Nervous system development relies on endosomal trafficking

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ABSTRACT
Accumulating findings have begun to unveil the important role of the endosomal machinery in the nervous system development. Endosomes have been linked to the differential segregation of cell fate determining molecules in asymmetrically dividing progenitors during neurogenesis. Additionally, the precise removal and reinsertion of membrane components through endocytic trafficking regulates the spatial and temporal distribution of signaling receptors and adhesion molecules, which determine the morphology and motility of migrating neurons. Emerging evidence suggests that the role of the endosomal sorting adaptors is dependent upon cell type and developmental stage. The repertoire of the signaling receptors and/or adhesion molecules sorted by the endosome during these processes remains to be explored. In this commentary, we will briefly address the progress in this research field.

ARTICLE HISTORY
Received 20 December 2016
Revised 6 March 2017
Accepted 30 March 2017

KEYWORDS
asymmetric mitosis; brain development; endosomal system; membrane trafficking; neuron migration; smad anchor for receptor activation (SARA)

Introduction
Early during neocortical development, apical radial glia (RG) divide symmetrically generating 2 identical daughter cells, thereby increasing the pool of progenitor stem cells. Later, RG divide asymmetrically producing another RG and an intermediate progenitor. The later will ultimately generate 2 post-mitotic multipolar neurons that migrate away from the ventricle border. These multipolar neurons later retract all their undifferentiated neurites and acquire a bipolar morphology, composed of a pia-directed leading process and a prospective axon that grows toward the ventricle. Once neurons have arrived at their final position within the cortical plate, the leading process branches and matures into the apical dendrite, and the axon elongates through the white matter until reaching its target cell.

There is an extensive literature concerning neurogenesis and neuronal migration during cortical development. Both processes are regulated under the control of cytoskeletal dynamics, internal and external signaling, and membrane trafficking (Reviewed in refs. 1 and 2). The endosomal system has emerged as a new multifactorial regulator in different nervous system development models, including brain cortex, cerebellum, spinal cord, and sensory organ precursor. Its machinery comprises a mosaic of interconnected membrane compartments in which both the endocytic and exocytic pathways intersect. Through the addition and removal of membrane signaling components at precise subcellular sites, the endosomal trafficking modules cell behavior and morphology of both progenitor cells and neurons.

In this commentary, we will briefly summarize the state of our knowledge of the mechanisms by which the endosomal system coordinates different aspects of nervous system development while focusing on discussing the recent advances in our understanding of the emerging role of Smad Anchor for Receptor Activation (SARA) in both neurogenesis and neuronal migration.

Cell fate determinant distribution during asymmetric mitosis
Several non-mutually exclusive models — centrosome dynamics, cell cycle length, asymmetric inheritance of cell fate determinants during mitosis — have been proposed to explain the asymmetric division of
cortical progenitors and other dividing progenitors.\textsuperscript{5,6,7} Emerging evidence shows that endosome-associated proteins can regulate the differential sorting of cell fate instructing molecules between daughter cells during asymmetric division (Table 1). A notable example is the endosome-bound adaptor protein Numb. Its overexpression enhances the proliferation of avian neuroepithelial progenitors.\textsuperscript{8} In line with this, a Numb knockout mouse line exhibits precocious neuronal differentiation in the mouse neocortex.\textsuperscript{9} However, in examining a different Numb knockout mouse model, Zilian et al., did not observe any premature neurogenesis in the forebrain. Instead, arrested neuronal differentiation was discernable in the hindbrain.\textsuperscript{10} Dissimilar results were also found in cultured cortical progenitors by Shen et al.\textsuperscript{11} While Numb is evenly distributed into the 2 daughters of a symmetric division, Numb accumulates into the daughter cell that acquires a neurogenic cell fate in asymmetric mitosis.\textsuperscript{11} These authors proposed that, in the Numb-predominant cell, the Notch signaling pathway is inhibited to promote neuronal differentiation. Recent studies suggested that the Numb-accumulating cell sequesters Notch into late endosomes, while the Numb-devoid cell recycles Notch back to the plasma membrane for signaling activation.\textsuperscript{12} These different findings raise the possibility that Numb’s endosomal trafficking role in asymmetric cell fate choice is context-dependent. Perhaps distinct cell determinant factor(s) besides Notch is sorted by Numb under different contexts.

SARA is also an endosome-bound adaptor protein. SARA specifically binds to the early endosome through its FYVE (Fab1p, YOTB, Vac1p, and EEA1) domain, and acts as a downstream effector of Rab5-mediated early endosomal fusion.\textsuperscript{13} Similar to Numb, the function of SARA during asymmetric divisions is also cell model dependent.

SARA, labeled by ectopic expression of fluorescently-tagged SARA, was first linked to asymmetric division in the Drosophila sensory organ precursor (SOP). Gonzalez-Gaitan and his colleagues noticed that during SOP mitosis internalized Notch receptor and its ligand Delta traffic through Sara+ endosomes.\textsuperscript{14} They showed that 15 times more SARA is segregated to the pIIa daughter than to the pIIb daughter cell. However, SARA mutant flies show normal internalized Notch and Delta asymmetric distribution into pIIa cells, as well as normal overall

| Table 1. Participation of endocytic components in asymmetric mitosis during nervous system development. |
|---------------------------------------------------------------|
| **Endocytic component** | **Associated phenotype**                                      | **Species** | **Reference** |
| Numb overexpression | Increased proliferation                                      | Chick       | [8]           |
| Numb knockout       | Increased cell cycle exit, and neuronal specification        | Mouse       | [9]           |
| Numb knockout       | Dispensable for neurogenesis. Delayed hindbrain neuronal differentiation |
| Numb asymmetric accumulation | Determines neuronal differentiation                      | Mouse       | [11]          |
| SARA asymmetric accumulation | Dispensable for SOP asymmetric mitosis and cell fate choice | Drosophila  | [14]          |
| SARA asymmetric accumulation | Maintains the proliferative state                          | Zebrafish   | [16]          |
| SARA symmetric distribution | Dispensable for proliferation and cell fate choice       | Mouse       | [17]          |

![Figure 1](image-url)  

**Figure 1.** Schematic diagrams depicting SARA’s distribution and roles in asymmetric mitosis of 3 different nervous systems. (A) SARA-positive endosomes (depicted in red compartments) accumulate in the pIIa cell during Drosophila SOP mitosis, although SARA mutant flies exhibit normal cell fate choice and SOP development. (B) In the zebrafish spinal cord, SARA endosomes predominantly accumulate to one of the daughter cells irrespectively if mitosis is symmetric or asymmetric. Only in the asymmetric lineage, SARA accumulation correlates with the progenitor type (P) fate. Either SARA KO or KD increases the proportion of neurons (N) at the expense of the progenitor type cell number. (C) In the mouse cortex, SARA endosomes distribute equally between daughter cells of apical progenitors. The proportion of progenitor (P) and neuron (N) daughter cells is not altered after SARA KD.
sensory organ development (Fig. 1A), indicating that SARA is dispensable in SOP asymmetric mitosis and binary cell fate choice.14 The same group later showed that the Notch receptor itself is required for the asymmetric segregation of SARA+ endosomes.15

In the zebrafish spinal cord system, SARA has been shown to be functionally critical to maintain the progenitor population number. Either knockout or knockdown of SARA leads to the generation of 2 post-mitotic neurons at the expense of self-renewing mitosis16 (Fig. 1B). In this model, SARA segregates differentially into the 2 daughter cells of either symmetric or asymmetric divisions. Interestingly, only in the asymmetric lineages, the daughter cell with accumulated SARA+ endosomes remains proliferative, while the other differentiates into a neuron. Nevertheless, it is not clear why SARA+ endosomes are also unevenly segregated during symmetric mitosis.

In mouse embryonic cortex, we recently showed that SARA labeled endosomes are distributed roughly equally into dividing apical progenitor cells, independently of the mitotic orientation angle (Fig. 1C; 17). In these experiments we examined endogenous SARA since ectopically overexpressed SARA may affect endosomal dynamics.13,18-20 Acute knockdown of SARA expression in the developing neocortex does not crucially affect the progenitor asymmetric division nor cell fate choice of daughter cells.17 Accordingly, SARA knockout mouse brains develop to normal size (unpublished).

Surface receptor treadmill is necessary for neuronal migration and morphogenesis

To reach their final destinations, the trajectories of migrating neurons are guided by chemotactic cue-transmitted signaling axes. Neuronal movement requires concerted cytoskeletal dynamics and membrane trafficking, which are coupled to attachment formation between the leading process and the extracellular matrix and/or neighboring cells, as well as detachment at the cell rear.21-23 It is generally thought that chemotactic surface receptors and adhesion molecules have to be sequestered from the cell surface, and later transcytosed and/or reinserted on different locations to ensure a vectorial movement.4 Compromised coordination of the trafficking events leads to migration defects that culminate in neuronal malpositioning within the cortex (Table 2), and perhaps aberrant synaptic contacts as well.24

Cerebellar granule cell precursors (GCPs) migrate along a gradient of brain-derived neurotrophic factor (BDNF). The chemotraction toward BDNF orients and stimulates neuronal migration in a process that relies on endosomal trafficking and signaling. Upon activation, the BDNF receptor TrkB in the leading process is endocytosed through a Numb-dependent pathway, which in turn stimulates local BDNF secretion. This feedback generates an asymmetric accumulation of signaling endosomes at the subcellular site with the highest level of BDNF. Thus, regulated TrkB endocytosis and resulting BDNF release coordinately determine the migratory direction of GCPs.25,26

By using the strategy to perturb known components of the endosomal trafficking regulation (i.e., Rab5, Rab11) or endocytosis (i.e., clathrin, dynamin), several investigators have highlighted the role of the endosomal trafficking in cortical neuron migration.27,23 In migrating neurons in which Rab5 or Rab11 GTpase are suppressed, N-cadherin which is normally found at the distal and tip region of the leading process, is delocalized to the soma and the proximal region of the leading process.27 In turn, the mislocalization of N-cadherin leads to migration arrest of postmitotic neurons in the intermediate zone (IZ) of the brain cortex. Similarly, interference with dynamin increases the

| Endocytic component | Plasma membrane receptor | Event | Effect | Reference |
|---------------------|--------------------------|-------|--------|-----------|
| Numb                | TrkB                     | GCP migration | Decreased neuronal migration | [25, 26] |
| Rab5/Rab11          | N-Cadherin               | Cortical neuron migration | Neuronal migration arrest at the IZ | [27] |
| Rab7                | N-Cadherin               | Terminal translocation, dendrite morphology | Increased soma-to-pia distance, thicker dendritic shaft | [27] |
| Dynamin/Clathrin    | β1-integrin/Focal Adhesion Kinase | Cortical neuron migration | Neuronal migration delay | [23] |
| Arf6/FIP3           | N-Cadherin               | Cortical neuron migration | Neuronal migration arrest at the IZ | [28] |
| SARA                | L1CAM                    | Multipolar-to-bipolar transition and migration | Neuronal horizontal orientation and migration delay | [17] |
accumulation of β1-integrin and focal adhesion kinase at the cell rear, and hence inhibits cell detachment and movement. Moreover, Rab7-dependent lysosomal degradation (of N-cadherin and perhaps other adhesion components) is important for the final phase of somal translocation and dendrite morphogenesis. ADP ribosylation factor 6 (Arf6) also regulates N-cadherin trafficking through Rab11 family-interacting protein 3 (FIP3). Knockdown of either Arf6 or FIP3 leads to cytoplasmic accumulation of N-cadherin and neuronal migration arrest at the IZ. These results highlight that unbalanced temporal-spatial distribution of adhesion molecules impairs both the location and morphogenesis of cortical neurons.

L1CAM (L1) is also a cell adhesion molecule best known for its importance in axon growth, guidance and fasciculation. We and others showed that either up- or down-regulating the expression level of L1 in cortical neurons disrupts their radial migration. Mutations in the L1 gene have been linked to hydrocephalus in several human congenital brain disorders. While in vitro studies have shown that the axonal plasma membrane distribution of L1 is regulated by a transcytotic pathway, how the surface expression of L1 is regulated in migrating neurons was unknown until recently. In the developing neocortex, our work showed that SARA knockdown led to an overall ~3-fold increase in surface L1 distribution. SARA knockdown, phenocopying L1 overexpression, delayed neuronal migration. This phenotype is concomitant with compromised multipolar-to-bipolar transition of migrating neurons; which get “stuck” at the IZ, and with soma and leading processes misalignment. We also found that SARA-silenced neurons with increased surface L1 preferably bind to their neighboring neurons instead of radial glial cells, as control neurons do.

In all, a handful of papers have provided the first pieces of evidence about how the endosomal system coordinates different aspects of nervous system development. Even though differential sorting has been implicated in asymmetric mitosis, it seems highly context-dependent. To better decipher the mechanisms underlying endosome-mediated cell fate specification, we will need to delineate the cargoes sorted out, while tracking endogenous endosomal components. This may also clarify contradicting findings coming from different systems. Finally, aside from neurons, other types of cells, such as astroglia, oligodendrocytes, and microglia, also migrate within the developing brain. Whether or not the endosome-mediated turnover of surface receptors also modulates the movement of these cells remains to be explored.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

**Funding**

This work was supported by NIH NEI (EY11307 and EY016805), Starr Foundation, and Research to Prevent Blindness to C-H S. C-H S is a recipient of Research to Prevent Blindness Stein Innovation Award. Travel grants from Journal of Cell Science and International Society for Neurochemistry were awarded to IM. We want to thank M. Lorenzatti and M. Otsu for assistance with Fig. 1.

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