

Review

Basal Body Assembly in Ciliates: The Power of Numbers

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Centrioles perform the dual functions of organizing both centrosomes and cilia. The biogenesis of nascent centrioles is an essential cellular event that is tightly coupled to the cell cycle so that each cell contains only two or four centrioles at any given point in the cell cycle. The assembly of centrioles and their analogs, basal bodies, is well characterized at the ultrastructural level whereby structural modules are built into a functional organelle. Genetic studies in model organisms combined with proteomic, bioinformatic and identifying ciliary disease gene orthologs have revealed a wealth of molecules requiring further analysis to determine their roles in centriole duplication, assembly and function. Nonetheless, at this stage, our understanding of how molecular components interact to build new centrioles and basal bodies is limited. The ciliates, *Tetrahymena* and *Paramecium*, historically have been the subject of cytological and genetic study of basal bodies. Recent advances in the ciliate genetic and molecular toolkit have placed these model organisms in a favorable position to study the molecular mechanisms of centriole and basal body assembly.

Key words: basal body, centriole, centrosome, cilia, ciliate, microtubule, *Paramecium*, *Tetrahymena*

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Since their discovery in the late 1800s, centrosome research has largely focused on chromosome segregation and the implications of defective segregation in tumorigenesis (1,2). Centrosomes are microtubule organizing centers (MTOCs) that are responsible for organizing the spindle poles that direct bipolar spindle assembly for mitotic chromosome segregation. In addition, centrosomes serve to direct vesicle and organelle transport, regulate cytokinesis and function as a signaling platform for proper cell cycle progression. At the centrosome core, a centriole pair organizes the surrounding matrix [pericentriolar material (PCM)] for microtubule nucleation and organization. Centrioles are nearly identical to basal bodies

that are required to assemble the axonemes of cilia and flagella (3). Contemporary studies propose that their function as basal bodies is at least as important as their centrosomal function (4) (reviewed in 5). Consistent with this, basal body and ciliary dysfunction are found in human diseases that exhibit maladies including kidney cysts, obesity, hypertension, altered left–right asymmetry of the body and renal and mucus clearance anomalies, collectively known as ciliopathies (5–9).

Basal bodies that organize cilia are derived through multiple mechanisms of assembly (10). In the case of primary cilia, centrioles migrate to the cell cortex and are converted to basal bodies to nucleate and assemble cilia (11,12). Alternatively, multiciliated epithelial cells undergo a massive amplification of centrioles, which then migrate to the cell cortex to become basal bodies that organize many cilia (10). The differences between centrioles and basal bodies lie in their distinct accessory structures that facilitate PCM recruitment and nucleation of microtubules for centrioles or cortical localization and cilia organization for basal bodies. Thus, while the organization (assembly process and core structure) of centrioles and basal bodies is likely to be identical, critical modifications enable their divergent functions within the cell (10).

The molecular events leading to the assembly of new centrioles and basal bodies are incompletely understood (13,14). Early structural studies in a number of model systems defined many of the conserved morphological stages leading to new organelles (reviewed in 13). In addition, genetic studies predominantly in *Chlamydomonas reinhardtii* and *Caenorhabditis elegans*, bioinformatic and proteomic studies and human disease genes found to affect basal bodies and cilia have identified many molecules that now require detailed cell biology studies to understand their function. In this review, we focus on the potential the ciliates *Tetrahymena* and *Paramecium* have as model systems for increasing our understanding of basal body and centriole assembly.

Ciliates

Tetrahymena and *Paramecium* are single-celled ciliated protists belonging to the alveolates group that also contains dinoflagellates and apicomplexans (15). Although both ciliates are related, they are distinct enough to be

classified in separate ciliate subclasses (16). While comparisons between the two are helpful, it is not uncommon to observe experimental differences (17), including some relevant to basal bodies. Moreover, *Paramecium*, but not *Tetrahymena*, has experienced recent genome duplications in which duplicated genes have been maintained, adding to the complexity in comparing these two organisms (18,19). Because these cells have highly organized microtubule-intensive cytoskeletons with many basal bodies, they provide opportunities for cell biologists to study the mechanisms of basal body assembly and ciliogenesis.

Tetrahymena has proven to be a valuable model system, the study of which has contributed to the discovery of dynein, telomere sequences, telomerase and ribozymes and the detailed analysis of posttranslational modifications of both histones and tubulin. *Tetrahymena* has several characteristics that make it a successful model system (Table 1). The combination of molecular techniques with advantageous cytology is beneficial for the study of basal body duplication and assembly. *Paramecium* also provides excellent cytology combined with the use of RNA interference (RNAi) to selectively inhibit translation.

Basal body structure and organization

Ciliate basal bodies primarily function to organize motile cilia. Unlike vertebrate centrioles, they do not organize centrosomes for chromosome segregation but are dedicated to the task of supporting cellular motility and cortical organization. *Tetrahymena* cells are 50 μm long and contain roughly 750 basal bodies at the outset of the cell cycle,

while the larger *Paramecium* cells are 120 μm long and contain approximately 4000 basal bodies (Figure 1) (17,20,21). Basal bodies in both ciliates are found in two cellular locations. Cortical rows of basal bodies (ciliary rows or kineties) run the length of the cells and nucleate cilia for motility. A second population of basal bodies is tightly organized within the oral apparatus or cellular feeding structure (22). In *Tetrahymena*, there are 18–21 longitudinal rows of basal bodies and 150 basal bodies in the oral apparatus (Figure 1). In *Paramecium*, there are approximately 70 longitudinal rows and approximately 1000 basal bodies in the oral apparatus (21,23).

Ciliate basal bodies comprise the same conserved structure found for centrioles and basal bodies in most other eukaryotes. Nine triplet microtubule blades are arranged in a barrel configuration (Figure 2), which extend from the proximal end, or base, containing the ninefold symmetric hub and spoke structures (cartwheels) to the transition zone where triplet microtubules are converted to the doublet microtubules of the ciliary axoneme. A mature basal body is approximately 200 nm in diameter and 550 nm in length (24), and the lumen is filled with an electron-dense column of material that is approximately 75 nm in diameter and 400 nm in length (Figure 2). Cartwheels in many organisms are lost upon centriole and basal body maturation (13,25); however, ciliates maintain robust basal body cartwheels through their life cycle (24,26,27).

While ciliate basal bodies contain the same core structure found in most centrioles and basal bodies, there are differences in the accessory structures that facilitate their function in supporting ciliary motility and organization of the cell cortex. These accessory structures are composed of both microtubule and nonmicrotubule components that are arranged in a polarized array for cortical organization (17,27,28). Three major accessory structures include the kinetodesmal fiber or striated rootlet and two microtubule bundles that are uniformly positioned at the posterior end (postciliary microtubules) and at the side (transverse microtubules) of basal bodies (Figure 2) (17). These structures are found for cortical basal bodies of the ciliary rows, whereas the densely packed basal bodies in the oral apparatus contain a modified version of these accessory structures (17,28). Cortical basal bodies are also involved in the nucleation of the centrin-based infraciliary lattice of *Paramecium* (29) and the more limited but probably homologous apical filamentous band of *Tetrahymena* (30).

Basal body assembly

In mammalian systems, the formation of new centrioles/basal bodies can be generalized into two modes: centriolar and acentriolar. The distinction between each of these mechanisms is generally classified by the fate of the basal bodies depending on whether they organize a single primary cilium or many cilia in multiciliated epithelia. In the centriolar pathway, centriole duplication is tightly

Table 1: *Tetrahymena* biology

Characteristics of <i>Tetrahymena</i> biology and study ^a
Approximately 3-h cell doubling time in axenic culture at 30°C
Growth to cell density of approximately 10 ⁶ cells/mL
750 basal bodies per cell
Nuclear dimorphism (deleterious mutations can be maintained, without phenotype, in the inactive germ line micronucleus before mating to produce the mutant phenotype in the transcriptionally active macronucleus during development)
RNAi (controlled expression)
Antisense gene silencing and antisense libraries for forward genetic approaches
Homologous recombination for genetic knock-ins and knock-outs
Transformation of both nuclei (by injection, electroporation and biolistics)
Tagged genes (endogenous and exogenous)
Regulated gene control
Long-term storage (liquid nitrogen)
Strain resources (Tetrahymena Stock Center; http://tetrahymena.vet.cornell.edu/)
Clonal genetic strains
Mendelian genetic inheritance
Synchronized mass mating
Genome sequence

^aAdapted from Turkewitz (100).

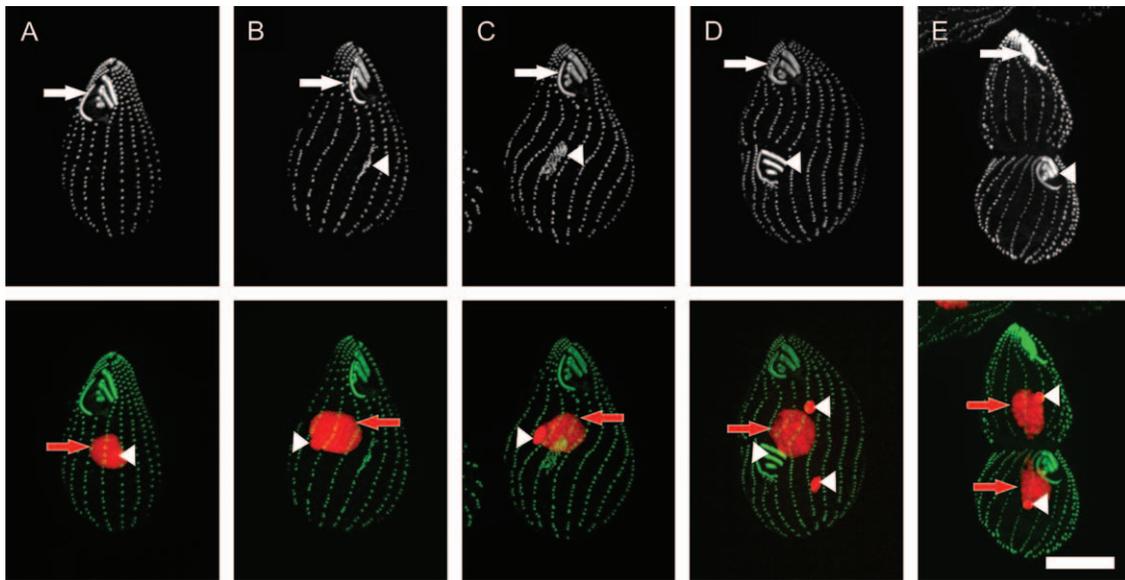


Figure 1: New basal body formation during the late stages of *Tetrahymena* cell division. *Tetrahymena thermophila* cells with basal bodies labeled by staining with anti-centrin (upper panels) and combined basal bodies [anti-centrin (green)] and DNA [Hoerscht 33342 (red)] (lower panels). A) Interphase cell with relatively uniform basal body spacing along the ciliary rows. Arrow denotes mature oral apparatus at the cell anterior. DNA staining shows both the larger macronucleus (red arrow) and the smaller dense micronucleus (white arrowhead). B) Basal bodies in the ciliary rows increase in density as a result of new basal body assembly. The development of the oral primordium (stomatogenesis) is initiated (arrowhead) at the cell median in preparation for cell division. C) New basal body assembly occurs at ciliary rows (most of the new basal body assembly is positioned immediately posterior to the division plane) and the oral primordium. The micronucleus begins to elongate into a prolate spheroid or rugby ball shape. D) The oral primordium organizes into the three membranelles and the crescent-shaped undulating membrane. The micronucleus has divided. E) The division plane is evident with both the micronucleus and the macronucleus separated between the mother and the daughter cells. Scale bar, 10 μm .

coupled with the cell cycle so that during DNA synthesis, each centriole pair separates and a new daughter centriole forms immediately adjacent to each mother centriole. The centriolar pathway maintains a constant number of centrosomes in each phase of the cell cycle, one in G1 and two in G2 and M, each containing a centriole pair. Here, the duplicated centriole pair function to organize centrosomes and then migrate to the cell cortex where the mature mother centriole acts as a basal body for primary cilia formation (11). Alternatively, multiciliated epithelial cells require a massive amplification of basal bodies that occurs by a combination of centriolar and acentriolar pathways. In contrast to the centriolar pathway described above, where only one new centriole is formed adjacent to the mother, several centrioles may form at a given time in close proximity to the mother centriole (10). The second acentriolar pathway is the *de novo* assembly of multiple centrioles around a matrix of electron-dense fibrous granules called deuterosomes (10). This second pathway enables massive centriole/basal body amplification. For both mechanisms, duplication and assembly occur when centrioles are resident within the cell interior before migrating to the cell cortex where they become basal bodies. In addition, the two pathways share structural assembly intermediates and molecular requirements, suggesting that they are conserved (13,31,32).

Consistent with centriolar assembly in mammalian cells, *Tetrahymena* and *Paramecium* basal bodies are assembled adjacent to an existing parent organelle. However, ciliate basal bodies duplicate and remain at the cell cortex. A significant advantage to studying new basal body assembly in *Tetrahymena* and *Paramecium* is the spatially constrained pattern by which new basal bodies are repeatedly assembled in ciliary rows (33,34). In these ciliates, 750 and 4000 basal bodies, respectively, must be assembled prior to each cell division to maintain the constant number of these organelles. Both circumferential and anterior-posterior gradients of proliferation exist at the cell cortex, so that new basal bodies are generally assembled in the medial region of *Tetrahymena* and *Paramecium* cells (21,33,34). Furthermore, new basal bodies are always positioned anterior to and in close proximity to the existing organelle, creating pairs of old and new basal bodies (Figure 1) (21,24,26). As basal bodies mature, they separate and move in the anterior direction away from the mother basal body along the ciliary row. Most of these basal bodies eventually become ciliated (21,30), with a variable lag in ciliogenesis that is dependent on where they are located in the cell (33,35,36). Furthermore, newly assembled basal bodies have the capacity to nucleate new basal body assembly soon after, within a single cell cycle, suggesting that the maturation into an organelle that is competent to

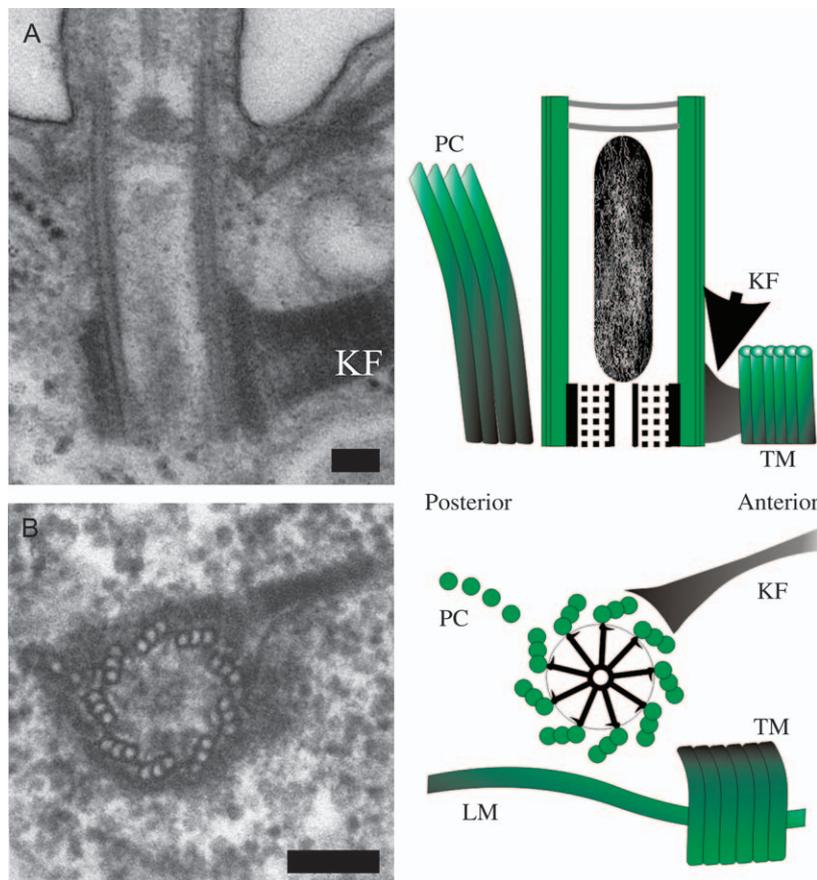


Figure 2: *Tetrahymena* basal body structure. A) Longitudinal section electron micrograph through a single basal body showing basal body cylinder structure and KF. The cartoon identifies a longitudinal basal body section with accessory structures. The cartwheel structure is at the base of the organelle. Several of the accessory structures surrounding the basal bodies are indicated. B) Cross-section electron micrograph through the basal body structure, KF and postciliary microtubules. The cartoon reveals a cross-section through the cartwheel structure and several of the accessory structures surrounding basal bodies. Labeled structures are as follows: LM, longitudinal microtubule; PC, postciliary microtubule bands; KF, kinetodesmal fiber; TM, transverse microtubules. The anterior and posterior positioning relative to the cellular geometry is indicated. Scale bars, 100 nm.

form daughters can occur fairly rapidly in ciliates (21,27). Thus, the competence to assemble individual basal bodies is not tightly coupled to the cell cycle, but instead, the time and place of new basal body formation are subject to regional controls of basal body populations (21).

The centriolar duplication of mammalian centrioles occurs exactly once during the cell cycle, such that a licensing mechanism that is coupled to the cell cycle must exist for tight regulation of new duplication. Models suggest that the linkage between the orthogonally paired new and old centrioles is important for tight control of assembly (37). Furthermore, defects in centrioles and centrosomes activate a checkpoint response, suggesting that centriole duplication and activity are tightly coupled to the cell cycle (38). The timing of basal body assembly in ciliates is consistent with the cell cycle. The majority of *Tetrahymena* basal bodies are formed as cells prepare for cell division (33,39), and similarly, in *Paramecium*, new duplication occurs in the final 30 min of the cell cycle (40). However, the regulation of duplication does not appear to be constrained by the cell cycle as found for mammalian cells in which newly assembled centrioles do not reduplicate until the following cell cycle (41). The lack of either a checkpoint response to basal body impairment or cell cycle-based regulation at the level of individual basal bodies is an

advantage for dissecting the mechanisms of basal body assembly in ciliates (42).

In addition to basal body assembly along the ciliary rows, new basal bodies are rapidly amplified at the oral primordium, which will become the oral apparatus of the daughter cell (23,43). Here, new basal bodies are assembled in 'clusters' either close to (*Paramecium*) or distant from (*Tetrahymena*) the mature parental oral apparatus. In *Tetrahymena*, these basal bodies duplicate from basal bodies contained in the ciliary rows before becoming grouped into four discrete clusters that make up the oral apparatus (Figure 1). While this amplification requires prolific assembly of new basal bodies, no acentriolar or *de novo* assembly has been observed in either *Tetrahymena* or *Paramecium* (24,44). However, *de novo* basal body assembly does occur during excystment and likely during oral development in the spirotrich ciliate, *Oxytricha* (45,46). Most of the basal bodies of the new oral apparatus nucleate cilia immediately during oral apparatus development (23,43).

Structural order of assembly

Upon initiation of duplication, the assembly intermediates leading to these structurally complex organelles have been thoroughly reviewed (13,14,32,47), and the current model is that the cartwheel defines the ninefold symmetry that is

the hallmark of centrioles, basal bodies and cilia (13) (Figure 3). In *Tetrahymena*, new basal bodies are assembled by the formation of a new organelle perpendicular and at the base or proximal end of an existing mature basal body (mother) (Figure 3A). The initial observed structure contains short singlet microtubules and the cartwheel (24). At the base of the cartwheel is an amorphous electron-dense disk known as the generative disk. As the new basal body matures into a probasal body, doublet and triplet microtubules are assembled. It is important to note that the early stages leading to cartwheel and singlet microtubules were not captured by this work. In *Paramecium*, Dippell described a detailed series of intermediate stages,

suggesting that the first structure responsible for the nine-fold symmetry is the generative disk and not the cartwheel. Singlet or 'A' microtubules develop from the generative disk in a sequential and clockwise fashion (26). Then, in a non-sequential manner, doublet or 'B' tubules form coincidentally with cartwheel assembly. Finally, triplet microtubules are formed. Thus, cartwheels may not be the morphological prerequisite for the ninefold symmetry. Subsequent work, however, suggests that cartwheels assemble prior to the A-tubules in *Paramecium* (27) (Figure 3B). The effort to resolve this and understand the stages of assembly in greater detail has been hampered by a deficiency of knowledge about molecules that function in this pathway.

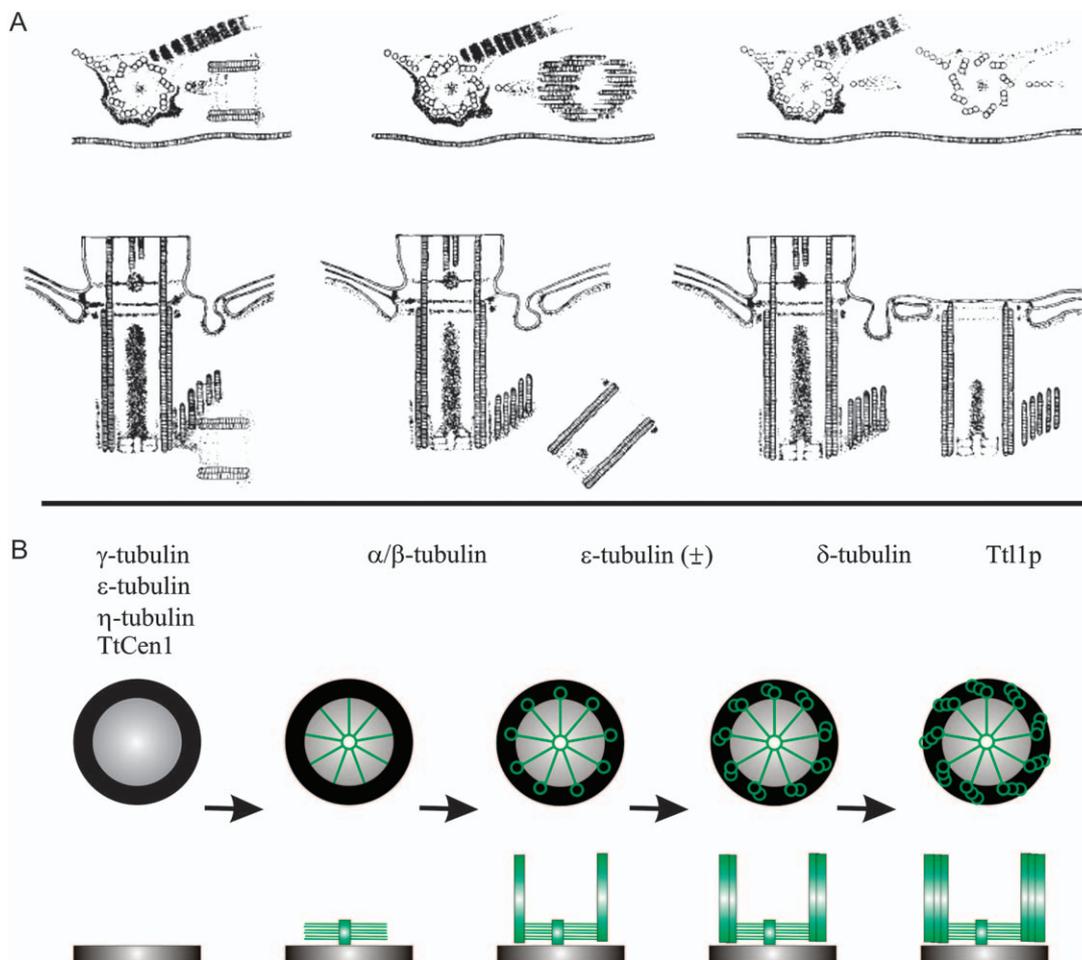


Figure 3: Stages of early basal body assembly. A) Drawing depicts longitudinal sections of basal body formation in *Tetrahymena*. Basal bodies form on the anterior side (relative to the cellular geometry) of the parent organelle. Nascent assembly is initiated at the proximal end and perpendicular to the existing parent basal body to form a short probasal body. The probasal body then separates and tilts parallel to the parent organelle and inserts into the plasma membrane. This image is from (24), and is displayed under the terms of a Creative Commons License (Attribution-NonCommercial-Share Alike 3.0 Unported license, as described at <http://creativecommons.org/licenses/by-nc-sa/3.0/>). B) Cartoon depicts the steps to new basal body assembly based on combined studies from *Tetrahymena* and *Paramecium*. Assembly begins with the formation of the generative disk, followed by cartwheel assembly, the first structural evidence for ninefold symmetry. Singlet microtubules are assembled at the ends of each cartwheel spoke, followed by the B- and C-tubules to generate doublet and triplet microtubules, respectively. Genes involved in each transition stage are indicated, based upon the molecular components described in this review. (\pm) indicates partial ϵ -tubulin depletion. Note that this is not an exhaustive list of the known molecular components. Top panels, cross-section; bottom panels, longitudinal section.

Work in *Chlamydomonas* and *C. elegans* has advanced the field through forward genetic analysis and RNAi screens, which identified new proteins important for centriole and basal body assembly (47,48). Mutations in some of these genes affect cartwheels and also disrupt the ninefold symmetry in *Chlamydomonas* and *Drosophila* centrioles (49–51). More recently, the development of powerful molecular biology techniques and sequenced genomes in ciliates has opened up this unique genetic and cytological system for detailed basal body studies. Advances in electron microscopy (EM), namely tomography, may enable a detailed morphological analysis of normal stages of assembly and establish structural precursors that lead to the observed ninefold symmetry. This technology has significantly advanced *C. elegans* centriole research (52). Finally, the ability to assemble basal body cartwheels from *Tetrahymena* basal body lysates, *in vitro*, suggests that ciliates could become a formidable model system in defining the key structures and molecules for cartwheel assembly (53).

From structure to molecules

EM studies of centrioles and basal bodies, including those of ciliates, show the conserved fine structure and intermediate stages of assembly of these organelles. More recent investigations in a number of model systems have identified conserved and nonconserved molecules that comprise centrioles and basal bodies. In addition, the identification of proteins associated with human ciliary disease brings an extensive number of new and important targets for functional analysis with respect to basal body assembly and function.

Basal body proteome

Proteomic, genomic and bioinformatic studies have defined many of the molecular components that comprise centrosomes, centrioles, basal bodies and cilia (54–62). The use of multiple strategies and organisms allows for the assembly of a comprehensive list of both overlapping and unique components. The large number of putative basal body, centriolar and ciliary proteins indicates the complexity of MTOCs and cilia.

To identify the molecules that comprise centrioles and basal bodies, two analogous proteomic studies were performed in *Chlamydomonas* and *Tetrahymena* (54,63). The extensive list of candidate *Tetrahymena* basal body proteins was reduced to a manageable list by using a comparative strategy. Proteins were prioritized based on whether they contained protein motifs associated with microtubules, are conserved to vertebrate proteins, are present in other similar proteomes and/or are known to have human disease relevance (63). High-priority proteins were green fluorescent protein tagged to determine whether they localize to *Tetrahymena* basal bodies. Twenty-four *Tetrahymena* proteins were localized to basal bodies in this study. Finally, proteins were localized using

immunolectron microscopy to define their position within the ultrastructure of the basal bodies. The localization of basal body components to discrete domains provides significant information in determining how these proteins may function at basal bodies. For example, proteins that localize to the distal portions are likely responsible for ciliary formation and function, while proteins that reside at the generative disk or cartwheel structures may be early assembling components critical for new basal body assembly. Indeed, the *Tetrahymena* Sas6a protein, which is essential for new centriole and basal body assembly in all organisms studied thus far, localized to the central hub of the cartwheel (63). Three novel cartwheel proteins were also identified in this study (Poc1, Bbc29 and Bbc82).

Molecular studies of ciliate basal body assembly

Tubulins

Tubulin modifications

The major structural unit of centrioles and basal bodies is the triplet microtubule structure composed of $\alpha\beta$ -tubulin dimers. The *Tetrahymena* genome contains one α -tubulin gene and two redundant β -tubulin genes, while *Paramecium* contains five α -tubulin genes and three β -tubulin genes, with minimal divergence between corresponding genes within each of these ciliates (18,19). Ciliates build a large number of complex tubulin-containing structures with varying degrees of microtubule stability and a wide range of functions using this limited number of tubulin genes (64). Tubulin posttranslational modifications may be responsible for establishing different classes of microtubules within these structures by affecting microtubule structure and/or the binding of microtubule-associated proteins (65–68). Specifically, centriole and basal body microtubules must be regulated to form a stable structure that is conservatively maintained once it is assembled (69,70,101).

Mammalian centrioles are disrupted by antibodies that target glutamylated tubulin, suggesting that this modification is required to stabilize the triplet microtubules (71). Furthermore, tubulin glutamylation is found at both *Tetrahymena* and *Paramecium* basal bodies (72,73). To test the possibility that glutamylation is required for new basal body assembly and stabilization, Wloga et al. generated mutants in the *Tetrahymena* α -tubulin glutamylase that is specific to basal bodies, Ttl1p (74). While the mutants had no effect on new basal body assembly, glutamylation was required for proper basal body maturation, stabilization and insertion into the cortex (74). In addition to glutamylation, tubulin acetylation, phosphorylation, tyrosination/detyrosination and glycylation of α - and/or β -tubulin have been identified in ciliates (reviewed in 65,66). However, the functional significance of these modifications for basal body assembly remains unclear. As histone modifications have proven to be important for nucleosome function, tubulin modifications may also be important for proper basal body assembly, maintenance and function (67).

Tubulin isoforms

Beyond the canonical α - and β -tubulins and their modifications, a new frontier in MTOC study was initiated by the discovery of additional tubulin family members. γ -Tubulin was found as an extragenic suppressor of a heat-sensitive β -tubulin mutation that causes stable microtubules in the mold *Aspergillus* (75). In ciliates, *Tetrahymena* contain one γ -tubulin, while *Paramecium* has two nearly identical γ -tubulins that are essential for viability and basal body assembly. Using the advantage of well-defined spatial patterns of basal body duplication that are not tightly coupled to the cell cycle, Ruiz et al. found that new basal body assembly is lost in *Paramecium* when the two γ -tubulin genes are disrupted (76). In ciliates, these defects can be identified by the lack of basal body pairs assembled near the cell equator prior to cell division (Figure 4). *Tetrahymena* also requires γ -tubulin for basal body duplication and for maintenance of existing organelles (77). Reintroduction of γ -tubulin expression following its depletion results in the rapid assembly of new basal bodies even though existing organelles appear not to be present. This surprising result suggests that *de novo* basal body formation might be able to occur in *Tetrahymena* cells without basal bodies. Two important experiments may further define how γ -tubulin functions in basal body assembly and maintenance and whether *de novo* assembly can occur in *Tetrahymena*. First, the localization of γ -tubulin within the ultrastructure of newly assembling and existing basal bodies would define where this protein acts in *Tetrahymena* basal body assembly. Second, detailed EM

of cells depleted of γ -tubulin and thus basal bodies may determine whether residual elements of the basal bodies remain that may function to nucleate new basal body assembly. Alternatively, these basal bodies may form and organize in the absence of existing structures as previously found for *de novo* assembly in other organisms, including certain ciliates (45). Finally, a novel mechanism for γ -tubulin regulation of basal body assembly was identified using site-directed mutagenesis in *Tetrahymena* (78). γ -Tubulin mutations commonly resulted in conditional phenotypes with defective basal body assembly. However, mutations targeted to the nucleotide-binding domain (NBD) were typically lethal, except for two that caused excessive basal body proliferation outside of the normal cortical organization as well as in the cytoplasm (78). These results suggest that γ -tubulin is an important initiator of basal body duplication in a manner regulated by the NBD.

Additional tubulins that are responsible for proper centriole and basal body assembly have been identified using both forward genetic and genome database searches (reviewed in 79). Intensive work in *Chlamydomonas*, as well as in *Paramecium*, has contributed to our functional understanding of these alternative tubulin proteins (79). First localized at mammalian centrosomes (80), ϵ -tubulin in *Paramecium* localizes to cartwheels and along the length of the microtubule triplets of the basal body (42). Loss of ϵ -tubulin is lethal and inhibits centriole and basal body duplication (42,81). Partial depletion of ϵ -tubulin causes a loss of B- and C-tubules in both *Chlamydomonas* and *Paramecium* (42,82),

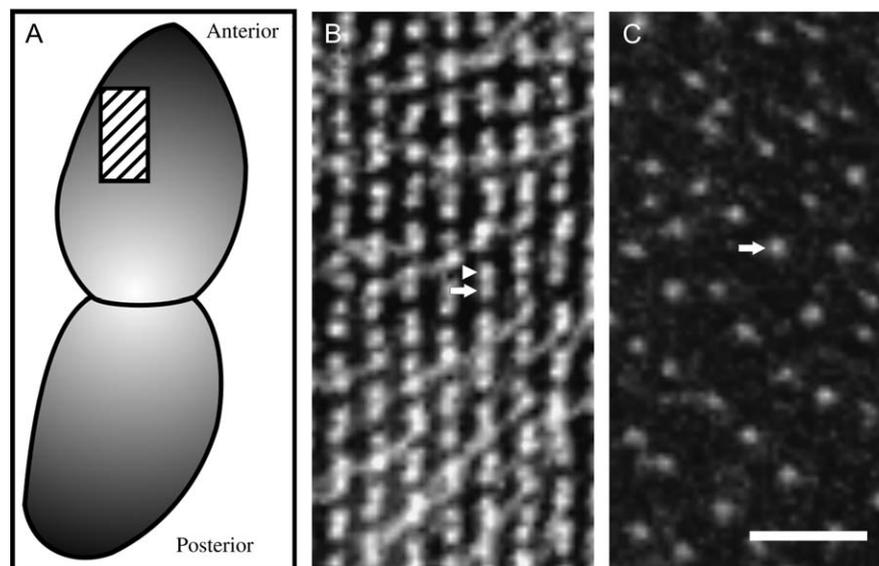


Figure 4: Loss of basal body duplication in *Paramecium* cells. A) The cartoon represents a dividing *Paramecium* cell at the stages visualized, and box indicates the anterior region of the dorsal side taken from both wild-type (B) and ϵ -tubulin-depleted (C) cells. Basal bodies (and some cilia) are stained with anti- α -tubulin antibodies (ID5). B) In wild-type cells, basal body doublets are easily visualized in close proximity to each other. Each doublet contains a mature basal body (arrow, posterior with associated cilia detectable at some basal bodies) and an immature basal body (arrowhead, anterior). C) Cells depleted of ϵ -tubulin by RNAi for 72 h do not have pairs of duplicated basal bodies (arrow). Images were kindly provided by Dr Pascale Dupuis-Williams (Université Evry Val d'Essonne, France). Scale bar, 2 μ m.

suggesting that B- and C-tubule formation are required to form and stabilize new centrioles and basal bodies.

Paramecium δ -tubulin is not required for basal body duplication but is responsible for either the formation or the maintenance of the C-tubule of the microtubule triplet (83,84). Accessory structures required to maintain cortical organization are lost in the absence of δ -tubulin, suggesting that C-tubules are responsible for linking these structures to the basal body cylinder. Basal bodies without C-tubules maintain the capacity to nucleate cilia or flagella.

Finally, forward genetic strategies identified a *Paramecium*-specific tubulin superfamily member, η -tubulin that was originally found as a conditional mutation (*sm19*) that, at restrictive temperature, inhibited basal body duplication (85). η -Tubulin is a divergent tubulin that genetically interacts with β -tubulin in a manner suggesting that the proteins directly interact (86). Furthermore, γ -tubulin is mislocalized in η -tubulin mutants (85). Thus, η -tubulin may be important for tethering γ -tubulin to the basal body for proper basal body assembly.

Centrin

First identified in green algae, centrin is responsible for contraction of calcium-sensitive striated flagellar rootlets and for centriole, basal body and spindle pole body duplication (87). Budding yeast centrin, Cdc31p, is required for the initiation of yeast spindle pole body duplication (88,89). There are an extensive number of centrin-like proteins, especially in ciliates.

Divergent results are reported for whether or not centrins are required for centriole and basal body assembly. Two human centrins, Centrin2 and Centrin3, are reported to be required for centrosome duplication (90,91). Similarly, loss of centrin function is found to abrogate spindle pole body, centriole and basal body duplication in *Chlamydomonas*, *Marsilea vestita*, *Schizosaccharomyces pombe*, *Leishmania* and *Tetrahymena* (91–96). These studies indicate that centrin is responsible for centriole and basal body assembly, whether using the centriolar or the acentriolar assembly pathways. However, recent RNAi experiments in human osteosarcoma (U2OS) cells found no role for Centrin2 (CrVfl2) or Centrin3 (ScCdc31) in normal or Plk4-induced pro-centriole assembly (97,98). Ciliate studies also contribute to this discrepancy. *Paramecium* Centrin2 (HsCen2/CrVfl2) or Centrin3 (HsCen3/ScCdc31) depletion by RNAi feeding suggests that, at early time-points, within the first cell cycle of RNAi treatment, these proteins are primarily responsible for basal body positioning and not for duplication (99). New basal body assembly occurs normally; however, basal bodies are not deposited into the cell cortex causing basal bodies to become internalized. This results in a dilution of the total number of basal bodies before the terminal phenotype of small rounded cells with internalized basal bodies (99). In contrast, a complete genome knockout of the *Tetrahymena*

centrin, TtCen1 (HsCen2/CrVfl2), inhibits both basal body duplication and maintenance so that basal bodies cannot be detected by either immunofluorescence or EM (96). The answers to two lingering questions could explain this discrepancy. First, it is not known whether or not the total cellular centrin protein levels are significantly reduced in the *Paramecium* studies. A low level of *Tetrahymena* centrin remains detectable even 96 h after induction of the knock-out allele, suggesting that centrin is stable for multiple cell cycles (96). Following centrin RNAi in *Paramecium*, old basal bodies retain centrin, and it is possible that this is enough to facilitate new basal body assembly. Second, ciliates have a number of centrin-like proteins, raising the possibility that there are redundant functions for centrins to facilitate basal body assembly (96,99).

Conclusions

The utility of ciliates for the detailed structural and functional analyses of nascent basal body assembly is well documented. The importance of extending this work beyond tubulins and centrins is more apparent as the tools required for detailed molecular study in ciliates becomes available. In particular, analysis of cartwheel proteins identified by proteomic studies may determine how early stages of basal body assembly occur. Now that many basal body proteins have been identified [many of which are conserved (Table 2)], and techniques are readily available to either reduce their abundance or introduce null, mutant or tagged alleles, ciliates are well positioned as a model organism to lead to insights into the functions of these proteins in basal body biology.

One possibility for the dissonance between the structural studies of basal body assembly in ciliates is that the early stages of basal body assembly may not proceed through a linear pathway but are rather accomplished through modular assembly of distinct components that ultimately leads to a functional organelle. We still do not know how the structures or components assemble together to generate the ninefold symmetric structure that is important for ciliary motility. To begin to understand this process, initial studies have focused on the gross morphology to determine those proteins that are essential to build the pro-centriole/basal body structure. As we understand the assembly in greater detail, such research will benefit by determining subtle defects and changes in the overall protein assembly dynamics of the basal body and centriole. Ciliates, with their increasingly sophisticated techniques for molecular analysis, are in an opportune position for detailed study of basal body assembly and function.

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Table 2: Proteins for centriole and/or basal body duplication and assembly^a

Protein	Phenotype ^b	Ciliate conservation ^c
SAK/PLK4	No duplication or reduplication	No
ZYG1	No duplication or reduplication	No
MPS1	No spindle pole body or centrosome duplication ^d	No
PLK1	No overduplication in S-phase arrest	Yes (01443850)
PLK2	No overduplication in S-phase arrest	Yes (00191790)
SPD-2/CEP192	No duplication ^d	No
SAS-6	No duplication or reduplication	Yes (00388200/00137600)
SAS-5	No duplication	No
SAS-4/CPAP	No duplication	Yes (00382220)
γ -Tubulin	No duplication	Yes (00079520)
δ -Tubulin	C-tubule formation disrupted (doublets)	Yes (00335970)
ϵ -Tubulin	No duplication (singlets)	Yes (00118700)
BLD10/CEP135	No duplication	Yes (01164140)
SFI1	No duplication	Yes (00999030)
Centrin2/VFL2	No duplication ^d	Yes (00384910)
Centrin3/CDC31	No duplication ^d	Yes (00523060)
SCF complex (SKP1)	Increased centrosome number	Yes (00426320)
p53	Centrosome amplification	No
CP110	No duplication	No
Centrobin	No duplication	No
Nucleophosmin	No duplication	No
Separase	No centriole separation	Yes (00297160)
Ana1	Reduced centrosome number	No
Ana2	Reduced centrosome number	No
Ana3	Reduced centrosome number	No

^aList of proteins for centriole and/or basal body assembly is adapted from Strnad and Gonczy (13) and Bettencourt-Dias and Glover (14).

^bBased on gene knockdown, mutants and/or protein inhibition (reviewed in 13,14).

^cConservation is predicted based on reciprocal BLAST scores and sequence alignments. Accession numbers for *Tetrahymena thermophila* (THERM_#) are provided. Links to the *Tetrahymena* genome site (ciliate.org) can be used to identify the *Paramecium* orthologs.

^dProteins where a defect in centriole or centrosome duplication has been observed but contradictory results are also observed in the literature.

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