

Basal body/centriole assembly and continuity

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The long-standing interest in centrioles and basal bodies stems from the evolutionary conservation of their structural design and from their dual mode of assembly (templated versus *de novo*), revealed by electron microscopic studies nearly four decades ago and unique for a subcellular organelle. Molecular dissection of the assembly pathway during the past few years has recently progressed, essentially through direct and reverse genetic approaches. These studies revealed essential roles for centrin and the γ -, δ -, ϵ - and η -tubulins in assembly or as specific signals for centriole duplication. Identification of further components of basal bodies and centrioles might help to unravel the two assembly pathways and their regulation.

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Abbreviation

RNAi RNA interference

Introduction

Centrioles and basal bodies display the same highly complex archetypal design known as the ‘centriolar structure’. This structure comprises a large proteinic assembly of nine triplets of microtubules, arranged with a ninefold symmetry into a cylinder of 100–250 nm in diameter and variable length, depending on the organism (Figure 1). Since its first appearance in eukaryotes, this geometry has been found to be strikingly conserved (Figure 2). Rare deviant forms occur in some phyla, however. For example, *Caenorhabditis elegans* centrioles have nine singlets instead of nine triplets of microtubules [1], and *Mastigamoeba* or *Drosophila* embryos have nine doublets [2,3]. Furthermore, some species display basal bodies with six or 10 triplets [4,5].

A basal body, anchored below the plasma membrane, generates a cilium or a flagellum, whereas centrioles, always found in pairs, organise the centrosome near the nucleus. However, at precise stages of cellular or devel-

opmental cycles, one centriole of the pair becomes a basal body to form a primary cilium in mammalian cells or in the sperm flagellum. Conversely, basal bodies that anchor the flagella in interphase turn into centrioles to drive mitosis, as in *Chlamydomonas*.

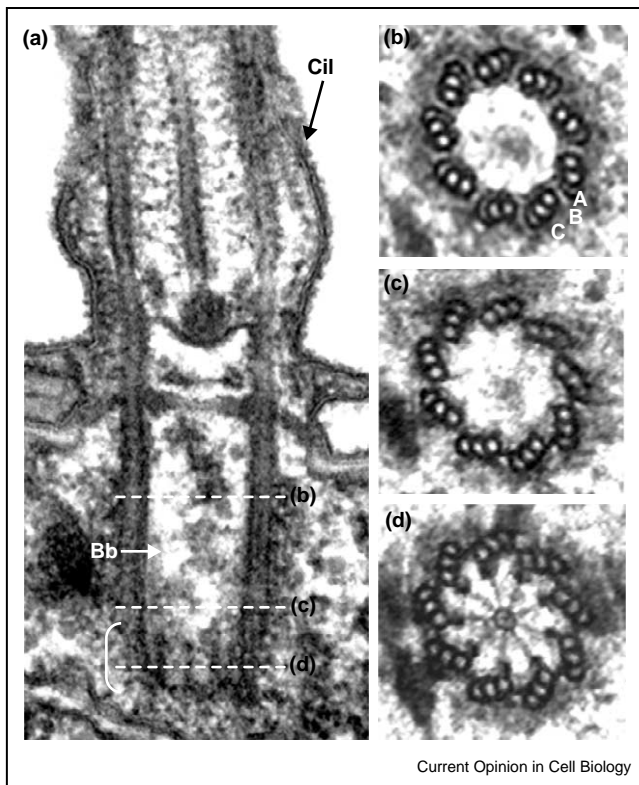
This aptitude to shuttle between cytoplasm and plasma membrane may reflect an ancestral property. Under both functional versions, the organelle exhibits the dual capacity to somehow ‘duplicate’ and it does this at a precise time in the cell cycle. Nevertheless, in various organisms the centriolar structure is not permanent. In the amoebae of *Naegleria*, for example, or during the first divisions of the mouse embryo, centrioles/basal bodies are not detected, but they appear *de novo* at a precise phase of the life cycle. Understanding these phenomena relies on biochemical characterisation of the organelle and the development of experimental approaches. As the organelle is often flanked by appendages [6,7] and tightly coated by pericentriolar material or ‘peribasal body’ material, which target many regulatory molecules [8], its biochemical dissection remains delicate.

In this review, we will discuss how significant progress has been made over the past two years, mainly through either a direct genetic approach in models such as *Chlamydomonas* or *Paramecium*, where mutations affecting basal bodies could be screened, or via a reverse genetic approach, using RNA interference (RNAi).

Proteinic constituents of centrioles/basal bodies

In addition to the microtubular cylinder, various precisely organised filaments, fibres and dense material of unknown composition complete the centriolar design (Figure 2). The major proteins of the microtubule scaffold itself have been identified after purification from ciliary/flagellar axonemes: the $\alpha\beta$ -tubulin dimers, the tightly associated tektins [9] and Sp77 and Sp83 [10]. A post-translational modification of $\alpha\beta$ -tubulins — polyglutamylation — appears as an early marker of centriole/basal body assembly [11,12] and could be important for the stability of the centriolar structure [13]. Table 1 lists the handful of presumed core proteins (i.e. proteins localised on or tightly bound to the centriolar cylinder or within the cylinder) or those specifically required for assembly and/or function of the structure. In *Chlamydomonas*, FLA10p, a kinesin-II protein [14] present in nascent basal bodies [14,15*], is only required for basal body function, since null alleles of the gene block flagellar assembly but leave the structure of basal bodies unaffected [16]. This is also observed in *Tetrahymena* [17]. It is not known if the same

Figure 1



The centriolar structure. The archetypal design is illustrated by thin sections through ciliary basal bodies of *Paramecium*. (a) Longitudinal section through a basal body (Bb) and its cilium (Cil), showing the continuity between basal body and ciliary microtubules. (b–d) Cross sections at different levels along the basal body, from its distal end (b) to its proximal end (d), characterised by an organised array of filaments, the ‘cartwheel’. Several piled up cartwheel discs can be observed in the proximal part of the basal body (bracket in [a]). Other fibrous material are consistently present: in the lumen of the microtubule cylinder, as a dense material lining the inner surface of the cylinder and as links between tubule C of a triplet and tubule A of the adjacent triplet. Courtesy of N Garreau de Loubresse.

remark applies to p210, which localises in the transition region between basal body and flagellum in the green algae *Spermatozopsis similis*. Like FLA10p, however, it is present on nascent basal bodies [18]. Cenexin [19] is a maturation marker of centrioles that is not detected by anticenexin antibodies on basal bodies, and might not be a structural component of the centriolar structure.

γ -Tubulin and centrin(s) seem to be essential proteins. γ -Tubulin is present in the lumen and is tightly bound to the microtubules of the centriolar cylinder [20,21] as well as to the pericentriolar material. Centrins, with different isotypes expressed both in mammals [22] and *Paramecium* [23], are homologues of the spindle pole body component CDC31p. They localise to the centriole/basal body lumen and to the links between paired centrioles/basal bodies [23–25,26*].

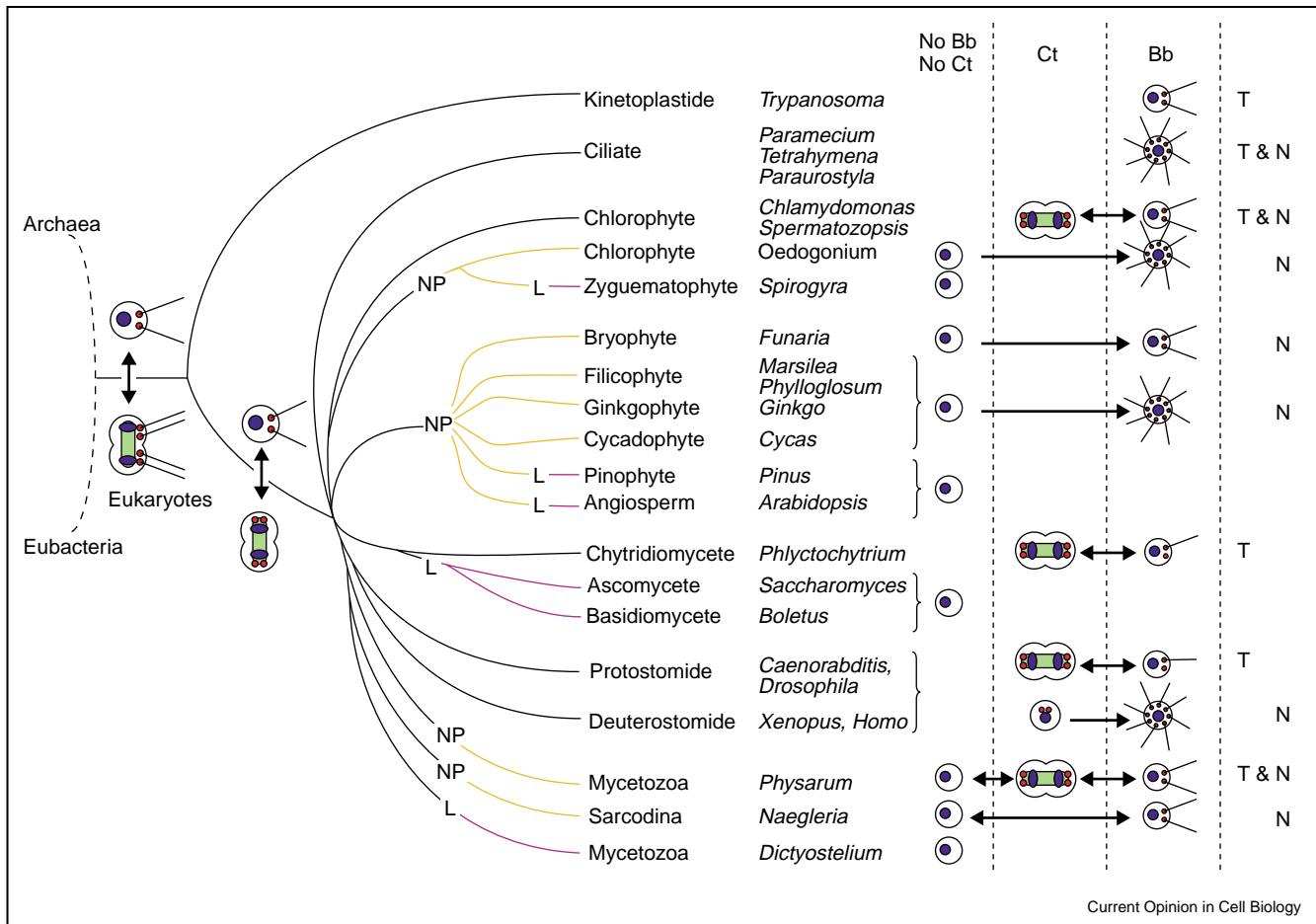
The recently identified tubulin subfamilies (for reviews, see [27,28]) are all involved in the biogenesis of basal bodies/centrioles. While δ - and ϵ -tubulins are found in protozoans and vertebrates, ζ - and η -tubulins seem restricted to a single species or phylum. The sole ϵ subfamily appears monophyletic. δ -Tubulins have identical functions across organisms — genetically ascertained in *Chlamydomonas* [29] and *Paramecium* [30*]. Despite this, their evolutionary status is not clear, and they may have evolved from different ancestral tubulins. It is therefore conceivable that, even though homologues of η - or ζ -tubulins seem absent from the sequenced vertebrate genomes, their function may be fulfilled by one of the divergent tubulins present in the human genome and not yet studied. VFL1p [31] and Bld10p [32], products of two *Chlamydomonas* genes identified by mutations, present interesting localisations: in a rotationally asymmetric pattern at the distal ends of the basal bodies, and at the cartwheel, respectively.

Two assembly pathways?

Ultrastructural studies have led to the belief that assembly of a new centriolar structure can proceed via two partially distinct mechanisms (Figure 3) — commonly referred to as the ‘templated’ and ‘*de novo*’ pathways [15*] (i.e. in the presence or absence of a pre-existing one). Where the ultrastructure of *de novo* assembly steps has been analysed, cartwheel-like structures (see Figure 1d) have been observed [33,34]. A similar scaffold imparting the ninefold symmetry might then be a common step along both templated and *de novo* pathways. Despite their phenomenological significance, the terms ‘templated’ versus ‘*de novo*’ are somewhat misleading. They imply the established existence of a template, provided by the mother organelle, which would be required in one case and dispensable in the other. Furthermore, they imply that, regardless of the pathway, the end products are identical and this may not always be true, since basal bodies formed *de novo* in many cases (plant gametes or ciliated epithelia) have no progeny. This functional difference is striking in certain annelids that form basal bodies in parallel in two types of spermatozoa, eusperm (for fertilisation) by the templated mode and parasperm (for enhanced motility of the eusperm) by the *de novo* mode [35].

The ability of cells that normally use the templated mode to re-form basal bodies/centrioles *de novo* has been assayed in different systems, with contrasting results. Two new approaches have recently been devised, however. In *Chlamydomonas*, where the *vfl2* mutation yields aflagellate cells thought to be devoid of pro-basal bodies, return to the flagellate phenotype was observed [15*]. In CHO cells, after centrosome ablation by laser microsurgery, *de novo* formation of pericentriolar material, followed by reappearance of centrioles, was observed [36**]. Interestingly, in the latter case random numbers of centrioles were produced. It is therefore possible, as

Figure 2



A phylogenetic tree of the centriolar structure. Basal bodies (Bb) and centrioles (Ct) are exclusively eukaryotic organelles. It is believed that the ancestral unicellular eukaryotes possessed two basal bodies with a unique function — that is, they nucleate the flagella and duplicate at each division, a situation perpetuated in organisms such as *Trypanosoma*. Later on in the course of evolution, basal bodies also participate in the organisation of the mitotic spindle, a dual function illustrated by *Chlamydomonas* in which the two basal bodies lose their flagella at the onset of mitosis and become the centrioles of two centrosomes. In organisms that retain basal bodies or centrioles as permanent organelles, duplicating at each division, two major situations are found. First, cells possess many basal bodies that are not involved in mitosis, as in ciliates. Alternatively, the typical alternance basal body/centriole is conserved, but the centriole predominates. This occurs in mammals (i.e. one centriole becomes a basal body in order to generate the so-called primary cilium and the spermatozoon flagellum) and in *Physarum* (i.e. centrioles turn into basal bodies only after induction of the transient and facultative flagellate form). In plants, however, centrioles and basal bodies were either totally lost (L) or are lost as permanent organelles (NP) that are only retained to generate male gamete flagella (e.g. as in ferns, *Cycas* and *Ginkgo*). Finally, sporadic, ‘late’ total loss of the organelle occurs in fungi and some protists, such as *Dictyostelium*. Interestingly, across these different evolutionary routes (except in branches characterised by complete loss), neogenesis (N) of centrioles/basal bodies is observed either as the sole mode of assembly or as an alternative to templated assembly (T). Neogenesis is restricted to particular physiological or developmental conditions (see text for full details). White circles and dumbbells represent interphase and dividing cells, respectively; blue circles correspond to nuclei, red small circles to basal bodies/centrioles. Green bars represent mitotic spindles, and cilia/flagella are shown as straight lines emanating from the centrioles/basal bodies.

proposed in the *Chlamydomonas* study [15^{*}], that the templated pathway is simply the kinetically dominant pathway, repressing the default *de novo* pathway. However, owing to a present inability to detect the earliest stages of assembly, it remains difficult to definitely rule out the possibility that the absence of a young pro-basal body/pro-centriole is not merely a lack of the ability to detect them with the available immunofluorescent probes.

Beyond speculation on the initial trigger for these two pathways, their regulation remains a major difference. In the templated pathway, the timing and steps of duplication are tightly controlled by the cell cycle, with specific adjustments according to cell type [37], while completely different physiological conditions trigger the naturally occurring *de novo* pathway. Regardless of the differences between the two modes, two objectives have to be reached: dissect the steps of the assembly line(s), and

Table 1

Proteins specifically involved in assembly/duplication of centrioles and/or the basal body.

Protein	Centriole	Basal body	Experimental evidence	Organism	References
α/β -Tubulin	+	+	Cytological/biochemical/genetic	All eukaryotes	
Tektins	+	+	Cytological/biochemical	All eukaryotes?	
Sp77, Sp83	+	+	Cytological/biochemical	All eukaryotes?	
γ -Tubulin	+	+	Cytological/genetic	<i>Paramecium</i> <i>Tetrahymena</i>	[20,21,51,52*]
δ -Tubulin	+	+	Genetic	<i>Chlamydomonas</i> <i>Paramecium</i>	[29,30*]
ϵ -Tubulin	+	+	Genetic	<i>Chlamydomonas</i> <i>Paramecium</i> <i>Trypanosoma</i>	[44**,45**]
η -Tubulin		+	Genetic	<i>Paramecium</i>	[56]
Centrin (s)	+	+	Cytological/genetic	Mammalian cells <i>Chlamydomonas</i> <i>Paramecium</i>	[22–25,26*,43**]
Cenexin	+		Cytological	Chicken, human	[19]
VFL1p		+	Cytological	<i>Chlamydomonas</i>	[31]
p210		+	Cytological	<i>Spermatozopsis</i>	[18]
CNap1p	+		Cytological	KE37 cells	[65]
FLA10p	+	+	Cytological/genetic	<i>Chlamydomonas</i>	[14]
Bld10p		+	Genetic/cytological	<i>Chlamydomonas</i>	[32]

Only the following proteins are listed: true core proteins, such as $\alpha\beta$ -tubulins; proteins shown, using electron microscopy immunolocalisation, to be localised on or tightly bound to the centriolar cylinder or within the cylinder; and proteins shown, in particular by genetic evidence, to be specifically required for assembly or structural integrity of the structure, to the exclusion of more general regulatory factors and signals involved in the cell and centrosome cycle.

identify what is provided by the mother organelle in the templated mode.

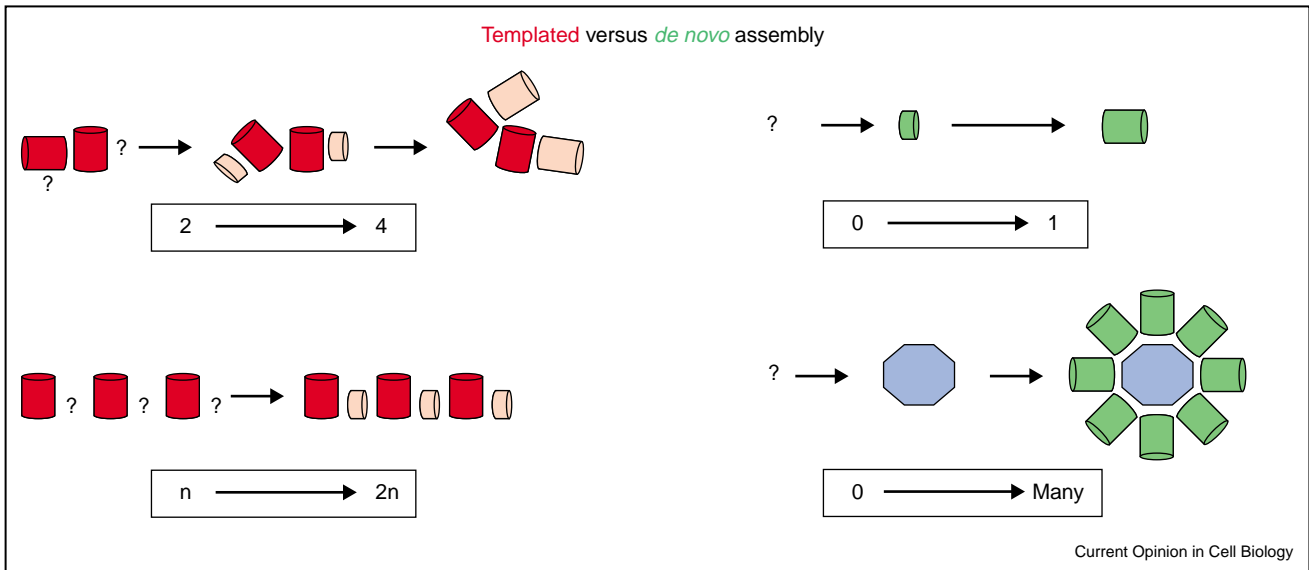
The assembly line(s)

A role of centrin in the assembly of centriolar structures, suggested by the function of *cdc31* in budding yeast and the effects of the *cpf2* mutations in *Chlamydomonas*, has been sought in a range of organisms. In the early steps of *de novo* assembly in *Naegleria* [38] and *Marsilea* [39*], a spot of centrin, as well as γ -tubulin and pericentrin [40], was detected by immunofluorescence, before appearance of $\alpha\beta$ -tubulin, which accompanies the development of the basal bodies. However, centrin RNAi experiments in *Marsilea* remained inconclusive, as centrin depletion blocked the divisions that subtend appearance and development of the blepharoplast [41], the massive precursor of the neofomed basal bodies (see Figure 3). Expression of the human centrin HsCen3p blocked spindle pole body duplication in yeast and centrosome duplication in *Xenopus* embryos [22]. Direct evidence from RNAi experiments showing that centrin-2, whose phosphorylation is crucial for the centriole duplication cycle [42], is indeed required for centriole duplication has recently been obtained in HeLa cells [43**]. This does not preclude a role for other centrin isoforms, however: centrin RNAi experiments in *Chlamydomonas* indicate a role in duplication and distribution of basal bodies (B Koblentz *et al.*, personal communication). The recent ability to express human centrins in *Paramecium* (F Ruiz, personal communication) opens further experimental possibilities for a refined analysis of centrin function.

New data published recently concerns the development of the pro-basal body/pro-centriole and in particular of the microtubule scaffold. In this scaffold (Figure 1b–d), each triplet comprises a complete microtubule (the innermost, called the A tubule) fused to two incomplete microtubules (tubules B and C, the outermost). The genetic study of δ - and ϵ -tubulins [29,30*,44**,45**] have confirmed convergent ultrastructural data [46,47]: A tubules assemble first, then B tubules and finally C tubules (Figure 4). It is not clear yet whether ϵ - and δ -tubulins are directly instrumental in shaping/priming the B and C tubules, respectively; or if they are involved in the stabilisation of the nascent organelle, as suggested in *Chlamydomonas* by revertants of the bald-2 mutant [48]. This ontogeny is consistent with the existence, in certain invertebrate species (devoid of δ - and ϵ -tubulins), of centrioles ‘arrested’ at a nine-singlet or nine-doublet stage. Genetic and cytological data on *Chlamydomonas* [44**] and *Paramecium* [45**] demonstrate that ϵ -tubulin, which localises at basal bodies and centrioles [49], is necessary to the cohesion of the centriolar structure. Little is known of the upstream events, except that centrin and γ -tubulin concentrate at the assembly site and that a pre-pattern for the ninefold symmetry is set before microtubule assembly. This is supported by electron micrographic studies and by the observation that, in different mutational contexts, absence of one or more triplets does not alter the underlying ninefold symmetry [30*,48,50].

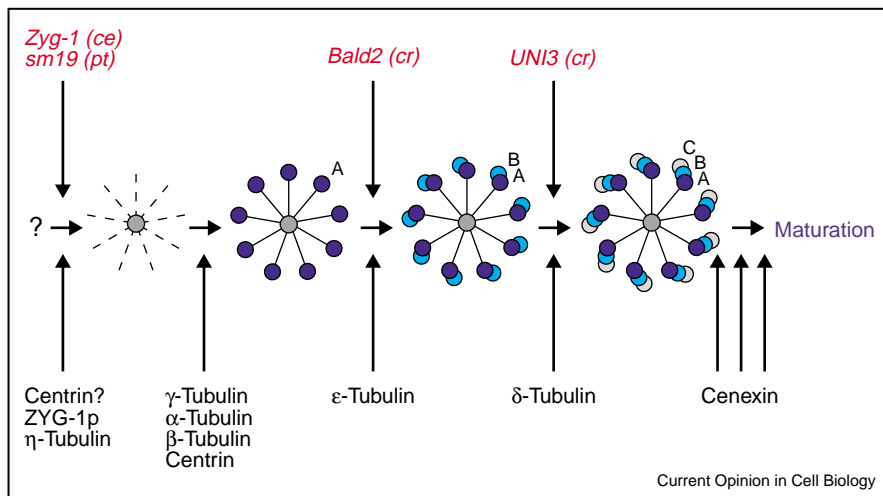
γ -Tubulin might have multiple functions. For example, in *Paramecium* [51] and *Tetrahymena* [52*] it is required for

Figure 3



De novo (neogenesis) versus templed basal body/centriole assembly. The templed assembly process results in centriole/basal-body doubling. The question marks symbolise unknown information that indicates the place of assembly of the new centriole or basal body. Whether in pairs (centrioles in centrosomes, basal bodies in *Chlamydomonas*) or arranged in large ensembles (in the ciliate cortex), each centriole/basal body (dark pink) promotes the 'budding' of a new centriole/basal body (light pink), close to and at right angles with the pre-existing one. The pro-centriole/pro-basal body (short, light pink cylinders) is the first morphological sign of assembly. *De novo* assembly. Two types of situations are recorded here. Few, generally two, centrioles or basal bodies (green) are assembled in the absence of a pre-existing centriole (*Physarum*, mouse egg) or basal body (*Naegleria*). It is not known whether in these cases the *de novo* assembly leads to two centrioles/basal bodies or to a single centriole/basal body which subsequently duplicates by the templed mechanism. By contrast, when many basal bodies are to be assembled, a structure (called blepharoplast in Ferns, *Gingko*, *Cycas*, and deuterosome in ciliated animal epithelial cells) develops (blue) from which basal bodies are assembled and then migrate to the cell membrane. The similarities, if any, between the sites of *de novo* and templed basal body/centriole assembly remain unknown. Again, the question marks illustrate unknown initial information.

Figure 4



The assembly line(s). This oversimplified scheme is not a metabolic pathway. It artificially separates steps that most probably overlap. The recorded steps correspond to the present state of knowledge. A black box (question mark) still shields the very initial steps; the nine-branched star symbolises the required prepattern; the sequential addition of tubules A, B and C is now attested by the effects of genetic blocks at nine-singlet and nine-doublet stages. The vertical arrows for $\alpha\beta$ -, γ -tubulins and centrin only mark their first point of entry into the assembly line. The significant mutational blocks are indicated in red. *ce*, *Caenorhabditis elegans*; *cr*, *Chlamydomonas reinhardtii*; *pt*, *Paramecium tetraurelia*.

basal body duplication, and in *Tetrahymena* [52*] and *Drosophila* it might be necessary for stabilisation of centriolar microtubules (Raynaud-Massina *et al.*, personal communication). γ -tubulin gene silencing in other cellular models essentially blocks cell division, and hence any further centriole duplication. The fact that in *Paramecium* or *Tetrahymena* the block of basal body duplication appears prominent probably reflects specific dynamic properties of these microtubule arrays, just as γ -tubulin RNAi experiments in *C. elegans* have revealed different properties of aster microtubules at different developmental stages [53,54].

Further upstream, other factors, distinct from the general cell cycle signals, seem specifically to control an on/off switch, for example the kinase Zyg-1p [55**] in *C. elegans*, or η -tubulin [56], which is thought to be a target for a mitotic signal in *Paramecium* (Ruiz *et al.*, personal communication).

Role of the pre-existing organelle

Does the mother organelle actually provide a template? The idea was first suggested from studies on ciliates before the electron microscopy era, and was later sustained by the lurking possibility, now ruled out [57], that basal bodies contained nucleic acids. The only way to demonstrate that the mother organelle acts as a template or that there is any structural continuity would be to create mutants that affect symmetry and follow their hereditary transmission.

The hypothesis that there is template cannot be rejected, because in various experimental or natural situations, absence/ablation of centrioles leaves the egg or cell unable to assemble the missing organelle. However, because the daughter organelle develops at a distance, the template cannot be the mother itself and must be a mobile structure. A logical hypothesis [58] proposed that cartwheel discs (see Figure 1), assembled within the centriolar structure, could be released to either initiate assembly (templated mode) or to store the structural information in a form that could well escape detection, as long as its molecular components are unknown (apparent *de novo* mode). Interestingly, the first protein localised at the cartwheel has just been characterised in *Chlamydomonas* [32].

But even the existence of a structural template does not account for the fact that the mother organelle provides a positional signal, which is critical, since the precise positioning of the daughter organelle and its links with the mother will later ensure the precise spatial distribution of mother and daughter. A circumferential polarity of the organelle or its immediate environment is well documented in protists [59] and likely to operate also in metazoan cells. This implies that a mother organelle displays a single site supporting daughter assembly. Recent data

[60*] challenge this notion: under *cdk1* inactivation in *Drosophila* wing discs, two successive daughters can develop at different sites around the mother. Whether this observation reflects an intrinsic general potential of centriolar structures or an immature condition [2] of these particular centrioles remains to be elucidated. Maturation is important for functional competence, nucleation/positioning of appendages and may be required for the organisation of space around a centriole or basal body.

Regardless of its *de novo* or templated origin, positioning of a newly formed centriolar structure is crucial for its function. The templated way, obeying the characteristic orthogonal configuration and channelled by links between mother and daughter, ensures transmission of the spatial information for cytoskeleton organisation, as well documented in protists [59] and also for whole cell polarities, as shown in *Trypanosoma* [61**]. The continuity of centriole/basal body lineage relays this information across cell divisions.

In the *de novo* pathway, the presence of a pre-determined site for assembly and/or insertion of newly formed basal bodies is suggested in different systems. For example, in *Naegleria*, proteins involved in basal body development concentrate at a single submembrane site. In other organisms when the templates have disappeared during encystment or sexual processes, as in *Chlamydomonas* [62] or certain ciliates [63,64], new basal bodies are always formed just below the plasma membrane, and something ensures their precise location and orientation. Even though no sign of a vestigial organelle can then be detected, at least in ciliates, the cell cortex retains a precise memory of the number and localisation of the pre-existing organelles. It has been suggested that such surface-stored elements might be involved in the resurgence of centrioles in the mouse embryo [65]. The major properties of centriolar structures — duplication, localisation and links between mother and daughter — thus need to be tightly regulated. This is manifest from many current studies on factors such as CNap-1 and on the interplay between kinases and phosphatases controlling duplication and disorientation of centrioles [66,67].

Centriole/basal body life cycle

Centrioles and basal bodies issued from the templated pathway are stable entities [68] that assemble, mature and in turn produce successive daughter organelles. Centriole length and time to maturation and competence for templating a new organelle all appear to be strictly regulated properties. For example, de-regulation of length control could be induced in *Drosophila* wing discs upon *cdk1* inactivation [60*]. It is not known whether centriolar structures senesce and/or are renewed at some stage during successive cell generations. The continuity of the centriolar lineage is sustained not only across mitotic divisions but extends across sexual reproduction, follow-

ing a uniparental transmission, and is achieved by a variety of mechanisms. This suggests that the two centrioles of the pair are not homologous and that the functional entity is the pair of centrioles. Indeed the two centrioles — or the two or more basal bodies in biflagellates or pluriflagellates — are of different ‘ages’, the younger maturing over one or more cell cycles. In ciliates also, all the basal bodies — of different ages — form together the functional structure for cell morphogenesis. Maturation is accompanied by physical and chemical changes, and by adjunction of appendages or new molecular markers such as cenexin [19,69]. It is also accompanied by an aptitude to carry out different cytoskeletal functions (e.g. nucleation of a primary cilium, control of cytokinesis [70] and organisation of space, and in particular differences in microtubule nucleation) [6,71]. It is significant that impairment of basal body development by mutation [29] or inactivation of δ - or ϵ -tubulin [30*,44**] also impairs the organisation of the basal body appendages.

Conclusions and perspectives

Most of the problems discussed here have been raised for over 40 years, and answers are just beginning to be found as adequate experimental approaches mature. Some of the significant progress in the biology of centriolar structures results from the characterisation or refined analysis of long-known mutations such as *bld2* in *Chlamydomonas*, or *Zyg1* in *C. elegans*. Other important steps have been accomplished by applying RNAi technology to known candidate genes, such as those expressing centrin or γ -tubulin. New core proteins and regulatory factors have been identified, and the next step should be to understand their function and identify their partners.

With completion of genome sequences, development of proteomics and the continuing search for new mutants in favourable organisms, within a very few years we can expect to receive answers to many of the questions raised regarding assembly and continuity of centriolar structures.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
 - of outstanding interest
1. O’Connell K: **The centrosome of the early *C. elegans* embryo: inheritance, assembly, replication, and developmental roles.** *Curr Top Dev Biol* 2000, **49**:365-384.
 2. Callaini G, Whitfield W, Riparbelli M: **Centriole and centrosome dynamics during the embryonic cell cycles that follow the formation of the cellular blastoderm in *Drosophila*.** *Exp Cell Res* 1997, **234**:183-190.
 3. Simpson AGB, Bernard C, Fenchel T, Patterson DJ: **The organization of *Mastigamoeba schizophrenia* n. sp: More evidence of ultrastructural idiosyncrasy and simplicity in pelobionts protists.** *Eur J Protozool* 1997, **33**:87-98.
 4. Schrevel J, Besse C: **A functional flagella with a 6 + 0 pattern.** *J Cell Biol* 1975, **66**:492-507.
 5. Mansir A, Justine J: **The microtubular system and posttranslationally modified tubulin during spermatogenesis in a parasitic nematode with amoeboid and aflagellate spermatozoa.** *Mol Reprod Dev* 1998, **49**:150-167.
 6. Bornens M: **Centrosome composition and microtubule anchoring mechanisms.** *Curr Opin Cell Biol* 2002, **14**:25-34.
 7. Preble A, Giddings T Jr, Dutcher S: **Basal bodies and centrioles: their function and structure.** *Curr Top Dev Biol* 2000, **49**:207-233.
 8. Doxsey S: **Centrosomes as command centres for cellular control.** *Nat Cell Biol* 2001, **3**:E105-108.
 9. Stephens R, Lemieux N: **Tektins as structural determinants in basal bodies.** *Cell Motil Cytoskeleton* 1998, **40**:379-392.
 10. Hinchcliffe E, Linck R: **Two proteins isolated from sea urchin sperm flagella: structural components common to the stable microtubules of axonemes and centrioles.** *J Cell Sci* 1998, **111**:585-595.
 11. Million K, Larcher J, Laoukili J, Bourguignon D, Marano F, Tournier F: **Polyglutamylation and polyglycylation of alpha- and beta-tubulins during *in vitro* ciliated cell differentiation of human respiratory epithelial cells.** *J Cell Sci* 1999, **112**:4357-4366.
 12. Lehtreck K, Geimer S: **Distribution of polyglutamylated tubulin in the flagellar apparatus of green flagellates.** *Cell Motil Cytoskeleton* 2000, **47**:219-235.
 13. Bobiniec Y, Khodjakov A, Mir L, Rieder C, Edde B, Bornens M: **Centriole disassembly *in vivo* and its effect on centrosome structure and function in vertebrate cells.** *J Cell Biol* 1998, **143**:1575-1589.
 14. Vashishtha M, Walther Z, Hall J: **The kinesin-homologous protein encoded by the *Chlamydomonas* FLA10 gene is associated with basal bodies and centrioles.** *J Cell Sci* 1996, **109**:541-549.
 15. Marshall W, Vucica Y, Rosenbaum J: **Kinetics and regulation of *de novo* centriole assembly. Implications for the mechanism of centriole duplication.** *Curr Biol* 2001, **11**:308-317.
This interesting study takes advantage of the *Chlamydomonas vfl1* mutation known to perturb basal body distribution at division and to produce cells devoid of flagella. In such cells, lack of basal bodies was deduced from immunofluorescence observation of the absence of labeling by antibodies against different basal body components. Reappearance of flagella in 50% of individual cells followed over one or two divisions and return to 100% flagellated cells after around five divisions in small populations derived from single aflagellate cells indicate that cells probably devoid of basal bodies can re-form them *de novo*. While not definitive, these experiments and their discussion undoubtedly rekindle an old debate.
 16. Matsuura K, Lefebvre P, Kamiya R, Hirono M: **Kinesin-II is not essential for mitosis and cell growth in *Chlamydomonas*.** *Cell Motil Cytoskel* 2002, **52**:195-201.
 17. Brown J, Marsala C, Kosoy R, Gaertig J: **Kinesin-II is preferentially targeted to assembling cilia and is required for ciliogenesis and normal cytokinesis in *Tetrahymena*.** *Mol Biol Cell* 1999, **10**:3081-3096.
 18. Lehtreck K, Bornens M: **Basal body replication in green algae — when and where does it start?** *Eur J Cell Biol* 2001, **80**:631-641.
 19. Lange B, Gull K: **A molecular marker for centriole maturation in the mammalian cell cycle.** *J Cell Biol* 1995, **130**:919-927.
 20. Fuller S, Gowen B, Reinsch S, Sawyer A, Buendia B, Wepf R, Karsenti E: **The core of the mammalian centriole contains gamma-tubulin.** *Curr Biol* 1995, **5**:1384-1393.
 21. Moudjou M, Bordes N, Paintrand M, Bornens M: **Gamma-tubulin in mammalian cells: the centrosomal and the cytosolic forms.** *J Cell Sci* 1996, **109**:875-887.

22. Middendorp S, Kuntziger T, Abraham Y, Holmes S, Bordes N, Paintrand M, Paoletti A, Bornens M: **A role for centrin 3 in centrosome reproduction.** *J Cell Biol* 2000, **148**:405-416.
23. Klotz C, Garreau DLN, Ruiz F, Beisson J: **Genetic evidence for a role of centrin-associated proteins in the organization and dynamics of the infraciliary lattice in *Paramecium*.** *Cell Motil Cytoskeleton* 1997, **38**:172-186.
24. Paoletti A, Moudjou M, Paintrand M, Salisbury J, Bornens M: **Most of centrin in animal cells is not centrosome-associated and centrosomal centrin is confined to the distal lumen of centrioles.** *J Cell Sci* 1996, **109**:3089-3102.
25. Laoukili J, Perret E, Middendorp S, Houcine O, Guennou C, Marano F, Bornens M, Tournier F: **Differential expression and cellular distribution of centrin isoforms during human ciliated cell differentiation *in vitro*.** *J Cell Sci* 2000, **113**:1355-1364.
26. Ruiz-Binder N, Geimer S, Melkonian M: ***In vivo* localization of centrin in the green alga *Chlamydomonas reinhardtii*.** *Cell Motil Cytoskel* 2002, **52**:43-55.
- This accurate immunofluorescence and electron microscopy study used an integrated single copy of centrin-GFP and demonstrated the global functionality of the incorporated tagged centrin. The authors also showed the presence of ultrastructural defects undetected by fluorescence microscopy.
27. McKean P, Vaughan S, Gull K: **The extended tubulin superfamily.** *J Cell Sci* 2001, **114**:2723-2733.
28. Dutcher S: **The tubulin fraternity: alpha to eta.** *Curr Opin Cell Biol* 2001, **13**:49-54.
29. Dutcher S, Trabuco E: **The UNI3 gene is required for assembly of basal bodies of *Chlamydomonas* and encodes delta-tubulin, a new member of the tubulin superfamily.** *Mol Biol Cell* 1998, **9**:1293-1308.
30. Garreau de Loubresse N, Ruiz F, Beisson J, Klotz C: **Role of delta-tubulin and the C-tubule in assembly of *Paramecium* basal bodies.** *BMC Cell Biol* 2001, **2**:4.
- The effects of silencing the δ -tubulin gene of *Paramecium* were examined using both immunofluorescence and electron microscopy. The authors showed a generalised loss of the C tubule in basal body cross sections, as well as secondary tubule loss and alterations in cortical cytoskeleton.
31. Silflow C, LaVoie M, Tam L, Tousey S, Sanders M, Wu W, Borodovsky M, Lefebvre PA: **The Vfl1 Protein in *Chlamydomonas* localizes in a rotationally asymmetric pattern at the distal ends of the basal bodies.** *J Cell Biol* 2001, **153**:63-74.
32. Matsuura K, Lefebvre PA, Kamiya R, Hirono M: **A novel component of the flagellar basal body essential for assembly [abstract].** 10th International Conference on the Cell and Molecular Biology of *Chlamydomonas*, June 11-16 2002, Vancouver, Canada.
33. Mizukami I, Gall J: **Centriole replication. II. Sperm formation in the fern, *Marsilea*, and the cycad, *Zamia*.** *J Cell Biol* 1966, **29**:97-111.
34. Renzaglia K, Maden A: **Microtubule organizing centers and the origin of centrioles during spermatogenesis in the pteridophyte *Phylloglossum*.** *Microsc Res Tech* 2000, **49**:496-505.
35. Ferraguti M, Fascio U, Boi S: **Mass production of basal bodies in paraspermiogenesis of Tubificinae (Annelida, Oligochaeta).** *Biol Cell* 2002, **94**:109-115.
36. Khodjakov A, Rieder C, Sluder G, Cassels G, Sibon O, Wang C: ***De novo* formation of centrosomes in vertebrate cells arrested during S phase.** *J Cell Biol* 2002, **158**:1161-1181.
- Using CHO cells arrested in S phase by hydroxyurea and a new method (laser irradiation) to eliminate the centrosome, the authors followed, by differential interference contrast (DIC) time-lapse microscopy and three-dimensional fluorescence of green fluorescent protein (GFP)- γ -tubulin, re-formation of a centrosomal cloud followed by the reappearance of centrioles. Most interestingly, electron microscopic observations show that this *de novo* formation leads to multiple centrioles and seems to occur by the type of mechanism naturally at play during ciliogenesis in epithelial cells.
37. Hinchcliffe E, Sluder G: **Two for two: Cdk2 and its role in centrosome doubling.** *Oncogene* 2002, **21**:6154-6160.
38. Levy Y, Lai E, Remillard S, Fulton C: **Centrin is synthesized and assembled into basal bodies during *Naegleria* differentiation.** *Cell Motil Cytoskeleton* 1998, **40**:249-260.
39. Klink V, Wolniak S: **Centrin is necessary for the formation of the motile apparatus in spermatids of *Marsilea*.** *Mol Biol Cell* 2001, **12**:761-776.
- Although not directly conclusive with respect to a possible essential role of centrin in blepharoplast formation, this paper is of interest as it reports the development of an efficient RNA interference method by direct addition of double-stranded RNA to dry spores at the time of inhibition.
40. Suh M, Han J, No Y, Lee J: **Transient concentration of a gamma-tubulin-related protein with a pericentrin-related protein in the formation of basal bodies and flagella during the differentiation of *Naegleria gruberi*.** *Cell Motil Cytoskeleton* 2002, **52**:66-81.
41. Tsai C, Wolniak S: **Cell cycle arrest allows centrin translation but not basal body formation during spermiogenesis in *Marsilea*.** *J Cell Sci* 2001, **114**:4265-4272.
42. Lutz W, Lingle W, McCormick D, Greenwood T, Salisbury J: **Phosphorylation of centrin during the cell cycle and its role in centriole separation preceding centrosome duplication.** *J Biol Chem* 2001, **276**:20774-20780.
43. Salisbury J, Suino K, Busby R, Springett M: **Centrin-2 is required for centriole duplication in Mammalian cells.** *Curr Biol* 2002, **12**:1287-1292.
- A specific and high level of inhibition of centrin-2 expression in HeLa cells was obtained by transfection of a homologous hCetn-2 short interfering RNA. Immunofluorescence analysis revealed centriole duplication arrest, although cells could undergo two or three more divisions, leading to spindle poles with one or no centriole, confirmed by electron microscopy, or to multipolar mitoses, owing to a failure of completion of cytokinesis. These data are the first direct evidence for a role of centrin in centriole duplication. Furthermore, the fact that its phosphorylation was shown to play a role in centriole separation [42] provides an approach to the mode of action of hCetn-2.
44. Dutcher SK, Morissette NS, Preble A, Rackley C, Stanga J: **Epsilon-tubulin is an essential component of the centriole.** *Mol Biol Cell* 2002, **13**:in press.
- The long-known *bald-2* mutation, shown to affect basal body structure (i.e. it leads to a failure to assemble B and C tubules) was cloned and shown to cause a premature stop codon in the ϵ -tubulin gene of *Chlamydomonas*. Using immunofluorescence, ϵ -tubulin was shown to localise at basal bodies. This genetic demonstration constitutes a decisive step forward in the dissection of basal body assembly.
45. Dupuis-Williams P, Fleury-Aubusson A, Garreau de Loubresse N, Geoffroy H, Vayssié L, Galvani A, Espigat A, Rossier J: **Functional role of ϵ -tubulin in the assembly of the centriolar microtubule scaffold.** *J Cell Biol* 2002, **158**:1183-1193.
- The ϵ -tubulin-encoding gene was cloned in the course of a PCR search for new tubulins in the *Paramecium* genome and its function examined using gene silencing. Using specific antibodies against the *Paramecium* protein and double labelling with anti- $\alpha\beta$ - and anti- ϵ -tubulins, localisation on basal body microtubules — especially at both the proximal and distal ends of basal bodies — was demonstrated. RNA interference experiments performed using the two available methods (microinjection and feeding) yielded impaired basal body duplication and loss of microtubules, mostly B and C tubules among the triplets. These observations suggest that ϵ -tubulin is incorporated in the basal body microtubule shaft.
46. Dippell R: **The development of basal bodies in *Paramecium*.** *Proc Natl Acad Sci USA* 1968, **61**:461-468.
47. Anderson R, Brenner R: **The formation of basal bodies (centrioles) in the Rhesus monkey oviduct.** *J Cell Biol* 1971, **50**:10-34.
48. Preble A, Giddings T Jr, Dutcher S: **Extragenic bypass suppressors of mutations in the essential gene BLD2 promote assembly of basal bodies with abnormal microtubules in *Chlamydomonas reinhardtii*.** *Genetics* 2001, **157**:163-181.
49. Chang P, Stearns T: **Delta-tubulin and epsilon-tubulin: two new human centrosomal tubulins reveal new aspects of centrosome structure and function.** *Nat Cell Biol* 2000, **2**:30-35.
50. Ruiz F, Garreau de Loubresse N, Beisson J: **A mutation affecting basal body duplication and cell shape in *Paramecium*.** *J Cell Biol* 1987, **104**:417-430.
51. Ruiz F, Beisson J, Rossier J, Dupuis-Williams P: **Basal body duplication in *Paramecium* requires gamma-tubulin.** *Curr Biol* 1999, **9**:43-46.

52. Shang Y, Li B, Gorovski MA: ***Tetrahymena thermophila* contains a conventional γ -tubulin that is differentially required for the maintenance of different MTOCs.** *J Cell Biol* 2002, **158**:1195-1206.

The *Tetrahymena* *GTU1* gene, which encodes γ -tubulin, was placed under the control of an inducible/repressible promoter and the effects of γ -tubulin depletion examined. Anti-HA antibodies were used to localise the HA-tagged GTU1p. While overexpression had no detectable effect, depletion of γ -tubulin affected differently the diverse microtubular systems. Depletion affect basal bodies most prominently, which were not only blocked in their duplication capacity but also in their structural integrity. This perfect genetic approach awaits an ultrastructural analysis.

53. Sampaio P, Rebollo E, Varmark H, Sunkel C, Gonzalez C: **Organized microtubule arrays in gamma-tubulin-depleted *Drosophila* spermatocytes.** *Curr Biol* 2001, **11**:1788-1793.
54. Hannak E, Oegema K, Kirkham M, Gonczy P, Habermann B, Hyman A: **The kinetically dominant assembly pathway for centrosomal asters in *Caenorhabditis elegans* is gamma-tubulin dependent.** *J Cell Biol* 2002, **157**:591-602.
55. O'Connell K, Caron C, Kopish K, Hurd D, Kempfues K, Li Y, White JG: **The *C. elegans* *zyg-1* gene encodes a regulator of centrosome duplication with distinct maternal and paternal roles in the embryo.** *Cell* 2001, **105**:547-558.

The *ZYG-1* gene, long known to be required for early embryonic divisions, was cloned and its function examined by a combined cytological/genetic approach, using strong loss-of-function thermosensitive alleles. Cell cycle progression is not affected by the mutations; however, paternal *ZYG-1* is shown to be required for a bipolar spindle during the first embryonic cell cycle. Maternal *ZYG-1* is required for bipolar spindles in further embryonic divisions. In *ZYG-1* mutant embryos, cell cycle progression is normal, but mitosis is monopolar and an electron microscopy study demonstrates that a single centriole is present in these monopoles. This gene, which encodes a kinase, is the first shown to control specifically basal body duplication.

56. Ruiz F, Krzywicka A, Klotz C, Keller A, Cohen J, Koll F, Balavoine G, Beisson J: **The SM19 gene, required for duplication of basal bodies in *Paramecium*, encodes a novel tubulin, eta-tubulin.** *Curr Biol* 2000, **10**:1451-1454.
57. Marshall W, Rosenbaum J: **Are there nucleic acids in the centrosome?** *Curr Top Dev Biol* 2000, **49**:187-205.
58. Mignot J: **New hypothesis on the replication of centrioles and basal bodies.** *C R Acad Sci III* 1996, **319**:1093-1099.
59. Beisson J, Jerka-Dziedzic M: **Polarities of the centriolar structure: morphogenetic consequences.** *Biol Cell* 1999, **91**:367-378.
60. Vidwans SJ, Wong ML, O'Farrell PH: **Anomalous centriole configurations are detected in *Drosophila* wing discs upon *Cdk1* inactivation.** *J Cell Sci* 2003, in press.

A thermosensitive *cdk1* allele, known to prevent mitosis but to allow multiple rounds of S phases in wing discs, was used to examine centriole

behaviour. An electron microscopic study revealed centrioles longer than control ones — and, in particular, daughter centrioles that were longer than their mother. Moreover, triplets of centrioles with two daughters close to but at right angle with the mother were revealed at different sites around the periphery of the mother centrioles. Even if specific for particular cell types/centrioles, such anomalies show that length of a centriole and determination of the duplication site might be modulated by regulatory signals.

61. Moreira-Leite FF, Sherwin T, Kohl L, Gull K: **A trypanosome structure involved in transmitting cytoplasmic information during cell division.** *Science* 2001, **294**:610-612.
- Here, the authors describe a molecular machine, operating like a zipper moving away from the basal body, by which the helical path of the trypanosome flagellum around the cell body transmits its shape to the new flagellum as it elongates during cell division. This direct imprinting from the pre-existing to the newly formed basal body/flagellum thus ensures the maintenance of shape and polarities in the daughter cells.
62. Cavalier-Smith T: **Basal body and flagellar development during the vegetative cell cycle and the sexual cycle of *Chlamydomonas reinhardtii*.** *J Cell Sci* 1974, **16**:529-556.
63. Fleury A, Le GH, Iftode F, Laurent M, Bornens M: **A scaffold for basal body patterning revealed by a monoclonal antibody in the hypotrich ciliate *Paraurostyla weissei*.** *Dev Biol* 1993, **157**:285-302.
64. Grimes GW: **Analysis of the determinative difference between singlets and doublets of *Oxytricha fallax*.** *Genet Res Camb* 1973, **57**:57-66.
65. Calarco P: **Centrosome precursors in the acentriolar mouse oocyte.** *Microsc Res Tech* 2000, **49**:428-434.
66. Mayor T, Stierhof Y, Tanaka K, Fry A, Nigg E: **The centrosomal protein C-Nap1 is required for cell cycle-regulated centrosome cohesion.** *J Cell Biol* 2000, **151**:837-846.
67. Meraldi P, Nigg E: **Centrosome cohesion is regulated by a balance of kinase and phosphatase activities.** *J Cell Sci* 2001, **114**:3749-3757.
68. Kochanski R, Borisy G: **Mode of centriole duplication and distribution.** *J Cell Biol* 1990, **110**:1599-15605.
69. Lange B, Faragher A, March P, Gull K: **Centriole duplication and maturation in animal cells.** *Curr Top Dev Biol* 2000, **49**:235-249.
70. Piel M, Nordberg J, Euteneuer U, Bornens M: **Centrosome-dependent exit of cytokinesis in animal cells.** *Science* 2001, **291**:1550-1553.
71. Piel M, Meyer P, Khodjakov A, Rieder C, Bornens M: **The respective contributions of the mother and daughter centrioles to centrosome activity and behavior in vertebrate cells.** *J Cell Biol* 2000, **149**:317-330.