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SPOTLIGHT

SNAP to attention: A SNARE complex regulates neuronal progenitor polarity

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SNARE vesicle targeting complex controls the polarity of neuronal progenitors. Kunii et al. (2020. *J. Cell Biol.* https://doi.org/10.1083/jcb.201910080) show that the SNAP23–VAMP8–Syntaxin1B complex is required for membrane targeting of N-cadherin and formation of adherence junction complexes in radial glia neuronal progenitors, the major prerequisite of cell polarity establishment.

Proliferation and maintenance of neural stem cells is crucial for proper establishment of the brain cytoarchitecture during development and correct functioning of the mature brain. In the vertebrate developing the central nervous system (CNS), neural stem cells form a monolayer that enfolds the neural tube. The rostral, brain-forming part of the neural tube develops into a chain of ventricles, the brain ventricular system, connected with the central canal of the spinal cord (1). The ventricular system and central canal are filled with cerebrospinal fluid that immerses the layer of neural stem cells in the primary proliferative zone of the developing CNS, an area called the ventricular zone (VZ). Neural stem cells divide there and produce young neurons that, after exiting mitotic cycle, migrate out of the VZ to settle in their final location in the developing brain and differentiate into mature neurons that assemble neuronal circuits.

The cells of the VZ, also called neuroepithelium, are polarized like all epithelial cells. They have a basal side with which they are attached to the pial surface, while with their apical side they face the ventricular lumen. Radial glia cells (RGCs) that appear later in development are a class of polarized cells that are present in many places in the CNS but especially distinct in the forebrain. The degree of polarization of the RGC is even more pronounced than that of neuroepithelial cells, as their basal process is very long. RGC apical processes span the entire thickness of the developing neocortex and can extend as far as several millimeters in length in primates (2).

The polarity of RGCs is important for both their proliferation and for the postmitotic migration of young neurons. Detachment of their basal and/or apical processes results in abnormal proliferation and apoptosis, leading to abnormal cytoarchitecture of the postnatal brain (3). If only the basal process is detached from the pia, radial glia proliferation is undisturbed but neuronal migration is disrupted, also leading to defects in the proper construction of the brain (4).

It is not only the morphology of basal and apical processes that makes RGC a highly polarized cell. Presence of adherence junctions (adherence junctions complex; AJC) on the apical side of the cell membrane is another sign and determinant of asymmetry. Disruption of adherence junctions can cause disorders such as hydrocephalus and hemorrhage in humans (5).

Key proteins involved in the establishment and maintenance of AJC and polarity in RGCs are transmembrane N-cadherin and its cytoplasmic partner β-catenin (6). Disruption of the N-cadherin–β-catenin complex in the brain causes complete loss of both AJC and apico-basal polarity (6). Similarly, disruption of proteins that control N-cadherin trafficking, such as LLGL1, Dlg5, Numb and Numblike, can also cause failure of adherence junction formation (7).

In the current issue, Kunii et al. identified new players in this process, SNARE complex protein Snap23 and its binding partners (8). SNARE complex proteins NSF, soluble NSF attachment protein (SNAP), and its receptor SNARE were first identified as key proteins in targeted vesicular fusion events in the secretory pathway (9). Later, SNARE presynapse-specific homologues, VAMP (also known as synaptobrevin), and syntaxin and its binding partner SNAP-25 were shown to be the main controllers of the synaptic vesicles' fusion with presynaptic neuronal membrane (10).

Although it could be suspected that SNARE complex has a role in the polarity of the neuronal progenitors, knockout mice lacking components of the classical VAMP–syntaxin–SNAP25 complex did not show any abnormalities in the morphology of RGC. Kunii et al. hypothesized that other homologous SNARE complexes might control polarity of neuronal progenitors. To test this hypothesis, the authors inactivated a close homologue of Snap25, SNAP23, in the mouse CNS by conditional knockout. These

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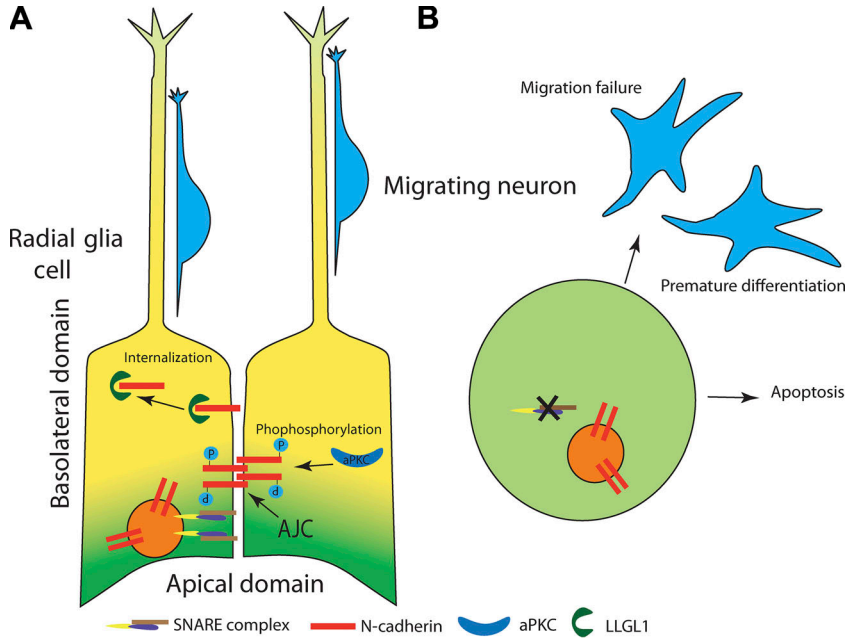

Figure 1. **Polarized organization of radial glia cells. (A)** N-cadherin membrane targeting is a key event in formation of AJC. SNARE complex targets N-cadherin to the plasma membrane, while LLGL1 removes it from the basolateral side. **(B)** If any of the three components of the SNARE complex is destroyed, AJC is not formed due to failure to deliver N-cadherin to the plasma membrane, preventing apico-basal polarization. This causes abnormal brain development. aPKC, atypical PKC.

mutant mice demonstrated severe hypoplasia and disorganization of the cytoarchitecture of the neocortex and hippocampus, defects in cerebellar development, hydrocephalus, and hemorrhaging. The authors suggested an explanation for why SNAP25 does not play a role in RGCs: its expression is mostly confined to neurons, while SNAP23 is expressed in both RGCs and postmitotic cells. Moreover, the authors found that SNAP23 localization is higher in the apical process than in the basal process. Consistent with this, apico-basal polarity of RGCs is defective in SNAP23 knockout animals, resulting in loss of these long processes—the main morphological feature of the radial glia.

Expectedly, disruption of radial glia polarity was accompanied by premature differentiation of RGC due to accelerated cell cycle exit as well as abnormal migration and increased apoptosis of their offspring neurons. This premature differentiation in turn results in exhaustion of the progenitor pool and ultimately leads to brain hypoplasia. At the cell architecture level, inactivation of SNAP23 in the RGC disrupts formation of the adherens junctions. This is accompanied by failure of targeted localization of N-cadherin and β-catenin to the apical end, as well as mislocalization of several other components of the AJC, such as Par3, ZO-1, Grb3, and Pals1.

This finding was substantiated by in vitro experiments wherein RGC with low levels of SNAP23 failed to attach to each other and had reduced levels of N-cadherin. The authors also reported protein-specific transport defects. Surface proteins like Ephrin-B1 and β-integrin did not make it to the plasma membrane, while membrane localization of other proteins such as low-density lipoprotein receptor (LDLR) and Na^+/K^+-ATPase was not affected.

Using another elegant approach, the authors showed that N-cadherin depletion from the plasma membrane is the key molecular event in SNAP23 deficiency that causes loss of polarity in RGC (Fig. 1). In this set of experiments, they deleted SNAP23 in the developing cortex using a combination of in utero DNA electroporation with CRISPR-Cas9. In genetic rescue experiments, the authors expressed either wild-type N-cadherin or a chimeric protein consisting of the extracellular domain of N-cadherin fused to the transmembrane and cytoplasmic domains of the LDLR protein. The authors found that transport of the wild-type protein was dependent on SNAP23, while the chimeric protein appeared to use an alternative pathway. Accordingly, only the chimeric protein could restore the RGC polarity and other phenotypes in the SNAP23 mutant phenotype.

To identify other members of SNARE complex that act with SNAP23 in the control of RGC polarity, the authors performed pulldown assays and precipitated several interacting partners, including homologues of VAMP and syntaxin. They then inactivated the identified candidates in the developing neocortex using siRNA. Inactivation of only two of them, VAMP8 and Stx1B, caused disruption of N-cadherin plasma membrane localization and AJC formation in radial glia (Fig. 1 B). In experiments using COS7 cells, the authors also found that VAMP8 preferentially colocalizes with vesicles transporting N-cadherin.

The study also raises a number of questions. For example, how is protein cargo specificity achieved? Which member of the SNARE complex can be replaced by a homologue without affecting specificity? Can SNAP25 fully replace SNAP23 in N-cadherin transport, or are other syntaxin homologues able to recapitulate Stx1B function? Another question to be addressed is whether localization of the SNAP23-containing complex is tightly regulated and, if so, how? It has been suggested that nonphosphorylated N-cadherin is removed from the basolateral side by Llgl1 via internalization (11). Conversely, atypical PKC phosphorylates N-cadherin on the apical side, preventing its interaction with Llgl1 and allowing N-cadherin retention on the apical membrane (Fig. 1 A; 11). Whether this is a mechanism that competes with SNAP23-mediated targeting remains to be clarified. It is also not clear whether the SNAP23-containing complex is required for targeting proteins located basolaterally such as Scripple-Lgl-Dgl.

In summary, this study uses a beautiful combination of mouse genetics and in vitro trafficking visualization and biochemistry to demonstrate an important role for the VAMP8–Stx1B–SNAP23 SNARE complex in the regulation of N-cadherin recruitment and neural progenitor polarity setting. Future work will undoubtedly provide further

insight into the mechanisms that regulate the function of this SNARE complex as well as the role of other SNARE proteins in this process.

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