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Sticky situations: recent advances in control of cell adhesion during neuronal migration

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Abstract

The migration of neurons along glial fibers from a germinal zone (GZ) to their final laminar positions is essential for morphogenesis of the developing brain, aberrations in this process are linked to profound neurodevelopmental and cognitive disorders. During this critical morphogenic movement, neurons must navigate complex migration paths, propelling their cell bodies through the dense cellular environment of the developing nervous system to their final destinations. It is not understood how neurons can successfully migrate along their glial guides through the myriad processes and cell bodies of neighboring neurons. Although much progress has been made in understanding the substrates (1–4), guidance mechanisms (5–7), cytoskeletal elements (8–10), and post-translational modifications (11–13) required for neuronal migration, we have yet to elucidate how neurons regulate their cellular interactions and adhesive specificity to follow the appropriate migratory pathways. Here I will examine recent developments in our understanding of the mechanisms controlling neuronal cell adhesion and how these mechanisms interact with crucial neurodevelopmental events, such as GZ exit, migration pathway selection, multipolar-to-radial transition, and final lamination.

Keywords

neuronal migration; glial-guided migration; endocytosis; Rap1; N-Cadherin; PAR complex

INTRODUCTION

Until the mid-1990s, adhesive mechanisms were the focus of efforts to understand the recognition and pathway-specific migration of neurons in the developing brain (14–16). Early classical ultrastructural electron microscopy (17) and correlated high-resolution time-lapse electron microscopy (18) provided tantalizing clues to the exquisite specificity of neuronal adhesion to migration substrates and the potential dynamic remodeling of neuron-glial junctions. The molecular cloning of adhesion receptors identified molecules that putatively mediate neuron-neuron or neuron-glial adhesive events (19–24). Moreover, gene targeting in the mouse showed that many of these molecules play key roles in migratory events *in vivo* during assembly of the brain's cortical regions (3, 25– 29). However, it proved difficult to elucidate the molecular mechanisms that control neuronal adhesive affinity or avidity by altering levels or types of adhesion molecules expressed at the cell surface. For example, the mechanisms proposed to alter cell surface adhesion receptor strength, such as carbohydrate modification of N-CAM (30–33) or transcription of *ASTN1* mRNA during cerebellar granule neuron (CGN) differentiation (16, 20), take much longer

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than the seconds to minutes needed to remodel neuronal junctions demonstrated by time-lapse/electron microscopy for neuron migrating along glial fibers (18). Moreover, few tools other than antibodies were available to observe the molecular components of junctions and manipulate adhesion receptor function.

Great progress in our ability to examine the molecular mechanisms controlling cell adhesion during neuronal migration have resulted from striking advances in *ex vivo* and *in vivo* manipulation of neurons, cell biology tools to alter receptor function, small-molecule inhibitors, and advanced time-lapse imaging. These tools have not only confirmed some early ultrastructure-based predictions about vesicle recycling and exocytosis but also implicated conserved polarity signaling pathways in adhesion receptor trafficking, linked conserved adhesion pathways to extrinsic signaling molecules like Reelin, and, for the first time, allowed direct visualization of adhesion receptor trafficking at the neuronal cell surface.

ENDOCYTOSIS AND NEURONAL ADHESION

Adhesion receptor trafficking has long been implicated in migration of motile cells, such as fibroblasts and leukocytes (34–38). Insertion of new adhesion receptors forward of the cell body is thought to generate traction that pulls cell components forward, while removal of adhesive elements in the rear may facilitate forward translocation. Thus, the balance between exocytosis and endocytosis and the site of adhesion receptor insertion and retrieval are major factors in the motility of non-neuronal cells.

Adhesion receptor trafficking was postulated as a general control mechanism of the substrate specificity of migrating neurons, as classical EM studies revealed clathrin-coated pits closely proximal to neuron-glial junctions of both radially and tangentially migrating neurons (17, 18, 39, 40). Not surprisingly, recent studies found that many molecules involved in neuron-neuron or neuron-glial adhesion, such as ASTN1, N-Cadherin, or integrins, are localized to the endocytic compartment near cell contact sites (41–43). Importantly, mechanistic studies clearly implicate endocytosis as a key regulator of neuronal migration (See Figure 1A and B). Segal and coworkers reported that TrkB-enriched signaling endosomes were required to orient CGN migration to the IGL in response to brain-derived neurotrophic factor (BDNF) (6, 44). Hatten and colleagues showed that the newly identified astrotactin 2 (ASTN2) protein is not a neuron-glial adhesion molecule like its homolog ASTN1; instead, it functions in CGN-glial junction formation by forming a complex with ASTN1 to regulate ASTN1 cell surface recruitment (41). Shieh et al. reported that multiple endocytic adaptor proteins are located primarily in the portion of the leading process just proximal to the neuronal cell body and that endocytic recycling of activated integrin receptors is required for the tangential migration of subventricular zone (SVZ)-a neurons (42). Interestingly, both of the latter studies found that small-molecule endocytosis inhibitors block neuronal migration, both *in vitro* and in *ex vivo* slice cultures; these results mesh nicely with findings that nonspecific inhibition of endocytosis via overexpression of dominant-negative Dynamin constructs inhibits migration. In addition, Shieh et. al. found that inhibition of endocytosis led to a acculation of adhesion receptors at the rear of the migrating neuron. Finally, Kawauchi and coworkers reported that endocytic trafficking pathways involving the Rab5, Rab7 and Rab11 proteins (small Ras-related GTPases regulating discrete stages of endocytosis) control neocortical radial migration. A Rab5/11 pathway by precisely regulating N-cadherin surface expression and Rab7 appears to controls the final somal translocation of pyramidal neurons within the cortical plate. (43). Thus, as in general cell motility models, endocytic trafficking of adhesion receptors dynamically orchestrates adhesive interactions required for neuronal migration.

POLARITY SIGNALING AND NEURONAL ADHESION

While the endocytosis studies described above provide some mechanistic insight into retrieval of adhesion receptors from the neuron surface, new reports suggest that conserved polarity signaling molecules, such as the partitioning defective (PAR) complex or the Rap1 GTPase regulate the extent of cell-surface adhesion receptor recruitment (Figure 1C). Moreover, fine-tuning of adhesion receptor function through polarity signaling molecules provides a new mechanism for the control of GZ exit, multipolar-to-radial migration, and final laminar positioning.

Reelin, Rap1, and N-Cadherin

The Reelin signaling pathway has long been a model for studies of the mechanisms controlling inside-out lamination of the vertebrate cerebral cortex (45, 46). Reelin is a large extracellular glycoprotein secreted by early-born cortical neurons that allows later-born neurons to migrate past them. Interestingly, although neurons can migrate along glial fibers in the absence of Reelin, its receptors (VLDLR/ApoER2) (47), or Dab1 (48, 49) (an essential signaling adaptor of the pathway), cortical layering appears to become inverted because of defective positioning within the cortical plate target area.

Despite tremendous progress in elucidating the genetics and biochemistry of Reelin pathway signaling, the precise cellular mechanism controlling selection of a final cortical plate position remains controversial. Appropriate positioning may be impeded when Reelin-deficient neurons fail to detach from glial fibers, via either a mechanism involving the α3 integrin receptor (50) or altered interactions between migrating neurons and those already positioned in the cortical plate (51, 52). Reports from the Cooper and Mueller laboratories shed new light on how Reelin signaling regulates cellular interactions (53, 54). Cortical pyramidal neurons normally migrate toward the cortical plate in three phases: multipolar migration, radial translocation along glial fibers, and somal translocation (55–57). Jossin et al. and Franco et al. used in utero electroporation, conditional mouse models, and time-lapse microscopy to elegantly show that deficiency in Reelin signaling perturbs the multipolar-to-radial migration transition and the terminal somal translocation phase within the cortical plate, without affecting migration along glial fibers. How does Reelin signaling mechanistically control these two events? Building on earlier studies from the Cooper laboratory suggesting that Reelin signaling activates the Rap1 small GTPase, Franco et al. and Yossin et al. argued compellingly that Rap1 is an essential downstream component of Reelin signaling during cerebral cortical development. First, Reelin stimulates Rap1 activation in cultured cortical neurons in both a Dab1- and VLDLR-dependent manner. Second, overexpression of Rap1GAP (a specific Rap1 inhibitor) and Rap1 silencing demonstrate that Rap1 activity is required for proper cortical plate targeting but not for migration along glial fibers; this phenotype closely resembles the deficits seen with Reeler and Dab1 loss of function. Finally, Rap1 gain of function partially rescues migration phenotypes induced by overexpression of a dominant-negative VLDR construct with altered Dab1 function and Reelin-induced Rap1 activation.

Rap1 is an ancient cell polarity signaling molecule with diverse activities. It regulates polarized cdc42 activation (in budding yeast) and cell-cell and cell-matrix interactions (in mammals) by modulating the adhesive activity of integrin or cadherin receptors (58, 59). The Cooper and Mueller laboratories report that N-Cadherin is likely a downstream functional target of the Rap1 arm of the Reelin signaling cascade. N-Cadherin, like Rap1, is required for cortical plate targeting, as N-Cadherin silencing or overexpression of a dominant-negative cadherin construct arrests migration deep within the cortical plate. Moreover, N-Cadherin gain of function rescues migration defects induced by Rap1 deficiency. How does Rap1 activity downstream of Reelin control N-Cadherin function?

Jossin et al. report that Rap1 is localized to transport vesicles in cortical neurons as well as other types of migrating cells. Both Rap1 loss of function (via overexpression of Rap1GAP) and perturbation of Reelin signaling (via overexpression of a dominant negative VLDR construct) significantly reduced N-Cadherin plasma membrane levels in cultured cortical neurons; Rap1GAP overexpression also inhibited binding of cortical neurons to N-Cadherin substrates. Thus, Rap1 signaling appears to control surface N-Cadherin levels as neurons transition between migratory phases during neocortical development.

Several intriguing questions about the Reelin/Rap1/N-Cadherin signaling pathway remain. First, while both the Cooper and Mueller groups agree that terminal translocation is defective when Reelin or Rap1 is perturbed, it is unknown how multipolar-radial transition ultimately affects cortical plate targeting. Second, Franco et al. propose that terminal translocation requires the attachment of cortical neurons to elements within the marginal zone, but it is unclear what these elements are and whether attachment is N-Cadherin-dependent. Finally, it is unknown how the presumed increase in N-Cadherin adhesion is integrated with 1) Reelin activity to decrease α3 integrin adhesion during cortical plate targeting and 2) Rab GTPase–dependent endocytic retrieval of N-Cadherin, which is important for glial-guided migration.

Siah, Pard3A, and JAM-C

The PAR complex, comprising the Pard3 and Par6 adaptor proteins, atypical PKCζ, and the CDC42 Rho GTPase, is perhaps the best-characterized evolutionarily conserved cell polarity signaling entity and is crucial for tight junction formation, mitotic spindle orientation, cell migration, and axon specification in various cell polarity models (60–62). While previous studies showed that Par6α is required to organize the cytoskeletal components that coordinate CGN nucleokinesis during migration along Bergman glial fibers (10, 63), the role of other PAR complex components and the identity of upstream regulators of polarity during migration are relatively unexplored. Recent examination of the regulation of PAR complex during neuronal differentiation revealed a surprising signaling pathway that controls adhesion receptor trafficking as CGNs exit their germinal zone niche (64). Developing CGNs are an excellent model for analyzing the mechanisms regulating GZ exit and for elucidating migration pathway selection, as they undergo two migration phases (65–67): tangential migration near the cerebellar surface followed by radial migration away from the EGL, during which CGNs cross the molecular layer (ML) and traverse to a final site within the internal granule layer (IGL). Interestingly, Pard3A expression is low in immature CGNs and increases as CGNs terminally differentiate, suggesting that it plays a role in GZ exit or the switch from tangential to radial migration. Indeed, systematic gain- or loss-of-function analyses in *ex vivo* cerebellar slices confirmed that Pard3A activity is necessary and sufficient for CGN GZ exit.

What controls Pard3A levels in differentiating CGNs? Famulski et al. used a two-hybrid screen to identify PAR complex–binding proteins and showed the seven in absentia homolog (Siah) family of E3 ubiquitin ligases to be key regulators of Pard3A. The interaction of Pard3A and Siah ubiquitin ligases requires Siah degron sequences within Pard3A and the Sina substrate-binding domain of Siah. Moreover, Siah ligases appear to antagonize Pard3A function, as Siah overexpression can induce proteosomal degradation of Pard3A, while levels of Pard6 or aPKCζ are unaffected. During cerebellar development, Siah expression is complementary to Pard3A expression: high in undifferentiated CGNs and extinguished during terminal differentiation. Systematic gain- or loss-of-function analyses in *ex vivo* cerebellar slices show that Siah activity is necessary and sufficient to maintain immature CGNs within the EGL and that Siah inhibition of GZ exit is dependent on its targeting of Pard3A for degradation, as Pard3A overexpression rescues any Siah-dependent inhibition of migration. Interestingly, long-term time-lapse imaging in cerebellar slices

revealed that excess Siah activity does not deter the motility of CGNs but restricts their movement to the EGL, blocking radial migration to the IGL. Thus, the apparent antagonistic relationship between Siah and Pard3A controls migration initiation and pathway selection in developing CGNs.

Regulation of cell adhesion through junctional adhesion molecule (JAM)-C is a major downstream function of Siah and Pard3A. JAM-C, a member of the immunoglobulin superfamily, is an essential component of epithelial tight junctions and is reported to interact directly with Pard3A via a JAM-C cytoplasmic PDZ binding motif (68–72). In epithelial cells, this interaction is essential for formation of tight junctions and-JAM-C recruitment to tight junctions. Interestingly, not only is JAM-C expressed in differentiating CGNs but JAM-C-mediated adhesion is also necessary and sufficient for CGN GZ exit. Moreover, Pard3A activity is essential to recruit JAM-C to neuron-neuron or neuron-glial cell contacts, and disruption of Pard3A/JAM-C interaction by overexpression of the Pard3A binding site in JAM-C blocks CGN movement to the IGL. Thus, Pard3A's activity in promoting GZ exit of differentiated CGNs through JAM-C recruitment to the plasma membrane facilitates the polarity-dependent integration of new neurons into the cerebellar cortex.

Several intriguing questions about the Siah/Pard3A/JAM-C pathway remain. First, it is currently unclear what upstream signals impinge on the Siah E3 ubiqutin ligase controls Pard3A turnover during neuronal differentiation. Interestingly, studies from other model systems suggest that Siah may be a downstream component of the Ras signaling pathway and a target of hypoxia signaling (73), suggesting that these pathways may intersect with polarity signaling during CGN development. Second, it is also unclear how Pard3A controls JAM-C surface recruitment. Recent studies in C. elegans and cultured fibroblasts implicate PAR3, aPKC and the NUMB polarity protein in surface receptor trafficking (74, 75). It will be interesting to determine if similar endocytosic mechanisms or perhaps exocytosis controls the PAR complex dependent recruitment of JAM-C during tangential to radial migration switch.

NEURONAL ADHESION DYNAMICS

In general models of cell motility, adhesion formation and disassembly drive migration by regulating how a cell binds to actomyosin and uses it to pull against migration substrates (76, 77). While various adhesion molecules are known to mediate binding of neurons to their glial or nonglial guides (1–3), we lack the tools to examine the basic features of neuronal adhesion in living cells in a dynamic, high-throughput fashion.

Two recent studies using fluorescence-labeled adhesion molecules and live cell imaging to examine CGN radial migration demonstrated that adhesion dynamics are linked to the saltatory movement of neurons along their glial guide. In the first study, Wilson et al. tracked neuron-glial attachment sites with an ASTN1-Venus fusion protein(41). During early CGN migration, when the neuron is presumably attached to the glial fiber, ASTN1-Venus was located in the leading aspect of the neuronal soma, where a specialized "interstitial junction" had previously been observed in correlated time-lapse microscopy and EM studies. As CGNs translocated along the glial fiber and the somal junction was released, ASTN1-Venus flowed from the soma to the proximal domain of the leading process, where a new adhesion site would form. In the second study, Famulski et al. developed a novel neuronal adhesion live imaging probe by fusing pHluorin (78) to the extracellular domain of JAM-C, a PAR complex–dependent adhesion molecule required for CGN radial migration (64). A similar approach has been to track SYNCAM1 trafficking at putative synaptic connections (79). pHluorin pH sensitivity ensures that JAM-C-pHluorin fluoresces only as the JAM-C extracellular domain traffics to cell contacts, allowing direct examination of cell

surface adhesion dynamics (Figure 2A). Surface JAM-C was observed at punctate junctions within the leading process and in larger adhesion plaques in the neuronal soma (Figure 2B). During the movement cycle, JAM-C puncta appeared in the f-actin–rich region at the base of the leading process, where actomyosin activity helps to power nucleokinesis. As the soma translocated forward, these leading-process junctions coalesced into the larger plaques seen in the leading portion of the soma. Surprisingly, the soma translocated past large adhesion plaques originally located in the leading somal pole, and the plaques subsequently disassembled within the trailing process. Interestingly, f-actin was heavily enriched at sites of surface JAM-C recruitment at two points of the cycle: after JAM-C puncta appeared in the leading process and at the time of their disassembly in the trailing process. The recruitment of f-actin to newly formed JAM-C puncta provides strong evidence that these locations are bona fide cell-cell adhesion sites and that the leading process is the locus of strong traction forces during migration. In future studies, it will be important to determine whether these adhesion dynamics observed in CGNs are applicable to other neuronal cell types. It will also be important to examine the precise relationship of actomyosin activity to neuron-glial adhesion sites in the leading process, as recent evidence suggests that actomyosin plays a direct role in the leading portion of the cell (10), where myosin II–based contractions generated forward of the nucleus are required for efficient adhesion and cell motility.

CONCLUSIONS

Over the past two decades, much progress has been made in identifying molecules that mediate the interaction of migrating neurons with glial or neuronal substrates on their journey to a final laminar position. Clearly, regulation of the affinity or avidity of the array of cell-surface adhesion receptors is crucial to guide a neuron’s migration path. An important challenge facing this field is to identify how cell-extrinsic cues cooperate with both the genetic programs controlling neuronal maturation and cell-intrinsic adhesion receptor trafficking mechanisms to regulate how immature neurons initiate, execute, and terminate this essential nervous system morphogenic event.

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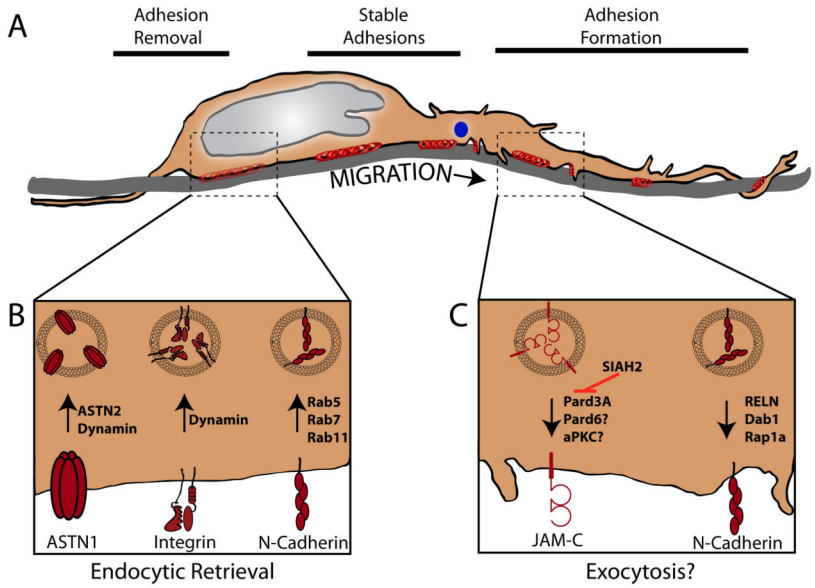

Figure 1.

A. During glial-guided of migration of CNS neurons in the developing mammalian brain neurons are polarized in the direction of migration (arrow). A leading process extends in the direction of migration, cytoplasmic organelles like the centrosome (blue circle) are often found where the leading process tapers into the neuronal cell body and the nucleus (grey oval) is located in rear the cell body. Similar to what is observed in other migrating cells, a gradient of adhesive contacts exist where neurons contact a migration substrate (glial fiber is shaded grey, adhesions are depicted in red): new adhesions are primarily located in the leading process, stable adhesions near the soma and adhesions are removed near the rear of the soma. **B.** Summary of recently reported signaling pathways that control adhesion receptor endocytosis: ASTN1 endocytosis is controlled by ASTN2 and dynamin in CGNs, Integrin receptor endocytosis occurs at the rear of migrating SVZa neurons via dynamin and N-Cadherin surface levels is regulated via endocytic recycling controlled by the Rab5, 7 and 11 GTPases. **C.** Summary of recently reported signaling pathways that control adhesion trafficking to the neuronal cell surface. JAM-C trafficking is facilitated by the Pard3A component of the PAR complex and is negatively regulated by the Siah E3 ubiquitin ligase. A RELN, Dab1 and Rap1A dependent pathway controls multipolar transition and final cortical plate positioning by regulating N-Cadherin. The precise mechanisms controlling receptor exocytosis are currently unclear.

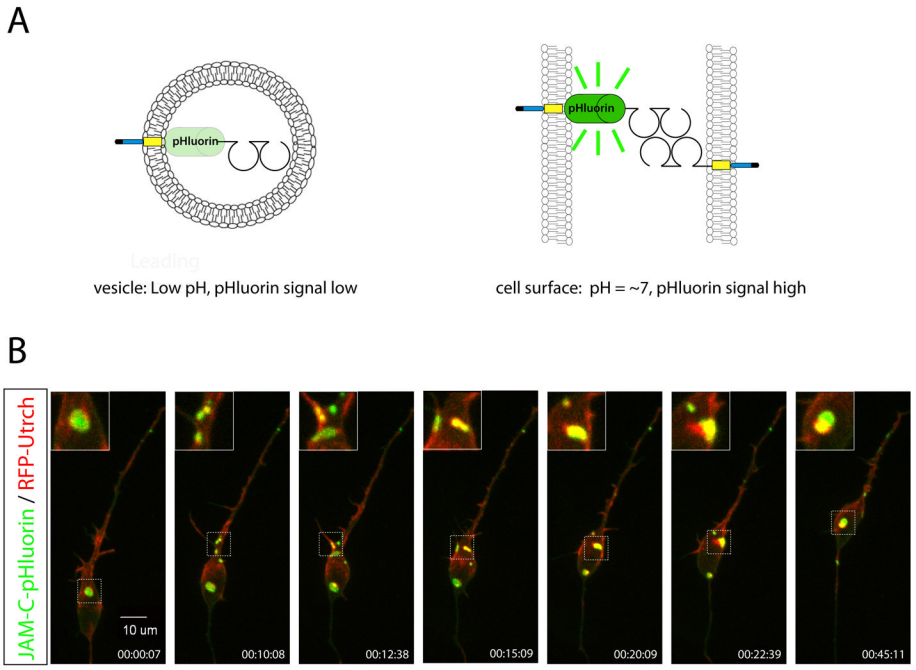

Figure 2.

A. Rational of JAM-C-pHluorin time lapse imaging probe. **B.** Time lapse spinning disk confocal imaging of CGNs nucleofected with JAM-C-pHluorin (green) and RFP-UTRCH (red), an F-actin probe. During CGN migration JAM-C-pHluorin puncta co-localize with UTRCH-RFP (yellow signal within enlarged insets). RFP-UTRCH quickly co-localized with newly formed JAM-C-pHluorin puncta, indicating that JAM-C-pHluorin puncta are sites of F-actin recruitment and therefore are bona fide cell-cell adhesion sites. Both panels reproduced with permission of Famulski et al.