



Brain Evolution and the Centrosome

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Abstract | Microtubules are cytoskeletal elements that are important for many cellular processes. Mitotic spindles that are formed for cell division are made up of microtubules. Centrosomes are cellular organelles that organize microtubules. In most cells cellular constituents are not distributed symmetrically within the cell and therefore cells exhibit distinct polarity and asymmetry. Therefore many cells have an apical side and a basal side. The microtubules also exhibit polarity along their proximal distal axis from their site of anchoring and nucleation. When a cell divides the centrosome also divides along with DNA replication. Thus each of the daughter cells will inherit a centrosome. Recent evidence suggests that the two centrosomes may not be equivalent and that the centrosome that a cell inherits may influence cell fate. In addition, during cell division the centrosomes are important for the alignment of the mitotic spindles and determining the plane of cell division. This in turn could impact the distribution of proteins in the dividing cell and thus confer different cell fates on the daughter cells. These aspects as they relate to neural stem cell division and regulation of neuron number are explored. In addition, the origins of the function of the mammalian centrosomes are traced.

Keywords: centrosome, microtubules, neural progenitors, asymmetric cell division.

1 Introduction

The origins of the nervous system lie deep in evolutionary space about 500 million years away where creatures such as comb jellies and sea anemones belonging to the phylum Ctenophora and Cnidaria had network of nerve cells that communicated with each other through synapses. This network of interconnected nerve cells presumably evolved into the dense interconnected neuronal network that is concentrated at the anterior end of the animal that we call the brain.^{1,2} How this happened is a matter of speculation but it is certain to have involved important changes in developmental patterning, such as the appearance of a hollow gut, formation of a second opening and inversion of the dorsoventral body axis.³ All chordates have a dorsal hollow neural tube and the nervous system arises from the neuroectoderm that is internalized during development by neurulation.³ The generalized vertebrate central nervous system has

a telencephalic and diencephalic forebrain region, a midbrain region, a hindbrain region and a spinal cord. These regions are patterned by patterns of gene expression during development that establishes a cartesian grid system in which the anterior-posterior axis and dorsal-ventral axis are specified. Positions along the grid have a unique pattern of gene expression and the neural development and patterning proceeds by decoding this unique gene expression pattern.³ One remarkable feature of the evolutionary lineage that led up to our own human brain is the huge expansion of the cerebral cortex that develops from the telencephalic forebrain region. The cerebral cortex of the human is many times folded in complex ways to accommodate an expanded surface area and it remains a modern iconic image of our claimed superiority as a species. In this review, we will discuss one mechanism that has been co-opted to give rise to this huge expansion of the cerebral cortex.

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2 Asymmetric Cell Division and Cellular Diversity

Evolution of multicellular organisms involved generation of cellular diversity leading to greater complexity. The origin of the mechanisms for generating cellular diversity can be traced back to the way unicellular eukaryotic organisms propagate themselves. This mechanism was then used to generate different kinds of cells and it is a variation of this same mechanism that has also been used to increase the size of the cortex in our species. When a cell divides, in the simplest instance, it makes a copy of itself. This is known as a symmetric cell division. But in order to achieve cellular diversity, the divided cells have to adopt different cell fates. Several possibilities exist for how this can be achieved. One possibility is that the cell divides such that the cellular components are not divided equally between the two daughter cells and hence they acquire different cell fates. This type of cell division is known as an **asymmetric cell division**. In addition, the environment in which a cell divides can also influence cell fate, so that even though cell division has been symmetric, exposure of the two daughter cells to different environments can lead to different cell fates.

3 Microtubule Organizing Centre

Cell division involves the duplication of genetic material followed by the segregation of genetic material into the two daughter cells, a process that requires physical motion and energy to accomplish. In the living cell this is provided by the **cytoskeleton**, proteins that provide scaffolding and maintain cell shape. In addition, the cytoskeleton also plays an important role in sensing signals, intracellular movement of proteins and for movement of the cell itself. Important cytoskeletal elements that participate in this process are **microtubules** and **actin**. In eukaryotes, the microtubules consist of heterodimers of two different tubulin protein subunits that interact laterally with each other to form a hollow tube. Subunits are added to this tube to lengthen it, a process known as polymerization. In a cell free system, the rate of microtubule polymerization is proportional to the amount of free tubulin present. However within the cell there are **microtubule organizing centres** that catalyse tubulin nucleation at concentrations that would be too low for spontaneous polymerization. One class of tubulin known as gamma tubulin forms a conserved ring structure that is important for it to function as a nucleator of microtubules.⁴ The gamma—tubulin ring complex consists of a complex of proteins including gamma-tubulin that stabilizes alpha and beta tubulin heterodimers

by binding to them and this provide the seed for polymerization of microtubules.⁵

4 Basal Body/Centriole and Axoneme/Cilium

The canonical **axoneme** consists of nine microtubule doublet placed radially and this classic structure evolved from the microtubules of the cytoplasm around the gamma-tubulin ring structure.⁶ The axoneme could initially have started out as a cytoskeletal protrusion with sensory function and as signalling molecules and receptors were specifically targeted to the protrusion this would have evolved into a sensory patch. The transformation from a sensory patch to an axoneme needed the organization of microtubules and molecules that provided directional force to the axoneme.⁷ With this modification the axoneme, apart from a sensory function, could now be used to perform a gliding like motion by the organism by anchoring the axoneme and pulling the cell body forward in a process known as gliding. In addition, the 9 fold symmetry of the axoneme may also enclose a pair of microtubules at the centre which would allow the axoneme to beat as in the case of the **flagella**. The base of the axoneme is called the **basal body** and this structure may have evolved simultaneously with the axoneme to initiate its growth. Therefore the primitive basal body performed a sensory and motile function. However, an alternative hypothesis for the origin of the basal body is that it was acquired by either endosymbiosis, or through viral integration.⁷

The addition of other proteins and structures to the basal body facilitated the interaction of the basal body with other cellular organelles. The axoneme too underwent changes to become the **cilium** which is a more complex version of the axoneme when the axoneme is covered by a membrane called a ciliary membrane to become a separate cellular compartment. The basal body evolved into the more complex **centriole** which became a separate cellular compartment. Its ultra structure allows a 9 fold symmetry composed of microtubule triplets (A, B, C tubules), each one connected to a central hub via a spoke and a pinhead. The basal body/centriole also provided a polarity to the cell and organization of other subcellular structures relative to the flagellum/cilium. An important aspect that contributed to the evolution of the cilia from the axoneme in eukaryotes was that along with the facilitation of microtubule polymerization by the basal body there was also an evolving repertoire of **molecular motors** that moved along microtubules and actin filaments by using ATP as an energy source. Molecular motors

fall into several classes and because of their protein structure these motors can move in only one direction along a microtubule or an actin filament.⁸ (Figure 1)

5 Centrioles in Cell Division

Adding molecular motors that move along microtubules radiating out of the basal body/centriole may have contributed to the basal bodies not always being attached to an axoneme or cilia but becoming free centrioles within the cell. All eukaryotic cells use microtubules for cell division. With the evolution of different motors, the centrioles could now interact with cytoplasmic microtubules and play a role in positioning of mitotic spindles during cell division.⁹ The mitotic spindles that segregate the genetic material are organized by the centrioles and consist of the *kinetochore spindles* that attach to the chromosomes, the *interpolar spindles* that lie between the two centrioles and the *astral spindles* that radiate out into the cytoplasm and eventually attach to the actin rich area beneath the plasma membrane known as the *cell cortex* or *cortical cytoskeleton*.¹⁰ Actin is a filament forming protein that utilizes energy to increase and decrease its filament length. The astral microtubules emanate from the centrioles and interact with the actin cortical cytoskeleton

to position the mitotic spindles and determine the plane of cell division.

6 Basal Body/Centriole Structural Asymmetry

One property of the basal body/centriole structure is that it is highly asymmetric along its proximo-distal axis. The tubulin cytoskeleton is polarized structurally because tubulin is polymerized and elongates by the hydrolysis of Guanosine-5'-triphosphate (GTP) only at one end called the plus end. This property along with the evolution of molecular motors that move only in one or the other direction gives the microtubules a directional asymmetry. Microtubules that radiate out from the centriole can thus provide a way of organizing and positioning other cellular organelles in an asymmetric fashion. Another aspect of asymmetry is that when a basal body/centriole duplicates during cell division the result is an 'older' centriole and a 'newer' recently duplicated centriole. These inherent asymmetries of the basal body/centriole are used to generate spatial organization within the cell that became critical to survival. This point is illustrated in the green alga *Chlamydomonas reinhardtii* in which the placement of the eye spot with respect to the flagella is important for its photo taxis behaviour.

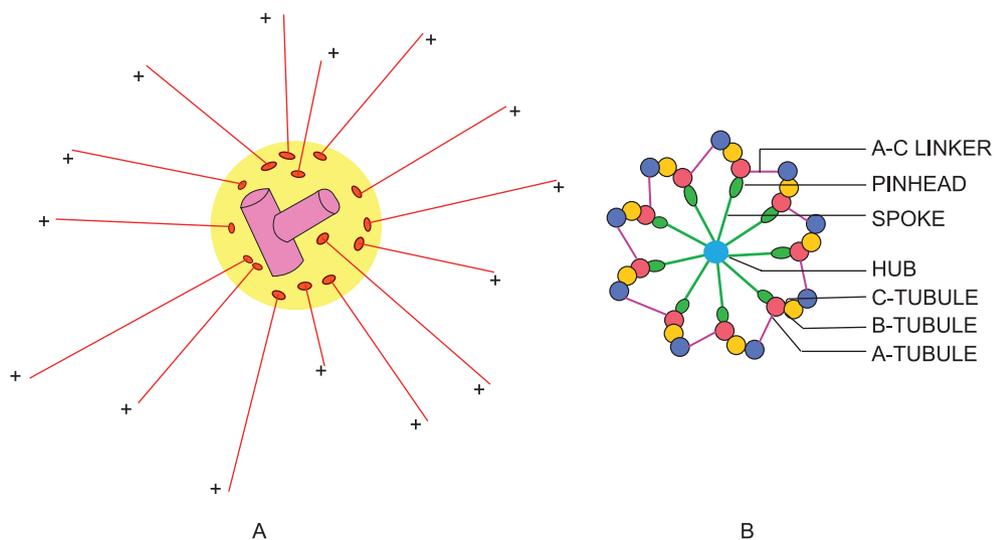


Figure 1: Centriole structure. (A) The centrioles (pink) are surrounded by an amorphous cloud of proteins called the pericentriolar material (green) and together they constitute a centrosome. Microtubules (brown lines) emanate from the centrosome and grow at the + end while the – end is attached to the centrosome via gamma tubulin ring complexes (red). Taken from Alberts, *et al.*, Molecular Biology of the Cell, Edition V. (B) The centriole is made up of repeated units of the cartwheel structure wherein a central hub is connected to nine microtubule triplets via spokes. At the end of each spoke is a pinhead which is attached to the A-tubule. The B and C tubules are incomplete. Each A-tubule is connected to its adjacent C-tubule by an A–C linker. The distal region of the centriole only contains the A-tubule and B-tubule (doublet region). Adapted from Azimzadeh and Marshall, 2010.

7 Using Basal Body Asymmetry to Propagate Cellular Organization

The unicellular green algae *Chlamydomonas reinhardtii* can exhibit phototactic behaviour. It has an eyespot that can sense light and it can move towards it by means of two flagella that are nucleated by basal bodies at its anterior end. In addition, because of molecular motors, there is bidirectional movement of proteins into the flagella, a phenomenon known as **intraflagellar transport**. Phototaxis requires the eyespot to occupy a precise position in the cell in relation to the flagella. This asymmetric position of the eyespot is dependent on the microtubules called **rootlets** that extend from the basal body into the cytoplasm to the posterior end. During *Chlamydomonas reinhardtii* cell division, the basal bodies divide and the older inherited basal body is identified as the 'older' mother and the newly formed basal body

is identified as the 'newer' daughter. This generational asymmetry is manifested in the fact that the two basal bodies do not have the same ultrastructure and protein composition. The asymmetry of the two basal bodies is transmitted throughout the cell organization giving the cell a polarity that is propagated through cell divisions in a stereotypic manner.¹¹ The basal bodies that give rise to the two flagella also anchor microtubule rootlets that extend into the cytoplasm. However the rootlets that arise from the older basal body and the ones that arise from the newer basal body are not identical. The new eyespot after cell division always forms near the plus end of one of the rootlet of the newer daughter basal body. Thus the rootlets themselves being not symmetrical are instructive in where the eyespot will form, and this asymmetry is because of the asymmetry of the basal body.¹² (Figure 2)

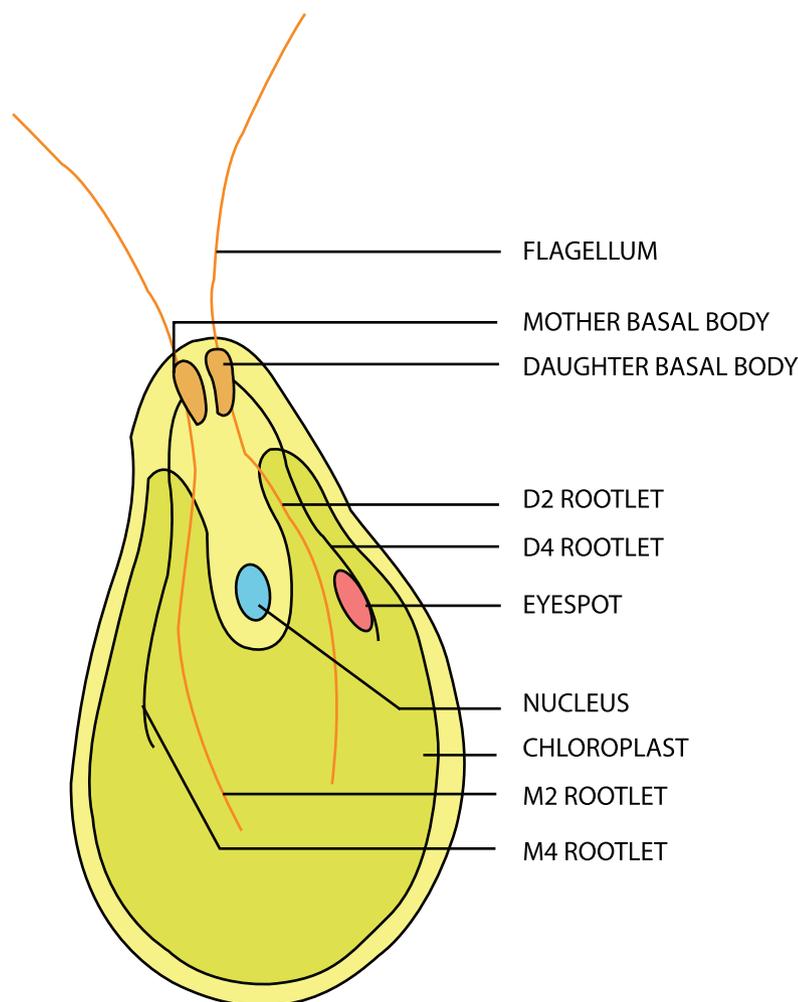


Figure 2: Eyespot asymmetry in *Chlamydomonas*. Two rootlets emanate from each basal body; M2 and M4 from the mother basal body and D2 and D4 from the daughter basal body. The eyespot always develops from one of the rootlets attached to the daughter basal body. Adapted from Boyd *et al*, 2011.

Therefore the proper placement of the eye spot and cellular organization is given by the inherent asymmetry of the two basal bodies and the microtubules that extend from it and also the proteins that are bound and transported along these microtubules. The cellular organization conferred by the old and new basal body that nucleate the two flagella and situate the eyespot towards one side also provides a mechanism for division of *Chlamydomonas reinhardtii* along a specific geometrical plane so that cellular components are equally partitioned.¹³ The mechanisms of intraflagellar transport and asymmetry between the two basal bodies propagate the asymmetry in eye placement in these algae. In addition, the directionality of the microtubules with respect to where they are attached to the centrosome via their minus ends directly influences polarity along the proximo-distal axis of the flagella.¹⁴

8 Using Spindle Pole Body Asymmetry to Achieve Asymmetric Cell Division

An important instance in the way the asymmetry of the two centrioles has been used is when the cell divides asymmetrically. *Saccharomyces cerevisiae* or yeast belongs to the kingdom Fungi and has been a fantastic model organism for dissecting many basic

biological processes. Among them is asymmetric cell division. Yeast belongs to the evolutionary lineages that have lost cilia or flagella and therefore also lack a basal body or centriole. However the equivalent of the centriole in the yeast is a structure known as the *spindle pole body* (SPB). SPB is the microtubule organizing centre in the yeast that is embedded in the nuclear membrane.¹⁵ As in the case of centrioles, SPB contain gamma-tubulin as well as other proteins that nucleate microtubules.¹⁵ SPB is capable of nucleating nuclear and cytoplasmic microtubules and the cytoplasmic microtubules are polarized with the plus end extending into the cytoplasm. Yeast reproduces asexually by a process known as budding. A new bud forms as a protrusion of the plasma membrane and the replicated chromosomes are moved into the bud and then the bud is then pinched off by constriction of the plasma membrane to generate a new daughter cell.¹⁶ This is an example of asymmetric cell division as the daughter cell is smaller and differs in several aspects from the mother cell.^{17,18} (Figure 3)

During budding, the SPB replicates and has two important functions. First, it has to pull the chromosomes apart and secondly it has to align the mitotic spindles in such a way that the

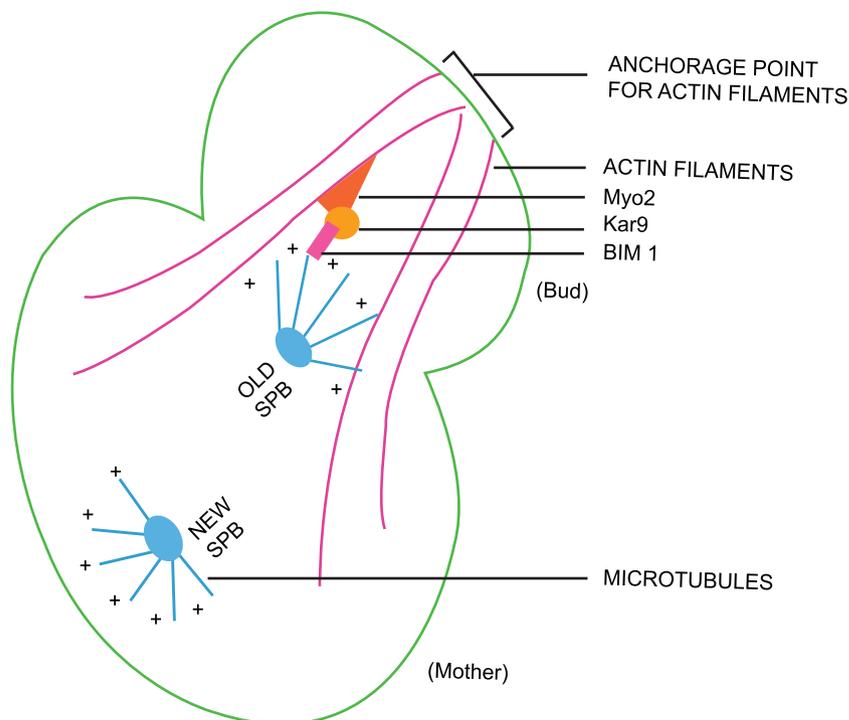


Figure 3: Asymmetric spindle pole inheritance in yeast. The old spindle pole body is always inherited by the daughter cell (bud). The Kar9-Bim1 complex, on the + end of the microtubules, is actively transported by Myo2 towards the neck of the bud on actin rails carrying the daughter spindle pole body along with it.

duplicated genetic material goes into the daughter cell which is aligned along the correct axis. In addition, the SPB that goes into the daughter cell also has the additional function of not allowing mitosis to proceed until the nucleus is within the daughter cell. As in the case of the basal bodies the SPB is also asymmetric, in that, the newer duplicated SPB and the older SPB are not structurally and functionally similar, and during the budding process the older SPB is always inherited by the daughter cell.^{19–21} One difference is that the two SPBs have unequal microtubule nucleation capacity with the older SPB being more efficient and hence inheritance of the older SPB allows for the microtubules to be anchored to the membrane of the bud so that the mitotic spindle can be properly oriented for budding.²² The way this process is accomplished is that the older astral microtubules of the mother SPB preferentially recruit a protein called Bim1 which then recruits Kar9.²³ This complex travels towards the plus end of the microtubule where it binds to type V myosin Myo2 which then allows this complex to be guided by actin cables to the neck of the bud.²⁴ The final stage of correct spindle positioning and anchoring to the daughter cell cortex requires the dyenin-dynactin motor complex. Inactive dyenin-dynactin is transported along the mother SPB microtubules into the daughter cell cortex where proteins present in the daughter cell cortex activate it thereby exerting a pulling force on the astral spindles and thus pulling the mother SPB into the daughter cell and correctly positioning the cell division plane.¹⁰ Although, the SPB is used for correct positioning of the mitotic spindles it does not seem to be involved in the determination of where the new daughter bud will form. This information is conveyed by the polarization of the actin cytoskeleton by a Rho GTPase family member Cdc42 that determines where the new bud will form.²⁵

9 Centrosome

The centrosome evolved from the centriole as a cytoplasmic organelle that was no longer associated with a cilium or flagellum. At the core of the centrosome are two centrioles with the conserved nine fold symmetrical ring structure. The centrosome provides polarity to a cell and regulates the spatial organization of the cytoskeleton. Apart from the two centrioles the centrosome is surrounded by a number of proteins known constituting the *pericentriolar material* (PCM). Both the centriolar core and the PCM have an ability to nucleate microtubules. During each cell division the centrosome divides once and there is an intrinsic difference between the older centrosome

and the newer centrosome as was the case with SPB and the basal bodies. Centrosome interaction with the microtubules can play a role in positioning the mitotic spindles and imparting polarity and asymmetry to the cell.²⁶

10 Centrosome and Cell Fate

In molluscs, belonging to the protostomes of the bilateria phyla, cleavages of the embryo are mostly asymmetric. It exhibits a stereotypical and spiral cleavage pattern in which the centrosome plays an important role in conferring asymmetrical cell fate of daughter cells.²⁷ In the mollusc *Ilyanassa obsoleta*, mRNA required for conferring cell fate in daughter cells is first localized to the centrosome by binding to proteins present in the PCM after directional transport towards the minus end of the microtubules. During cell division, the mRNA then moves towards the cell cortex by an actin dependent mechanism and the aster spindles are positioned such that the mRNA is asymmetrically inherited by one of the daughter cells.²⁸

The cleavage pattern of embryonic cells in the early evolutionary lineage of the flatworms is like that of the molluscs, however this mode of cleavage is lost in planarians in which the early embryo cleavages do not follow a specific cleavage pattern and is not stereotypical. Correspondingly, the centrosome that is present in the early lineage of the flatworms is subsequently lost in the planarians. This suggests that the centrosome is not required for the mechanics of cell division, a fundamental cellular process, but rather that it has acquired functions that are important for developmental processes such as patterning of the early embryo.²⁷ It is noteworthy in this respect that acentriolar flies have been engineered that are able to go through cell division, however these flies have defects in cell fate.²⁹

11 Centrosome and Establishment of Embryo Polarity

The *Caenorhabditis elegans* (round worm) zygote establishes asymmetries as it divides and patterns itself into an organism. During fertilization of the oocyte, the sperm contributes two things apart from the DNA. First, it contributes the centrioles and secondly it deposits a large amount of the protein CYK-4 which is a RhoGAP. The Rho family of GTPase are regulated by 2 classes of proteins—RhoGEF that activate Rho by triggering GDP/GTP exchange and RhoGAPs that inactivate Rho by catalysing GTP hydrolysis. The inactivation of Rho by CYK-4 results in the relaxation of the actin cytoskeleton that forms a mesh around the zygote. The result is that there is relaxation of

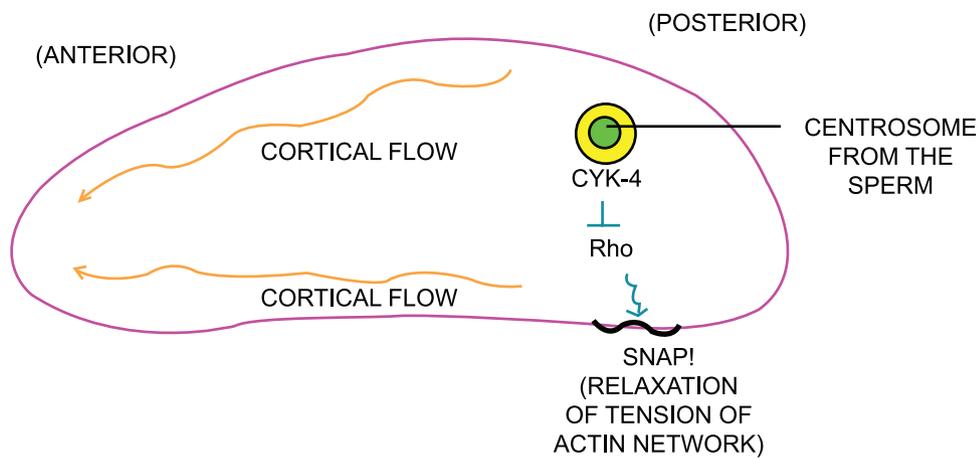


Figure 4: Generation of asymmetry in *C. elegans* embryo. (CYK-4) introduced by the sperm centrosome inhibits Rho activity causing a breakdown in the cortical actin network closest to the point of entry of the centrosome. This causes a contraction of the acto-myosin network leading to cortical flow and the concomitant distribution of Par proteins thereby generating a gradient of the same.

the actin cytoskeleton at the site of sperm entry and contraction at the opposite direction.³⁰ The polarized contraction of the actin network with the help of myosin sets up a force that allows for the movement of the cytoplasm known as **cortical flow** and results in the redistribution of organelles and proteins important for asymmetric cell fate as well as spindle axis alignment.³¹ This contraction of the actin network with the help of myosin is important for establishing the distribution of an important group of proteins called the **Par (partitioning defective) proteins** in the anterior part of the embryo.³² Par proteins are critical for establishing cell polarity and were discovered because mutations in these proteins caused polarity deficits.³³ It has been suggested that centrosome movement and spindle alignment follows cues established by cortical movement of actin.^{10,32} However, other studies suggest that the centrosome does not simply follow cues provided by the actin based cortical movement but in addition actively provides signals for cell polarity.³⁴ In fact some reports have suggested that the initial polarity signal could be proteins present in the centrosome that interact with the Par polarity proteins. In this case, the two centrosomes by means of directional traffic of proteins along microtubules could accrue proteins differentially during cell division and this asymmetry of the two centrosomes could then influence other cell polarity proteins including those associated with the actin cytoskeleton.³⁴ An additional contribution of the centrosome to cell asymmetry is by virtue of its position within the cell. Experiments have shown that cytoplasmic microtubules

constrain the motion of the centrosome within the egg and the resulting centrosome position within the egg is important in influencing where the initially symmetrical fertilized egg will begin to exhibit asymmetry. The cortical membrane closest to and overlying the centrosome will be the point where symmetry breaking is initiated that will ultimately result in an asymmetrical anterior and posterior half such that after cell division the two resulting daughter cells will be unequal. It is thought that a signal from the centrosome to the overlying cortex initiates this cell polarization event and once it is initiated the centrosome moves closer to associate with the cortex. This symmetry breaking in turn initiates cytoplasmic flows resulting in asymmetric localization of the Par proteins.^{31,35} (Figure 4)

12 Centrosome Size Asymmetry and Asymmetric Cell Division

The Par complex is also involved in setting up polarity in *Drosophila melanogaster* as in the case of *Caenorhabditis elegans*. The classic model system to study asymmetric cell division in *Drosophila* is the germline stem cell which gives rise to the gonialblasts. The stem cell remains attached to the mitotic niche known as the **hub** whereas the gonialblast leaves the hub to eventually become the sperm. The hub secretes a ligand Upd which activates the JAK-STAT (Janus kinase—signal transducer and activator of transcription) signaling pathway that keeps the stem cell from differentiating.³⁶ During this process of gonialblast cell division the older mother centrosome is inherited by the cell that remains attached to the hub

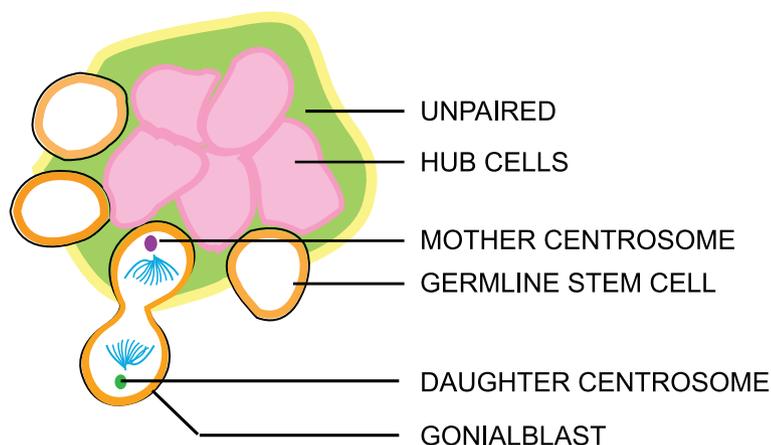


Figure 5: Centrosome inheritance in *Drosophila* male germline stem cells. Hub cells in the *Drosophila* testis secrete the protein 'unpaired' which keeps the cells closest to them in active proliferation. On division, the daughter cell abutting the hub cell always inherits the mother centrosome and the daughter cell which is farther away, and will eventually differentiate into a sperm, inherits the daughter centrosome.

and the daughter centrosome is inherited by the gonialblast.³⁷ This stereotypical inheritance of the centrosome is thought to be the result of centrosomal asymmetry, making the older centrosome more efficient in nucleating astral microtubules and therefore is able to attach to the hub more efficiently.³⁶ This differential ability to nucleate microtubules depends on the asymmetric localization of proteins such as centrosomin (Cnn) to the PCM.³⁸ The incorporation of Cnn is based on its interaction with other centrosome associated proteins and it is incorporated into the PCM closest to the centrosome first and therefore accumulates in a proximo distal manner. Once incorporated the Cnn moves distally into the surrounding PCM. Gamma-tubulin which is important for the stabilization and growth of the PCM can be bound by Cnn. Therefore, having more Cnn results in bigger centrosomes with larger PCM. In addition, because of more gamma-tubulin nucleation when more Cnn is present there can be asymmetric transport of other regulatory proteins.³⁹ In this way, the intrinsic asymmetry of the older mother centrosome and the newer daughter centrosome can contribute to different fates of the cells that inherit them. (Figure 5)

13 Centrosome Associated Kinases and Cell Fate

The presence of several centrosomally localized kinases also contributes to the asymmetry of the centrosomes. Localization of several polarity proteins depends on their phosphorylation status and this in turn is regulated by kinases such as Aurora A and Polo, both of which are associated with the centrosome.^{40,41} Polo is a key cell cycle regulator

and can phosphorylate Cnn leading to a stronger interaction of Cnn with other centriole proteins such as Asl/DSPd2 and therefore more accumulation of Cnn in the centrosome.³⁸ Furthermore, Polo localization can itself be asymmetric.⁴² Mutations of Polo lead to the loss of aPKC asymmetrical localization, an atypical protein kinase that is part of the Par complex. This results in misorientation of the spindles and overproduction of neuroblasts and suggests that localization of proteins that are important for cell fate is affected by loss of Polo. Polo kinase phosphorylates Pon (Partner of Numb) and this phosphorylation is important for the asymmetrical localization of Numb. Numb is an important cell fate determinant that inhibits Notch signalling and hence self renewal of neuroblasts.⁴³ In addition to its centrosomal location Polo is also found in the cytoplasm therefore it is possible that it is the cytoplasmic Polo that is responsible for phosphorylation dependent localization of proteins whereas centrosomal Polo is responsible for proper spindle orientation.⁴⁴ Aurora A is another kinase associated with the centrosome that is also important for the localization of Numb.⁴¹ Aurora A phosphorylates Par6 which changes the level of aPKC phosphorylation.⁴⁵ aPKC affects Numb localization and in the absence of Aurora A, aPKC and Numb are not properly localized and there is an excess of neuroblast production thus providing an additional pathway for affecting cell fate.^{46,47} As in the case of Polo, Aurora A is also found in the cytoplasm and therefore it has not been easy to dissect out the role of centrosomal localization of Aurora-A from its requirement in the cytoplasm.⁴¹ Thus several pathways lead to the asymmetrical localization

of Numb and several of these pathways involve modification by phosphorylation by kinases that are present on the centrosome.

14 Centrosome and the Division of Neuroblasts

In a cell division that is asymmetric, the distribution of proteins that are intrinsic cell fate determinants is polarized and the mitotic spindles are oriented such that cell division partitions the proteins asymmetrically.⁴⁸ In *Drosophila Melanogaster*, the **neuroblasts** that differentiate into neurons delaminate from the epithelium. The epithelial cells that make up this layer exhibit apical basal polarity. Likewise, the delaminating neuroblasts also exhibit apical basal cell polarity. The polarity proteins that are asymmetrically distributed and localized to the apical cortical surface include Bazooka/Par3-Par6/aPKC complex.⁴⁹ This complex further associates with other asymmetrically distributed proteins such as Inscutable (Insc), Pins and $G_{\alpha i}$. Initial polarity of proteins in the neuroblasts depends on the signals from the overlying epithelial cells from which they delaminate which recruits Bazooka/Par-3 through its interaction with membrane phosphoinositols. Bazooka/Par-3 then recruits Par6 and aPKC apically to form the apical polarity complex in the neuroblasts. Proteins that localize basally are Miranda, Prospero and Numb.^{16,50} However, part of the apical localization signal also derives from the centrosome, in particular the localization of aPKC which is dependent on centrosomally localized kinases.⁴⁵ Moreover, even if the apical basal polarity of the neuroblasts is established by cortical signals, upon delamination from the overlying epithelial layer, in subsequent cell divisions the centrosome seems to play an important role in the process of asymmetric partitioning of proteins and cell fate.⁵¹ Par complex and other polarity markers are not present after the first mitosis and therefore the initial cortical polarity is passed on during subsequent cell divisions by the centrosome through the astral microtubules that stay attached to the cortex.⁵² The neuroblast divides asymmetrically to give rise to two daughter cells. The apical cell is larger, inherits the apical membrane, remains a neuroblast capable of additional divisions whereas the basal cell is smaller and becomes a **ganglion mother cell**. In these divisions, the daughter centrosome always remains in the apical neuroblast whereas the older mother centrosome migrates into the ganglion mother cell, a correlation suggesting a role for centrosomes in asymmetric cell fate which includes unequal recruitment of PCM and increased motility of the centrosome

that moves into the cell that will be the ganglion mother cell. In addition, the proper alignment of the spindles is also dependent on one of the centrosomes always being attached to the apical surface.^{51,53} (Figure 6)

15 Information Flow from Centrosome to Astral Microtubules to Cell Cortex

From the previous example, it can be seen that astral microtubules that emanate from the centrosome to the actin rich cell cortex can play an instructive role in cell polarity. One pathway through which this occurs is a phenomenon known as **telophase rescue**. This is an important pathway for cell polarity that involves the kinesin motor heavy chain Khc-73 that moves towards the plus end of the microtubule and its interacting partner, the guanylate kinase Dlg. The binding of Khc-73 to Dlg results in the activation of Pins- $G_{\alpha i}$ pathway of cortical polarity. $G_{\alpha i}$ signalling is important for dynein motor activity and this activity is essential for proper spindle positions as it interacts with apical proteins through its association with Insc.^{10,54} Furthermore, the centrosome associated Aurora A kinase also phosphorylates Pins which is important for the recruitment of Dlg and Khc-73.⁵⁵

Another line of evidence for the role of centrosomal proteins in cell fate determination is the centriolar protein anastral spindle 2 (Ana2). This protein anchors the dynein light chain protein Cut up (Ctp) to the centrioles. Together Ana2 and Ctp are important for the spindle pole localization of Mushroom body defect (Mud), a protein that also interacts with Pins to orient the mitotic spindle.⁵⁶ Mud can directly interact with the dynein complex. Mud and Pins also can interact with the Par complex via the protein Insc. Therefore, in neuroblasts that express Insc, the Par complex (Baz/Par-6/aPKC) and the Pins complex ($G_{\alpha i}$ /Pins/Mud) are found on the apical surface. This is an interesting mechanism for generating unequal daughter sizes as both complexes exert a force on the mitotic spindle so that it is displaced anteriorly and results in a larger apical neuroblast and a smaller basal ganglion mother cell.¹⁶

16 Asymmetric Effects of Extrinsic Signalling Pathways on the Centrosome

The ancestral function of the centrioles was to nucleate microtubules for cilia formation but subsequently evolved into centrosomes that came to play an important role in cell division. Centrioles and basal bodies are common to all eumetazoa since both ctenophores and cnidarians possess

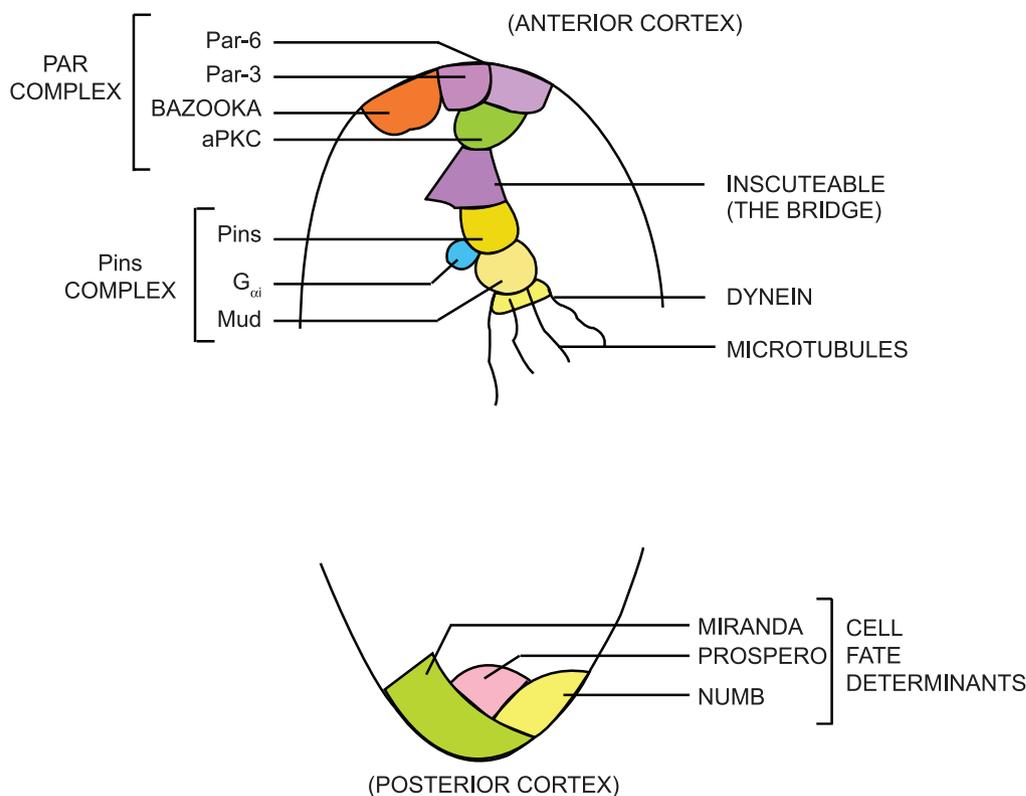


Figure 6: Arrangement of polarity proteins in *Drosophila* neuroblasts. The Par complex sets up a polarity in neuroblasts and the Pins complex, which directs spindle orientation, is made to align with the former by the protein 'inscuteable' in the anterior part of the cell. The cell fate determinants localize to the posterior part of the cell and the daughter cell inheriting these proteins tends to differentiate.

basal bodies and centrioles.⁵⁷ Most vertebrate cells have cilia that are either motile or nonmotile and in keeping with its evolutionary function the cilia participates in signal sensing in vertebrates. Therefore, evolutionarily conserved signalling pathways such as *Sonic Hedgehog* (Shh) and *Wnt* signalling are transduced by vertebrate cilia. In the case of Shh for example, the receptors for Shh are located on the cilia and this region is the main site for signal transduction of this pathway. Similarly, the cilium is also important for Wnt and its downstream effector β -catenin signalling.⁵⁸ As the centrosomes acquired an important function in asymmetric cell division, signals such as Shh and Wnt that are transduced at the cilia may have been co-opted to set up asymmetries during cell division.

We can therefore look at the evolutionary role of Wnt and see how this role has been co-opted during asymmetric cell division. The sea anemone *Nematostella vectensis* belongs to anthozoans which are basal members of Cnidaria. They possess two germ layers, an external ectodermal layer and an inner endodermal layer. Activation of Wnt signalling leads to the stabilization of β -catenin and

when this pathway is overstimulated it leads to an increase in endodermal precursors at the expense of ectodermal cells. This was due to the fact that β -catenin begins to be asymmetrically segregated in those cells that will give rise to the endoderm. Sea urchin embryos also have shown a similar role for β -catenin in cell fate determination suggesting that this may be common to all eumetazoans. Part of the asymmetry is due to selective degradation mechanisms for β -catenin and asymmetric localization leading to germ layer segregation.⁵⁹

The *Hydra*, belongs to Cnidaria, that is a basal branch of the bilateria tree, and has been studied as a model system for stem cell renewal and asymmetric cell division.¹ The *Hydra* has ciliary bodies and a centriolar structure although no direct link has been established between the role of centriolar structure and how it contributes to asymmetric cell division.⁶⁰ However, in the case of the species *Clytia hemisphaerica*, a cnidarian, it has been found that a highly conserved centriole associated protein, POC1, when disrupted, leads to cell cycle lengthening and arrests suggesting that the centriole was associated with cell cycle regulation very early in evolution.⁶¹

The body wall of the *Hydra* consists of an ectodermal and an endodermal layer and constitutes two different stem cell lineages. The third type of stem cell present in the *Hydra* is the interstitial cell that gives rise to cells of both germ cell and somatic cell lineages. The somatic cell lineages include nerve cells, nematocytes (stinging cells) and secretory cells. Activation of Wnt signalling in the *Hydra* led to an increase in interstitial cells accumulating β -catenin which was followed by a proliferative burst. This increase in cell division further led to an increase in the stem cell lineages of nematocytes and nerve cells.⁶² Although the interstitial cells had β -catenin, the differentiated lineages did not which suggests that its role in stem cell proliferation is an evolutionarily old one.

β -catenin is localized in between the centrosomes in many mammalian cells during interphase and it is important for establishing bipolar mitotic spindles. Separate binding sites also localize β -catenin to the centrosome during mitosis although the function of this localization is not clear. Over expression of β -catenin can lead to multiple centrosomes and abnormal spindles thus suggesting a role in the organization of mitotic spindles.⁶³ In epithelial cells, β -catenin is also found in *adherens junctions* that are specialized forms of cell-cell contact important for maintaining apical basal polarity.⁶⁴ Cytoplasmic dynein is important for the interaction of microtubules with the cortical actin cytoskeleton and dynein interacts with β -catenin and is a part of the adherens junction protein complex and therefore microtubules can be preferentially pulled to this site.⁶⁵ This may serve as a mechanism by which Wnt signalling may influence spindle position and cell fate. Although β -catenin is not asymmetrically localized to the centrosomes, asymmetric localization of other proteins that interact with β -catenin could nevertheless lead to asymmetric transduction of extracellular signals.⁶⁶ Chibby is one such protein that is present on the distal end of the mother centrosome. Chibby directly binds to β -catenin and represses its transcriptional effects. However the interaction of Chibby with another component of the centriolar appendage Cenexin relieves the inhibition of Chibby on β -catenin.⁶⁶ Therefore, Wnt signaling during cell division can lead to asymmetric effects on the two centrosomes and hence lead to asymmetric cell fates.

17 Involvement of the Centrosome in Cortical Size Increase

The high degree of encephalization of the human brain is the result of a sequence of evolutionary

changes that date back millions of years.⁶⁷ Clues to the genes that are important for regulating cortical size come from analysing abnormal brain development that result in smaller than normal cortical volume. *Primary microcephaly* is a recessive autosomal disorder in which the brain size is reduced and all 8 genetic loci that have so far been discovered code for proteins that localize to the centrosome at some point in their cell cycle.^{68,69} In addition, proteins that are known to be associated with microtubule organization and positioning of mitotic spindles also affect the number of neurons produced.⁷⁰ Therefore, mechanisms that involve the centrosome have had a major impact on the evolution of the cortex.⁷⁰ The ways in which the centrosome affects cortical development is being unravelled but the findings to date point to how evolutionarily conserved pathways are used to generate the neurons of the cortex.

During development, the *apical progenitors* are the *radial glial cells* that line the ventricles as a pseudostratified epithelial layer.⁷¹ The radial glial cells divide to give rise to neuronal progenitors and neurons.⁷¹ The radial glial cells have an apical basal polarity that is dependent on a conserved set of genes that also confer polarity in worms and flies.⁷² During radial glial cell division the mitotic spindle aligns with the polarity axis of the cell by mechanisms similar to that responsible for delaminating neuroblasts in the fly.⁷³ In mammalian neuroblasts the homologue of Pins is LGN and the Mud homologue is NuMA.⁷⁴

One important development that has allowed for the impressive expansion of the cerebral cortex to occur is that the neurogenic region of the developing cerebral cortex is not confined to the pseudo stratified epithelial layer lining the ventricles but rather extends up the *subventricular zone*.^{75,76} This region contains cells that are collectively known as *intermediate precursor cells* or *basal progenitors*. In primates, the subventricular zone is further divided into an inner subventricular zone and an outer subventricular zone. The outer subventricular zone of primates contain a class of progenitors that has been called the *outer radial glial (oRG) cells*, a type of basal progenitor cell.^{75,76} These cells have a basal process that attaches them to the pial surface. oRG cells go through self renewal as well as give rise to cells that are capable of transit amplification thereby increasing the neuronal output greatly.⁷² oRG cells have also been found in the lissencephalic cortex of mouse, however they are infrequent and do not go through transit rounds of amplification.⁷⁷ oRG cells arise from apical progenitors but have lost their apical process and have hence detached from

the ventricular zone. This is an important event since it allows for a large increase in radial surface area without a corresponding increase in the size of the ventricular zone.^{72,78}

Regulation of spindle orientation is important for governing daughter cell fate by pathways that are not fully understood in the context of the mammalian neocortex. However, mutations in *Insc* led to an increase in the number of planar cell divisions and abolished divisions in which the spindle was oriented vertically or obliquely with respect to the apical membrane. Correspondingly, there was an increase in the population of basal progenitors, cells that are derived from the apically located radial glial cells and divide to give rise to neurons.⁷⁹ (Figure 7)

In an example of evolutionary conservation apical progenitor radial glial cells, like the ancestral eukaryotic cells, contain cilia that are nucleated by the basal body/centriole.⁸⁰ The cilia of apical progenitor cells project into the ventricles and can potentially sense extracellular signals present in the ventricles that influences the way the apical progenitor will divide.⁸¹ When basal progenitors are formed, this is first preceded by the loss of the cilia in an apical location and instead cilia on the

delaminating basal cell is found in a basolateral position.⁸² It is tempting to speculate that the cues sensed by the cilia are critical for determination of cell fate and further that the centrioles play an important role both in the placement of the cilia and the orientation of the plane of cell division. (Figure 8)

The β -catenin signalling pathway also plays an important role in mammalian neurogenesis.^{83,84} β -catenin is localized to the centrosome and is involved in positioning the mitotic spindles and the plane of cell division.⁸⁵ Furthermore, production of basal precursor cells from radial glial cells as well as cell cycle exit is controlled by the level of β -catenin.^{86,87} Phosphorylation of β -catenin was shown to be important for this effect and it is hypothesized that the phosphorylated form of β -catenin stabilizes components of the centrosome that affects cell fate.⁸⁸

Mutations that affect centrosomal integrity have shed light on the importance of the centrosome in asymmetric cell division and cell fate. Mutations in the protein *dsas-4* results in acentriolar cells and interestingly seventy percent of the neuroblasts were able to polarize normally and undergo asymmetric cell division.⁸⁹ However, in

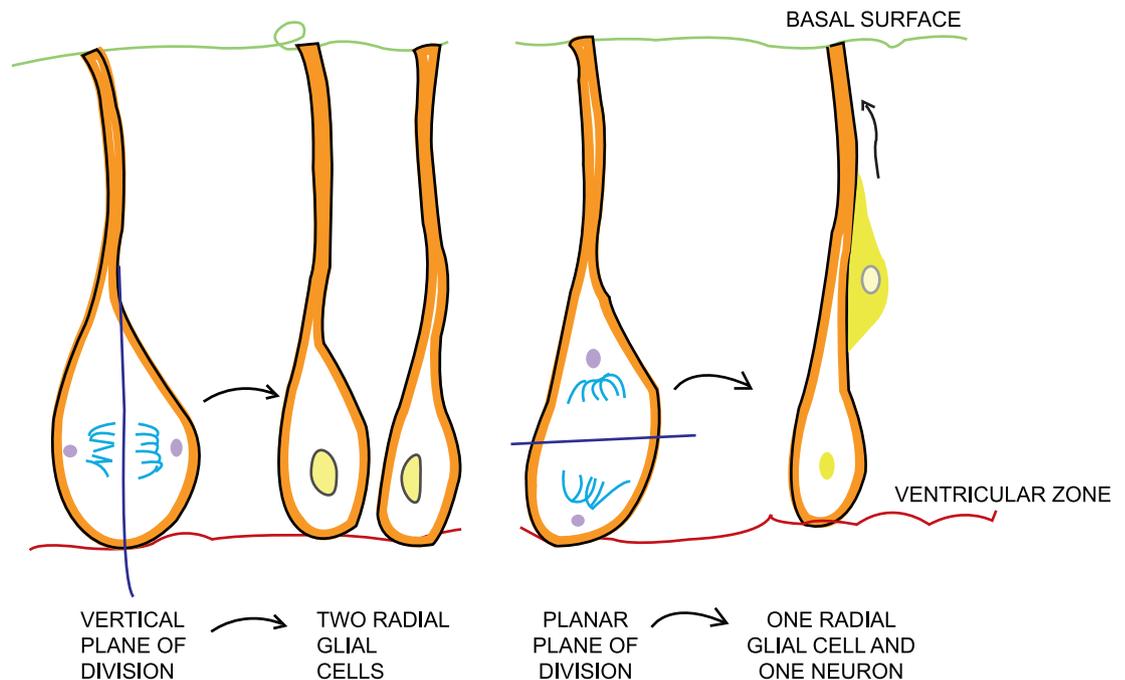


Figure 7: Spindle alignment and cell fate. A vertical plane of division usually leads to equal partitioning of cell fate determinants leading to both the daughter cells acquiring the same fate while a horizontal plane of division leads to compartmentalization of cell fate determinants into one of the daughter cells, causing one to remain proliferative while the other one differentiates. The cell destined for differentiation loses its apical connection close to the ventricular zone and starts migrating towards the basal surface. Adapted from Lancaster and Knoblich, 2012.

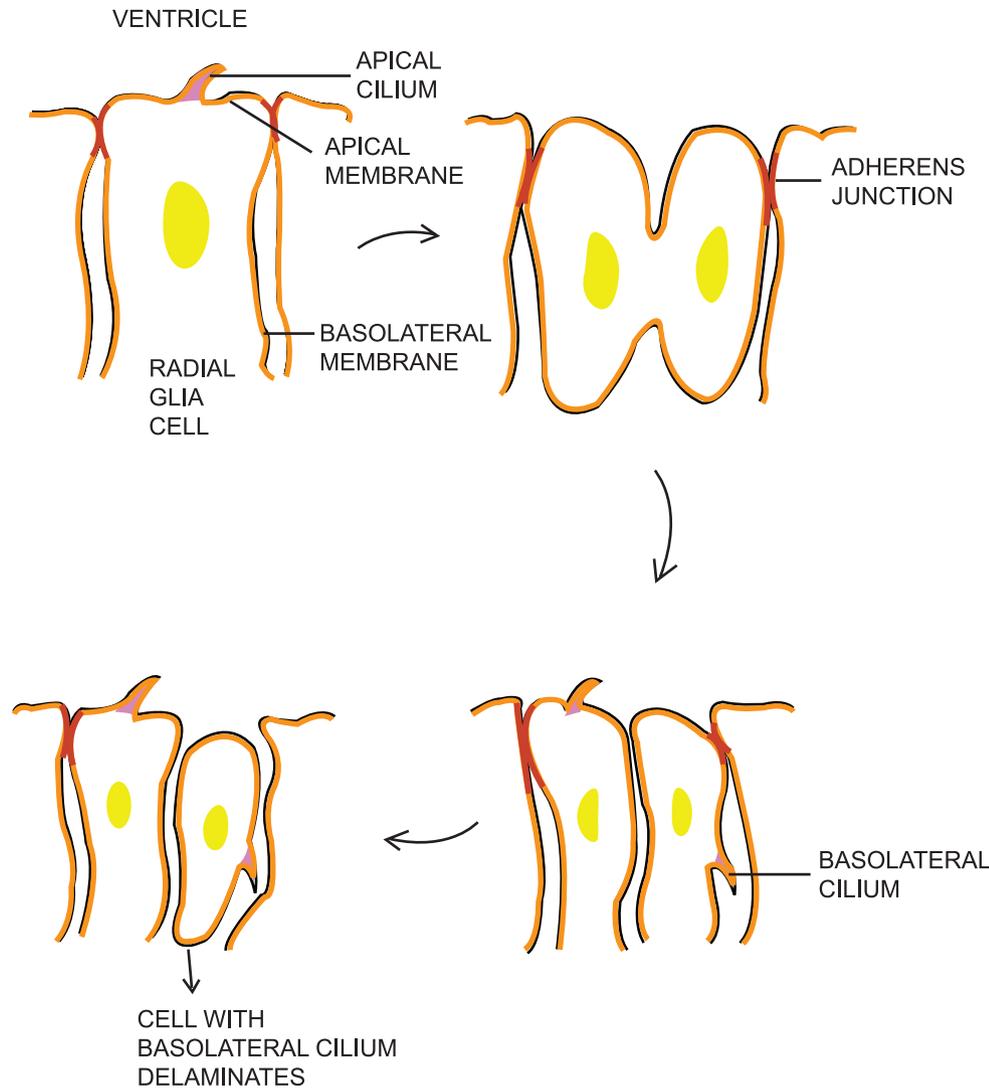


Figure 8: Change in position of cilia. Radial glial cells in the ventricular zone usually have a cilium protruding into the ventricular space from where they are thought to transduce mitogenic signals. A shift in the position of the cilium to a more basolateral position, and therefore away from the ventricle, is seen to be associated with delaminating cells. Adapted from Wilsch-Brauninger *et al*, 2012.

the remaining neuroblasts polarization was abnormal and spindles did not orient correctly.⁸⁹ Interestingly, implantation of pieces of *dsas-4* mutant brain into wild type host develops into a tumor.⁹⁰ In the case of *cnn* and *asl* mutants, both of which affect centrosome function it was found that a significant percentage of cells did not divide correctly.^{47,91} These results can either be taken to suggest that centrosomes are not important for spindle positioning and asymmetric cell division or perhaps more correctly that although there are parallel back up pathways that ensure fidelity of cell division in the absence of centrosome function these pathways are not enough to compensate for the essential role that the centrosome plays in asymmetric cell division. To fully appreciate the

role of the centrosome in cell division it would be important to look at other parameters even in the cells classified as normally dividing. This would include analysis of correct polarization and axon outgrowth of neurons in the absence of normal centrosomes.²⁹

18 Conclusion

This review highlights the conserved role of the basal body/centrioles in primitive cells and how these roles have been co-opted during the development of the cerebral cortex. The role of centrioles in forming cilia and its role in nucleating microtubules are evolutionarily conserved features that are present in mammalian neuroblasts. The asymmetric features of the mother and

daughter centrioles have been exploited in several evolutionary lineages to confer global polarity on cells and through asymmetric cell division this asymmetry influences cell fate. The present analysis suggests that positive selection of mutations related to the centrosome may have played a major role in the expansion of the cerebral cortex in the lineage leading up to humans. Future studies will be aimed at shedding light on the exact mechanism by which the centrosome is able to control cell division and cell fate in the mammalian cortex.

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