Trafficking in cell fate

Sandra Bajjalieh

The *hyh* mutation that underlies a form of congenital hydrocephalus has been traced to the gene encoding αSnap, a membrane trafficking protein. The mutation causes mis-sorting of apical proteins and precocious neurogenesis.

The sophisticated neural network that is the mammalian neocortex develops from a precisely timed series of cell divisions. Most cortical neurons arise from the ventricular zone, a region of polarized epithelium adjacent to the lateral cerebral ventricles. During neurogenesis these cells undergo a fixed number of cell divisions that give rise to additional progenitors and to cells that differentiate into neurons[1]. At the beginning of the process, 100% of cell divisions produce two new progenitors. As development progresses, an increasing percentage of new cells leave the cell cycle and differentiate into neurons. The newly formed neurons migrate up to form the lower, and then the upper, layers of the cortex. As the ratio of new progenitors to neurons dips below 50% and rapidly proceeds to 0%, there is a burst of neurogenesis that gives rise to the densely packed layers of the upper cortex[2].

Expanding the progenitor pool through early cell cycles is crucial to generating a sufficient number of neurons to properly form the cortex. Indeed, it has been proposed that a lengthening of this stage during evolution is what gave rise to larger cortices in primates[2]. In congenital hydrocephalus this process goes awry. The reduced cortical thickness and overabundance of deep layer neurons suggests a failure to generate or maintain normal numbers of progenitors. The study of congenital hydrocephalic mouse mutants is providing a window into the genetics of this pathology and the cellular events that regulate cell fate during neurogenesis.

Snap!

Two new reports, one by Chae *et al.*[3] on page 264 and one from Hong *et al.*[4], indicate that normal membrane trafficking is required for normal progenitor cell production. Both groups mapped the *hyh* mutation that gives rise to the hydrocephalus–with–hop gait phenotype in mice. Mice homozygous with respect to this recessive mutation are born with thin cortices that contain an abnormally high percentage of deep-layer (early-born) neurons, leading to hydrocephalus and death within two months of birth. Analyses of the genes in the region traced the mutation to the gene (*Napa*) that encodes the trafficking protein αSnap. The mutation is a single-base change that leads to the substitution of a highly conserved methionine with isoleucine at position 105, which is located on the surface of the protein. Chae *et al.* report that the mutation produces lower levels of *Napa* mRNA and both groups found lower levels of αSnap protein.

αSnap (soluble N-ethylmaleimide-sensitive factor (NSF) attachment protein) is a ubiquitous membrane trafficking protein present in all eukaryotic cells. Snap proteins bind Snap receptor (SNARE) complexes, the

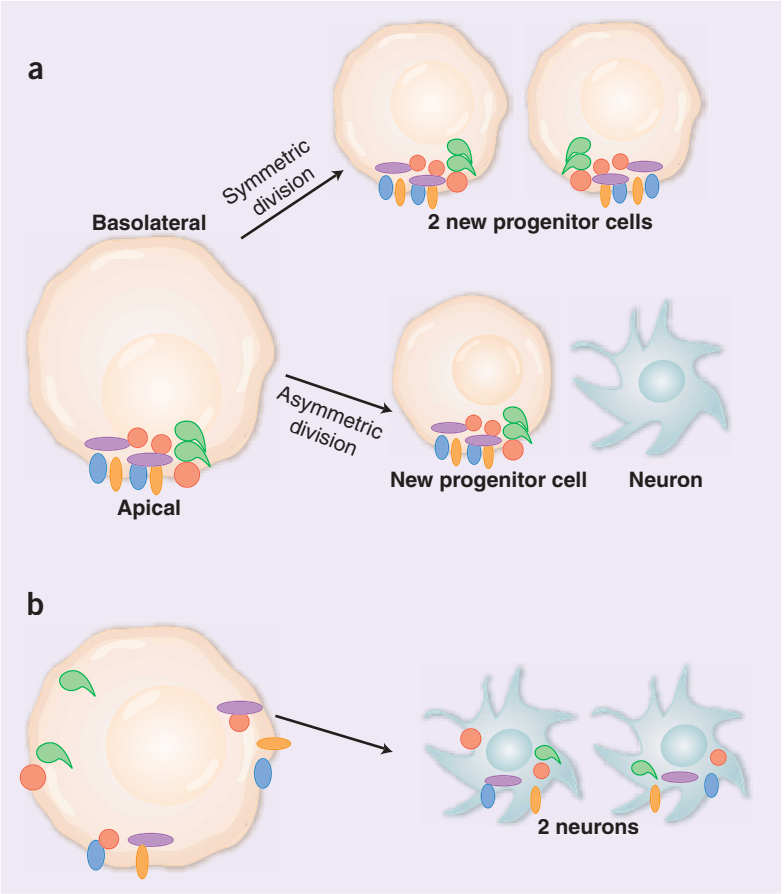

Figure 1 How trafficking might drive cell fate. (**a**) Normal progenitor cells concentrate soluble factors needed to maintain the progenitor fate at the apical surface, probably anchored to membrane proteins trafficked to the apical surface. Symmetric cell divisions result in two daughter cells that inherit half of the apical domain and half of the basolateral domain. These cells maintain a progenitor fate. Asymmetric divisions produce one daughter that receives the apical domain and one that receives the basolateral domain. This results in one new progenitor (the apical cell) and one cell that exits the cell cycle and becomes a neuron. (**b**) In *hyh* mutants, apical proteins are poorly trafficked, resulting in incomplete cell polarization. In this case, cell division doesn't produce cells with sufficient apical factors to maintain a progenitor fate, and both daughters leave the cell cycle to become neurons.

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helical bundles that form between transport vesicles and acceptor membranes and are essential for membrane fusion (reviewed in ref. 5). When it binds the SNARE complex, Snap recruits NSF, an ATPase that catalyzes the disruption of the complex. This frees individual SNARE proteins for another round of complex formation and membrane fusion.

αSnap is an essential protein whose levels must be carefully titrated. Chae *et al.* report that a null mutation in *Napa* is lethal in mice, and hypomorphic alleles in *Drosophila melanogaster* lead to reduced neurotransmission (M. Babcock, G.T. Macleod, J. Leither & L. Pallanck, personal communication). Overexpression of the protein blocks fusion of yeast vacuoles[6] and disrupts synapse formation and other aspects of development in *D. melanogaster* (M. Babcock, G.T. Macleod, J. Leither & L. Pallanck, personal communication).

The *hyh* mutation does not affect the gross structure of αSnap or its ability to bind SNARE complexes or to recruit and stimulate NSF-catalyzed disassembly of SNARE complexes. Thus, the mutant phenotype seems to be due to a simple reduction in αSnap levels. This suggests that αSnap has a dose-dependent action during ventricular zone proliferation that is essential to producing neural progenitors.

The importance of being apical

In early cortical development, two main types of cell division are observed[7]: symmetric and asymmetric. In symmetric cell divisions both daughters inherit the same complement of membrane and cytoplasm and give rise to two new progenitors. In asymmetric cell divisions one daughter inherits the apical half of the mother cell and remains a progenitor whereas the other daughter inherits the basolateral half and becomes a neuron. The differential partitioning of membrane domains and soluble factors in asymmetric cell divisions is proposed to contribute to differences in cell fate (**Fig. 1**).

Chae *et al.* report that in *hyh* mutants the apical localization of several soluble proteins implicated in cell fate is disrupted and that localization of VAMP7, a vesicular SNARE that participates in apical targeting[8], is less polarized than in wild-type mice. This suggests that the phenotype could result from a reduction in SNARE-mediated delivery of scaffolding proteins to the apical surface. It also supports a model in which becoming a progenitor is the non-default fate, one that must be actively maintained with sufficient apical signaling machinery. Thus, the decision that must be induced is to prolong proliferation, rather than to initiate differentiation.

Identifying the genes responsible for naturally occurring mutations often leads to surprising revelations about cellular processes and to new questions about old players. The seemingly pronounced sensitivity of cortical neurogenesis to levels of αSnap suggests that the shared machinery of membrane trafficking may not function equally throughout the cell. Future studies comparing apical to basolateral sorting in *hyh* mutant progenitor cells, examining the organization of other polarized tissues and determining whether other Snap isoforms can rescue the phenotype will expand our understanding of snap function. Analyses of other cell fate determinants in *hyh* mutants will help identify the role these molecules have in the decisions that produce complex cellular patterns. The findings certainly highlight the importance of distinct plasma membrane domains in polarized cells and suggest that they could be orchestrating cell fate determination during neurogenesis.

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Sox3 and sexual dysfunction: it's in the head

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Humans with mutations in *SOX3* have panhypopituitarism, but the developmental mechanisms underlying this defect are unknown. Conditional disruption of *Sox3* in mice now suggests that anterior pituitary development depends on *Sox3* expression in the overlying neural ectoderm, which establishes midline structures and regulates production of inductive BMP and FGF signals.

Thirteen years ago, Lovell-Badge and colleagues proved that *Sry* is the mammalian testis-determining gene by showing that *Sry* was sufficient to convert XX mouse embryos into phenotypic males[1]. *Sry* is the founding member of a large family of related genes called SOX genes (for SRY-related HMG-box). The SOX DNA-binding domain, called HMG for high mobility group, is the most highly conserved region of these proteins.

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Do other SOX genes also function in sex determination? Haploinsufficiency with respect to *SOX9* causes campomelic dysplasia, a syndrome characterized by skeletal abnormalities and ambiguous genitalia or sex reversal[2,3]. Mice with mutations in *Ods* and gain of *Sox9* function also have sex reversal, identifying the importance of *Sox9* gene dosage[4]. Another member of the SOX family, *Sox3*, is closely related to *Sry*, but its role in sex determination is controversial[5,6]. On page 247 Rizzoti *et al.* report that *Sox3*, unlike *Sry* or *Sox9*, is dispensable for directing the indifferent gonad to develop into an ovary or testis on a mixed genetic background[7]. Instead, *Sox3* seems to affect gonadal function indirectly through its roles in brain development and hypothalamic induction of anterior pituitary development.

Sox on the brain

The phenotype of *Sox3* mutant mice is variable and complex, with abnormalities throughout the hypothalamic-pituitary-gonadal axis (**Fig. 1**). Severely affected mice have craniofacial abnormalities, defects in the brain midline, profound growth insufficiency, male hypogonadism and lethality, whereas less affected mice are viable and fertile. The expression pattern of *Sox3* provided