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EDITED BY

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ASSISTANT EDITOR
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The Control of Microtubule Assembly *in Vivo*

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We dance round in a ring and suppose,
But the secret sits in the middle and knows.

Robert Frost (1949)

With the advent of electron microscopy, it was recognized that microtubules were ubiquitous components of eukaryotic cell organelles and were in fact participants in many of the most basic cellular processes, most notably as the spindle

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fibers of the mitotic apparatus and as the 9 + 2 axonéme tubules in cilia and flagella. Somewhat later they were found to be one of the main components of neural tissue, and it is now known that microtubule networks exist in the cytoplasm of most eukaryotic cells.

The exquisite and delicate control over the timing of appearance and positioning of microtubules and microtubule-containing organelles is spectacularly obvious in many cellular events, and the question of the nature of their regulation is thus a fascinating problem. But it is also a particularly frustrating problem because of the abundance of tantalizing data which approach the question but fall short of answering it. The secret, in this case, is all too elusive.

The vastness of the microtubule literature and the frequency with which it is reviewed are often noted [generally, as here, at the beginning of the very reviews in question: for example, see Porter (1966) for the first review of microtubule function, and Burnside (1975) for a more recent historical overview; reviews by Hepler and Palevitz (1974), Jacobs and Cavalier-Smith (1977), Newcomb (1969), Olmsted and Borisy (1973), Pickett-Heaps (1975b), Roberts (1974), Snyder and McIntosh (1976), and Stephens and Edds (1976) provide several viewpoints on microtubule structure and function in animals and plants.] What follows therefore is in no way a comprehensive review of the microtubule literature or even of that part of it which might be supposed to be included under the title. The representation of papers discussed is just that, a selective representation. In 1974 Roberts summed up rather nicely what was then known about the control of microtubule assembly *in vivo* by noting that it was "all rather hazy at the moment." Unfortunately, it is still rather hazy, but in the last several years much more information has been accumulated which both gives direct clues about the molecular mechanisms underlying the temporal and spatial regulation of microtubule assembly and makes the interpretations of some earlier observations (particularly on the role of microtubule-organizing centers, for example) more sound. This article, then, addresses the following questions. First, what exactly do microscopists see when they look at a cell in which microtubules are in the process of coming or going? Second, what kind of experiments are possible to examine microtubule assembly events in living cells? And third, what molecules in addition to the primary constituent of microtubules, tubulin, are involved in controlling these events?

I. The Secret: Observations of Microtubule Assembly *in Vivo*. The Importance of Microtubule-Organizing Centers

One of the surprises of the close look at cells allowed by electron microscopy was not only the ubiquity of microtubules but also the fact that very disparate structures were formed from them. For instance, the two most prevalent

microtubule-containing organelles, mitotic spindles and cilia and flagella, are very different in that the first is a labile structure repeatedly and rapidly assembled and disassembled at each cell division, while the others are stable structures with diverse accessory structural components, persistent under treatments such as cold, pressure, and various mitotic poisons (e.g, colchicine) which cause the mitotic spindle to disappear. The question of how the precise regulation of both the temporal appearance and spatial organization of microtubules is achieved immediately became the subject of an enormous amount of research. Crucial to this question is the point of origin of microtubules, and investigation of this revealed yet another microtubule-containing organelle. Light microscopy showed dense structures at the poles of many mitotic spindles and at the base of cilia and flagella—the centriole and the basal body, respectively. Electron microscope studies showed these two structures to be morphologically equivalent, consisting of a cylindrical structure with a basic pattern of nine triplet tubules arranged around a central "cartwheel" structure [see Fulton (1971) and Pitelka (1974) for historical and morphological reviews of these structures]. However, although the outer doublet tubules of the axoneme are direct extensions of the basal body triplet tubules, the microtubules in the mitotic apparatus are not continuous with centriole tubules, and many mitotic spindles, notably those in higher plants, have no centrioles at the poles at all. The polar mitotic microtubules in fact appear to arise out of amorphous electron-dense material which surrounds the centriole (or, particularly in anastral mitotic figures, the polar tubules appear, like the earth in Genesis, to arise out of nothing at all). Furthermore, in addition to the highly structured basal body and the seemingly structureless pericentriolar material there are diverse other structures from which microtubules arise. The useful term "microtubule-organizing center" was coined by Pickett-Heaps (1969) to denote the structures or material from which microtubules initiate.

A. The Morphogenesis of Basal Bodies and Centrioles

Some of the most elegant descriptions of the process of microtubule assembly *in vivo* are the original observations of the formation of centrioles and basal bodies which were made as soon as the technology of electron microscopy permitted. These observations have stood in the literature awaiting complete interpretation from biochemical data (but the biochemical technology in this area has not quite caught up yet). Early on, centrioles were postulated to be self-replicating organelles; the fact that new centrioles often arose close to mature ones, the difficulty of discerning intermediate forms, and the complexity of the organelle all seemed to indicate that they must be autonomous—"reproducing" by dividing—or at least that the foramtion of a new centriole required the presence of a mature one and was directed by it. Even after it was realized that they

could in fact arise *de novo* (Dirksen, 1961), the idea persisted. This problem has been discussed at length by Pickett-Heaps (1969, 1971, 1975a). In fact, centrioles and basal bldies *in vivo* arise out of electron-dense material of uncertain structure and composition, which may or may not be associated with a mature organelle.

Studies on basal body and centriole morphogenesis in various vertebrate tissues have yielded similar reconstructions of the development of the mature organelle from precursor structures which are in turn derived from amorphous or fibrous electron-dense masses in the cell interior. Dirksen and Crocker (1966) examined the formation of centrioles in differentiating ciliated cells of fetal rat tracheal epithelium. Subsequently, Dirksen (1971) described centriole morphogenesis in the ciliated epithelium of mouse oviduct, which took place in a brief period after birth but was not synchronized, even within a single cell. Centriole morphogenesis was similar in both tissues, proceeding through four sequential stages. First, in the center of the cell clusters of electron-dense fibrillar masses 60–80 nm in diameter appeared, which later became organized into larger aggregates 100–700 nm in diameter in cleared areas of the cytoplasm. The structure of these aggregates was difficult to ascertain because of their electron opacity; they were usually amorphous, but occasionally microtubules or a mature centriole was present. As shown in Fig. 1, these electron-dense masses then gave rise to procentrioles, becoming surrounded by as many as nine immature centrioles in various stages of development. The first stage in centriole development was the appearance of an annulus or disk of indistinct structure which later developed the typical centriolar cross section. The microtubules of the procentrioles were often connected to the central mass by fine strands. Concomitantly with centriole maturation the central mass of material either disappeared or became clearly hollow, suggesting that the centrioles had in fact been formed from this material. Occasionally procentrioles were found surrounding mature centrioles. In neither of these studies was Dirksen able to define clearly the relationship between mature centrioles and the amorphous material which appeared to be the earliest procentriole precursor.

Steinman (1968) followed the differentiation of ciliated cells in epidermis and trachea of *Xenopus laevis*. His electron micrographs show dense, amorphous masses around which are clustered smaller electron-dense masses which apparently become procentrioles, cylinders 150 nm in diameter with nine single tubules. These structures appeared deep in the cytoplasm of the cell near the nucleus; correlated with their disappearance multiple mature centrioles 200 nm in diameter with a typical cross-sectional structure appeared in the apical cytoplasm. The inference was that the procentrioles rapidly matured and migrated to the cell surface, although no intermediate forms were found. The centrioles then aligned at the apical surface, ciliary shafts grew out of them, and basal body accessory structures such as rootlets appeared. Small, electron-dense bodies close to the base of the basal body and the growing cilium were interpreted as

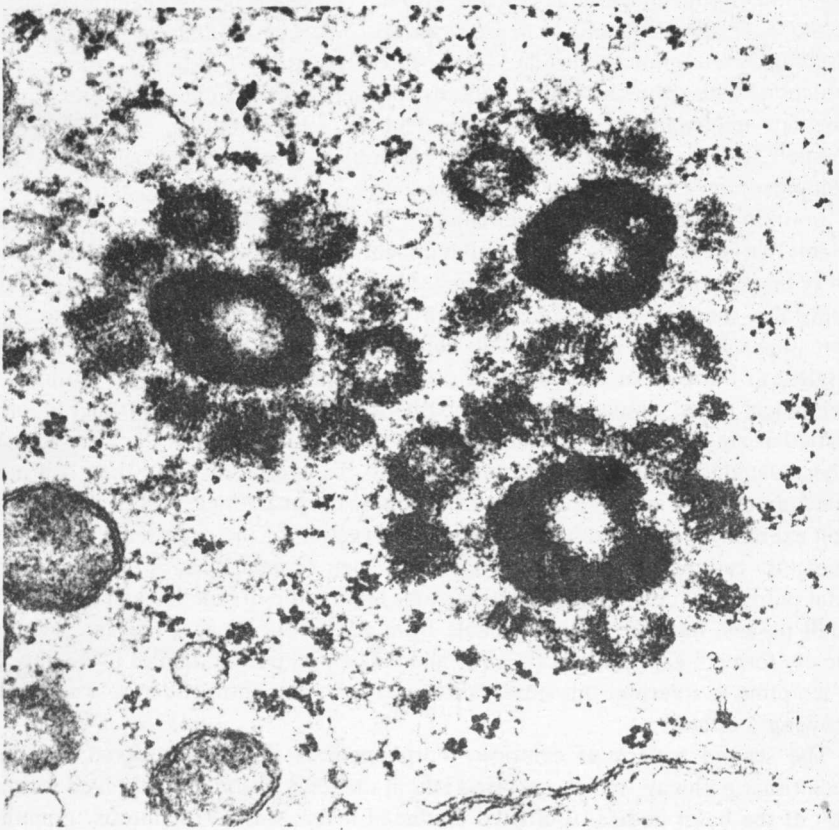

FIG. 1. Centriole morphogenesis in developing mouse oviduct epithelium. Three centriole generative complexes, each consisting of the central electron-dense precursor structure (clearly "hollow" at this stage of development) surrounded by nine developing procentrioles. Two procentrioles in the upper right complex were not in the plane of section. ×47,450. Reprinted from Dirksen (1971), with permission.

axoneme precursors; similar but less electron-dense material was interpreted as the source of the basal body rootlets. The various morphological events described did not occur in synchrony; a single cell often contained both basal bodies with growing axonemes and unaligned centrioles still in the supranuclear cytoplasm. Attachment of the centriole at the cell surface appeared to be the signal for assembly of the axoneme; Steinman saw only two cases in which an unaligned centriole bore an axoneme.

A similar pattern of centriole formation was observed by Kalnins and Porter (1969) during ciliagenesis in chick tracheal epithelium. Basal bodies formed in a

region of fibrous material associated with the cell centrioles; procentrioles arose in a cluster around a core of dense material sometimes containing a cylindrical structure resembling a centriole. The earliest procentrioles were short cylindrical structures without microtubules; tubules appeared first as singlets, progressing to doublets and triplets. The basal bodies then elongated, matured, and migrated to the cell surface, where the cilia were assembled. In this tissue basal body development appeared to be synchronous.

Sorokin (1968) studied the formation of centrioles and subsequent ciliagenesis in fetal rat lungs. He observed two patterns of morphogenesis. The first occurred early in development in interphase pulmonary cells of all types and involved the centrioles subsequently associated with the mitotic spindles. Usually one, but sometimes as many as eight, new centrioles arose directly adjacent (at right angles) to the wall of a preexisting centriole, first as procentrioles; during development these annular structures lengthened into cylinders. The triplet tubules started as singlet tubules to which the second and third tubule walls were added. These daughter centrioles were released into the cyoplasm when they had matured about halfway. An interesting and somewhat puzzling aspect of the regulation exerted in this system is Sorokin's observation of the occasional growth of transitory rudimentary cilia from one of the pair of centrioles in differentiating fetal pulmonary cells. These are transitory embryonic organelles and are rare in adult tissues; they are distinguishable from adult cilia because they are incompletely formed, especially at the tips, and the central pair of tubules is lacking. It is tempting to interpret this observation as a lapse in control, that is, a centriole growing a cilium.

The second pattern of centriole morphogenesis Sorokin observed was the acentriolar pathway, which occurred late in the fetal period and involved formation of the basal bodies of ciliated epithelial cells. Masses of fibrous, granular material accumulated in close proximity to Golgi elements in the apical cytoplasm. The fibrogranular areas increased in size and apparently condensed into spherical masses, around the periphery of which procentrioles arose. Ultimately the mature centrioles aligned in rows underneath the apical end of the cell membrane. The signal for ciliagenesis appeared to be placement of the basal bodies at the cell membrane; accessory structures such as satellites and roots appeared, and then the ciliary shaft grew out. The ciliated border cilia grew faster than the rudimentary cilia and had the complete 9 + 2 cross-sectional pattern. The mature basal bodies did not ususally have procentrioles associated with them, but Sorokin observed this in a few cases and concluded that the capacity for formation of an associated organelle is retained in basal bodies produced by the acentriolar pathway.

A similar dual set of pathways for centriole morphogenesis was observed by Anderson and Brenner (1971) in rhesus monkey oviduct. After ovariectomy, deciliation and loss of basal bodies occurred in ciliated epithelial cells of the

oviduct; estrogen treatment caused redifferentiation of these cells. The major pathway was acentriolar, in which basal bodies were formed from procentrioles generated from aggregates of fibers with no structural resemblance to centrioles. First, fibrous granules of 40–60 nm in diameter appeared in the apex of future ciliated cells. These granules usually were aggregated into sheets or spheres but sometimes were dispersed in the cytoplasm and sometimes associated with the nuclear membrane; occasionally a few microtubules were present among them. The fibrous material appeared to fuse to form the procentriolar ring. These investigators found the stages in procentriole development difficult to interpret. The central cartwheel structure formed before the triplet tubules; the A tubules then formed in sequence around the ring, but tubule growth after that was not synchronous. The transition from procentriole to basal body involved lengthening of the procentriolar cylinder and migration to the cell surface, followed by the addition of accessory structures and changes in the internal cartwheel arrangement. The ciliary shaft was assembled after the basal body reached the cell surface.

The second, minor, pathway Anderson and Brenner observed was the centriolar pattern, in which 1 to 10 procentrioles formed at right angles to walls of the preexisting pair of centrioles. As in the acentriolar pathway, these procentrioles also appeared to form from amorphous electron-dense material which in this case surrounded the walls of the mature centrioles and in which their bases were embedded. The maturation pattern of these centrioles was the same as the acentriolar pathway; mature centrioles were released into the cytoplasm and apparently migrated to the surface along with those formed through the acentriolar pathway. Since all procentrioles associated with any one mature centriole were always at the same stage of development, it was inferred that morphogenesis at any one site was synchronous. These workers also observed occasional formation of transitory rudimentary cilia, often with abnormal and incomplete microtubule patterns in cross section. These appeared several days before the main ciliature formed.

Dippell (1968) detailed the sequential assembly of basal body structure in *Paramecium,* as shown in Fig. 2. Basal body assembly in this ciliate is under very tight spaital and temporal control. New basal bodies form immediately anterior to an existing adult basal body but separated by a few hundred angstroms; the majority form in a 20-minute period, 50 minutes before the completion of cell division. Dippell did not find the diverse electron-dense aggregates reported in the vertebrate studies. The first structure she observed was the direct precursor of the basal body cylinder, a flat disk of dense, fibrous material with no discernible substructure. Microtubule assembly always started at a specific point and proceeded around the disk, forming a ring of nine singlet tubules. B-tubule assembly then started, often before completion of all the A tubules and not always sequentially. Dippell observed short fibers between adjacent tubules in the beginning stages, which later disappeared; she speculated that these fibers functioned in

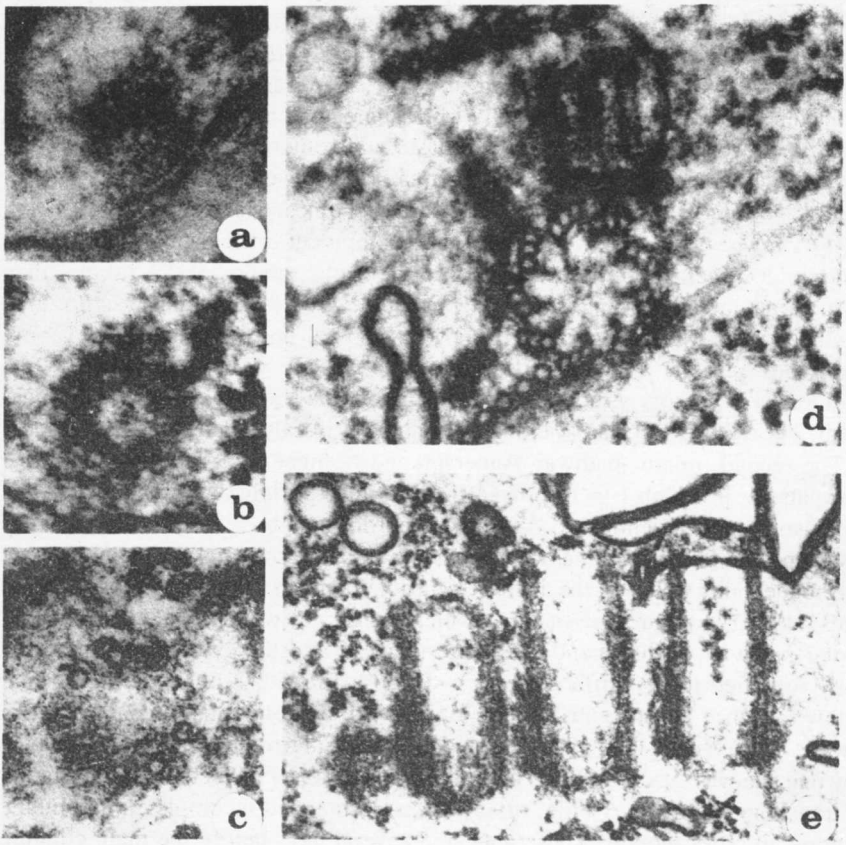

FIG. 2. Morphogenesis of basal bodies in *Paramedium*. (a) Formation of the first tubule. ×140,000. (b) A ring of singlet tubules partially completed. ×138,000. (c) Formation of the B tubules; doublets are partially completed. ×140,000. (d) Adult basal body (in cross section) with a new basal body anterior and at right angles. ×140,000. (e) Four generations of basal bodies. ×68,000. Reprinted from Dippell (1968), with permission.

development of the ninefold symmetry. After the triplet tubules formed, the other internal structures of the central cartwheel appeared. New basal bodies moved to the cell surface, where the cilia ultimately formed. Dippell often observed cytoplasmic microtubules associated with both growing and mature basal bodies; these were never continuous with basal body microtubules but inserted or originated in dense material around the basal body. The position of an existing basal body determined the position of the new one; Dippell occasionally ob-

served new basal bodies forming near immature ones that had not yet moved toward the cell surface.

A sequence of events similar to that reported by Dippell was observed by Johnson and Porter (1968) during formation of basal bodies at cell division in the unicellular biflagellate alga *Chlamydomonas reinhardii*. The earliest stage in basal body development they observed was a ring of nine singlet tubules. Cavalier-Smith (1974) reported that basal body morphogenesis in this organism, as in the vertebrate studies, could proceed through two alternative pathways. In vegetative cells flagella are resorbed prior to cell division, but the basal bodies remain attached to the plasma membrane. A new basal body arises close to the old one, physically attached to the wall of the old one by amorphous material. In zygotes the basal bodies and associated structures as well as the flagella disappear, and there is no trace of the flagellar apparatus throughout zygospore maturation; basal bodies are then assembled *de novo* close to the plasma membrane during zygospore germination. Basal body assembly is apparently the same in both the vegetative and sexual cell cycles, the first recognizable intermediate being the ring of nine singlet tubules around a central cartwheel structure. Cavalier-Smith also occasionally observed disklike structures which may have been earlier stages. The B and C tubules were apparently added on together, followed by elongation of the cylinder and finally the appearance of accessory structures such as roots and striated connections. As in other organisms, flagellar outgrowth began only after complete assembly of the basal body and its attachment to the plasma membrane. The cellular control over the number of flagella and accessory structures appears to be more stringent than control over the number of basal bodies; daughter cells may have four basal bodies but rarely more than one pair of flagella and associated structures.

During mitosis, when the basal bodies are free of flagella, their identification as such or as centrioles is ambiguous. However, although they may sometimes be physically close to the mitotic apparatus, they do not function as mitotic centers (Cavalier-Smith, 1974; Coss, 1974; Johnson and Porter, 1968). Johnson and Porter (1968), however, suggested that their position may be directly involved in determination of the plane of cell division.

Gould (1975) examined intermediate structures in preparations of basal bodies isolated from *Chlamydomonas*, as shown in Fig. 3. He also confirmed that the primary component of basal bodies is in fact the structural microtubule protein tubulin. Gould suggested that his results "revive" the possibility of the generation of new basal bodies by direct nucleation from an existing basal body. His electron micrographs showed that isolated basal body pairs have two probasal bodies attached to them through a complex of associated structures; just after cell division the probasal body consists of a ring or annulus of nine "dots" (rudimentary microtubules) connected to the mature basal body by fibers. This annulus

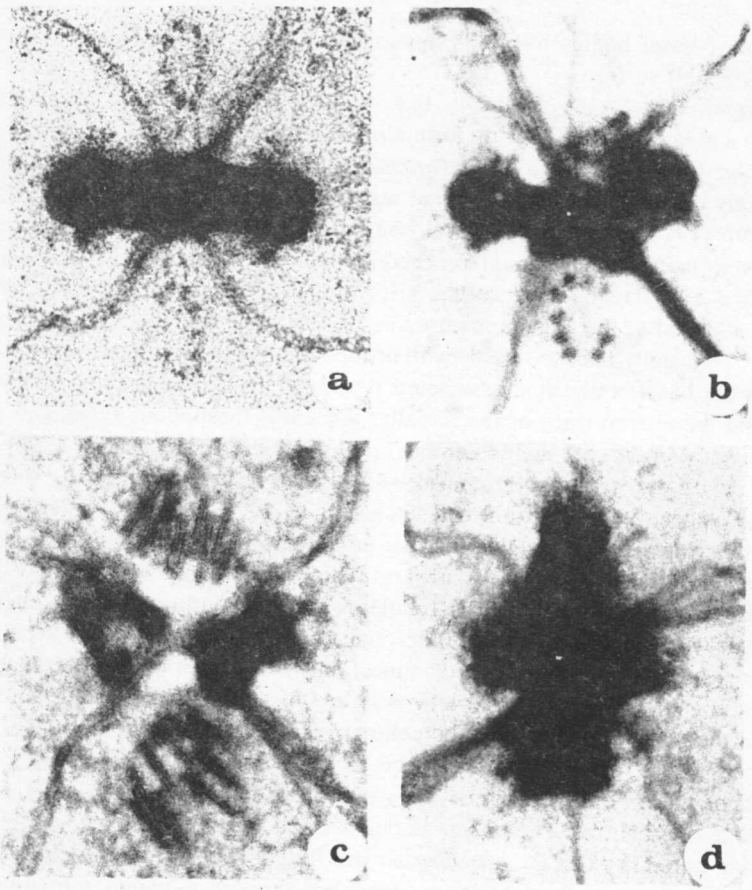

FIG. 3. Isolated basal bodies from *C. reinhardii*. Stages in morphogenesis. (a) Basal body pair isolated after cell division with two probasal bodies each consisting of an annulus of nine components, two of which are connected by long fibers to the proximal end of one of the mature basal bodies. ×33,000. (b and c) Basal body pairs isolated before cell division, showing elongation of the probasal bodies. ×33,000. (d) Basal bodies isolated at the onset of mitosis, showing completion of probasal body maturation. ×33,000. Reprinted from Gould (1975), with permission.

was not visible in thin sections of cells, either because it is too thin or possibly because it is unstable. Just before the next cell division the probasal body elongates and forms a new mature basal body; at this time Gould was able to distinguish that each dot is in fact a triplet microtubule, several of which are connected to the proximal end of the mature basal body by fibers. Gould interpreted his data to mean that development of the probasal bodies proceeds through simultaneous assembly of the A, B, and C tubules, but that the A tubules elongate some-

what faster than the others so that the growing end of the probasal body might appear in cross section to represent a nine-singlet tubule structure.

Basal body morphogenesis has been observed in a variety of other organisms; in many cases it appears to occur very rapidly without obvious intermediate forms. Millecchia and Rudzinska (1970) observed that basal body morphogenesis in the suctorian *Tokophyrya infusionum* proceeded similarly to that in *Paramecium*; new probasal bodies arose adjacent to mature ones, the A tubules being formed first. Allen (1969) reported that the earliest stage he observed in basal body morphogenesis in *Tetrahymena* was a structure containing nine singlet tubules. Fulton and Dingle (1971) reported that no structure resembling a centriole or basal body could be found in the ameboid form of the ameba-flagellate *Naegleria* but that during transformation to the flagellated morphology basal bodies appeared about 10 minutes before the flagella themselves. These investigators found no structural precursors, although it appeared that basal bodies arose within the cytoplasm and then moved to the cell membrane. Outka and Kluss (1967) similarly did not observe basal body precursors in amebas of the ameba-flagellate *Tetramitus rostratus*, but during the ameba-to-flagellate transformation they found basal body-like structures in association with the nuclear membrane or with dense bodies in the cytoplasm. More recently, Ash and Stephens (1975) found that, during ciliagenesis in the gill of the bay scallop, *Aequipecten irradians*, the formation of basal bodies combined two aspects of morphogenesis seen separately in other studies. First, as in vertebrate studies, they observed the appearance of a complex of dense granules but thereafter, as in *Naegleria*, mature basal bodies appeared very rapidly with no obvious organized intermediate stages. Basal body formation was not synchronous within a cell. As in other systems, the elaboration of accessory basal body structures and ciliagenesis was initiated after movement of the mature basal body to the cell surface.

The above-mentioned studies, although differing in detail, give a fairly unified idea of the sequential assembly of basal bodies and centrioles. There are, however, several examples of different—I am tempted to say stranger—modes of formation of basal bodies, particularly in the development of multiple flagellated plant sperm (see reviews by Hepler and Palevitz, 1974; Paolillo, 1975). For example, Mizukami and Gall (1966), and more recently Hepler (1976) and Myles and Hepler (1977), have described spermiogenesis in the fern *Marsilea*. *Marsilea* sperm have over 100 flagella, the basal bodies of which arise from the blepharoplast, a spherical structure 0.8 μm in diameter formed before the last cell division in developing spermatids from a solid sphere of material of moderate electron density and complex substructure. This structure separates into two blepharoplasts which appear to serve as microtubule-organizing centers during assembly of the mitotic spindle, although they do not remain as the focal points of the mitotic tutubles after prophase. During metaphase the blepharoplast becomes hollow, and at this time the walls can be seen to consist of radially