Neurogenesis in G minor

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The orientation of the mitotic spindle determines whether divisions of the polarized neural progenitors in the ventricular zone cause their expansion or lead to neurogenesis. A guanine nucleotide exchange factor for the small GTPase RhoA is now shown to tip this balance in favor of neurogenesis.

The number of neurons generated during embryonic development of the mammalian cerebral cortex depends on the number and kind of divisions that neural progenitors undergo[1,2]. The initial neural progenitors, the neuroepithelial cells and the related radial glial progenitors derived from them are highly elongated cells with pronounced apical-basal polarity whose cell bodies occupy the ventricular zone. These cells undergo mitosis at the ventricular surface (that is, their apical side), and for this reason are collectively referred to as apical progenitors. Apical progenitors increase in number by symmetric proliferative divisions, but switch to asymmetric differentiative divisions to generate neurons. The latter cell divisions yield an apical progenitor and either a neuron or an intermediate progenitor that in turn divides in a more basal location (hereafter referred to as basal/intermediate progenitor), typically in the subventricular zone, and in most cases, generating two neurons. In this issue, Gauthier-Fisher *et al.*[3] report a major step forward in elucidating the machinery that controls the symmetric versus asymmetric division of apical progenitors.

An important aspect of the cellular basis underlying the switch of apical progenitors from symmetric to asymmetric division is the orientation of the cleavage plane relative to their apical-basal axis[1]. This orientation determines whether cell-fate molecules localizing in a polarized fashion along the apical-basal axis of the progenitor in M phase are distributed equally (symmetric division) or unequally (asymmetric division) to the daughter cells. Therefore, for both daughter
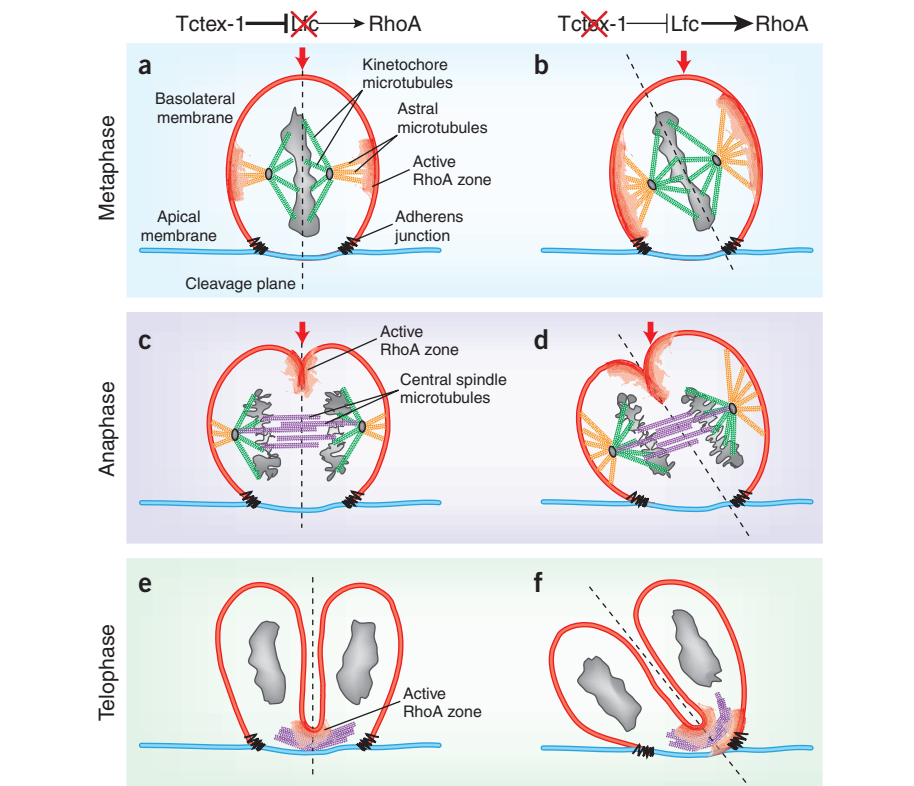
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Figure 1 Mitotic spindle positioning and cleavage furrow ingression in apical progenitors. (**a**–**f**) Schematic illustrating symmetric (**a**,**c**,**e**) and asymmetric (**b**,**d**,**f**) divisions of neural progenitors in metaphase (**a**,**b**), anaphase (**c**,**d**) and telophase (**e**,**f**) and the effects of Tctex-1 and Lfc on RhoA-mediated spindle pole positioning and cleavage furrow ingression. Red arrows in **a**–**d** indicate the basal-most position of the cell body; the basal process[12] is not shown for clarity. Note the vertical orientation of the metaphase plate in **a**, the horizontal orientation of the mitotic spindle in **a** and **c** and the vertical ingression of the cleavage furrow in **e**. Note the oblique orientation of the metaphase plate in **b**, of the mitotic spindle in **b** and **d** and of the ingression of the cleavage furrow in **f**. Note the larger active RhoA zone at the lateral cell cortex in **b** as compared with **a**, at the basal cell cortex in **d** as compared with **c** and the additional active RhoA zone at the adherens junctions in **f** as compared with **e**.

cells to remain apical progenitors, the cleavage plane needs to be oriented parallel to the apical-basal axis (vertical cleavage plane) to ensure an equal distribution of polarized cell-fate determinants (**Fig. 1**)[1,2,4–6]. In contrast, if the orientation of the cleavage plane deviates from the apical-basal axis such that polarized cell-fate determinants are inherited unequally by the daughter cells (**Fig. 1**), their fate is likely to be different.

In all cells, the cleavage plane is normally oriented perpendicular to the axis of the mitotic spindle, and the mitotic microtubules are thought to be important in determining the position of the contractile actomyosin ring, which drives cleavage furrow ingression (**Fig. 1**). In the neuroepithelium, perturbation of proteins known to control the orientation of the mitotic spindle, such as Nde1 (ref. 7), Aspm[4], LGN[5,6] and Lis1 (ref. 8), shifts apical progenitor divisions from symmetric to asymmetric and may affect the extent of neurogenesis. However, it remains unclear how the dynamics of spindle microtubules and the contractile actomyosin ring are coordinated to achieve not only a certain orientation of the mitotic spindle relative to the apical-basal cell axis, but also the proper ingression of the cleavage furrow given this spindle orientation.

The assembly of F-actin and myosin II into the actomyosin contractile ring depends on the small GTPase RhoA[9]. Similar to other small GTPases, RhoA exists in an active, GTP-bound form and an inactive, GDP-bound form; these two states are brought about by guanine nucleotide exchange factors (GEFs) and GTPase activating proteins, respectively. Gauthier-Fisher et al.[3] studied a RhoA GEF called Lfc, the mouse homolog of human GEF-H1 (**Fig. 2**)[10], which activates RhoA, but not the related small GTPases Rac1 and Cdc42. Notably, studies with non-neural cells have shown that Lfc/GEF-H1 is associated with spindle microtubules[10] and is therefore present in a strategic position, both spatially and temporally, to control the RhoA-mediated dynamics of the contractile actomyosin ring during M phase.

Gauthier-Fisher et al.[3] found that Lfc is expressed during embryonic cortical development in apical progenitors in the ventricular zone, basal/intermediate progenitors in the subventricular zone and neurons. When the authors carried out RNAi-mediated knockdown of Lfc in apical progenitors by in utero electroporation, they saw decreased generation of neurons and basal/intermediate progenitors from apical progenitors. This reduced neurogenesis was not a result of a premature switch to gliogenesis. Instead, Lfc knockdown increased the pool of apical progenitors. Thus, these data nicely demonstrate that Lfc normally promotes those apical progenitor divisions that generate basal/intermediate progenitors and neurons (the latter originating from apical progenitors either directly or indirectly, that is, via basal/intermediate progenitors).

In non-neural cells, Lfc binds to a protein called Tctex-1 (**Fig. 2**), which is a component of the dynein motor complex and which also binds to the βγ-subunit heterodimer of G proteins (M.A. Greeve, A. Brunet, C. Wu, C.J. Bakal, N. Fine and R. Rottapel, unpublished data). As Lfc is negatively regulated by Tctex-1, Gauthier-Fisher et al.[3] also investigated the consequences of Tctex-1 knockdown in apical progenitors. They found that the lack of Tctex-1 in apical progenitors increased the generation of neurons and decreased the pool of apical progenitors. Moreover, knockdown of both Tctex-1 and Lfc revealed that the pro-neurogenic effect of Tctex-1 knockdown is mediated by Lfc. When considered with the previous observation of negative regulation of Lfc activity by Tctex-1 (M.A. Greeve, unpublished data), these data indicate that the extent to which apical progenitors generate neurons (or neurogenic basal progenitors) versus apical progenitors is controlled by the levels of active Lfc. Specifically, Tctex-1–bound, inactive Lfc promotes apical progenitor expansion at the expense of neurogenesis, whereas Tctex-1–free, active Lfc promotes neurogenesis at the expense apical progenitor expansion.

How may Lfc exert these effects? In mitotic non-neural cells, Lfc/GEF-H1 localizes to the astral and kinetochore microtubules (being enriched at their tips) and to the midbody region in telophase[10,11]. Lfc activates RhoA, which promotes contractile ring dynamics, and is involved in mitotic spindle positioning[11] and cleavage furrow ingression[10]. Given these observations in non-neural cells, Gauthier-Fisher et al.[3] examined whether depletion of either Lfc or Tctex-1 affects mitotic spindle orientation in apical progenitors. Notably, knockdown of Lfc increased the proportion of mitotic spindles oriented parallel to the ventricular surface (horizontal mitotic spindle). This presumably would result in an increased proportion of cleavage furrows ingressing along the apical-basal axis, which should favor symmetric proliferative divisions of apical progenitors (**Fig. 1**). Knockdown of the Lfc inhibitor Tctex-1 had the opposite effect (less horizontal mitotic spindles, that is, more oblique cleavage furrows and asymmetric neurogenic divisions; **Fig. 1**). These consequences of Lfc and Tctex-1 depletion were already observed at metaphase, indicative of effects on mitotic spindle positioning. However, they were enhanced in magnitude at anaphase and telophase, suggesting that Lfc action continues during the late stage of mitosis and while the cleavage furrow is ingressing.

The study by Gauthier-Fisher et al.[3] thus comprehensively analyzes Lfc and Tctex-1 function in neurogenesis. It does, however, raise
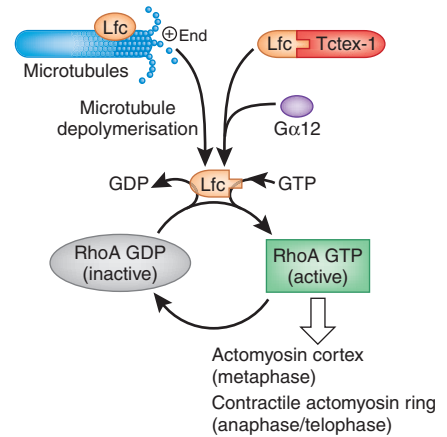

Figure 2 Cartoon illustrating the activation of RhoA by Lfc. Active Lfc is locally released by either microtubule depolymerization or dissociation from Tctex-1, which is promoted by Gα12. Lfc in turn promotes a guanine nucleotide exchange of the small GTPase RhoA where the active GTP-bound form regulates the actomyosin cortex and the contractile actomyosin ring.

the question as to the cellular mechanism(s) underlying the alterations in mitotic spindle orientation and, consequently, of cleavage furrow ingression in apical progenitors. There are four scenarios that could explain this, none of which are mutually exclusive. All these scenarios make the assumption that Tctex-1–free Lfc is the active component and exerts its effects via activation of RhoA. The first two involve mitotic spindle positioning, and the last two implicate cleavage furrow ingression.

In the first scenario, which we call actomyosin cell cortex competence, higher levels of Lfc on astral microtubules may lead to a greater area of the actomyosin cortex becoming competent to interact with these mitotic microtubules (**Fig. 1a,b**). This would increase the options for mitotic spindle positioning and thus the probability that the mitotic spindle no longer shows a perfectly horizontal orientation, which in turn would favor asymmetric neurogenic division.

The second possibility is that Lfc affects spindle length. Until metaphase, the shape of the cell body of mitotic apical progenitors is usually not perfectly spherical but is instead egg shaped, with the long axis being parallel to the apical-basal axis[12]. This implies that a horizontal mitotic spindle will be shorter than one with an oblique or even vertical orientation. Extrapolating from the observation that in non-neural cells interfering with Lfc function in prometaphase results in a shorter mitotic spindle[11], the possibility arises that mitotic spindles in Lfc knockdown apical progenitors may be shorter, thus favoring a

horizontal spindle orientation and symmetric proliferative divisions (**Fig. 1c,d**).

A third scenario is that initiation of cleavage furrow ingression is mislocalized. A hallmark of cleavage furrow ingression in apical progenitors is the typical initiation at only one cortical side (the basal side of the cell body) and the basal-to-apical direction with midbody ormation at the apical-most side of the cell[2,12]. Mislocalized Lfc-mediated RhoA activation at the cell cortex may result in a shifted initiation site. Consequently, cleavage furrow ingression would deviate more often from the apical-basal axis, lowering the probability of a perfectly vertical cleavage plane (**Fig. 1c,d**). A loss of unidirectionality, with the cleavage furrow ingressing from both sides (as is the case in non-epithelial cells) is also possible. This would favor asymmetric neurogenic division of apical progenitors.

Finally, the cleavage furrow may be attracted to junctional complexes, causing changes in the orientation of the cleavage plane. Lfc physically interacts with the G protein subunit Gα12 (M.A. Greeve, unpublished data), which is expressed ubiquitously. Notably, Gα12 is found at junctional complexes in epithelial cells directly interacting with ZO-1 (ref. 13), which in apical progenitors localizes to the adherens junctions at the apical-most end of their lateral membrane. Consistent with the localization of Lfc/GEF-H1 to junctional complexes in non-neural epithelial cells[14], one may therefore envision that there is Lfc-mediated RhoA activation at adherens junctions, which in turn may promote actomyosin ring contraction toward them (**Fig. 1e,f**). This would result in a cleavage plane slightly deviating from the apical-basal axis, which in turn would favor asymmetric neurogenic division of apical progenitors.

It will be exciting to see the extent to which future investigations endorse these scenarios. Irrespective of their outcome, the study by Gauthier-Fisher et al.[3] provides very strong support for the recently challenged[5,6] view that mitotic spindle and cleavage plane orientation are crucial for the decision of whether apical progenitors expand by symmetric divisions or engage in neurogenesis by generating basal/intermediate progenitors and neurons by asymmetric divisions.

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Pool rules

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A study in this issue uses a new technique to show that synaptic vesicles released spontaneously and those released in response to action potentials are drawn from distinct, non-overlapping pools that coexist in presynaptic terminals.

A synaptic vesicle is a synaptic vesicle is a synaptic vesicle, or is it? The critical observation that spontaneous synaptic potentials were the same size as the smallest responses evoked by nerve stimulation led to the revolutionary hypothesis that synaptic responses are "built up of small all-or-none quanta which are identical in size and shape with the spontaneously occurring miniature potentials"[1]. We now know that the basic unit, the quantum, of neurotransmission is the response to the amount of transmitter packaged in a single synaptic vesicle. Until recently, we all assumed that the synaptic vesicles that were released spontaneously were the same as the synaptic vesicles that were released on stimulation. But it's starting to look like we were wrong. In this issue, Fredj and Burrone[2] use a new genetically encoded probe to show that two distinct and non-overlapping pools of vesicles coexist in presynaptic terminals. Vesicles capable of undergoing evoked release come from the recycling pool. They are released on depolarization, endocytosed and re-readied for evoked release. Spontaneously released vesicles come from the resting pool, whose function had previously been obscure. These new 'pool rules' will undoubtedly spark investigation into other potential differences among synaptic vesicles.

Our first hint that perhaps all synaptic vesicles are not created equal came from an optical imaging study[3] that demonstrated that an identical stimulation protocol released fluorescent dye at different rates, depending on whether the dye had been loaded into vesicles that had fused spontaneously or in response to stimulation. This same study also reported that the selective poisoning of spontaneously released vesicles left evoked release unaffected. Together, these data suggested that spontaneous and evoked release draw from separate pools of vesicles in the presynaptic terminal[3]. A couple of years later, however, this notion was challenged by another study[4], which argued that experimental artifacts might account for these earlier observations and found, using more sophisticated techniques, that both spontaneous and evoked release drew from the same vesicle pool.

Fredj and Burrone[2], however, have revisited this debate with a new tool and come down on the side of separate pools for spontaneous and evoked release. The important technical advance of their study is the development of a biotinylated variant of the synaptic vesicle protein VAMP2. This genetically encoded probe allows for the irreversible and saturable labeling of synaptic vesicles that release in the presence of extracellular streptavidin molecules tagged with fluorescent Alexa dye. Because Alexa dyes are available in a rainbow of hues and are readily washed out, it is possible to sequentially label vesicles released under different conditions with different colors. Fredj and Burrone[2] were able to label vesicles released in response to long trains of action potentials. Critically, there did not appear to be any effect of streptavidin binding on subsequent release or endocytosis of labeled synaptic vesicles. By delivering several bouts of the train stimulus, all of the streptavidin binding sites that became available during evoked release could be saturated. Further stimulation did not increase the intensity of fluorescent labeling. Similar saturable labeling could also be achieved with multiple rounds of direct depolarization using

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