phenotype of this mutation, the loss of several species of inner-arm dyneins, indicates that actin is required for assembly of these motor protein complexes. Remarkably, when rabbit muscle skeletal actin labeled with a fluorescent tag was introduced into *ida5* mutant cells by electroporation, a significant fraction of the cells recovered motility and incorporated the fluorescent actin into the axoneme (Hayashi et al., 2001). This result suggests new opportunities for introducing proteins and other molecules into *C. reinhardtii* cells and for determining the role of actin in inner arm dynein assembly.

The outer arm dyneins, which are spaced along the axoneme with a 24-nm period, contain a single isoform with three catalytic heavy chains, two intermediate chains, and eight light chains. Mutations that affect outer dynein arm components generally reduce beat frequency. The intermediate and light chains appear to play roles in regulating dynein activity and in binding dynein to its cargo. A docking complex containing three subunits is essential for assembly of the outer arm dyneins onto the A tubule.

HOW IS THE ACTIVITY OF DYNEIN ARMS REGULATED?

The flagellar bending patterns observed in living cells require precise control of dynein activity around the circumference and along the length of the axoneme. Structural asymmetries within the axoneme, such as by the lack of the outer dynein arm on the microtubule doublet termed "1" may reflect asymmetry of dynein function (Fig. 2; Hoops and Witman, 1983). The two flagella are positioned with 180° rotational symmetry, doublets "1" facing each other, such that the flagella beat with effective strokes in opposite directions.

Much evidence has accumulated indicating a role for the central pair apparatus and the radial spokes in controlling dynein arm activity. The radial spokes project inward from the B tubule of each doublet, with a pair of spokes within each 96-nm longitudinal repeat (Fig. 2). The spokeheads appear to interact with projections from the central pair microtubules. Mutations that lead to complete or partial loss of the radial spokes result in paralyzed flagella. The radial spokes contain at least 22 proteins, of which five are located in the rounded spokehead structure (for review, see Mitchell, 2000; Porter and Sale, 2000). The protein encoded by the *PF14* locus, RSP3, was recently shown to be an A-kinase anchor protein, which localizes protein kinase A by binding the regulatory subunit. RSP3 is positioned at the base of the radial spoke stalk near the inner dynein arms, placing the protein kinase A it binds in a position to regulate the activity of these motor proteins (Gaillard et al., 2001). Recently, calmodulin and the dynein light chain LC8 were found to be associated with the radial spoke stalk, suggesting that calmodulin plays a role in calcium control of flagellar beating modulated by the radial spokes (Yang et al., 2001).

The central pair apparatus consists of the C1 and C2 microtubules and a set of attached projections that appear to interact with radial spokes (for review, see Smith and Lefebvre, 1997; Mitchell, 2000). The central pair rotates during the beat cycle in *C. reinhardtii* and some other organisms. It has been proposed that the rotating central pair projections function like a distributor to activate sequentially subsets of radial spokes and dynein arms (Omoto et al., 1999). Insertional mutations in genes encoding components of the central pair apparatus have facilitated cloning of several of these genes. Mutants lacking central pair components have paralyzed flagella. Several bypass suppressor mutations restore motility to the radial spoke and central pair mutants without restoring the missing structures. The suppressor mutations identify genes encoding outer arm dynein heavy chains, inner dynein arm components, or components of the

dynein regulatory complex, a set of seven proteins located within the inner arm 96-nm repeat (for review, see Porter and Sale, 2000). Partial restoration of motility by the suppressor mutations suggests that they identify components of a control system that inhibits dynein activity.

THE BASAL BODY APPARATUS AS MTOC

Basal bodies and centrioles are conserved in structure; both are cylindrical organelles 200 to 300 nm in diameter and 400 to 700 nm in length, with walls composed of nine triplet microtubules. In *C. reinhardtii* as in many other cell types, the basal bodies play interchangeable roles as components of microtubule organizing centers (MTOCs) and as nucleation sites for assembly of axonemes (for review, see Marshall and Rosenbaum, 2000; Preble et al., 2000). In interphase *C. reinhardtii* cells, the two mature basal bodies are docked at the anterior end of the cell. The A and B tubules of the axonemal doublet microtubules are continuous with the A and B tubules of the basal body triplet microtubules. The basal bodies are maintained at an angle of approximately 90°, connected by proximal and distal striated fibers (Fig. 3). Near the proximal end of each basal body is a probasal body; these can first be detected during metaphase of the previous mitosis. In its role as an MTOC, the basal body apparatus organizes the assembly of four microtubule rootlets, two containing two microtubules and two containing four microtubules, which extend into the cell beneath the plasma membrane. These rootlets play a role in positioning of cellular organelles, including the eyespot, contractile vacuoles, and organelles involved in mating. In addition, the four-membered rootlets are involved in positioning the cleavage furrow, a role similar to that of the preprophase band microtubules in cytokinesis of plant cells (Preble et al., 2000). SF-assemblin, a 30-kD protein that forms noncontractile striated fibers, is associated with the proximal ends of the four microtubule rootlets and may function to stabilize these microtubules (Lechtreck and Silflow, 1997). During mitosis, the basal bodies assume the role of centrioles. Prior to mitosis, the two flagella are resorbed, along with the striated fibers, leaving the two basal bodies attached to the plasma membrane but free to move apart, each with its probasal body, to positions near the poles of the mitotic spindle. The probasal bodies elongate during early prophase, resulting in two pairs of basal bodies that segregate to the two daughter cells. Intact basal bodies are not essential in *C. reinhardtii*, as revealed by the phenotype of the *bld2* mutation that lacks intact basal bodies (for review, see Preble et al., 2000). In addition to the absence of flagella, the cells have defects in positioning of the microtubule rootlets, the mitotic spindle, and the cleavage furrow. The phenotype of *bld2* cells indicates that the basal bodies are important for the proper function of the MTOC.

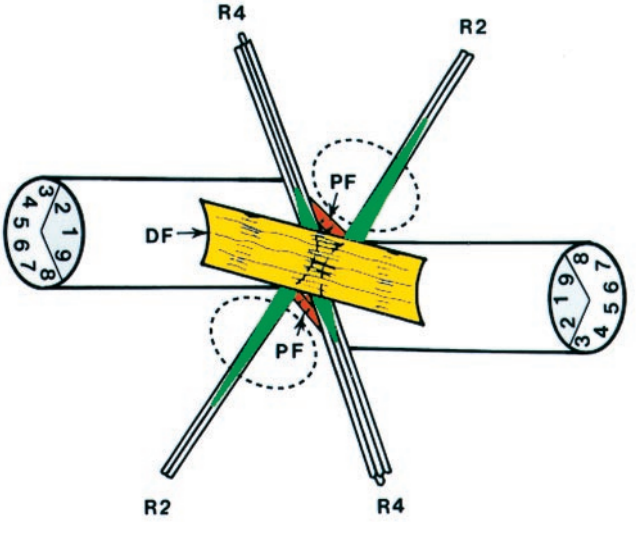

Figure 3. Schematic diagram of the basal body apparatus of *C. reinhardtii* as seen from above. Numbers identify the relative positions of triplet microtubules that form the walls of the basal bodies. Wedge-shaped shaded area indicates the position of the Vfl1 protein at the distal end of each basal body. R2, R4 represent two- and four-membered microtubule rootlets, respectively. DF, Distal striated fiber; PF, proximal fibers. Green fibers associated with the rootlets indicate SF-assemblin fibers. Dotted ovals indicate the positions of probasal bodies. (Adapted from Hoops and Witman [1983] with copyright permission of the Rockefeller University Press).

BASAL BODY REPLICATION AND MATURATION

In common with centriole replication in dividing animal cells, the primary mode of basal body replication in *C. reinhardtii* is semiconservative (for review, see Preble et al., 2000). Each mature basal body gives rise to a single probasal body, positioned at a specific site near the proximal end of the mature organelle. At cytokinesis, the pair segregates to a daughter cell that now contains two basal bodies differing in age by at least one cell cycle. The age of a centriole/basal body has structural and functional significance. For example, in animal cells, only the older centriole is capable of nucleating the growth of a primary cilium. In some biflagellate algal cells, the morphology of each flagellum depends on the age of the basal body from which it grows; more than one cell cycle is required for a basal body to reach maturity, a phenomenon known as flagellar transformation (Beech et al., 1991). In *C. reinhardtii*, the two basal bodies are positioned in a stereotyped pattern, with the younger basal body located in a cis-position with respect to the eyespot. Only subtle differences in motility distinguish the flagella assembled by the two basal bodies (Mitchell, 2000). However, *C. reinhardtii* provides a unique opportunity to study the fascinating process of basal body maturation because of a class of "*uni*flagellate" mutations. Cells with *uni* mutations fail to assemble flagella preferentially on the

younger basal body, resulting in cells with a single flagellum trans to the eyespot (for review, see Preble et al., 2000). The gene identified by the *uni3* mutation was found to encode δ-tubulin, a new member of the tubulin superfamily (for review, see Dutcher, 2001). Basal bodies in *uni3* mutant cells are defective in the assembly of the C-tubule of the triplet microtubule, suggesting a role for δ-tubulin in forming these specialized microtubules.

STRUCTURAL ASYMMETRY OF BASAL BODIES

Casual observation of the ultrastructure of the basal body cylinder suggests that it has 9-fold rotational symmetry. However, on closer examination, rotational asymmetry appears to be a universal feature of basal bodies and centrioles (for review, see Beisson and Jerka-Dziadosz, 1999). The importance of these asymmetric properties for the proper replication and function of the basal body apparatus has been demonstrated by recent genetic and biochemical studies of *C. reinhardtii* and the related alga *Spermatozipsis similis* (for review, see Preble et al., 2000). Several distinct fibrous structures associate laterally with specific microtubule triplets of the basal bodies (Fig. 3). The distal fiber attaches to triplet microtubules 9, 1, and 2 of each basal body, resulting in the positioning of the two basal bodies, and the two flagella assembled from them, in 180° rotational symmetry (Hoops and Witman, 1983). The distal fiber contains centrin, a calcium-binding protein that is also a component of the flagellar transition zone. Centrin-based fibers also bind to microtubule triplets 7 and 8 of each basal body and extend toward the nucleus, providing a link between these organelles. Centrin is also a component of the flagellar transition zone. Centrin confers contractile properties to these fibers, which play a role in maintenance of the spatial orientation of basal bodies, segregation of basal bodies to daughter cells, and flagellar excision, as shown by the phenotype of mutations in the *VFL2* gene encoding centrin. What is the molecular basis of rotational asymmetry and why is it important for basal body function? The cloning of the *VFL1* gene in *C. reinhardtii* has revealed a molecular marker for the asymmetry inherent to the basal bodies. (Silflow et al., 2001). Mutations in this gene result in cell populations with a variable number of flagella and in defective basal body positioning. The gene product localizes within the basal body at its distal end, with a rotationally asymmetric position near triplet microtubule number 1 (see immunofluorescence localization image on the cover). The connection of the distal striated fiber to the same side of the basal body suggests that the VFL1 protein may be involved in connecting the distal fiber to the basal bodies to ensure their proper positioning in the cell and segregation during the cell cycle.

HOW IS FLAGELLAR ASSEMBLY CONTROLLED?

The regulation of flagellar assembly may be at least as complex as the regulation of flagellar beating (for review, see Asleson and Lefebvre, 1998). For example, when one of the two flagella is amputated, the other one immediately begins to resorb into the cell, while the replacement for the amputated flagellum begins to regrow. At some point, the two flagella reach the same length, at which time the resorption stops, and both flagella regrow to normal length with rapid kinetics. It is clear that the cell is seeking to maintain two flagella of equal length. The dynamic nature of flagella is underscored by results from in vivo labeling experiments showing that significant exchange of proteins occurs in steady-state flagella (Song and Dentler, 2001).

Mutants with short or long flagella demonstrate that the length to which flagella assemble is directly controlled by the action of a large number of gene products. Short flagella mutants grow flagella of 30% to 50% of normal length (12 μm). Long flagella mutants can grow flagella up to three times normal length (up to 36 μm) or more. None of these mutant cells swim very well. Length control mutants show dramatic behavior in the dikaryon experiments described above. When mutants with short flagella are mated to wild-type cells, the short flagella rapidly (within 15 min) grow out to wild-type length. When mutants with long flagella are mated to wild-type cells, the long flagella resorb to wild-type length within 30 min. Most importantly, in neither case do the flagella simply average the length of the four flagella of the initial dikaryon cell. Rather, the wild-type length is "enforced" on the mutant flagella. These results indicate that cells are somehow able to detect the length of their flagella and maintain that length at a genetically determined value.

WHAT BIOCHEMICAL MECHANISMS REGULATE FLAGELLAR ASSEMBLY?

Although a detailed molecular model describing flagellar assembly is not yet available, such a model will need to incorporate some striking recent findings regarding a new type of flagellar motility not involved in swimming. This motility, called intraflagellar transport (IFT) involves the rapid movement of particles toward the tip (anterograde movement) or the base (retrograde movement) of the flagella between the flagellar membrane and the outer doublet microtubules (Fig. 4; for review, see Rosenbaum et al., 1999). The particle movement is remarkably fast, up to 2 μm s^{-1} toward the flagellar tip and 3.5 μm s^{-1} toward the base. The particles themselves, called rafts, are aggregates of lollipop-shaped subunits, consisting of at least 15 polypeptides.

One possible role for IFT is to carry flagellar proteins to the distal end of the growing axoneme, which is the site of assembly of flagellar proteins. To date,

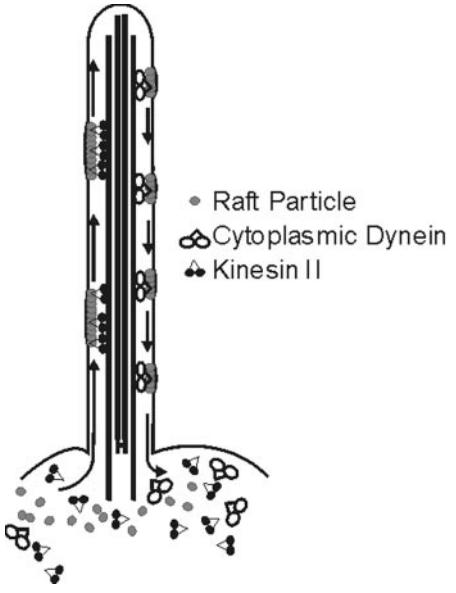

Figure 4. Model for intraflagellar transport. Transport of IFT (raft) particles toward the microtubule plus ends at the flagellar tip is dependent on kinesin-II motors. Particle transport toward the base of the flagellum utilizes minus end-directed cytoplasmic dynein motors. (Adapted from Pazour et al. [1999] by copyright permission of the Rockefeller University Press).

however, none of the proteins that comprise the raft particles has been shown to be an axonemal structural protein. The association of the proteins with the raft particles is too loose to survive isolation and purification or the movement of the particles serves some other role in the flagella. It is possible, for example, that the movement of the rafts might represent part of a signaling mechanism used to monitor the state of the flagella.

Genetic experiments show that the movement of the raft particles is essential for assembling and maintaining *C. reinhardtii* flagella (Iomini et al., 2001). Mutations in the *FLA10* gene encoding the anterograde motor, a heterotrimeric kinesin-II, lead to a flagella-less phenotype (for review, see Rosenbaum et al., 1999). Because the *fla10* mutation is temperature sensitive, it has been possible to show that kinesin-II is needed to maintain and to initially assemble the flagella because *fla10* mutants at the nonpermissive temperature slowly resorb their flagella. Mutations in the retrograde motor, DHC1B, a member of the cytoplasmic dynein superfamily, also cause defective assembly, although mutants with DHC1B defects retain a small stub about 10% of the normal flagellar length. This stub is filled with raft particles. One possible interpretation of this phenotype is that retrograde particle transport is required to return the anterograde motor and associated proteins to the cell body to allow anterograde motility to occur. Cytoplasmic dynein may also play a role in the cytoplasm by transporting flagellar proteins to the base of the flagellum in preparation for assembly. It is interesting that although cytoplasmic dynein has been described in many metazoan and protistan systems, database searches have returned no evidence of cytoplasmic dynein genes from higher plant genomes (Lawrence et al., 2001).

IFT proteins are found in animals as well. Genetic analysis has shown that they are involved in the assembly and maintenance of cilia in animal systems. A number of mutants in the roundworm *Caenorhabditis elegans* with defects in the assembly of a set of sensory cilia have been shown to affect proteins with close homologs in *C. reinhardtii* IFT genes (for review, see Rosenbaum et al., 1999). These proteins may also play a role in human disease. Mutations in the *Tg737* gene in mouse and human causes defects in the assembly of primary cilia in renal cells, resulting in polycystic kidney disease. This gene is homologous to a *C. reinhardtii* IFT gene (Pazour et al., 2000).

SUMMARY

Recent discoveries have underscored the importance of eukaryotic cilia/flagella, not only in motility, but also in development and in sensory processes. The advantages of *C. reinhardtii* genetics together with the available molecular tools have placed this model system in a unique position to provide insights into fundamental molecular mechanisms for the assembly and function of these organelles. The significance of cilia, flagella, and basal bodies for plant systems extends beyond those taxa that produce flagellated gametes. For example, study of the remarkable de novo assembly of hundreds of proteins to form these organelles may uncover principles of self-assembly that will be universally applicable. Even in angiosperms and gymnosperms that jettisoned the organelles in evolution, proteins found in flagella and basal bodies have been retained, including cytoskeletal proteins and molecular motor proteins. Comparison of the functions of these proteins in plant cells lacking centrioles and in plant cells that retain centrioles as basal bodies will provide insights into the evolution of plant cells and plant genomes.

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