Common Themes in Centriole and Centrosome Movements

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Abstract

Centrioles are found in nearly all eukaryotic cells and are required for growth and maintenance of the radial array of microtubules, the mitotic spindle, and cilia and flagella. Different types of microtubule structures are often required at different places in a given cell; centrioles must move around to nucleate these varied structures. Here we draw together recent data on diverse centriole movements to decipher common themes in how centrioles move. Par proteins establish and maintain the required cellular asymmetry. The actin cytoskeleton facilitates movement of multiple basal bodies. Microtubule forces acting on the cell cortex, and nuclear-cytoskeletal links, are important for positioning individual centrosomes, and during cell division. Knowledge of these common mechanisms can inform the study of centriole movements across biology.

Introduction

In metazoan cells the major microtubule organising centre (MTOC) of the cell is the centrosome (Figure 1 and glossary). It is composed of a pair of microtubule-based centrioles surrounded by a pericentriolar matrix (PCM). Centrioles were once thought to be static organelles located in the centre of the cell, hence their name. In fact, they move around the cell to fulfil their functions and correct centriole and centrosome positioning is vital for many biological processes. There are now many examples of centriole/centrosome movements in various physiological contexts and many different cell types. We will use the term centrosome in situations where the centriole pair and PCM all move together, and centriole where only a single centriole moves. Basal body is the term often used for a centriole that assembles a cilium or flagellum. While many advances have been made over the last few years in understanding what controls centrosome, centriole and basal body position, often, these fields are investigated in isolation. If the data from these disparate fields are analysed

36 together, it becomes apparent that there are common themes in both the mechanics of
37 movement and the regulatory mechanisms involved. Here, we first provide a brief overview
38 of the contexts in which centriole, centrosome and basal body movements are seen, and then
39 elaborate on how some of the common themes that are emerging across eukaryotic biology
40 are applied in each case.
41
42 Centrosome location is critical for many biological processes (Figure 2-4) and also impacts
43 the position of other organelles. The centrosome and the nucleus are closely associated, and
44 the Golgi apparatus is also found near the centrosome [1] enabling polarization of membrane
45 trafficking and secretory machineries [2-3].While centrosomes are not absolutely required to
46 organise the mitotic spindle [4-6], their position is important in symmetric and asymmetric
47 cell divisions (Figure 3) as movement of the two centrosomes to opposite sides of the nucleus
48 defines both the axis of division, and spindle position. In *Caenorhabditis elegans*, centrosome
49 positioning is key to the polarity establishment required during asymmetric cell divisions and
50 defines the anterior-posterior axis of the embryo [7]. The African trypanosome does not use
51 its centrioles (located at the base of the flagellum and termed basal bodies) to organise the
52 mitotic spindle; however, basal body positioning and segregation control cell morphogenesis
53 by influencing cytoskeletal construction and directly positioning the kinetoplast
54 (mitochondrial genome) [8] (Figure 3).
55
56 Centriole/centrosome position also contributes to the spatial organisation of many cells in
57 G1/interphase. In the biflagellate green alga *Chlamydomonas reinhardtii*
58 centriole/centrosome position maintains overall cell geometry [9], and in metazoans it
59 organises the radial microtubule array during interphase (Figure 2). In many types of
60 migrating cells, centrosome position between the nucleus and the leading edge is key to

61 migration [10-13]. Formation of the specialised immunological and virological synapses
62 involves centrosome re-orientation. During immunological synapse formation, the
63 centrosome migrates to the contact site between the T-cell and the antigen presenting cell
64 (Figure 4), where, in cytotoxic T-cells, it is involved in directed secretion of lytic granules
65 [14]. During virological synapse formation, which mediates the cell-cell transfer of viral
66 particles between an infected cell and a target cell, the centrosome of the infected cell
67 likewise re-orients towards the site of contact between the cells [15]. Finally, while centrioles
68 are not absolutely essential for cell division [16], they are critical for ciliogenesis. Humans
69 build motile and sensory cilia, and ciliogenesis of both kinds requires centriole/basal body
70 movement to the cell surface [17] (Figures 2 and 4). Most branches of the tree of life are
71 ciliate, and in many cases failure to build a cilium is incompatible with life. Even in
72 *Drosophila*, which can develop without centrioles, death eventually occurs due to the lack of
73 cilia on sensory neurons [16].
74
75 Thus, there is a considerable diversity of centriole/centrosome movements in biology. All of
76 the studied mechanisms of centriole movement require the actin or microtubule
77 cytoskeletons, or both. Are there common mechanisms that apply across these varied
78 processes or are there specialised mechanisms to facilitate movement in each physiological
79 context?
80
81 ***The microtubule cytoskeleton and centriole movement***
82 The position of the centrosome in both migrating and non-migrating interphase cells requires
83 a polarised radial microtubule array [18-19]. During cell migration, centrosome position is
84 actin-independent [10, 12], while the cortical pool of the microtubule minus-end directed
85 motor cytoplasmic dynein is implicated in centrosome position in several different cell types

[10, 12-13, 20]. During astrocyte migration, the small GTPase Cdc42 controls centrosome and Golgi re-orientation towards the direction of migration through the microtubule cytoskeleton and cytoplasmic dynein [10, 21]; however, in fibroblasts, the centrosome remains at the cell centre while the nucleus moves rearward [20]. Nonetheless, centrosome maintenance at this position is dependent on microtubules and dynein as inhibition of either causes a rearward centrosome displacement [20].

Microtubules and cytoplasmic dynein are also implicated in centrosome positioning in non-migrating cells [19, 22-24], suggesting that, when the centrosome needs to be central within the cell, its position is actively maintained using microtubules and dynein to stabilise the centrosome-associated microtubule array. In interphase cells, the centriole pair do not necessarily remain together. The older, mature, centriole can remain stationary while the younger, immature centriole moves around the cell [25]. The Rho-associated kinase p160Rock, is proposed to regulate the central position of the mature centriole [26]. p160Rock is a major regulator of myosin II but it has many different substrates [27] and during cell migration it can mediate both microtubule-dependent centrosome re-orientation [28] and actin rearrangements; consequently, the way in which it affects centrosome position remains unclear.

In summary, microtubules and cytoplasmic dynein regulate centrosome position when a single centrosome needs to be positioned in the centre of the cell. How about when centrioles need to move away from the centre, or if there are multiple centrioles to move? In these cases, several studies have highlighted a primary role for the actin cytoskeleton in centriole positioning.

Actin in centrosome/centriole movements

While the first cell division of many organisms is symmetric, that of *C. elegans* is asymmetric. During the first division of the *C. elegans* zygote, the cell divides asymmetrically along its anterior–posterior axis to give rise to two cells that are committed to different cell fates. The centriole pair are key factors in the polarity establishment required during these divisions. They are derived from the sperm cell rather than the oocyte and become embedded in the actin cortex underlying the plasma membrane by a mechanism that may not involve interactions with microtubules. RNAi ablation of tubulin does not prevent polarity establishment and is not required for centriole-cortex interaction [7, 29], suggesting that another cytoskeletal polymer mediates centriole position. However, another study found that tubulin disruption delays polarity induction, which requires a small centrosomal microtubule aster [30]; therefore the precise role of microtubules in this process remains to be clarified.

The trachea, oviduct and ependymal epithelium of mammals are composed of a multi-ciliated epithelium where each cell may have hundreds of cilia, each grown from a basal body. These form *de novo* and migrate simultaneously to the apical cell surface. Drugs that target microtubules do not directly stop basal body movement in oviduct [31], although the contribution of microtubules to basal body movements in other cell types is unclear. In contrast, much evidence from several cell types implicates the actin cytoskeleton in basal body migration and docking at the cell surface. Actin and myosin associate with either basal bodies, or the material surrounding them [32-34] and basal body migration is blocked by treatment with inhibitors of actin [35] or myosin [36]. Basal body docking with the cell membrane is also actin dependent. The Wnt planar cell polarity pathway and its effectors are implicated in membrane trafficking during ciliogenesis and the formation of an actin array

136 essential for basal body docking [37-39] [40]. Finally, myosin II localises to basal body
137 accessory structures in multi-ciliated epithelia [32], and is needed for basal body migration
138 [41].
139
140 There are emerging similarities in the structure and function of basal bodies of cilia and
141 centrioles at the immunological synapse [42], such as the requirement for intraflagellar
142 transport components. These were previously thought of as cilium assembly and maintenance
143 proteins [43], but recent data have also demonstrated a role in polarised recycling at the
144 immunological synapse [44]. This suggests that there may be similar principles guiding
145 centriole movement and membrane trafficking in both cases. During immunological synapse
146 formation, receptor-mediated engagement between the immune cell and its target triggers a
147 transient aggregation of actin across the nascent synapse [45]. The polarisation of the
148 centriole pair to the synapse is accompanied by concomitant actin clearance from the inner
149 part of the forming synapse, to produce an outer ring of actin [14, 46] and it has been
150 suggested that the forces generated by actin clearance are used to move the centrioles forward
151 [2].Centrioles are always docked at the centre of the synapse and it is unclear whether this is
152 due to radial actin reorganisation that localises them to this region by default, or whether
153 there are other actin binding proteins that regulate the site of docking. Much less is known
154 about the role of the cytoskeleton in virological synapse formation, although it is an actin-
155 dependent process [47] and integrity of both the actin and microtubule cytoskeletons is
156 crucial [48-49].
157
158 In general it is over-simplistic to consider the actin and microtubule cytoskeletons in
159 isolation. Extensive cross-talk exists and many biological processes are carried out by actin

and microtubules acting together. Much research has shown that interactions of microtubules that are anchored into the actin cortex are often responsible for centriole positioning.

Microtubule interactions with the actin cortex and centriole and centrosome positioning

Interaction between microtubules and the actin cytoskeleton at the cell cortex are essential for maintaining the physical position of the centrosome within the cell and for orchestrating placement of the duplicated centrosomes during symmetric and asymmetric cell divisions. Pulling forces of microtubules anchored to the cell cortex provide a mechanism for centrosome positioning [11, 50]. Studies of male germline stem cells in *Drosophila* have revealed differential centrosome behaviour during the asymmetric cell divisions that characterise development. After centrosome duplication, the older of the two centrosomes retains a well-defined microtubule array and remains in place, while the younger centrosome nucleates few microtubules and migrates away to set up the symmetrical plane of the mitotic spindle [51].

The pulling forces are provided by dynein on microtubules that extend to and are anchored into the cell cortex regulated by both dynein and the actin motor myosin II. Myosin II is one of several proteins that organise the cortex, and cortical organisation is critical for providing the mechanical support needed for centrosome positioning. The importance of cortical rigidity has been highlighted by recent studies that showed that myosin II- and moesin-dependent cortical rigidity are required for spindle positioning [52-54]. Moreover, cell shape plays an important part in spindle orientation, which is highly dependent on cell-substrate adhesions [55] that are communicated to the cytoskeleton via integrins and actin-microtubule linkers including EB1 and myosin X [56-57].

Taken together, these studies provide evidence for a conserved pathway that explains centrosome movement in cells containing a centralised centrosome with a radial array of microtubules that are in contact with the plasma membrane. However, not all eukaryotic cells have a radial array of microtubules emanating from a centralised MTOC. Protists such as the African trypanosome represent the polar opposite, with microtubules arranged as a subpellicular sheet underlying the plasma membrane [58]. Basal bodies extend a microtubule axoneme for the flagellum in this organism, but not a radial array of microtubules within the cell body. Despite these differences, microtubules do provide the mechanism for basal body movements and segregation during cell division [59] and these microtubules are closely associated with the plasma membrane in the same way that microtubules associate with the actin cortex underlying the plasma membrane in other organisms.

Thus, the idea that microtubule interactions with a cortical cytoskeleton are used to move single or paired centrioles/centrosomes is conserved across eukaryotic biology.

The role of the nuclear envelope in centriole movements.

It is increasingly recognised that the nucleus, as well as the cortex, plays a role in positioning centrioles. In many cells, the centrioles are tightly associated with the nuclear envelope. This connection is observed in lower eukaryotes as a physical linkage of striated fibres called the basal body-nucleus connector (rhizoplast, [60]). During the cell division cycle of *Chlamydomonas*, the two flagella are cleaved from the cell. The two pairs of centrioles move from the apical surface to the poles of the spindle during mitosis, and after division return to the apical surface and grow two new flagella. The basal body-nucleus connector exists as striated fibres that connect both pairs of centrioles, which are in turn connected to the nucleus by centrin-containing rhizoplasts [61]. Is the nucleus, the centriole or both involved in the

movement to and from the apical surface? In cells lacking the rhizoplast connection, the nucleus is mispositioned, but the mature centriole is correctly localised, indicating that nuclear mis-positioning has little impact on mature centriole positioning [9] and suggesting that the centriole regulates nuclear positioning rather than vice versa. In a migrating mammalian cell the opposite appears true. Studies on centrosome reorientation in migrating fibroblasts suggest that the centrosome might remain relatively central while the nucleus moves rearwards [20]. Centrosome position is maintained by dynein-mediated cortical tethering of microtubules [62]; however, it is unclear whether centrosome rotation drives nuclear movement or vice versa.

In higher eukaryotes, the link between the nuclear envelope and the centrosome is essential for development [63] and is robust enough to withstand cell lysis and nuclear isolation [64]. This linkage is required for nuclear migration and the control of cell cycle timing [64] and in *Drosophila* there is evidence that the centrosomes can reach the cell cortex during development with the aid of the nucleus [65].

In the organisms studied to date, the centrosome-nucleus linkage is mediated by proteins containing paired KASH (Klarsicht-anchorage protein1-Syne homology) and SUN (Sad1-UNC84) domains (Figure 5). First discovered in *C. elegans*, proteins with these domains are found across eukaryotes and localise to the nuclear envelope and centrosomes [63, 66-68]. Multiple SUN and KASH proteins exist that provide links between the nuclear envelope and cytoskeletal polymers [69-70] (Figure 5). One of the diverse roles of the SUN-KASH complex is the regulation of centrosome position. The *C. elegans* KASH protein ZYG-12 anchors the centrosome to the nuclear envelope during embryogenesis [63]. ZYG-12 is not found in mammalian cells; however several proteins fulfill the role of nucleus-centrosome

linkers including the nuclear membrane protein emerin [71], and the multi-isoform KASH protein Nesprin 2 [66]. Several Nesprin isoforms contain calponin-homology domains that allow them to bind actin, and these are implicated in positioning the centrosome during ciliogenesis of sensory cilia [72], suggesting that actin-dependent nuclear re-positioning or rotation may re-orient the centrosome apically. A novel epithelial-specific Nesprin isoform, Nesprin 4, interacts with the microtubule motor kinesin-1, and this link is proposed to contribute to nucleus and centrosome positioning in interphase cells [73]. It will be interesting to see if Nesprin 4 and kinesins are also involved in centrosome/centriole positioning during ciliogenesis as Nesprin-microtubule links are also important in cell migration. In migrating neurons, a SUN1/2-Nesprin1/2 complex acts with the lissencephaly-associated proteins Lis1 and Doublecortin to couple the centrosome and nucleus through cytoplasmic dynein [66, 74]

Regulation of centriole movements

Given that centrioles and centrosomes can track around the nuclear envelope, change their position relative to the nucleus, embed themselves in the cortex, or move around the cell, how do they know where to go? In the absence of other cues, cell-cell contacts are the main mediators of centrosome positioning [75-76]. The extracellular signals that trigger centrosome and centriole movements are varied; however there is now much evidence from a variety of systems that these signals converge on the Par (partitioning) proteins [77] and the Rho family of small GTPases.

The Par proteins are a key set of polarity proteins that were identified in screens for mutants affecting the first asymmetric cell division in *C. elegans* [78-79]. In both *C. elegans* and *Drosophila*, the Par complex acts through the Rho family of small GTPases and the actin

cytoskeleton to establish the cortical polarity that is needed for spindle positioning [50, 80]. Once this initial polarity is established, the same mechanisms act together with microtubule-cortical interactions to produce the forces that result in the asymmetrically placed spindle. External signalling cues from neighbouring epithelial cells are needed to regulate the localisation of polarity markers – and hence the axis of the mitotic spindle - during asymmetric cell division in *Drosophila* neuroblasts [81]. Two Rho GTPases act together to regulate polarity establishment in *C. elegans*. Rho1 mediates the centrosome-dependent cortical actomyosin rearrangements that lead to contractile asymmetry within the cortex. Cdc42 mediates the link between the cortex and Par6 proteins, and coordinates Par protein segregation as the cortical asymmetry develops [80]. During cell migration, integrin signalling through Cdc42 to Par6 and aPKC is required for the microtubule-dependent centrosome localisation observed during astrocyte migration [10, 21] and blocking Cdc42 prevents macrophage polarization towards a chemotactic signal [82]. A Par3-Par6-aPKC complex stabilises microtubule-dependent cell polarity during keratinocyte migration, although its role in centrosome movement is unclear [83]. During development, aPKC is needed during neuronal repolarization [84] and Pard3 controls centrosome positioning during neurulation [85]. During ciliogenesis of multi-ciliated epithelia, the Par3-Par6-aPKC polarity complex localises to cilia and regulates ciliogenesis via association with kinesin-II [86], one of the motors required to build cilia by intraflagellar transport [43]. Rho is not needed for centriole re-orientation during virological synapse formation, however, inhibition of Rac and Cdc42 prevents centrioles from re-orientating [49]. Cdc42 and Par proteins are also implicated in immunological synapse formation. Cdc42 inhibition blocks centrosome re-orientation [87], while Par3 is recruited to the synapse [88] and overexpression of a dominant-negative form of Par1b blocks centrosome re-orientation [89], suggesting that Par3 localisation is functionally relevant to immunological synapse formation. The signalling

285 events that regulate the Par proteins in this case are unclear, however strength of signalling
286 via the T cell receptor is important [90] and when more than one contact is present, the
287 centrosome can oscillate between the possible targets [91] until the decision is made to kill
288 the target that produces the strongest signal [92]. It is therefore reasonable to suggest that this
289 provides the required external cue.
290
291 ***Concluding remarks***
292 While the importance of the cytoskeleton, polarity proteins, and the nuclear envelope in
293 centriole movements has long been recognised in several different fields, the idea of common
294 themes has been slower to emerge. Research carried out over the last few years has
295 highlighted that, even though centrioles and centrosomes are positioned to achieve very
296 different outcomes, much of the basic machinery that is used is remarkably similar. It seems
297 likely that disparate signalling events might converge on the recruitment of the Par proteins to
298 establish and maintain the asymmetry that is a key feature of these centrosome re-orientation
299 events. In general, where multiple centrioles need to be moved, there is a requirement for the
300 actin cytoskeleton, while microtubule forces acting on the cell cortex are particularly
301 important for positioning individual centrosomes, and during cell division. Finally, the
302 involvement of KASH proteins in multiple centrosome positioning contexts suggests that
303 they too may represent a conserved mechanism for regulating centrosome location, and their
304 potential roles in mediating other centrosome movements warrants investigation. A challenge
305 for the future is to identify the polarity cues that regulate centrosome position in organisms
306 outside the metazoa that lack the Par proteins. It will be interesting to see if there are
307 conserved mechanisms to set up asymmetry in these systems. These might include examples
308 of cytotaxis such as those that are involved in polarity replication during trypanosome
309 morphogenesis [93] or the inheritance of cortical organisation in ciliates [94].

Several proteins that are implicated in control of centrosome/centriole positioning have been linked to human inherited disease. Lissencephaly, or "smooth brain," is a brain malformation disorder caused by abnormal neuronal migration early in development. Two of the underlying proteins, Lis1 and Doublecortin, mediate the centrosome-nucleus linkage [74] and it seems likely that polarity problems caused by disruption of this link might contribute to the disease. Other neuronal migration disorders can also result in structurally abnormal or missing areas of the brain including midline defects such as agenesis of the corpus callosum and hypoplasia of the cerebellar vermis. Many the ciliopathies, or diseases of cilium dysfunction, present with midline defects as part of the phenotype, and two ciliopathies, Meckel-Gruber syndrome and hydrolethalus syndrome, have been linked to centrosome/basal body-positioning defects [95-96]. How these fit in to the pathways and processes described here remains to be seen, however, the Meckel-Gruber syndrome proteins are implicated in planar cell polarity signaling [97-98] and may regulate centrosome re-orientation during ciliogenesis through actin cytoskeleton remodeling and maintaining the centrosome-nuclear envelope connection [72]. Finally, there are other diseases that have been linked to centrosome dysfunction [99] and it will be fascinating to discover if centriole/centrosome position is also compromised in these cases.

As more details of the molecular control of polarity establishment are uncovered, it will become possible to understand which of the activities in *C. elegans* represent general principles in polarity establishment, and which are specialized to the particular case of embryonic polarity establishment. Despite the likelihood of cell-type specific specializations, analysis reveals a commonality in the mechanisms used to move centrioles and centrosomes throughout eukaryotic biology. Notwithstanding the very different contexts in which centriole

movements are observed, these commonalities have the potential to contribute to our understanding of centriole movements in less well-studied systems.

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545
546
547 ***Figure Legends***

548 **Fig 1: Centriole duplication cycle.** During interphase/G1 centrioles function in organizing
549 microtubules, and in many eukaryotic cells the mature centriole assembles a primary
550 cilium/flagellum. The centriole pair must duplicate only once during the cell cycle and this
551 begins at the G1/S-phase transition with a pro-centriole assembled orthogonal to each mature
552 centriole. Recent studies have dissected the molecules required for initial pro-centriole
553 assembly in *C. elegans* early embryos and humans (pro-centriole assembly: *C. elegans*: SPD-
554 2, ZYG-1 Sas-6, Sas-5, Sas-4, α-, β-, and γ-tubulin; Humans: Cep192, Plk4/Sak, Sas-6, Sas-
555 4, CPAP, α-, β-, γ-, δ-, ε-tubulin, CP110, and Cep135. Regulatory molecules: Cdk1/Cyclin B,
556 Aurora-A, Plks. Control of centriole duplication: Separase, Plk1, Plk4, SAS-6.) [99]. Pro-
557 centriole elongation continues through G2 until there are two pairs of centrioles that migrate
558 to the pole of the spindle. Following mitosis the tight association and orthogonal orientation
559 of the mature centriole and pro-centriole is no longer apparent. This is a stage termed
560 'disengagement' and is a crucial stage in the control of centriole duplication [100]. (a)
561 Microtubule arrays/primary cilium functions. (b) New centriole formation. (c) New centriole

562 elongation. (d) Centrosome segregation. (e) Centrosomes move to spindle poles. (f)
563 Centrosome inheritance to daughter cells.
564
565 **Fig 2: Centrosome migration in G1/interphase cells.** In many animal cells the centrosome
566 migrates from a central position within the cell to the cell cortex, where a primary cilium
567 assembles from the mature centriole. The primary cilium acts as an antenna for the cell that
568 senses the environment and is needed to transduce certain signaling pathways. Migration of
569 the centrosome can involve interaction with radial microtubules and actomyosin at the cell
570 cortex, however, the role of the cortex in primary cilium formation is not known. (a)
571 G1/interphase cell. The centrosome is located centrally within the cell. Central location is
572 maintained by microtubules/dynein and regulated by p160Rock. (b) The centrosome moves
573 to the cell surface in some cell types via interaction of microtubules with the actomyosin cell
574 cortex. (c) A microtubule-based primary cilium is assembled from the mature centriole.
575
576 **Fig 3: Centrosome/basal body migration during cell division.** (a). Symmetric &
577 asymmetric cell division. Migration of the duplicated centrosomes to the opposite poles of the
578 spindle requires actin-microtubule interactions with the cell cortex (left). The Par proteins are
579 important in modulating these interactions in order to promote asymmetric positioning
580 (right). (b). Interphase African trypanosome cell with a single flagellum assembled from the
581 mature basal body (left). G1/S-phase basal body duplication occurs and a new flagellum
582 assembles alongside the old flagellum. Migration is microtubule-dependent via subpellicular
583 microtubules at the cell cortex (middle). Intriguingly, actin and myosin II are not involved in
584 either basal body migration or cytokinesis in *T. brucei* (right). (c). Bi-flagellated interphase
585 *C. reinhardtii* cell (left). Flagella are cleaved and the centrioles migrate to the poles of the
586 mitotic spindle via a nucleus-centriole connector (rhizoplast; middle). Centrioles return to

587 the cell cortex and two new flagella are assembled for each daughter cell prior to cytokinesis
588 (right).
589
590 **Fig. 4: Centriole/basal body migration in terminally differentiated cells.** (a). The process
591 of ciliogenesis produces thousands of motile or immotile cilia on many specialized terminally
592 differentiated cells. Large numbers of basal bodies are formed within a single cell (left).
593 Basal bodies migrate and dock with the cell membrane. Movement requires actomyosin, and
594 is regulated by GTPase RhoA (middle). Motile or immotile cilia are assembled from the
595 docked basal bodies (right). Basal bodies form via a combination of the centriolar and
596 acentriolar pathways (see text box). The role of the existing centrosome is unknown (b).
597 Cytotoxic T-cells form an immunological synapse to facilitate killing a target cell.
598 Centrosome migration is required during the early stages of synapse formation and occurs by
599 interactions between microtubules and the cell cortex. Recognition of a target cell by a
600 cytotoxic T-cell and assembly of the synapse (left; arrow points to nascent synapse).
601 Movement of the centrosome to the synapse requires both microtubules and actomyosin
602 (middle). The centrosome docks at the plasma membrane of the immunological synapse and
603 lytic granules (black) travel along microtubules to the synapse to kill the target cell (right).
604
605 **Fig 5: SUN and KASH domain proteins couple the nucleus to the actin and microtubule**
606 **cytoskeletons.** The SUN domain-KASH domain interaction occurs within the space between
607 the inner and outer nuclear membranes. Many different KASH-domain proteins exist and can
608 provide a physical linkage between the nuclear lamina and the cytoskeleton. KASH proteins
609 with an N-terminal actin-binding domain link the actin cytoskeleton to the nucleus. Other
610 KASH proteins link microtubules to the nucleus via interactions with kinesin or dynein. The

611 SUN-KASH interaction is evolutionarily conserved and ha many roles within cells,
612 including nuclear migration and centrosome orientation.
613
614 Figure I
615 Transmission electron micrograph of a centriole pair from a mouse kidney cell. Scale bar:
616 100nm.
617
618 **Glossary:**
619 **Actin motors:** myosins are actin motors that carry cargo along actin and are ATP-dependent.
620 With the exception of myosin VI all other myosins studied to date are plus-end directed.
621 **Astral microtubules:** extend out from each centrosome at opposite poles of the mitotic
622 spindle pole to the cell cortex and are required for mitotic spindle orientation.
623 **Basal body:** a microtubule organizing centre that subtends a cilium or flagellum.
624 **Cell cortex:** a specialized area of the cell underlying the plasma membrane that is required
625 for mechanical support of cell shape and form. Microtubules (called cortical microtubules),
626 actin (called cortical actin) or both are found at the cell cortex in a wide range of eukaryotic
627 cells.
628 **Centriole:** a microtubule-based barrel-shaped structure generally composed of 9 triplet
629 microtubules that is found in many cells (Figure I).
630 **Centrosome:** the major microtubule organizing centre in mammalian cells. It organizes
631 radial arrays of microtubules, mitotic spindle microtubules and astral microtubules, and
632 contains a pair of centrioles.
633 **Immunological/Virological synapse:** named for their similarity to classical neurological
634 synapses, the immunological synapse is the interface between an antigen-presenting cell and

635 a lymphocyte, while the virological synapse is the interface between infected cells and target
636 cells that can mediate cell-cell spread of viruses.
637 **Microtubule motors:** kinesin motors move along microtubules towards the plus-end of
638 microtubules and dynein motors move towards the minus-end of microtubules. Both are
639 ATP-dependent motors.
640 **Pericentriolar material:** the matrix that surrounds the centrioles within the centrosome. It
641 contains proteins responsible for microtubule nucleation and anchoring and plays a role in
642 centrosome duplication.
643
644 **Box 1. Basal body production during ciliogenesis**
645 Many metazoan organisms build two types of cilium: non-motile sensory, or primary, cilia
646 and motile cilia. Each assembles from a basal body, which is analogous to the mitotic
647 centrioles.
648
649 • **Primary cilia** are solitary organelles that assemble from a basal body derived from the
650 pre-existing mature centriole, which moves to the cell surface and docks before extending
651 the ciliary axoneme.
652
653 In contrast, there can be hundreds of motile cilia on a single cell and each needs a basal body.
654 Basal body formation is linked to differentiation rather than proliferation and multiple basal
655 bodies are formed in the cytoplasm and then simultaneously migrate to the cell surface. Basal
656 bodies are formed *de novo* by a combination of the centriolar pathway and the acentriolar
657 pathway, both of which can occur in a single cell.

- In the **centriolar pathway**, new basal bodies are produced around an existing centriole template, just as observed during cell cycle-dependent centriole duplication. However, more than one new basal body can form around a single centriole.
- In the **acentriolar pathway**, basal body formation is not templated. Here, multiple basal bodies form around an intermediary structure called a deuterosome rather than around an existing centriole.

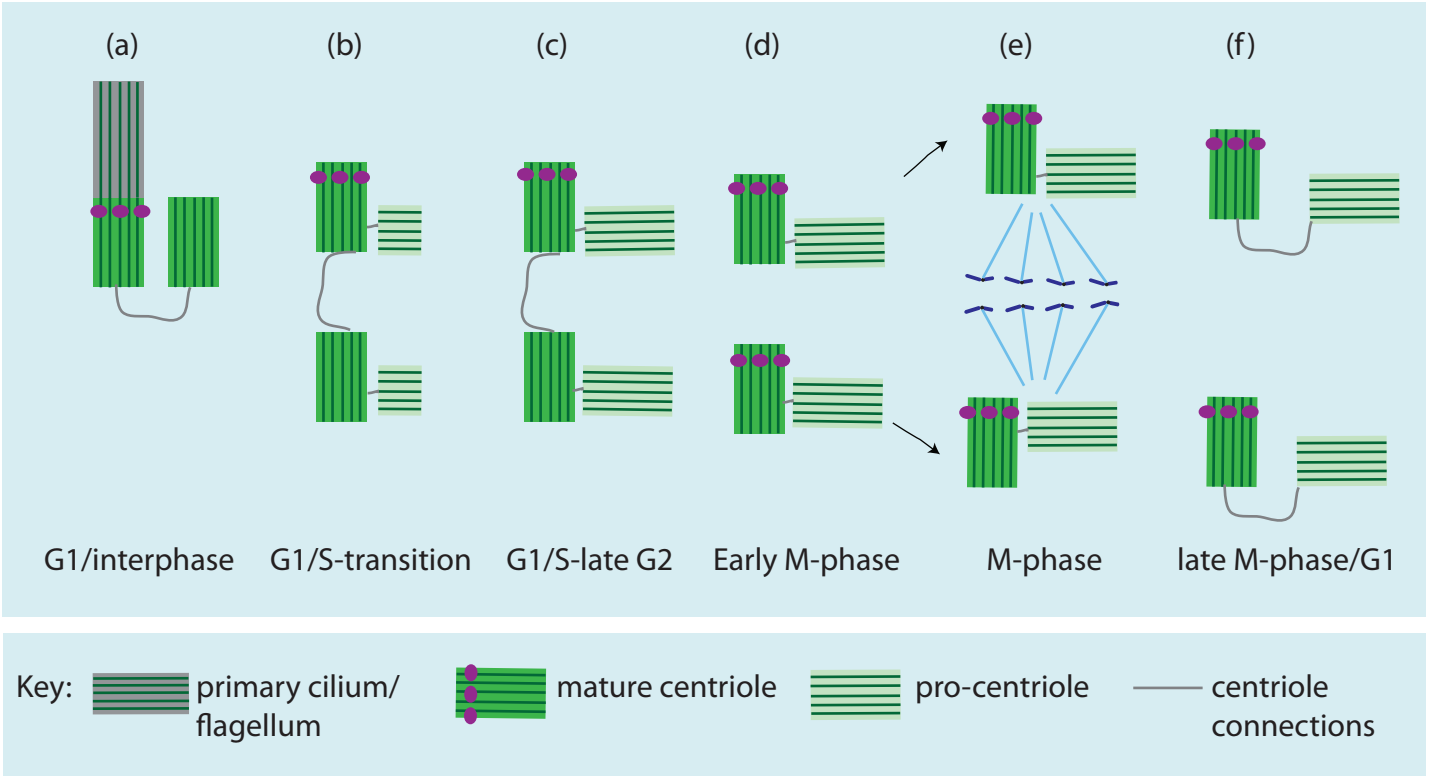
(a)
(b)
(c)
(d)
(e)
(f)
G1/interphase
G1/S-transition
G1/S-late G2
Early M-phase
M-phase
late M-phase/G1
Key:
primary cilium/ flagellum
mature centriole
pro-centriole
centriole connections

Figure 1

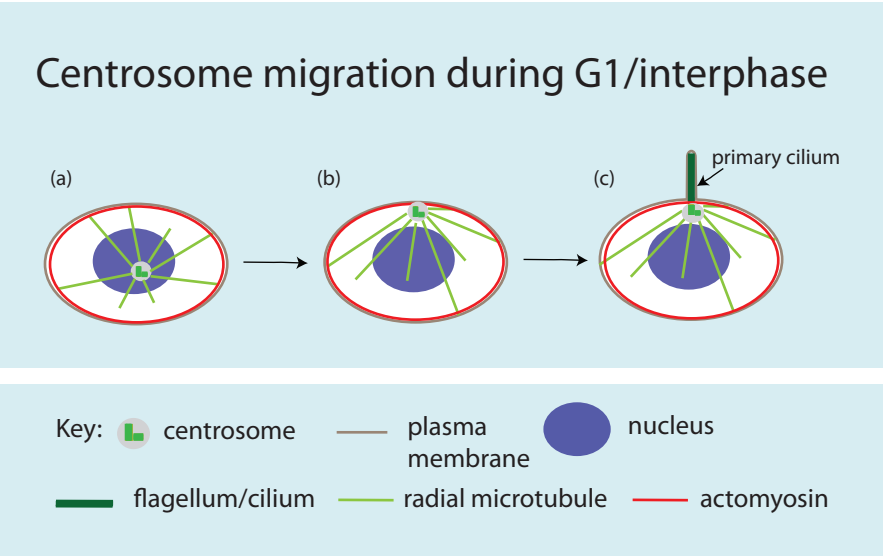
Centrosome migration during G1/interphase
(a)
(b)
(c)
primary cilium
Key:
centrosome
plasma membrane
nucleus
flagellum/cilium
radial microtubule
actomyosin

Figure 2

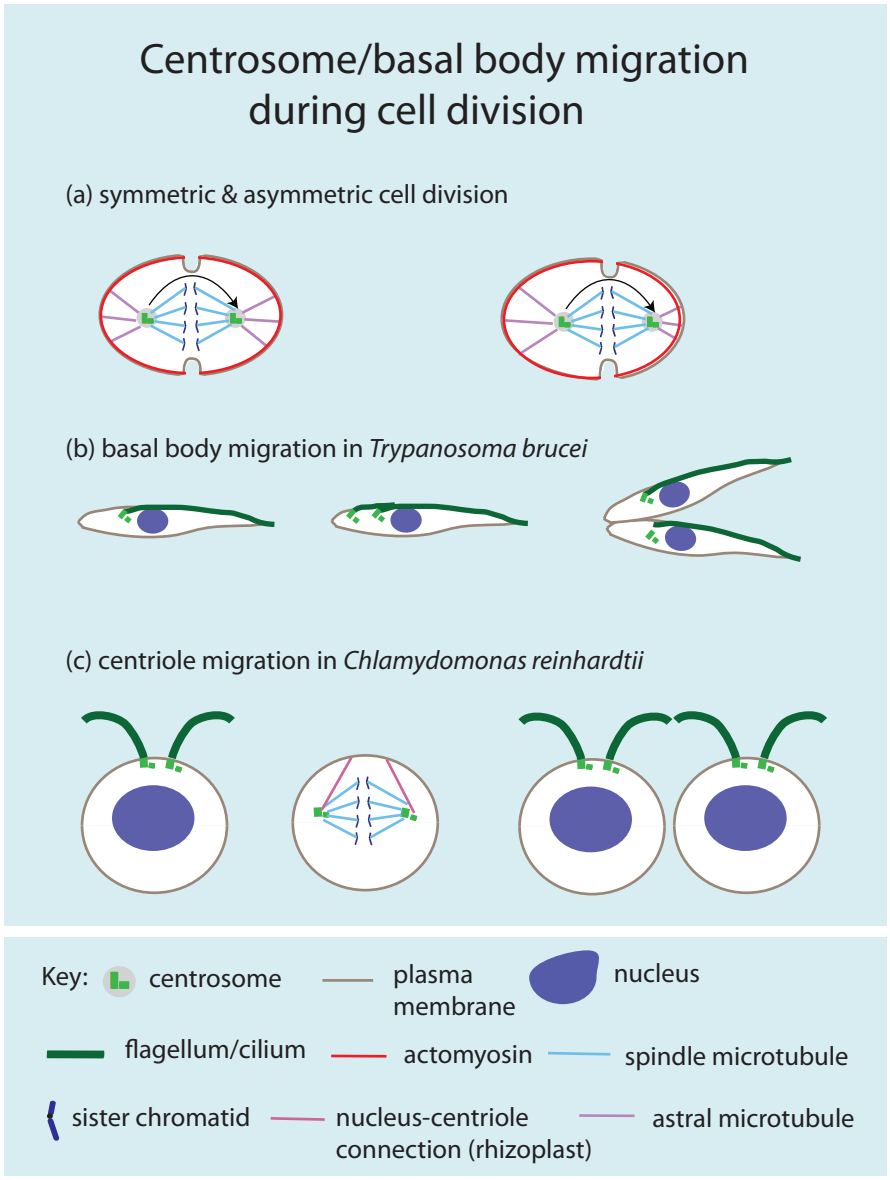
Centrosome/basal body migration during cell division
(a) symmetric & asymmetric cell division
(b) basal body migration in *Trypanosoma brucei*
(c) centriole migration in *Chlamydomonas reinhardtii*
Key:
centrosome
plasma membrane
nucleus
flagellum/cilium
actomyosin
spindle microtubule
sister chromatid
nucleus-centriole connection (rhizoplast)
astral microtubule

Figure 3

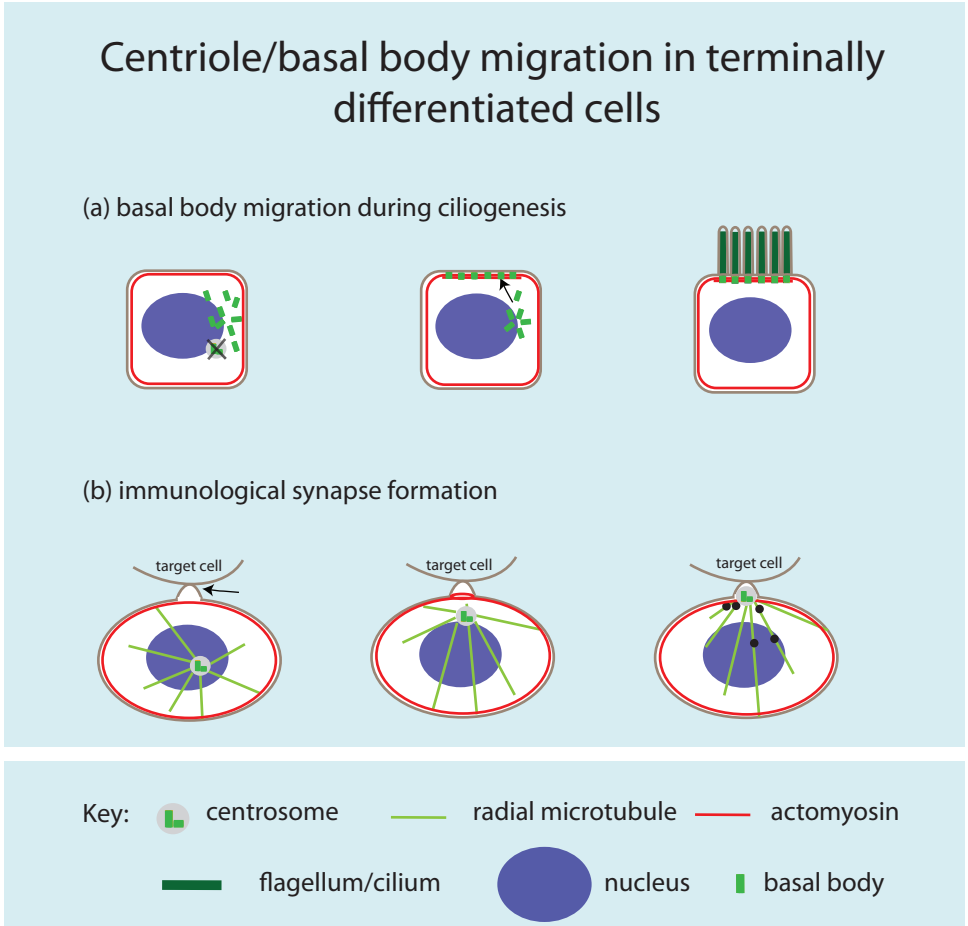
Centriole/basal body migration in terminally differentiated cells
(a) basal body migration during ciliogenesis
(b) immunological synapse formation
target cell
target cell
target cell
Key:
centrosome
radial microtubule
actomyosin
flagellum/cilium
nucleus
basal body

Figure 4

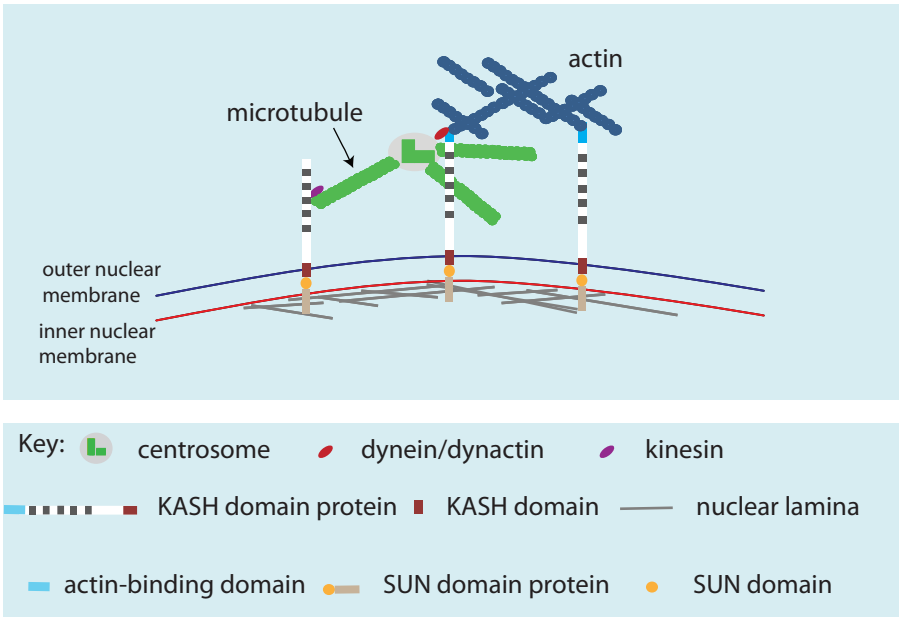
actin
microtubule
outer nuclear membrane
inner nuclear membrane
Key:
centrosome
dynein/dynactin
kinesin
KASH domain protein
KASH domain
nuclear lamina
actin-binding domain
SUN domain protein
SUN domain

Figure 5