Centrosome heterogeneity in stem cells regulates cell diversity

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Abstract

Stem cells are at the source of creating cellular diversity. Multiple mechanisms, including basic cell biological processes, regulate their fate. The centrosome is at the core of many stem cell functions and recent work highlights the association of distinct proteins at the centrosome in stem cell differentiation. As showcased by a novel centrosome protein regulating neural stem cell differentiation, it is timely to review the heterogeneity of the centrosome at protein and RNA levels and how this impacts their function in stem and progenitor cells. Together with evidence for heterogeneity of other organelles so far considered as similar between cells, we call for exploring the cell type-specific composition of organelles as a way to expand protein function in development with relevance to regenerative medicine.

Timeliness to review centrosome heterogeneity in stem cell function

Stem cells generate all organs in our body and, typically, their progeny, the transit-amplifying progenitors (TAPs), both amplify and diversify the cell types in a given tissue. Stem cells can generate this diversity indirectly by generating distinct TAPs that produce different progeny and allow their amplification, such as in the hematopoietic system. Stem cells also generate cell diversity directly (e.g., by asymmetric cell division) in a temporal order giving rise to different cell types in a sequential manner [e.g., neural stem cells (NSCs)] generating different types of neurons first and then glia [1]. However, also in the nervous system, TAPs became more frequent and diverse in phylogeny (see Glossary), culminating in the large zone of basal progenitors (BPs) [the outer subventricular zone (SVZ)] in the human cerebral cortex (Box 1) [2]. Thus, the regulation of stem cell behaviors and the mechanisms governing the production of distinct progeny and diverse TAPs bring about organ size and cellular diversity.

Centrosomes have been considered a homogeneous organelle and mainly contextualized with cell division, migration, and polarization. Recent work showed that centrosomes contain different components that impact stem cell behavior. One such example is the protein AKNA, which is only in the differentiating subset of NSCs that leave the stem cell niche [3]. This is the case in interphase, that is, without effects on the mode of cell division, but with profound effects on the generation of TAPs, as will be discussed later. These new findings and new technology, such as single-cell approaches and more refined proteomics techniques allowing deeper insights into cellular and organellar heterogeneity, call for a short review on organellar heterogeneity in governing cell function. Here, we

will discuss how centrosome heterogeneity regulates stem cell behaviors, focusing largely on the nervous system because these processes are particularly well examined and understood in neurogenesis. This will bring us to elaborate on differences in protein composition at centrosomes affecting microtubule organizing center (MTOC) activity and stem cell differentiation before discussing centrosomal RNAs (cenRNAs) as a possible source of centrosome heterogeneity that could impact stem cells behaviors. As an outlook, we close by linking centrosome heterogeneity to disease etiology and call for consideration of organellar heterogeneity as a general principle to amplify and diversify protein function, highlighting the need for more comprehensive organellar proteomics in a cell type-specific manner.

Centrosome differences in vertebrate (stem) cells

The centrosome is made of a linked pair of centrioles surrounded by pericentriolar material (PCM) (Box 2). Centrosomes nucleate and organize microtubules (MTs) in most not- terminally differentiated cells alone or with other organelles like the Golgi apparatus [4] and also act as the basal body of primary and motile cilia. The centriole's substructures require a specific set of proteins to fulfill their duties (Table 1) with both centrioles exhibiting structural and functional differences (discussed in [101]), which causes a first level of heterogeneity when cells divide.

Centrosomes are affected by mitotic kinases prior to and during cell division [5] and the path- ways are conserved in evolution. These commonalities may have led us to oversee potential cell and context-dependent differences and think of it as an organelle with homogeneous composition. When a cell initiates cell division, the PCM grows and increases MT nucleation, while centriole cohesion factors are disassembled to separate sister centrosomes. 'Mobile' distal appendage (DA) components regulating centriole docking to the cell membrane are also released before cell division to facilitate cilia disassembly [6]. As for DAs, subdistal appendage (SDA) 'mobile' factors are also disassembled during mitosis, as shown by the disappearance of proteins required for MT anchoring, such as AKNA, NINEIN, and CEP170, among others [3,7]. It is not clear why this is the case, but it may be to avoid having mitotic spindles with asymmetric MT organization. Of notice, mobile appendage components are farthest to the centriolar wall. DA and SDA 'core components' (e.g., CEP83 and ODF2, respectively) are detectable at mitotic centrosomes [6], are nearest to the centriole wall, and have scaffolding functions. Thus, fractions, but not the whole appendage structure, are removed from centrioles in mitosis. This plays a key role in rebuilding DAs and reforming a primary cilium soon after division in the cell inheriting the older centrosome [6,8]. In murine NSCs, the mother centrosome is kept more often by the future NSC, while the differentiating progeny inherits the daughter centriole [9]. Intriguingly, the future NSC forms a primary cilium sooner than its differentiating sister [8], eventually resulting in asymmetric ciliary signaling. Not completely removing SDAs in mitosis suggests that the cell inheriting the older centrosomes could organize centrosomal MTs sooner than its counterpart does, with implications in downstream cellular processes associated with MT organization and cell fate specification, like polarization, delamination, and migration. Asymmetrically dividing Drosophila neuroblasts get the daughter centriole-containing centrosome, which has intrinsically stronger MTOC activity [10]. The daughter centriole-containing centrosome is attached early to the neuroblasts' apical cell cortex, which could regulate asymmetric segregation of fate determinants and niche allocation (reviewed in [11]). It remains to be directly demonstrated if the asymmetric inheritance of centrosomes also leads to asymmetric MTOC activity. However, spindle size asymmetry is a key component of asymmetric cell division in mammalian NSCs, with the daughter cell originating from the larger spindle giving rise to a neuron, while the cell with smaller spindle will generate a progenitor [12]. Thus, asymmetry in mother and daughter centriole inheritance and spindle size are a first layer of centrosome heterogeneity in a cell's life, regulating its behavior and fate. However, recent work also unraveled differences between

self-renewing and differentiating NSCs in centrosome composition in interphase [3], underlining the fact that different (yet related) cells have different needs for this organelle. Notably, interphase centrosome composition can differ in proteins that mediate its MTOC activity, thereby influencing movement [3,13,14]. This essential function will be discussed in the following section.

Centrosome MTOC activity affects stem cell differentiation

Centrosomes participate in MT dynamics and organization in mammalian progenitor cells and are the main MTOCs in most stem cells [3,15–18]. Importantly, centrosomes also incorporate specific proteins to regulate their MT nucleating capacity according to cell type and cell cycle phase [3]. Changes in centrosome MTOC activity in different cells are well known (reviewed in [19,20]), including the decrease in the ability of the centrosome to organize and nucleate MTs during differentiation [3,15–18,21,22]. This is the case for gut and muscle progenitors as well as skin stem cells [16,18,22]. Notably, some epithelial-type stem cells, such as NSCs, first upregulate centrosomal MTOC activity to promote delamination and differentiation and only subsequently reduce it as they further mature [3]. However, the direct role of centrosomal MTOC activity per se in fate determination and the underlying mechanisms is just starting to be elucidated [3,14,16,17].

Inactivation of centrosomal MTOC activity is achieved mainly in three ways: (i) by downregulating the expression of centrosomal MT organizers (in the PCM or at SDAs) [3,16,23]; (ii) by relocalization of MT organizers and nucleators to alternative noncentrosomal MT organizing centers [14,17,18,24]; and (iii) by completely removing centrosomes as in oocytes [25]. Removing whole centrosomes is an extreme case, as most differentiated cells conserve them. Therefore, centrosomes must serve other functions besides organizing MTs, such as cilia formation. As reducing MTOC activity is a prominent functional change of centrosomes during differentiation, the question arises if this is solely an accompanying process or if it directly modulates stem cell fate. Cellular processes essential for stem and progenitor cell homeostasis such as motility, adhesion, division, and cell signaling (many essential signaling molecules bind MTs) are MT-dependent. Altering centrosomal MTOC activity could thus directly regulate stem cell fate and behaviors, but in most cases it is not clear how. We next review recent key discoveries in mammalian NSCs and encourage readers to consult these studies [15–17,26,27] for other stem and progenitor cell systems.

During embryonic neurogenesis, centrosomal MTOC activity controls NSC delamination and differentiation, BP polarity, and neuronal migration. Centrosomes of the epithelial-like NSCs are located in the apical process and nucleate MTs in apical and basal directions [3,28]. Apical MTs form a ring around the junctions of the cell soma or process to stabilize them [29,30]. Similar to other epithelial cells [24,31], some of these MTs may be re-anchored to adherens junction by CAMSAP-family of proteins [32] and their interactors. Perturbation of SDA and DA proteins affects the organization of apical MTs, leading to ectopic delamination and abnormal differentiation [3,9,14,30]. Thus, centrosomal MTOC activity is essential to maintain NSCs physically in their niche (the ventricular zone) and control cell fate. Impairment of centrosomal MTOC activity stiffens the apical membrane, thereby activating the YAP pathway, which induces cell proliferation and a higher number of BPs [30]. RHOA signaling is another candidate that is likely affected by MTOC activity by altering the activation status of MT-bound effectors such as GEF-H1 [33,34]. Indeed, similar to NINEIN loss-of-function, RHOA loss disrupt cell junctions and leads to NSC delamination [35,36]. Thus, centrosomal MTOC activity is present in NSCs and also maintains their NSC identity by retaining them in the niche and regulating specific signaling pathways, directly or indirectly.

MTs are organized in varicosities of the basal process of NSCs by the minus-end MT stabilizing proteins CAMSAP [32]. That means NSC centrosomes are not the only organizing center in these cells. However, this changes once NSCs decide to differentiate, as they increase centrosomal MTOC activity [29]. NSCs do so by upregulating the expression of AKNA, a new centrosomal protein, which strongly organizes MTs at SDAs [3]. Similar to other canonical MT anchoring factors, such as NINEIN and CEP170, AKNA also localizes at the proximal ends (PEs), where it could contribute to MT nucleation. Indeed, AKNA overexpression increases MT nucleation in vitro and in vivo by recruiting the nucleation machinery [3]. The increase in AKNA protein levels leading to more potent centrosomal MTOC activity and MT nucleation induces cell junction weakening (e.g., by recruiting CAMSAP proteins to the centrosome), retraction of the apical processes, and delamination [3,29]. This shows that, besides maintaining NSC integrity, changes in centrosomal MTOC activity and MT nucleation also regulate early (possibly the first) steps of NSC differentiation. AKNA levels are highest in BPs, indicating that BPs have intense centrosomal MTOC activity and MT nucleation. This needs to be down- regulated for cells to repolarize and move to the cortical plate (CP), thereby regulating the duration that BPs spend in the SVZ [3]. As mentioned earlier, neurons inactivate centrosomes as they mature and reorganize MTs in noncentrosomal locations [21,23]. This seems to happen gradually, concomitantly to the downregulation of AKNA, as centrosomal MTOC is still required to a certain extent in migrating neurons to couple the nucleus to the centrosomes and allow nuclear translocation during migration (reviewed in [37,38]). Blocking centrosomal MTOC inactivation in neurons by keeping AKNA expression blocks BPs from leaving their niche [3]. Thus, as in NSCs, the rate of centrosomal MTOC activity controls BP and neuronal differentiation and behavior.

Centrosome protein heterogeneity regulates MTOC activity in stem cells

Not all centrosomes are equal, in particular regarding the protein composition. Initial proteomics studies of purified centrosomes established a defined set of core centrosomal proteins [39,40]. This pool of proteins expanded since researchers analyzed other types of cells and it seems clear that different cells can: (i) express different centrosome factors, or (ii) localize them to noncentrosomal locations, often to fulfill other roles. AKNA is one of many good examples [41–43], as it is highly expressed in lymphoid cells and neural progenitors, but not in fibroblasts (our own observation) or very lowly in epithelial cells [3]. Hence, it is paramount to investigate centrosomal proteins in the correct biological context to understand their cellular and molecular functions correctly.

In embryonic NSCs, the protein composition of SDAs can differ if they self-renew or differentiate. Self-renewing or proliferating NSCs decorate SDAs with NINEIN, and loss-of-function experiments indicate that MT anchoring at SDAs by NINEIN is necessary for stem cell maintenance [9,13,44]. In contrast, differentiating NSCs decorate SDAs with AKNA, and perturbation indicates that intense MT organization at SDAs drives stem cell delamination and differentiation [3]. Intense centrosomal MTOC activity is required to retract the apical process and reposition the centrosome towards nonapical locations [3]. Therefore, centrosomes with SDAs containing different proteins differ in their cellular function. NINEIN changes localization from centrosomes to MTs upon NSC differentiation via alternative splicing [14]. DCTN1/p150Glued is another SDA-associated protein that is alternatively spliced [14], which could also change its MT anchoring properties. These data suggest a model in which AKNA may take over the role of NINEIN in MT anchoring at SDAs in differentiating NSCs and BPs, while NINEIN coordinates the initial steps in gradually reorganizing MTs to noncentrosomal places in neurons.

Notably, the delamination mechanisms from an epithelial layer are not nervous system-specific, but also apply to other epithelial cells undergoing epithelial–mesenchymal transition (EMT). In these cells, AKNA localizes to the centrosome and recruits CAMSAP3 from cell junctions to the centrosome, thereby weakening the junctional complexes while promoting centrosomal MT nucleation and anchoring [3]. Reducing AKNA levels during EMT retains CAMSAP3 at junctional complexes and retains epithelial junctions. Thus, the cell type-specific composition of SDAs, with or without AKNA, potently influences centrosome functions and stem cell behavior. Notably, skin stem cells also delaminate, in this case from the basement membrane, to move towards suprabasal layers where they further differentiate [22,45]. However, the centrosome-related molecular mechanisms and regulators of this are just starting to be identified. Gut and muscle progenitor cells switch from centrosomal to noncentrosomal MT organization as they differentiate or mature [15,19]. However, these cells do not undergo a delamination process and the molecular regulators for this switch are also largely unknown. Therefore, changes in centrosomal MTOC activity happen in cell differentiation, even if delamination is not involved.

The expression of NINEIN and AKNA in NSCs is regulated by the transcription factors PAX6 [13,46] and SOX4 [47] (and our own observations), respectively. In PAX6 mutant NSCs, NINEIN expression is downregulated, while AKNA levels are elevated, and cells show precocious delamination due, in part, to aberrant cell adhesion at the apical surface. SOX4 promotes NSC differentiation to BPs [48]; knock-down of SOX4 reduces AKNA levels while overexpression increases them. Thus, stem cell- specific centrosomal proteins controlling MTOC activity are regulated at the expression level by context-dependent genetic programs and are not a passive differentiation effect.

The examples mentioned earlier in NSCs and other cells, such as epidermal, epithelial, and muscle stem/progenitor cells [15,16,18], highlight the key theme of the review, namely that the changes in centrosome protein composition occurring during differentiation are an active coordinated process required for controlling stem cell fates and behavior. Beyond protein specificity, there may be another layer of regulation and diversity at the RNA level, which we discuss next.

RNA contribution to centrosomal functions and differences

The presence of mRNA at or near centrosomes was observed in the mid-1960s [49,50]. First examples of specific mRNAs and mRNA-binding proteins were detected two to three decades ago [51–53] and since then have been validated in several model organisms, including immortalized mammalian cell lines. However, in primary stem cells this phenomenon has just started to be explored [54]. Nevertheless, high-throughput identification of cenRNAs and their partners, as well as the characterization of their dynamics in living cells at a quantitative level, remained a challenge. This has been overcome, at least for individual RNAs, thanks to single-molecule fluorescent in situ hybridization, transgenesis, live imaging, and machine learning for automatic quantification of cellular features [55,56], and has allowed investigation of the role of specific RNAs in more detail. Recent work showed that centrosomal mRNAs are moved to the centrosome by active polysome transport

[57] and may be translated at centrosomes or while moving towards them. Notably, distribution analyses of RNAs have revealed that centrosomes contain highly specific RNAs (e.g., amongst 602 genes encoding for centrosomal proteins screened only six had mRNAs concentrated at the centrosome in HeLa cells) and in low copy numbers [56–58]. This high degree of specificity [57] implies precise functional roles and argues against a purely structural role contributing to the formation of the membraneless centrosome compartment aided by RNA concentration [56,59].

Indeed, despite their low quantities, they are essential for the proper function of the organelle, as demonstrated by RNA digestion, RNA interference, or translation inhibition [54,60]. The mRNAs so far detected at centrosomes mostly encode centrosome- and MT-associated proteins with scaffolding roles (e.g., Plp/Pcnt) [56,61], MT organizing activity (e.g., Ninein, Cep350) [54,57], or MT nucleating and polymerizing functions (e.g., Aspm, Cyclin-b, Cen/CDR2/CDR2L) [54–56,61,62]. Importantly, the centrosome localization of Cen RNA has been shown to be functionally relevant, as mitotic aberrations occur in its absence [56]. Also, ribosomal RNAs have been observed at centrosomes and may help maintain spindle integrity and MT nucleation in coordination with RNA-binding proteins like RAE1, MASKIN, FUBP2, and FMRP [56,60,63]. Importantly, RNAs are found in interphase centrosomes or mitotic spindles, indicating specific roles during the cell cycle. For example, Pcnt mRNA accumulates and is translated in early mitotic spindles to enhance centrosome maturation [61], while Ninein mRNA is found at centrosomes in interphase, when SDAs are built to anchor MTs [54]. It has been observed that RNAs are loaded on polysomes or kept inactive in P-bodies, exon-junction protein complexes, and RNA export-factors until they reach the organelle and are translated there [54,60,64]. Yet, since there is no comprehensive RNA-seq data from the interphase centrosome, we do not yet know how diverse the RNA at the centrosome may be in different cell types and which other RNAs beyond those encoding for centrosomal proteins may be at the centrosome.

Could RNA contribute to specializing or diversifying centrosomes? One possibility could be enriching transcripts around one centriole or part of this to set it apart from the other centriole. This has been observed by Ryder and colleagues in fruit fly embryos [56] for Centrocortin, the Drosophila melanogaster homolog of mammalian CDR2 and CDR2L proteins [65], which is biased to the mother centrosome. Notably, the mother centrosome is richer in PCM and MT-nucleators PCNT/PLP and CDK5RAP2/Cnn [66,67]. This suggests that the mother centriole could nucleate more MTs during or after mitosis in these cells and, as in neuroblasts, could segregate molecules asymmetrically to the daughter cells. Another possibility would be delivering RNAs with specific modifications in the polyadenylation format or the splice pattern [51,54,57,62]. First, this could help mark and sort proteins from a bigger pool to be delivered precisely to the centrosome, such as cycling proteins or kinases. Second, differentially spliced transcripts could promote loading centrosomes with variations of a protein once translated there to regulate its activity. This could be the case for NINEIN, DCNT1, AKAP9, DYNC1I2, KIF2A in NSCs and neurons [14] and the brain-specific MT-associated protein kinase SAD-A (see 'Note added in proof' section). The centrosomes containing one or the other splice variant may thus behave differently. Finally, proteins translated at centrosomes may not be subject to the same post-transcriptional modification as in the endoplasmic reticulum and Golgi. As with splice variants, a different post-translational modification could also affect protein function and, in turn, centrosomal behavior in different cell types.

Sequencing cenRNAs in different (stem) cell types will substantially help understand the exciting and emerging field of RNA localization and function at the centrosomes. Hassine and colleagues have taken the first steps by performing transcriptomic analysis of purified mitotic spindles in one cell line, showing that thousands of RNAs of different classes are enriched there and STAUFEN1 regulates the localization of many of them [68]. It is worth noting that using crude preparations of purified centrosomes requires a large number of cells and can be contaminated with noncentrosomal proteins and nucleic acids, making it difficult to adapt this approach for stem cells, in particular those with low cell numbers, which calls for other methods. These could be APEX-mediated biotinylation, which allows the monitoring of RNAs at many organellar sites [69], or pulling down RNA-binding proteins in cellular fractions to at least minimize contaminations from other sites. Possibly, a combination of ultrastructural sections combined with sequencing approaches may also be a promising approach [70].

Concluding remarks

Here, we discussed centrosome heterogeneity as a regulator of stem cell function during mitosis and in interphase. This is particularly relevant as these processes affect cellular and functional diversity in ontogeny and phylogeny. Protein and RNA composition of centrosomes can differ between cell cycle stages without major changes in cell identity, as in amplifying NSCs early in development and in commonly used cell lines. However, currently, we lack information in any primary stem and progenitor cells (with the exception of [16]). The aforementioned considerations should motivate comprehensive studies of the centrosome proteome and transcriptome in primary (stem) cells, as we now learn about the key roles of differentially regulated centrosomal factors, to then combine with functional assays using cell type-specific fluorescent reporters [17].

Learning about organelle heterogeneity is important to answer relevant questions in developmental biology and to comprehend diseases better. Metastasis formation is one of the greatest challenges in treating cancer and the centrosome and cytoskeleton play key roles (see Outstanding questions). Their distinct composition in different cancers may shed some light on their metastasis mechanisms. Furthermore, localization of a ubiquitous protein in a specific organelle in one tissue or cell type may help prioritize gene variants found in patients with a specific disease phenotype. Some ubiquitous splicing proteins are found specifically at the centrosome [71] in NSCs (A. O'Neill et al.), allowing prioritization of mutations in patients with neuronal ectopias, that derive from cells failing to delaminate and/or migrate towards their correct positions. Given the insights into delamination mechanisms and specific types of migration in the developing cortex, one may envision manipulating MT and centrosome dynamics to counteract those defects. It is encouraging that at least in one pioneering example, ectopic neurons could be instructed to resume migration by doublecortin overexpression [72]. Excitingly, such approaches could now also be tested in human models of heterotopia [73], possibly using small molecules already used in therapies in humans (e.g., taxanes, eribulin, and vinca alkaloids), which, at specific doses, can fine tune MT dynamics. Thus, exploring and understanding centrosome heterogeneity allows targeted manipulation towards novel, highly specific, therapeutic approaches.

Beyond the centrosome, could organelar heterogeneity be a general principle to multiply and specify functions of proteins to generate large cellular diversity in ontogeny and phylogeny?

The mitochondria of glia and neurons differ by about a fifth of their proteome in vivo and in vitro [74,75] and this accounts for differences in fatty acid metabolism, calcium buffering, and protection against damage by reactive oxygen species directly affecting cell fate (e.g., in glia-to-neuron reprogramming [75] and neurogenesis [76,77]). Recent work showed that self-renewing NSCs have high levels of the nuclear factor Trnp1 [78] that regulates the size and function of nucleoli, another organelle with functions in most cells. This promotes proliferation, self-renewal, and protein synthesis in self-renewing NSCs [79] as opposed to differentiating NSCs that lose Trnp1 [78], and ultimately affects brain size and folding [78,80]. Also the cytoskeleton shows enormous protein diversity. MTs, for exam- ple, are made of alpha and beta tubulin dimers, for which there are up to nine isoforms each in humans. The combination of isoforms, which is highly cell type-specific, together with different post-translational modifications, potently regulates MT dynamics [81], which in turn influences differentiation, migration, and delamination of stem and progenitor cells [3,17,26,82–84]. These considerations call for unbiased proteome analysis of organelles in a cell type-specific manner, for example, using fractionation of the proteome, enriching different organelles in distinct fractions [85]. This is now possible due to much- increased sensitivity in mass spectrometry and the unprecedented access to the entire diversity of human cell types from induced pluripotent stem cells. Thus, the future looks bright for cell biology to unravel the cell type-specific functions of organelles beyond their typical roles.

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520

Highlights

- Near ubiquitous organelles such as centrosomes differ structurally and functionally in mammalian stem cells and their progeny, expanding the concept of universal cellular functions.

- Cell type-specific spatiotemporal localization of proteins and RNAs control heterogeneity of centrosomes and other organelles.

- Differential centrosomal microtubule organizing center (MTOC) activity controls stem cell behavior, adding a layer of regulation to diversify cell types during ontology and phylogeny.

- Understanding cell type-specific composition and function of organelles (e.g., the centrosome) opens new approaches to target specific cells in disease, such as metastasis or neurodevelopmental disorders.

Glossary

Cerebral cortex: the dorsal region of the telencephalon that expanded particularly in mammalian phylogeny. It can be smooth (e.g., in mice) or folded/ gyrified (e.g., in ferrets and human).

Microtubules (MTs): protein polymers of alpha- and beta-tubulin that serve as a platform over which many complexes move within the cell. MTs also separate chromosomes during cell division. MT dynamics refers, broadly speaking, to the growing and shrinking behavior of MTs.

Neuronal ectopia: foci of misplaced neurons within the cerebral cortex. The ectopia arise during brain development and are partly caused by mutations in genes controlling cell delamination and migration.

Nucleoli: the largest substructure of the nucleus, where, among other processes, ribosomal RNA synthesis takes place.

Ontogeny: the developmental history of an organism.

Organelles: specialized subunits within a cell. They can be spatially segregated by membranes or by different liquid phases. Examples are the mitochondria, the Golgi apparatus, the endoplasmic reticulum, centrosomes, phagosomes, etc.

Phylogeny: the evolutionary history of an organism and the relationship among or within species.

Polysome: a group of ribosomes bound to a molecule of mRNA, which then can cotranslate polypeptides out of the same molecule in tandem.

Proteome: the entire set of proteins present in a cell or tissue. The proteome of an organelle indicates correspondingly all proteins in that organelle.

Radial glia cells: the neural stem cells of the developing brain and spinal cord. They are a specialized type of epithelial cells.

Subventricular zone (SVZ): the region in the developing brain directly above the ventricular zone (where RGCs reside) and below the intermediate zone and cortical plate (where neurons are located). The SVZ is the niche of basal progenitors.

570 Taxanes, eribulin, and vinca alkaloids: antimitotic drugs usually used in chemotherapeutic approaches
571 to eliminate cancer cells. Taxanes like paclitaxel/Taxol and docetaxel/Taxotere prevent MT
572 depolymerization by stabilizing GDP-bound tubulin in MTs. Eribulin/Halaven blocks MT polymerization
573 by binding sites at the plus ends of existing microtubules. Vinca alkaloids, such as vincristine und
574 vinblastine, prevent MT polymerization by binding and blocking tubulin heterodimers.

575

Box 1. Neural progenitors define the architecture of the forebrain.

The basic principles governing the formation of the mammalian forebrain, particularly the cerebral cortex, are conserved in phylogeny [2]. Radial glial cells (RGCs), the neural stem cells, line the ventricular zone (VZ) and directly contact the ventricle through their apical process containing the primary cilium and the centrosome. RGCs are bound to each other via cell junctions at apical processes, thereby forming a polarized epithelium. RGCs divide in the VZ to self-renew or give rise to differentiating progeny during the neurogenic period. This progeny can be a neuron that will immediately move out of the VZ to the cortical plate (CP) where they mature, using the basal process of the RGC as a guide and support. Alternatively, RGCs give rise to intermediate transient-amplifying basal progenitors (BPs), which will sit directly above the VZ to make one or more rounds of division, thereby forming a new layer termed the subventricular zone (SVZ). The multipolar BPs then transform into bipolar neurons that leave the SVZ and head towards the CP to differentiate further and mature. The repolarization process is essential to control the time that BPs spend within the SVZ [3,92], which supports cell expansion and, ultimately, neuronal output. At the peak of cortical neurogenesis, most neurons are produced via BPs. In species with folded brains, such as primates, these become even more frequent and diverse, culminating in additional and larger SVZs (inner and outer SVZ).

Moreover, in this period, a least in rodents, nine in ten RGC divisions are symmetric (i.e., giving rise to two RGCs). Hours later, one or both daughter cells (now called differentiating RGCs) delaminate towards the SVZ and transform into BPs [93]. Daughter cells that do not differentiate but divide are known as proliferating RGCs. Proliferating and differentiating RGCs can be identified by the expression of BTG2/TIS21 [94] and, as more recently shown, by the expression of centrosomal proteins and the dynamics of microtubules [3].

Box 2. Can SDAs contribute to MT nucleation and growth?

SDAs look like conical-shaped stems ending in a rounded head in electron microscopy photographs, which some scientists think may contain MT nucleators. Gamma-tubulin has been shown convincingly at SDAs at least by five studies [88,95–98]. Schweizer and colleagues show MTs emanating from the distal part of centrioles in MT regrowth assays (see Figure 1B arrowheads in [95]). Furthermore, SLAIN2 interacts with core SDA components NINEIN, ODF2, CEP170, CEP128, CNTRL, and EB1/MAPRE1 [99]. EB1 is an SDA protein but also a MT plus-end tracking (+TIP) factor like SLAIN2 involved in MT growth and stabilization via interactions with the MT polymerase ch-TOG/CKAP5, cytoplasmic linker proteins (CLIPs), and CLIP-associated proteins (CLASPs). Thus, SDAs could potentially attract MT nucleation and growth machineries.

609 **Table 1. Centrosome associated proteins discussed in this review.**

610 Abbreviations: DA, distal appendages; DC, daughter centriole; MC, mother centriole; MTs,
611 microtubules; PCM, pericentriolar material; SDA, subdistal appendages.

Protein	Localization	Functions
CDK5RAP2	PCM	PCM scaffold protein, centrosomal γ-tubulin localization, MT nucleation
CEP152	PCM	PCM scaffold protein
CEP192	PCM	PCM scaffold protein
NEDD1	PCM	MT organization/anchoring and nucleation, centrosomal γ-tubulin localization
PCNT	PCM	PCM scaffold protein
TUBG	PCM	MT nucleation, MT minus-end capping
CEP135	PE	Centriole–centriole cohesion
C-NAP1	PE	Centriole–centriole cohesion
CEP68	Linker fibers	Centriole–centriole cohesion
CEP250	Linker fibers	Centriole–centriole cohesion
ROOTLETIN	Linker fibers	Centriole–centriole cohesion
CEP83	DA	Dock MC to cell membrane, role in primary cilia formation
CEP89	DA	Role in primary cilia formation
CEP164	DA	Dock MC to cell membrane, role in primary cilia formation
LRRC45	DA + PE	Role in primary cilia formation, centriole–centriole cohesion
SCLT1	DA	Role in primary cilia formation
AKNA	SDA + PE + MTs	MT organization/anchoring, nucleation, polymerization
CCDC68	SDA+ PE	MT organization/anchoring
CCDC120	SDA + PE	MT organization/anchoring
CEP128	SDA	MT organization/anchoring
CEP170	SDA, MTs	MT organization/anchoring
CEP350/CAP350	SDA and DC	MT organization/anchoring
CNTRL	SDA	MT organization/anchoring
DCTN1	SDA + PE + MTs	MT organization/anchoring
NINEIN	SDA + PE + MTs	MT organization/anchoring and nucleation
ODF2	SDA	MT organization/anchoring and nucleation and role in primary cilia formation
EB1	SDA + MTs	MT organization/anchoring, role in primary cilia formation, MT growth and stabilization
CEP120	DC + PCM	Regulation of PCM assembly
CTROB	DC	Regulation of centriole duplication
CAMSAP	MTs + PCM	MT minus-end capping, MT organization/anchoring and nucleation

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613

Outstanding questions

Can we modify the composition of organelles to control their behavior and thereby instruct (stem) cells to produce a desired cell type for use e.g., in regenerative therapies?

Which factors (e.g., proteins, RNAs) regulate organellar heterogeneity, in which quantities, and at what time points?

Are there other yet-uncovered processes related to or coordinated by RNAs taking place at centrosomes, such as RNA metabolism, RNA inhibition, assembly of ribonucleoproteins, or even splicing itself?

How large is centrosome diversity in cancer and metastasis?

Note added in proof

While this review was in proof stage, A. O'Neill et al. (Science, in press) showed a high degree of centrosome proteome differences between cell types and during neural stem cell to neuron differentiation with a striking abundance of distinct RNA-binding proteins with relevance to neurodevelopmental disease.

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Declaration of interests

The authors declare no competing interests.