EXTRACELLULAR AND INTRACELLULAR SIGNALING FOR NEURONAL POLARITY

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I. INTRODUCTION

The establishment of cell polarity is crucial for the development of tissues and organs (205, 311, 317). Various types of cell polarity are found in the human body, including apico-basal polarity in epithelial cells, front-rear polarity in migrating cells, and axon-dendrite polarity in neurons. In general, neurons extend two distinct types of neurites: dendrites and axons. Axons and dendrites contain different types of proteins and organelles and thus differ in both function and morphology (FIGURE 1). Dendrites are relatively short and thick, and in most excitatory neurons, they possess dendritic spines. Functionally, dendrites receive chemical signals from other neurons through neurotransmitter receptors. Axons are typically long and thin, and axon terminals contain synaptic vesicles for synaptic transmission to other neurons. Thus neurons are polarized both functionally and morphologically (52). Many scientists are fascinated by the fundamental issue in neuroscience: the mechanisms of neuronal polarization. Although Santiago Ramón y Cajal discovered the basic morphological features of neurons using “la reazione near,” Golgi’s method, in the early 20th century, there were few studies of neuronal polarization until the late 1980s (52). Dr. Banker and colleagues published the first report containing the phrase “neuronal polarity” (41). These authors subsequently described the process of neuronal morphogenesis under culture conditions (66) and divided the developmental stages of cultured hippocampal neurons according to their morphology (stages 1 to 5). Many researchers have attempted to examine neuronal polarization based on these definitions. In contrast to studies using cultured neurons, studies of neuronal polarity in vivo remain under development. In recent years, in utero electroporation methods for genetic manipulation of the rodent brain have become feasible (285, 328). This approach allows us to examine neuronal polarity in the mouse cerebral cortex. In vivo, neuronal polarity includes both axon-dendrite polarity and neuronal migration.

II. DEVELOPMENT OF NEURONAL POLARIZATION IN VITRO AND IN VIVO

A. Neuronal Polarization in Cultured Neurons

Cultured neurons dissociated from embryonic hippocampus have frequently been used to examine neuronal polar-
ity. In that system, five developmental stages have been defined (66) (FIGURE 1). First, hippocampal neurons form several thin filopodia shortly after the dissociated neurons are seeded (stage 1). Next, these neurons extend multiple immature neurites, described as minor processes (stage 2). These neurites undergo repeated random growth and retraction, and there are no morphological differences among them. One day after seeding, neurons begin to show polarized morphology: several minor neurites and one neurite that is much longer than the others (stage 3). The longest neurite eventually becomes the axon. Therefore, the earliest event of neuronal polarization in cultured neurons is axon initiation. Then, within 7 days, the remaining neurites develop into dendrites (stage 4). Finally, the neurons form
polarization processes in vivo are roughly separated into the “inheritance of polarity” mode and the “establishment of polarity” mode (FIGURE 1). One of the best examples of the “inheritance of polarity” mode is vertebrate retinal ganglion cells (and possibly neurons in the early developing cerebral cortex). Because these neurons are directly generated from neuroepithelial cells, they can inherit the apico-basal polarity of the neuroepithelial cells (275). In retinal ganglion cells, the dendrites and axon are differentiated from the apical process and the basal process, respectively (383). The multipolar (MP) cells do not appear during this developmental process. Interestingly, impairment of the retinal neuroepithelial cell polarity induces the abnormal emergence of MP cells (383).

In mid to late cerebral cortical development, most of the pyramidal neurons are generated from neural stem cells defined as radial glial cells in the ventricular zone (VZ) (213, 248, 333) via intermediate (basal) progenitor cells in the subventricular zone (SVZ) (214, 249, 369). Because the intermediate progenitor cells lose their polarity, the neurons cannot inherit their polarity and thus must “establish” their polarity again to become mature neurons. First, the newly generated neurons extend multiple neurites that are identical to those in stage 2 cultured neurons, at which point they are defined as MP cells in the lower portion of the intermediate zone (IZ) (119, 214, 237, 249, 287, 329). Almost 60% of MP cells initially extend a tangential trailing process, which ultimately develops into an axon, and then generate a leading process, which later develops into a dendrite (119, 237). These neurons are defined as bipolar (BP) cells. Fewer than 30% of MP cells initially generate a leading process and then extend a trailing process. BP cells migrate toward the cortical plate (CP) and develop into mature pyramidal neurons. The MP-to-BP transition is a critical step in neuronal polarization in vivo (119, 158, 233, 237).

Most in vivo cells undergo the same initial step of neuronal polarization as cultured neurons: trailing process (future axon) formation (119, 237). Recently, several researchers discovered a new type of basal (intermediate) progenitor cell termed basal radial glial cells, specifically in animals with gyrified brains (33, 76, 198, 338). This type of progenitor cell possesses clear polarity even when the cell body is detached from the apical surface. Because these cells are thought to be involved in brain evolution, it may be interesting to analyze the polarization process in neurons derived from the basal radial glia cells.

In addition to axon-dendrite formation, neuronal polarization in vivo includes a different, but not completely separate, process: migration (231, 283) (FIGURE 1). Generally, leading process formation is essential for migration toward the proper destination. Therefore, the MP-to-BP transition is also critical for neuronal migration (158, 167). Neuronal migration in vivo is roughly separated into two modes: somal translocation and locomotion (230). During the somal translocation mode, neurons extend long processes toward the direction of movement, and they then translocate their somata using these processes. After the translocation of the somata, the remaining process at the opposite end will retract. In contrast, migrating neurons during the locomotion mode extend relatively short leading processes. Migrating neurons extend the leading process a short distance toward the direction of movement and then translocate the somata. The neurons repeat this cycle during locomotion. In the early developing cerebral cortex, most neurons migrate according to the somal translocation mode. During the mid to late embryonic stages, neurons migrate from the IZ to the CP according to a locomotion mode and then convert to the somal translocation mode at the end of migration. In general, somal translocation is radial glia-independent, whereas locomotion is radial glia-dependent (230).

C. Microenvironment of Neuronal Polarization

Multiple cellular processes, such as cell migration and proliferation, occur within specific microenvironments in vivo. The development of cells in vivo is distinct from in vitro cell
Neural polarity in vivo

Neurotrophins (233) and transforming growth factor-β (TGF-β) (376) regulate neuronal polarization in vivo in an autocrine or paracrine manner (see below). ECM molecules also regulate neuronal polarization. In the developing retina, retinal ganglion cells are generated directly from neuroepithelial cells. Shortly after cell division, the retinal ganglion cells reextend a basal process toward the basal surface, where laminin is expressed. Once the basal process contacts laminin, the process is stabilized and ultimately develops into an axon (276). Another microenvironmental cue is cell-to-cell interaction (158, 168, 237). One such example is the radial glial scaffold, which is involved in neuronal migration. As described above, BP cells in the upper portion of the IZ migrate toward the CP according to the locomotion mode. Several types of junctions, such as gap junctions and N-cadherin-mediated adherens junctions, are thought to be involved in this process (81, 158, 168). Interestingly, recent studies suggest that N-cadherin-mediated cell adhesion is important not only for neuronal migration but also for the MP-to-BP transition (158, 168). Inhibiting the N-cadherin-mediated radial glial cell-neuron interaction impairs the MP-to-BP transition. In addition, leading process formation is abnormal in neurons transfected with a dominant-negative mutant N-cadherin (92). These results indicate that N-cadherin-mediated interaction may regulate the MP-to-BP transition, particularly leading process formation. Although these studies suggest that the radial glial cell-neuron interaction is also important for neuronal polarization (92, 158), this connection remains to be proven. Another example of a cell-to-cell interaction is the interaction between pioneering axons and neurons. Recently, we proposed the “Touch and Go” model for neuronal polarization in vivo (237). As described in the previous section, the newly generated neurons develop MP morphology in the lower IZ. In the lower IZ, there are many tangentially oriented axons, called pioneering axons, arising from the early-born cortical neurons. Once one of the neurites of a MP cell “touches” a pioneering axon, this interaction induces the stabilization and rapid extension (“go”) of the contacting neurite. The contacting neurite eventually becomes an axon. TAG-1 (transient axonal glycoprotein-1), a member of the immunoglobulin superfamily of cell adhesion molecules, is involved in this process (237). One possible mechanism underlying this process is that physical contacts increase the tension of immature neurites. Mechanical tension induces neurite elongation, thereby specifying the axon (187). MP cells repeatedly extend and retract immature neurites, which could stabilize a single neurite via cell-to-cell interactions, thereby increasing neurite tension and inducing axon specification. Another possible mechanism underlying this process is that the cell-cell interactions activate signaling molecules that promote axon specification. The downstream signal of TAG-1 is discussed in the next section. Both of these mechanisms are likely involved in axon specification according to the “Touch and Go” mechanism.

The importance of the microenvironment for neuronal polarization in vivo raises a question: How do neurons in culture establish their polarity without that microenvironment? The answer is very simple: the neurons create certain microenvironmental cues by themselves. Neurons in culture secrete neurotrophins such as brain-derived neurotrophic factor (BDNF) and neurotrophin (NT)-3 (106, 233). Neurotrophins act as autocrine or paracrine microenvironmental cues for neuronal polarization in culture (233). Given that neurons determine their polarity in a stochastic manner, these auto- or paracrine factors supply basic stimuli to each minor process. Without neurotrophin signals, neurons fail to polarize (233). This result suggests that microenvironmental cues are essential for neuronal polarization even in culture. The microenvironmental signals in vivo are more...
complicated than in culture. These microenvironments are differentially expressed in space and time and might be important for the location and timing of neuronal polarization (axon initiation).

III. MECHANISMS OF NEURONAL POLARIZATION

Although many researchers are now interested in the mechanisms of neuronal polarization in vivo, much of the basic knowledge comes from studies using dissociated neurons. Therefore, we begin by reviewing some fundamental mechanisms that are presumably shared across in vivo and in vitro systems and then discuss the current knowledge about signaling pathways for neuronal polarization in vivo.

A. Basic Knowledge About Neuronal Polarization: Lessons From Dissociated Neurons

1. Cytoskeletal organization

One principal element of neuronal polarity is cytoskeletal organization. Almost all signals modulate cytoskeletal organization, thus determining cell morphology and motility (11, 19, 193, 367). In particular, growth cone dynamics are regulated by two cytoskeletal components, microtubules and actin filaments (59, 196) (FIGURE 3). The growth cone is divided into three regions based on their cytoskeletal components (196). The central (C) domain mainly contains microtubules, and the peripheral (P) domain is rich in actin filaments and contains filopodia and lamellipodia-like structures. Between these domains, there is an acto-myosin contractile structure termed the transition (T) zone. The three steps of growth cone dynamics that result in neurite outgrowth are as follows: protrusion, engulfment, and consolidation (99). Membrane protrusion is primarily driven by filamentous actin (F-actin) polymerization. Engulfment results from microtubule polymerization and microtubule-mediated transport of membranous organelles and vesicles. Consolidation is the process of F-actin depolymerization at the neck (proximal region). In this section, we focus on microtubule and actin filament dynamics.

A) MICROTUBULES. Microtubules are important regulators of the polarity of many cell types (73, 193, 323). Microtubules are composed of α-tubulin and β-tubulin heterodimers and consist of two distinct ends: the plus and minus ends (FIGURE 4). The plus end is a crucial site for tubulin polymerization. The minus end is often anchored to a microtubule-organizing center (MTOC), such as the centrosome and the Golgi complex.

The first two reports that described the orientation of microtubules in neurons were published in 1981 (39, 123). These studies independently reported that the microtubules in axons are uniformly oriented, with distally directed plus

![FIG. 3. The cytoskeletal structures in neurons. The dendrites and axons have different microtubule orientations; dendritic microtubules show mixed orientation, and axonal microtubules show plus-end-distal orientation. Growth cones are divided into three regions based on their cytoskeletal components. The central (C) domain mainly contains microtubules, and the peripheral (P) domain is enriched in actin filaments and contains filopodia and lamellipodia-like structures. The transition (T) zone contains an acto-myosin contractile structure.](http://physrev.physiology.org/)

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ends in the peripheral nervous system, such as the frog olfactory nerve and the cat lumbar colonic nerve. Then, Baas and colleagues (16, 17) found that the orientation of microtubules in axons is plus-end-distal and that the orientation of microtubules in dendrites is mixed in cultured rat hippocampal neurons. Based on their results and those of modern studies using EB3-GFP to label the plus ends in living cells (319, 368), microtubule orientation during neuronal polarization was analyzed in detail. At stage 2, microtubule orientation in minor neurites is uniformly plus-end-distal. At stage 3, microtubule orientation in axons is plus-end-distal and remains plus-end-distal in minor neurites, identical to that in stage 2 neurons. As dendrites develop (after stage 4), both plus- and minus-end-distal microtubules are detected in dendrites; however, only plus-end-distal microtubules are detected in axons (FIGURE 3). Alternatively, microtubule orientation during neuronal polarization in vivo is complex (286). A recent study using the EB3-GFP labeling method and an embryonic cortical slice culture system demonstrated that the orientation of microtubules in MP cell neurites is plus-end-distal, identical to that of stage 2 cultured neurons, whereas the orientation of microtubules in the trailing process (nascent axon) is mixed (287). Because the orientation of microtubules in mature axons in the cerebral cortex remains unclear, we cannot determine whether this difference is dependent on the envi-
The orientation of microtubules primarily relies on the MTOCs (27, 197). One major MTOC is the centrosome. Typically, centrosomes are composed of two centrioles and pericentriolar material, such as a \( \gamma \)-tubulin ring complex (\( \gamma \)-TuRC) (197). \( \gamma \)-Tubulin nucleates microtubule polymerization; therefore, microtubules are tethered to the \( \gamma \)-TuRC via their minus ends. In addition, the aPKC-dependent activation of Aurora-A kinase phosphorylates Nde1I, most likely inducing nucleation at MTOCs (223). This sequential event induces neurite elongation in dorsal root ganglia neurons. Although the position of the centrosome has been proposed to be a primal regulator of axon specification (56, 190), recent studies using cultured neurons and in vivo models demonstrated that the position of the centrosome is a result, not a cause, of axon specification (287, 320). These studies showed that axon specification occurs independently from the position of the centrosome. In addition, axon elongation occurs normally in cultured neurons with experimentally ablated centrosomes (320). The finding that noncentrosomal microtubules are dominant in neurons suggests the pivotal function of noncentrosomal MTOCs and microtubules (16). In general, the noncentrosomal microtubule array is generated via three mechanisms: release from centrosomes, capture by noncentrosomal MTOCs, or interaction with minus end-binding proteins followed by transport and assembly (27). Microtubules are released from centrosomes via the microtubule-severing protein katanin (118, 208). Inhibiting katanin function in cultured neurons results in the impairment of axon extension (2). Subsequently, the noncentrosomal microtubules are captured by noncentrosomal MTOCs, such as the cortical membrane, the nuclear envelope, and the Golgi complex. In neurons, it has been shown that the Golgi complex in dendrites, referred to as the Golgi outpost, acts as an MTOC and regulates dendritic branching (255). However, Golgi outposts and other MTOCs, such as the \( \gamma \)-TuRC, have not been found in axons (18). Thus the free minus ends might be capped by minus end-binding proteins, such as ninein (23, 34, 216), or by the calmodulin-regulated spectrin-associated protein (CAMSAP)/Nezha/Patronin family of proteins (22, 155, 334, 375) to stabilize the microtubules. Finally, the noncentrosomal microtubules are transported to the proper subcellular region and aligned in their proper orientation. However, the mechanism by which microtubule orientation is determined remains unclear.

Previous studies demonstrated that the stability of microtubules in axons is different from that in minor neurites or dendrites (20, 116, 368). The posttranslational modification of tubulin, primarily \( \alpha \)-tubulin, affects microtubule dynamics (90). Several types of tubulin posttranslational modifications have been identified: tyrosination, acetylation, polyglutamination, polyglycylation, palmitoylation, phosphorylation, and polyamination (Figure 4). Of these modifications, the roles of tyrosination, acetylation, and polyamination of tubulin have been analyzed in detail in neurons; thus, in this review, we focus on these modifications. The COOH terminus of \( \alpha \)-tubulin is tyrosinated by tubulin tyrosine ligase (TTL) (229, 279, 296) and presumably detyrosinated by CCP2 (AGBL2) (284). The tyrosination of tubulin recruits microtubule depolymerizers, such as kinase-13 (Kif2A, 2B, and 2C), thereby contributing to the shorter lifetime of tyrosinated microtubules (266). TTL knockout mice exhibit abnormal layer formation and axon formation in the cerebral cortex (71), and enhanced axon specification and elongation are detected in TTL knockout cultured neurons (178). In contrast, TTL overexpression in cultured neurons inhibits axon outgrowth (271). In addition, Kif2A knockout mice also exhibit abnormal layer formation in the cerebral cortex, and enhanced axon elongation and branching are detected in Kif2A knockout cultured neurons (136). Therefore, the tubulin tyrosination/detyrosination cycle is an important regulator of neuronal polarization, including migration and axon specification. Interestingly, removing the penultimate glutamate residue from \( \alpha \)-tubulin forms \( \Delta \)-tubulin, which cannot be tyrosinated, resulting in exit from the tyrosination/detyrosination cycle (261). Microtubules composed of \( \Delta \)-tubulin are the major component of the brain and neurons (262). Unlike detyrosinated tubulin, \( \Delta \)-tubulin is abundant in growth cones.

Tubulin acetylation is a result, not a cause, of microtubule stabilization (363). Unlike most posttranslational modifications of tubulin, which occur outside the microtubule, acetylation occurs on residues within tubulin (90). \( \alpha \)-Tubulin is known to be acetylated by MEC17/\( \alpha \)TAT in \( C. \) elegans neurons (3, 306) and also in the mouse central nervous system (161) and to be deacetylated by histone deacetylase 6 (HDAC6) and by the NAD-dependent deacetylase sirtuin-2 (146, 250). Although increasing tubulin acetylation via trichostatin A treatment or HDAC6 knockout results in decreased axon elongation, HDAC6 overexpression does not affect axon formation (336). Interestingly, trichostatin A treatment or HDAC6 knockout impairs axon initial segment (AIS) formation; thus tubulin acetylation may be involved in maintaining neuronal polarity.

Recently, a study suggested a novel mechanism for modulating microtubule stability in axons (316). This study showed that polyamination of tubulin, which is mediated by transglutaminase, increases its stability. Because \( Ca^{2+} \) activates transglutaminase, neurotrophin-induced \( Ca^{2+} \) release might regulate axonal microtubule stability via polyamination. Although transglutaminase regulates neurite extensions in neuroblastoma cells, its functions in neurons and in vivo remain unknown.
Another class of regulators of microtubule stability is microtubule-associated proteins (MAPs). In neurons, MAP2, a dendrite-specific MAP, and tau, an axon-specific MAP, prevent the interaction between microtubule-severing proteins and microtubules, thereby contributing to microtubule stability (68, 160). These proteins may also contribute to axon-dendrite polarity in cultured neurons (42, 43, 74). A more recent study suggests that one polarity protein, partitioning-defective 3 (Par3), regulates the stability of microtubules (47). Briefly, Par3 oligomerization induces conformational changes in Par3; subsequently, Par3 binds to and stabilizes microtubules. The details of this function of Par3 are described below. Doublecortin (Dcx) has been identified as a causal gene of human X-linked lissencephaly and double cortex syndrome (60, 98). Dcx is expressed in migrating neurons (80) and regulates migration and the MP-to-BP transition in the developing cerebral cortex (21, 203, 292). Dcx binds to the lattice of microtubules and promotes microtubule nucleation and stability (79, 219). The interaction between Dcx and microtubules is mediated by “Dcx domains,” which may cross-link two tubulin subunits on the same or different microtubules. Dcx activity is partially regulated by Dcx phosphorylation. Dcx phosphorylation by several kinases, such as Cdk5, cAMP-dependent protein kinase (PKA), and MARK, reduces the affinity of Dcx to microtubules (104, 293).

**B) ACTIN FILAMENTS.** The dynamic organization of the actin cytoskeleton directs the morphology of growth cones (59, 196). Actin filaments contain two distinct ends: the barbed and pointed ends (FIGURE 4). In the growth cone, ATP-actin is added to the barbed end, which is directed toward the cell membrane, and ATP hydrolysis and ADP-actin disassembly occur at the pointed end. Therefore, these actin filament dynamics cause retrograde actin flow from the peripheral region to the central region of the growth cone. The formation of highly branched arrays of actin filaments is important for lamellipodia organization (326). The branching of actin filaments is catalyzed by the actin-nucleating Arp2/3 complex (5). The Arp2/3 complex binds to the side of the actin filament and initiates new actin filament assembly. Although much is known regarding the roles of actin filament dynamics in the migration of nonneuronal cells (193), our current understanding of the mechanism by which actin filament dynamics regulate neuronal polarity is far from complete. In this review, we focus on the role of actin filaments in axon formation.

A previous study demonstrated that the actin cytoskeleton is more dynamic in the growth cone of the future axon than in other minor neurites (37). Cultured neurons treated with cytochalasin D, an actin-depolymerizing drug, extend multiple axons. These results suggest that an appropriate level of actin instability at growth cones is critical for axon formation. Consistent with this insight, the actin-severing protein family ADF/cofilin (25, 242) regulates axon formation by creating space for microtubules to penetrate into the peripheral regions of growth cones (77). In contrast, it has been previously shown that the Ena/VASP family of barbed end protector proteins (96, 112) is required for neurite formation by regulating actin dynamics and by promoting actin bundle formation in the peripheral regions of growth cones of neurons cultured on poly-β-lysine (PDL) (111). Actin bundle formation is important for microtubule penetration into the peripheral regions of growth cones. In Ena/VASP mutant mice, axon formation in the cerebral cortex is severely defective (184). Activating integrin signals rescue the phenotypes of Ena/VASP mutant neurons. When neurons are cultured on laminin, these neurons require Arp2/3 rather than Ena/VASP for axon elongation (111). Inhibiting Arp2/3 in neurons increases axon elongation in the presence of PDL (267) but inhibits axon elongation in the presence of laminin (111). Therefore, the coordinated regulation of actin filament and microtubule dynamics is essential for axon formation; however, neurons may select the machinery for this process based on the surrounding microenvironment.

**2. Intracellular transport**

The intracellular transport of cytosolic proteins, membranous structures, and organelles is a critical regulator of cell polarity. Motor proteins, including the myosin, dynein, and kinesin families of proteins, are responsible for the anterograde and retrograde transport of cargo (131, 238, 295). In general, myosins are actin-dependent motors, whereas dyneins and kinesins are microtubule-dependent motors. In this section, we survey the mechanism by which cargo is selectively transported to the appropriate subcellular region by motor proteins (FIGURE 5).

**A) MOTOR PROTEINS.** Fifteen kinesin protein families, consisting of 45 mammalian kinesin superfamilies proteins (KIFs), exist. The most characterized kinesin is kinesin-1. Kinesin-1 is a complex of two kinesin heavy chains (KHCs, also called KIF5), and two kinesin light chains (KLCs) (32, 183). KIF5 contains a motor domain and an alpha-helical coiled-coil domain. Of the 45 identified KIFs, KIF1, KIF2, KIF3, KIF4, KIF5, KIF13, KIF17, KIF26, KIF20B, and KIFC2 are known to play important roles in cargo transport in neurons. Kinesins move toward the plus-end of microtubules, and microtubules are plus-end-distal in axons; thus kinesins are responsible for most of the anterograde cargo transport in axons (131, 238, 295).

The kinesin family of proteins selectively transports various types of cargos, such as organelles and cytosolic, membrane-bound, and cytoskeletal proteins. For example, the anterograde transport of mitochondria into axons is mediated by kinesin-1 (97, 110, 322). Vesicles containing the neurotrophin receptor TrkB are transported toward the distal end of the axon by kinesin-1 (12). Kinesin-4 (KIF4) transports membrane proteins such as the cell adhesion molecule L1, which is involved in various developmental
processes in neurons, toward the distal end of the axon (265, 289). Cytoskeletal proteins, such as neurofilaments and tubulin, are transported into axons by kinesin-1 (174, 177, 340, 370, 371). Selective transport by kinesins is important for synaptic transmission in mature neurons. Kinesin-3 regulates the transport of synaptic vesicle precursors into axon terminals (114, 246, 253, 382).

The kinesin family of proteins also transports specific proteins that regulate axon formation. For instance, Par3 is transported to the tips of developing axons by kinesin-2 (KIF3A) (88, 244). Collapsin response mediator protein-2 (CRMP-2) is transported to the distal ends of nascent axons by kinesin-1 through binding with KLC (12, 166, 174, 348). Both Par3 and CRMP-2 are predominantly localized at the distal ends of axons and play pivotal roles in axon specification. Disrupted-in-schizophrenia 1 (DISC1), a susceptibility gene for major psychiatric disorders, including schizophrenia (50, 153), is transported by kinesin-1 to axon growth cones (309, 339). This transport is important for axon elongation. Kinesin-6 (KIF20B), a mitotic kinesin, interacts with Shootin1 and regulates its transport toward the tips of axons (290). In cooperation with L1 and actin, Shootin1 induces axon specification in cultured neurons and in vivo, generating a driving force in growth cones (307, 343, 345). This traction force is regulated by the PAK-mediated
phosphorylation of Shootin1 (343). Because Shootin1 is transported toward the tips of axons by a growth cone-like structure, termed wave, the mechanism by which neurons select between these two independent mechanisms to transport Shootin1 is interesting. PIP₂-containing vesicles are transported by guanylate kinase-associated kinesin (GAKIN)/KIF13B (139). GAKIN/KIF13B is known to regulate axon specification in cultured neurons (139), and its activity is regulated by MARK2 (379).

The kinesin family of proteins also regulates cargo transport into dendrites. In dendrites, there are several types of neurotransmitter receptors. Of these, the N-methyl-D-aspartate receptor (NMDA-R), α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate receptor (AMPA-R), and γ-amino butyric acid receptor (GABAR) are transported by kinesin-1 (KIF5) or kinesin-2 (KIF17) (300, 301, 350). In addition, postsynaptic proteins, such as postsynaptic density-95 (PSD-95), are transported by kinesin-3 (KIF1B) (217).

The dynein family members are classified into two groups: axonemal and cytoplasmic dyneins (353). Cytoplasmic dyneins move along the microtubules toward the minus-end; thus these motors are referred to as minus-end-directed motors. Cytoplasmic dyneins contain two dynein heavy chains, light intermediate chains, and a complex of intermediate and light chains. Because detailed information regarding the structure and basic function of dyneins is reviewed in an excellent article (353); in this review, we focus on the role of dyneins in neuronal development. The activity of dyneins is known to be regulated by dynactin and LIS1/NDEL (297, 312, 360). LIS1 was originally identified as a causal gene of type I lissencephaly (282). The suppression of dynein or LIS1 expression impairs neuronal migration in the developing cerebral cortex (133, 335, 346). On the bases of these results, a dynein-based neuronal migration model has been proposed (347). Briefly, dynein-based neuronal migration consists of three steps. First, leading process extension occurs. As the leading process elongates, the centrosome moves toward the leading process and causes “swallowing” at the proximal region of the leading process. Second, the nucleus translocates to this swelled region (defined as nucleokinesis). Finally, the cell soma catches up to the nucleus. Because it had been believed that the centrosome is always ahead of the nucleus and that the nucleus is surrounded by centrosome-derived microtubules, the forward movement of the nucleus was thought to be induced by the dynein complexes. However, recent studies demonstrated that the centrosome-nucleus positioning is sometimes reversed (287, 352). Therefore, the simplified dynein-based model requires revision (286). One possible mechanism is based on the acto-myosin-dependent driving force to translocate the nucleus (29, 294).

**B) THE MECHANISMS OF SELECTIVE TRANSPORT**. Selective targeting of different cargo to a specific subcellular region is crucial for the polarized distribution of molecules. Selective transport is established via three mechanisms: the preferential and selective binding of motor proteins to cargo adaptors, the loading and unloading of cargo, and selection at the AIS (see below) (131, 238, 295).

**C) PREFERENCES OF KINESINS**. Although most kinesins move toward the plus-end of microtubules, dendrite- or axon-specific kinesins have been identified. In polarized neurons (stage 3 and stage 4 neurons), the motor domains of KIF5, KIF3, KIF13A, and KIF21A preferentially accumulate at the tips of axons rather than in dendrites (144, 157, 234). Although KIF17 immunoreactivity is selectively localized to dendrites in vivo and in cultured neurons (300), the motor domain of KIF17 accumulates in axons (144). Because the tail domains of KIF proteins regulate the motor activity of KIFs by interacting with the head domain (63), the tail domain likely regulates the selective localization of KIF proteins in some cases. In addition, KIF5 transports the AMPA-R subunit GluR2 to dendrites by interacting with the adapter protein glutamate receptor-interacting protein (GRIP1) (301), suggesting that the interaction between the cargo and the motor protein plays an important role in selective transport (132). In nonpolarized neurons (stage 2 neurons), the motor domains of KIF5 and KIF17 accumulate in a subset of neurites (144). However, the motor domains of other KIF proteins fail to accumulate or localize to most neurites (144). Therefore, how do KIF proteins determine their selectivity? The evidence suggests that tubulin posttranslational modifications determine the axon-dendrite preference of KIFs. The KIF5 motor domain has been suggested to preferentially recognize axonal microtubules (178). Interestingly, the affinity of KIF5 for microtubules is enhanced or reduced by tubulin posttranscriptional modifications, such as acetylation and tyrosination, respectively (178, 280). As described above, acetylated and detyrosinated microtubules are enriched in axons, whereas tyrosinated microtubules are abundant in dendrites of polarized neurons (116, 368). Therefore, tubulin acetylation at axons may induce the selective transport of KIF5 to axons, and tubulin tyrosination may exclude KIF5 from dendrites (178, 280). Previous studies demonstrated that taxol treatment, which induces the acetylation, detyrosination, and polyglutamination of tubulin, results in the accumulation of the KIF5 motor domain at the tips of minor neurites as well as at the tips of axons (116). However, specifically increasing tubulin acetylation using trichostatin A or tubacin does not alter the axon-dendrite preference of KIF5 (116). A more recent study suggests that the KIF5 motor domain preferentially binds to GTP-tubulin (235). These results indicate that the axon-dendrite preference of KIF5 is controlled by multiple posttranslational modifications and/or by binding to GTP/GDP-tubulin.

**D) CARGO ADAPTORS**. Most kinesins interact with their specific cargos via specific cargo adaptors (131, 238, 295) (FIGURE
Several cargo adaptors involved in selective transport have been identified. In this review, we discuss several well-characterized cargo adaptors, c-Jun N-terminal kinase (JNK)-interacting proteins (JIPs), nuclear distribution gene C homolog (NUDC), Par3, DISC1, and CRMP-2.

JIPs are scaffolding proteins for the JNK signaling pathways (61). JIPs also act as cargo adaptors to transport cargo into axons (181). JIPs serve as bridges between KIF5 and cargos such as phosphorylated β-amyloid precursor protein (APP) (150) and apolipoprotein E receptor 2 (ApoER2), which is a Reelin receptor (354). In Drosophila, JIP regulates axon-directed cargo transport in a KIF5-dependent manner (35, 40, 140, 141). In contrast, JIP1 or JIP1/JIP2 knockout mice do not exhibit any abnormalities in axon-directed cargo transport (171, 366). Because there are four mammalian isoforms of JIP, the differences between insects and mammals may be caused by the functional redundancy of the mammalian JIPs. Knockdown of JIP1 inhibits polarization of cultured neurons (54). In contrast, knockdown of JIP3 does not affect polarization but inhibits axon elongation in cultured neurons and in vivo (145).

NUDC is a subunit of the dynein complex and is known to regulate nuclear movement during interphase in Aspergillus nidulans (256). The NUD family of proteins, including NUDC, NDEL1, and LIS1 (NUDF), regulates nuclear translocation during neuronal migration by interacting with dynein (14, 44, 225, 310). NUDC binds to KLC, thereby indirectly linking the dynein complex to KIF5 and regulating the anterograde transport of dynein in fibroblasts (372). However, the roles of NUDC in kinesin-dependent cargo transport in cultured neurons and also in vivo remain unclear.

Par3 forms a complex with Par6 and aPKC and accumulates at the distal end of growing axons (244, 305). Because Par3 interacts directly with kinesin-2 (KIF3A) (88, 244), Par3 is a cargo adaptor between the Par complex and kinesin-2, thereby regulating the transport of the complex toward the tips of nascent axons.

DISC1 acts as a cargo adaptor for kinesin-1 (KIF5) by interacting with NDEL1, LIS1, growth factor receptor-bound protein 2 (Grb2), Girdin, cAMP-specific 3’5’-cyclic phosphodiesterase 4B (PDE4B), glycogen synthase kinase-3β (GSK-3β), and fasciculation and elongation protein zeta-1 (Fez1) (70, 162, 172, 204, 212, 309, 339). DISC1 controls the axonal transport of NDEL1/LIS1, Grb2, and Girdin (70, 309, 339). These interactions are important for axon elongation.

CRMP-2 was originally identified as a mediator of semaphorin-3A signaling in the nervous system (102) and is specifically localized to axons (148). CRMP-2 interacts with the KLC of kinesin-1, thus regulating the axonal transport of tubulin heterodimers and the Sra1-WAVE1 complex as a cargo adaptor (166, 174). Similarly, UNC-33, a homolog of CRMP-2 in C. elegans, links tubulin heterodimers to KLC-2 of kinesin-1 (348). An additional study demonstrated that CRMP-2 controls the transport of TrkB in a kinesin-1-dependent manner (12). In this process, CRMP-2 links TrkB to kinesin-1 through Slp1 and Rab27 (12). Therefore, CRMP-2 acts as a cargo adaptor and thereby regulates axon formation by transporting the critical proteins involved in remodeling microtubules and actin filaments and in trafficking growth factor receptors.

E) LOADING AND UNLOADING OF CARGO. To selectively transport specific cargos to specific regions, the critical events are the loading and unloading of cargo (FIGURE 5). Previous studies demonstrated that several signals, including protein kinases, Rab GTPases, and Ca²⁺, regulate the loading and unloading of cargo (131, 238, 295).

The phosphorylation of cargo adaptors and kinesins has been suggested to regulate the loading and unloading of cargo from kinesins. GSK-3β phosphorylates CRMP-2 and interrupts its interactions with both tubulin (378) and the KLC of kinesin-1 (12), thereby inducing the unloading of cargo from the cargo adaptor (CRMP-2) and kinesin-1. GSK-3β activity might thus regulate CRMP-2 activity, indirectly controlling the anterograde transport of specific cargos, such as tubulin, the Sra1-WAVE1 complex, and Trks, during axon formation. GSK-3β also phosphorylates KLC and may induce the degradation of KLC. Membrane-bound organelles are unloaded from kinesin-1 upon GSK-3β-dependent phosphorylation of KLC (222). Rho-associated kinase (Rho-kinase; ROCK, ROK), an effector of the small GTPase RhoA, also phosphorylates CRMP-2, thereby inhibiting the interaction of CRMP-2 with tubulin and with the KLC of kinesin-1 (13).

Calcium/calmodulin-dependent protein kinase II (CaMKII) phosphorylates the tail domain of KIF17 (kinesin-2) (107). Kinesin-2 (KIF17) transports NMDA-Rs as cargo via the adaptor proteins LIN10 or Mint1. The unloading of cargo from KIF17 is regulated by KIF17 phosphorylation (107). CaMKII also phosphorylates liprin-α1, which is a cargo adaptor for leukocyte antigen-related tyrosine phosphatase (LAR-RPTP) (138). Phosphorylation of liprin-α1 induces its degradation via the ubiquitin-proteasome system, thereby inducing the unloading of LAR-RPTP-containing cargo from kinesin-3 (KIF1A). Because the activity of CaMKII is regulated by NMDA-R-dependent Ca²⁺ influx, the unloading of the NMDA-R and other cargo from kinesins may play an important role in the activity-dependent recruitment of NMDA-Rs and other molecules to the postsynaptic membrane.

Activating the JNK signaling pathway leads to the dissociation of JIPs from KLC (kinesin-1), thereby inducing the
unloading of cargo in Drosophila (141). The activation of
DLK and MKK7, which are components of JNK pathway
kinases, has been shown to inhibit the interaction between
kinesin-1 and APLIP1, a Drosophila homolog of JIP1. Al-
though many studies have demonstrated that phosphoryla-
tion regulates the loading and unloading of cargo, as de-
scribed above, only a few studies have demonstrated the
significance of the loading and unloading of cargo to neu-
ronal polarity.

Recently, one study demonstrated that Par3 phosphory-
ation at serine 1116 by extracellular signal-regulated kinase (ERK) disrupts the binding of Par3 to KIF3A (88).
Because phosphorylated Par3 accumulates at the tips of
axons in cultured neurons, this phosphorylation might
regulate the unloading of the Par complex from KIF3A.
The knockdown of Par3 impairs axon specification and
the MP-to-BP transition in cultured neurons and in vivo,
respectively. Unlike wild-type Par3, a phosphomimetic mu-
tant of Par3 (S1116D) did not rescue the Par3 knockdown
phenotype. Therefore, the appropriate unloading of the Par
complex is critical for neuronal polarization both in cul-
tured neurons and in vivo (88).

The Rab family of GTPases regulates vesicle trafficking at
various steps (87, 318, 331, 381). Rab GTPases serve as
molecular switches by cycling between their GDP-bound
inactive and GTP-bound active forms. Rab GTPase activity
is regulated by Rab GDP/GTP exchange factors (GEFs),
Rab GTPase-activating proteins (GAPs), and Rab guanine
nucleotide dissociation inhibitors (GDIs) (87, 318, 331,
381). Rab GEFs convert GDP-bound Rab GTPases to
their corresponding GTP-bound form, thus activating
Rab GTPases. In contrast, Rab GAPs convert the GTP-
bound form to the GDP-bound form, thus inactivating
Rab GTPases (87, 318). GTP-bound Rab GTPases inter-
act with and activate their specific effectors. Previous
studies have shown that Rab GTPases regulate the load-
ing and unloading of specific cargo from kinesins or from
cargo adaptors (10, 246).

A previous study suggested that Rab3 plays an important
role in the loading and unloading of synaptic vesicle precur-
sors (246). GTP-bound Rab3, but not GDP-bound Rab3,
can interact with DENN/MADD. Because DENN/MADD
binds to kinesin-3 (KIF1A/KIF1B) as a cargo adaptor, only
GDP-bound Rab3 can link Rab3-containing vesicles to ki-
nesin (246). Therefore, Rab3 activity regulates the loading
and unloading of cargo. Because a previous study suggested
that DENN/MADD acts as a GEF on Rab3 (373), the
DENN/MADD-Rab3 complex may sustain Rab3 activity
and facilitate the stable transport of Rab3-containing ves-
icles. Vesicle unloading may be regulated by Rab3-specific
GAPs. Interestingly, Rab3-GAPs regulate synaptic trans-
mision (288), suggesting that Rab3 is important for vesicle
transport to regulate synaptic function.

Rab27, the most similar isoform to Rab3, also regulates the
trafficking of a variety of secretory vesicles in many types of
cells, including neurons (86). Rab27 is localized to TrkB-
containing vesicles and regulates the anterograde transport
of Trks in axons (10). The GTP-bound form of Rab27 can
interact with an effector of Rab27, synaptotagmin-like pro-
tein 1 (Slp1). Slp1 directly binds to the cytoplasmic region
of TrkB in a manner dependent on activated Rab27. Slp1
also interacts with CRMP-2. Thus the Slp1/Rab27/CRMP-2
complex directly links TrkB-containing vesicles to
kinesin-1. Because the formation of this complex depends
on the GTP/GDP-binding state of Rab27, Rab27 may reg-
ulate the loading and unloading of TrkB-containing vesi-
cles. Further studies are required to understand the mecha-
nisms by which Rab27 activity is regulated in neurons.

The motility and localization of mitochondria are regulated
by the intracellular Ca\(^{2+}\) concentration (45, 135). Mitoch-
donria are transported by kinesin-1 (KIF5) via the MIRO-
Milton adaptor protein complex in Drosophila (97, 135)
and in mammalian neurons (38, 82, 199, 313). In-
creased Ca\(^{2+}\) levels induce conformational changes in
MIRO through its EF hand motifs to bind to KIF5 (359).
This MIRO-KIF5 interaction interferes with the interaction
between KIF5 and microtubules, arresting mitochondrial
movement. Another potential mechanism of mitochondrial
unloading is that elevated Ca\(^{2+}\) concentrations may de-
crease the affinity of the MIRO-Milton complex for KIF5,
thereby inhibiting mitochondrial movement (200). Further
studies are required for understanding the precise mecha-
nisms of the Ca\(^{2+}\)-induced unloading of cargo.

3. Intracellular signal molecules

Various types of intracellular signaling molecules have been
identified as regulators of neuronal polarity. In this section,
we introduce the roles of the Rho family of small GTPases,
kinases, scaffolding proteins, transcription factors, and epi-
genetic modifiers in neuronal polarization (FIGURE 6).

A) THE RHO AND RAS FAMILIES OF SMALL GTPASES. Rac1, Cdc42,
and RhoA, members of the well-understood Rho family of
small GTPases, play crucial roles in the morphogenesis of
various cells by reorganizing the cytoskeleton (113). Be-
cause Rho GTPases cycle between GDP-bound inactive and
GTP-bound active states, these molecules act as molecular
switches. The active forms of Rho GTPases interact with
and activate a variety of downstream effectors, and they
mainly regulate cytoskeletal reorganization.

Neurons electrooporated with constitutively active (CA)
Rac1, dominant-negative (DN) Rac1, or Rac1 GEFs, such as
STEF/Tiam1 or P-Rex1, exhibit retarded migration and a
loss of leading and trailing processes, suggesting that the
balance of Rac activity is required for the MP-to-BP transi-
tion and migration in vivo (167, 179, 380). Because several
Rac isoforms are expressed in the neocortex, the defects in
neuronal polarity are milder than in DN Rac1-expression studies (191). In contrast, cerebellar granule neurons do not express other Rac isoforms. The cerebellar granule neurons of Rac1-deficient mice display impaired neuronal migration and axon formation both in vivo and in vitro (330). These phenotypes might be caused by the mislocalization of the WAVE complex and the subsequently reduced actin dynamics.

Cdc42 plays a critical role in axon formation upstream of the Par3-Par6-aPKC complex in cultured neurons (298) and in vivo (93). PI3K activates Cdc42 by producing PIP3, Cdc42 interacts with Par6 (156, 157, 195, 273), and Par3 mediates Cdc42-induced Rac activation via the specific Rac GEFs Tiam1 and STEF/Tiam2 (115, 245). Because activated Rac can bind to PI3K, this mechanism is thought to be a positive-feedback loop for axon specification.

RhoA is generally associated with actin cytoskeleton re-modeling and myosin-based contractility. In neurons, RhoA usually acts as an inhibitory molecule for the formation of axons and neurites. The CA form of RhoA inhibits neurite formation, whereas a DN form of RhoA enhances neurite outgrowth (53, 298). Inhibiting the activity of Rho-kinase (ROCK), an effector of RhoA, induces the formation of multiple axons in cultured neurons (49, 53). Rho-kinase phosphorylates myosin light chain, myosin phosphatase, CRMP-2, Tau, MAP2, and LIM kinase and regulates actin filament and microtubule dynamics and protein trafficking (6, 7, 13, 173, 201).
Ras, one of the most common oncogenes in humans, is known to regulate axon specification in cultured neurons (252, 377). Ras activity is particularly high in the growth cones of nascent axons (75). Ras activation induces membrane targeting of integrin-linked kinase (ILK), which, in turn, activates Akt and inactivates GSK-3β, thereby stimulating axon formation. Because ILK is a pseudokinase, ILK may act as a scaffold for Ras/Akt/GSK-3β (272). Ras also activates ERK2 through Raf and MEK (9, 62, 180, 185, 236, 263, 355), thereby regulating neuronal polarization (88, 252, 377).

Rap1, a member of the Ras superfamily of GTPases, is also required for appropriate neuronal polarity (298). Rap1 activity is highest in the longest neurite (232). Neurons deficient in the Rap1 GEF C3G (Rapgef1, Grf2) display MP morphology and are arrested in migration in brain slice cultures (357). A recent study demonstrated that Reelin, an extracellular matrix glycoprotein that helps to regulate the processes of neuronal migration and positioning during the organization of the cortex, stimulates the activation of Rap1, regulating the polarity of migrating neurons in the developing mouse cortex via the regulation of N-cadherin (81, 158).

B) KINASES. I) Ca2+/calmodulin-dependent protein kinase kinases, CaMKK/CaMKI. Calcium is known to be involved in axon formation by mediating responses to axon guidance molecules. The Ca2+ effector CaMKK and its target CaMKI have been implicated in axon elongation in cultured hippocampal and cortical neurons (55, 351, 362). CaMKI phosphorylates and activates MARK2 and promotes neurite outgrowth (351). A rapid increase in Ca2+ induced by NT-3 in an inositol 1,4,5-trisphosphate (IP3)-dependent manner leads to the local activation of CaMKK in the growth cone (233). Inhibiting neurotrophin receptors or CaMKK attenuated NT-3-induced axon specification in cultured neurons and the MP-to-BP transition in vivo (233). Another report showed that treatment with muscimol, a GABA_A receptor agonist, promoted axonal growth via the CaMKK/CaMKI pathway, suggesting that distinct upstream factors may regulate CaMKK activity under different circumstances (1).

II) The mitogen-activated protein kinase (MAPK) family. The MEK-ERK2 pathway and the upstream molecule Ras regulate neuronal polarization (88, 252, 377). The overexpression of dominant-negative or constitutively active mutants of MEK1 inhibits proper neuronal polarization (88), suggesting that the spatial and temporal regulation of the MEK-ERK2 pathway is critical for the neuronal polarization. ERK2 is localized not only in the cell soma but also in the growth cone, and it regulates the interaction between Par3 and KIF3A as described above (88).

JNK, a member of the MAPK family that was initially described as a stress-activated protein kinase, was identified based on its ability to phosphorylate c-Jun (186). JNK phosphorylates microtubule regulators, such as DCX, MAP1B, MAP2, and SCG10/stathmin-2. Activation of JNK and the JNK binding partner JIP is required for axon initiation in vitro (54, 254). JNK activity is elevated in the IZ during cortical development (337). The targeted deletion of the JNK activator mkk4 (358) or the electroporation of neurons with dominant-negative JNK inhibits radial migration (167). Recent studies showed that JNK regulates the MP-to-BP transition and neuronal migration via SCG10/stathmin-2 phosphorylation during cortical development (337, 365). JNK1 and dual leucine zipper kinase (DLK) double-knockout mice exhibited severe defects in axon formation in the cerebral cortex (129).

C) SCAFFOLD PROTEINS. Several important regulators of neuronal polarity play roles as scaffold proteins. In this review, we focus on the major scaffold proteins for neuronal polarity, CRMP-2 and Par3.

I) CRMP-2. CRMP-2 is one of at least five CRMP isoforms and historically was independently identified as Ulip2/CRMP-62/TOAD-64/DRP-2 (102). CRMP-2 is highly expressed in the developing nervous system. Mutations in UNC-33, a C. elegans homolog of CRMPs, lead to severely uncoordinated movement and abnormalities in axon guidance (121, 348). The functions of CRMP-2 in neuronal polarization have been well-studied using cultured neurons. Previous studies showed that CRMP-2 is enriched in the growing axons of hippocampal neurons and that CRMP-2 overexpression induces primary axon elongation and multiple axon formation, whereas inhibiting CRMP-2 function impairs axon formation in cultured hippocampal neurons (148). CRMP-2 interacts with tubulin heterodimers and promotes microtubule assembly in vitro (85). CRMP-2 also regulates the endocytosis of specific adhesion molecules, including L1, by interacting with Numb (243) and by reorganizing actin filaments via the Sra-1/WAVE1 complex (166), and it induces the trafficking of vesicles containing TrkB by interacting with the Slp1/Rab27 complex (12). Thus CRMP-2 promotes neurite elongation and axon specification by regulating microtubule assembly, adhesion molecule endocytosis, actin filament reorganization, and axonal protein trafficking.

CRMP-2 activity is regulated by COOH-terminal phosphorylation. GSK-3β phosphorylates CRMP-2 at Thr-514 and Ser-518 following the priming phosphorylation at Ser-522 by cyclin-dependent kinase 5 (Cdk5), thereby inactivating the binding of CRMP-2 to tubulin dimers (378). In cultured hippocampal neurons, the nonphosphorylated CRMP-2 level is enriched in axon growth cones. The expression of a CA form of GSK-3β inhibits axon formation, whereas the nonphosphorylatable form of CRMP-2 coun-
teracts the inhibitory effects of GSK-3β, suggesting that GSK-3β regulates neuronal polarity via phosphorylating CRMP-2. Neurotrophic factors, including NT3 and BDNF, inhibit GSK-3β activity and, therefore, CRMP-2 phosphorylation. Depleting CRMP-2 prevents NT3-induced axon outgrowth in cultured hippocampal neurons (378). Taken together, regulating GSK-3β and CRMP-2 activities is important for neuronal polarization in vitro. The details of the upstream GSK-3β signaling pathway are presented below (see cell extrinsic signals).

The function of CRMP-2 in neuronal polarization in vivo has recently been analyzed. In the developing neocortex, inhibiting CRMP-2 function via gene knockdown or via the expression of a dominant-negative mutant delays neuronal migration and impairs the MP-to-BP transition (324). A recent study demonstrated that the phosphorylation status of CRMP-2 affects the MP-to-BP transition (151). There are few in vivo studies of CRMP-2 using knockout mice. In contrast to the studies using cultured neurons, there are no apparent axonal abnormalities in knock-in mice expressing the phosphorylation-deficient form of CRMP-2 (CRMP-2-S522A) (374). However, these knock-in mice exhibit increased numbers of primary dendrites. This phenotype is enhanced by crossing with the CRMP-1-knockout mice (374). Because neurons express all CRMPs (CRMP-1 through CRMP-5) during brain development, functional compensation may occur in individual CRMP knockout models. Further studies using individual and multiple conventional or conditional CRMP knockout mice are required.

II) Par3. The Par genes were initially identified via genetic screens for regulators of cytoplasmic partitioning in early embryos of C. elegans (170). Six par genes were identified based on this screen. Par1 and Par4 encode serine threonine kinases, as described above (109, 227). Par2 contains a RING finger domain that may function in the ubiquitination pathway (218). Par3 and Par6 contain PDZ domains, suggesting that these proteins act as scaffold proteins (72, 361). Par5 is a member of the 14-3-3 family of proteins, which bind to phosphorylated serine and threonine residues (228).

Par3 forms a conserved protein complex with Par6 and atypical protein kinase C (aPKC), which is defined as the Par complex. The Par complex regulates multiple polarity events in animals, including asymmetric cell division, tight junction formation, and directional migration (67, 100, 152, 176, 325). During neuronal development, the Par complex is mainly localized at the tips of nascent axons in a KIF3A-dependent manner, and it participates in axon formation (244, 305), implying that the transport of the Par complex by kinesin plays an important role in axon formation, as shown in hippocampal neurons (244). In this process, Par3 acts as a cargo adaptor by linking KIF3A with the Par complex. A recent study demonstrated that the Par3-KIF3A interaction is regulated by the ERK2-dependent phosphorylation of Par3 and is important for neuronal polarization (88). More recently, two studies showed that Par3 regulates neuronal polarization in vivo. In the developing cerebral cortex, knockdown of Par3 impairs the MP-to-BP transition (47, 88). Interestingly, unlike WT Par3, mutant Par3 that does not interact with KIF3A fails to rescue the knockdown phenotype, suggesting that Par3-mediated protein transport is also important for neuronal polarization in vivo (88).

The Par complex is a key factor in the Rac1/Cdc42 signaling pathway. Cdc42 plays a critical role in axon formation upstream of the Par complex (298). Activated Cdc42 interacts with Par6 via the semi-Cdc42/Rac interactive binding (CRIB) domain of Par6, which then associates with aPKC and Par3, leading to aPKC activation (156, 157, 195, 273). Par3 directly interacts with Tiam1/STE, the Rac-specific GEFs, and forms a complex with aPKC, Par6, and Cdc42, thereby mediating Cdc42-induced Rac1 activation (211, 245). Because Cdc42 is activated by PI3K and the active form of Rac1 can activate PI3K, a positive-feedback loop composed of PI3K, Cdc42, the Par complex, and Rac1 has been considered responsible for neuronal polarization. This pathway promotes axon formation, presumably via actin filament remodeling. In addition, Par3 acts as a microtubule-associated protein that binds to and bundles microtubules in a conformation-dependent manner (47). In the closed conformation, the intramolecular interaction between the NH2 and COOH termini of Par3 impairs its microtubule regulatory activity. As Par3 accumulates, intermolecular oligomerization promotes an open conformation that directly binds to, bundles, and stabilizes microtubules via its NH2 terminus. This microtubule regulatory activity of Par3 is crucial for neuronal polarization (47).

B. Signals That Regulate Neuronal Polarization In Vivo

Polarization of neurons in vivo is precisely regulated by their microenvironment (extracellular signals) and the timing of gene expression. The extracellular molecules, including secreted factors, such as neurotrophins, TGF-β, Wnts, IGF-I, semaphorin, and Reelin; ECM components, such as laminin; and cell adhesion molecules, such as TAG-1 and N-cadherin, are known to regulate neuronal polarization in cultured neurons and also in vivo (81, 92, 127, 158, 233, 237, 269, 376) (FIGURE 3). Of these, we first review neurotrophins, TGF-β, Reelin, TAG-1, and N-cadherin, which are known to regulate neuronal polarization in vivo. Generally, these extracellular signals use similar intracellular signal molecules, such as CaMK, LKB1, and Rac1, to induce neuronal polarization. In some cases, extracellular signals such as semaphorins antagonize the intracellular signal pathways that are known to initiate axon formation in cul-
ture and reciprocally induce dendrite formation (303). Thus the key components that drive the polarization machinery are shared by neurons in culture and in vivo. However, the extracellular molecules that trigger the shared intracellular molecules are diverse among the developmental stages and types of neurons. This diversity generates redundancy and may ensure proper neuronal polarization in vivo.

Given that transcription factors and epigenetic modifiers determine the timing of neuronal differentiation and morphogenesis (206), we next review how the regulation of gene expression contributes to neuronal polarization in vivo.

1. Extracellular signals

A) NEUROTROPHINS. The neurotrophins consist of four members: nerve growth factor (NGF), BDNF, NT-3, and neurotrophin-4 (NT-4). Among them, BDNF and NT-3 are highly expressed in the central nervous system and stimulate axon specification and axon elongation (221, 233, 302). Neurotrophins activate specific receptor tropomyosin receptor kinases (Trks) and induce Ras and phosphoinositide 3-kinase (PI3K) activation, thereby increasing the production of phosphatidylinositol 3,4,5-trisphosphate (PIP₃) (281). PIP₃ is enriched at the tips of nascent axons, and treatment of cultured neurons with PI3K inhibitors disturbs axon specification (210, 305). PIP₃ indirectly activates Akt, thereby inactivating GSK-3β via its phosphorylation at serine 9. GSK-3β inactivation leads to the activation of production of phosphatidylinositol 3,4,5-trisphosphate (PIP₃) (281). PIP₃ is enriched at the tips of nascent axons, and treatment of cultured neurons with PI3K inhibitors disturbs axon specification (210, 305). PIP₃ indirectly activates Akt, thereby inactivating GSK-3β via its phosphorylation at serine 9. GSK-3β inactivation leads to the activation of production of phosphatidylinositol 3,4,5-trisphosphate (PIP₃) (281). PIP₃ is enriched at the tips of nascent axons, and treatment of cultured neurons with PI3K inhibitors disturbs axon specification (210, 305).

B) TGF-β SUPERFAMILY PROTEINS. The TGF-β superfamily consists of TGF-β, activin, and bone morphogenetic protein (BMP) and regulates a wide variety of cellular processes. Upon ligand binding, the TGF-β superfamily receptors form heterotetrameric complexes composed of type I and type II serine/threonine kinases. Among them, BMP, suppresses CRMP-2 transcription (109), and its mammalian ortholog, microtubule affinity-regulating kinase (MARK), was originally identified as a modulator of microtubule dynamics (69). MARK2 phosphorylates MAPs, including tau, MAP2/4, Tau1, and DCX, and changes their affinity to microtubules (31, 69, 293). MARK2 is negatively regulated by aPKC-dependent phosphorylation (48). Neurons electroporated with MARK2 or DCX shRNA remain in the IZ and exhibit an MP morphology in vivo (291, 292). Given that increasing or decreasing the expression of MARK2 alters neuronal migration and the MP-to-BP transition, the appropriate regulation of MARK2 expression is essential for neuronal polarization in vivo (291).

Neurotrophins also activate LKB1 through cAMP and PKA or p90RSK activation (302). LKB1, a mammalian ortholog of the serine/threonine kinase Par4 (107), is known to regulate cell polarity in various cell types (16). Emx-Cre-mediated conditional knockout of the LKB1 gene in mice leads to the absence of axons from cortical pyramidal neurons without affecting their migration (28). Knockdown of LKB1 prevents axon specification both in cultured neurons and in vivo (302) and prevents neuronal migration in vivo (13). LKB1 activity is regulated by cAMP and PKA (301, 302). In contrast, semaphorin-3A (Sema3A), a secreted chemorepulsive protein, elevates the cGMP level but reduces the cAMP level reciprocally, thereby suppressing the PKA-dependent phosphorylation of LKB1 (303). Once LKB1 is activated via PKA or p90RSK-dependent phosphorylation, LKB1 interacts with the pseudokinase STRAD and phosphatides several downstream kinases, such as NUAK, AMPK MARK2, and the SAD kinases (197). Of these kinases, SAD kinases and MARK2 likely mediate the function of LKB1 in neuronal polarization. SAD kinases, which belong to the MARK family, phosphorylate Tau-1 at S262, which is highly phosphorylated in dendrites but not in axons (173). SAD-A and SAD-B kinase double-knockout mice exhibited abnormally oriented dendrites and a loss of axons in vivo (173). Suppression of other kinases, such as NUAK and AMPK, did not affect neuronal polarization (52, 366), but overactivation of AMPK decreased the axon length in vivo (8). Notably, NUAK regulates terminal axon branching both in cultured neurons and in vivo (52). Taken together, the neurotrophins precisely regulate the activity of several protein kinases. However, these kinases may not simply work in parallel. Therefore, it will be important to show when and where each of these molecules works during the polarization process.

Because TGF-β2 expression is restricted to the VZ, there might be a gradient of TGF-β2 concentration along the ventricular-to-pial-surface axis (376). However, this simple radial gradient cannot explain the direction of axon initialization because more than half of the MP cells initially extend axons tangentially rather than radially toward the ventricle (119, 237, 287). Therefore, the TGF-β gradient likely acts as a basal factor for axon specification, and other cues, such as cell adhesion molecules, may select one immature neurite as an axon, as described below (237). Another TGF-β superfamily member, BMP, suppresses CRMP-2 transcription via SMAD during brain development (324). Therefore, there are two TGF-β signaling pathways that regulate neuronal polarization: the Par6/Smurf1-mediated local protein deg-
radiation of RhoA and the SMAD-dependent transcriptional regulation of CRMP-2 expression.

C) REELIN. Reelin is a glycoprotein that is secreted by Cajal-Retzius cells into the marginal zone of the embryonic cerebral cortex (341). Because cortical layer formation is severely affected in Reelin-deficient mice, this molecule is thought to be an important regulator of cortical development. Reelin acts through its receptor, apolipoprotein E receptor 2 (ApoER2), as well as very-low-density lipoprotein receptor (VLDLR), and activates several intracellular signaling molecules via the adaptor protein disabled-1 (Dab1). Recent studies suggested that Reelin signaling regulates the MP-to-BP transition and axon formation (81, 158). Reelin stimulation induces Dab1 phosphorylation and the binding of Crk-family proteins (CrkL) and C3G, a Rap1GEF, to Dab1 (24). This complex activates Rap1 in a Reelin-dependent manner. Dab1- or Rap1-deficient neurons exhibited MP morphology in vivo, suggesting that Reelin-Rap1 signaling is important for the MP-to-BP transition (81, 158). A previous study suggested that Rap1 activation was required for the appropriate targeting of cadherin proteins to maturing cell-cell interactions (134). Indeed, Rap1 regulates the surface expression of N-cadherin in neurons (158), possibly through p120 catenin (142). Because N-cadherin has been shown to be important for the MP-to-BP transition and migration (81, 91, 158, 168), the function of Reelin in these events may be partially mediated by N-cadherin. The detailed roles of N-cadherin are described in the following section.

The effect of Reelin on axon formation has been examined using cultured neurons but remains controversial. One study showed that stimulating cultured neurons with full-length Reelin increased growth cone motility and induced axon formation via Cdc42 activation (189). Another study showed that stimulating one minor neurite with the cleaved form of Reelin did not induce axon formation, unlike other axon formation-inducing factors, such as neurotrophins, TGF-β, and Wnts (233). These results raise the possibility that the status of posttranslational processing is important for its function in axon formation, as described previously (159).

D) CELL ADHESION MOLECULES. I) TAG-1. TAG-1 is a GPI-anchored immunoglobulin superfamily cell adhesion molecule that is known to regulate axon fasciculation and guidance (163, 308). TAG-1 protein expression is highest in the lower IZ, where the MP-to-BP transition and axon specification occur (237). In the lower IZ, TAG-1 is expressed in both MP cells and pioneering axons. TAG-1 can mediate cell-to-cell interactions between these cells. Suppression of TAG-1 expression in MP cells by shRNA disrupts the MP-to-BP transition and axon specification. WT TAG-1 rescued these phenotypes, but the cell adhesion-deficient mutants of TAG-1 did not (237).

As with other GPI-anchored proteins, TAG-1 can directly activate intracellular signal molecules (163, 308). The outside-in signaling of TAG-1 is mediated by two possible mechanisms: one involving transmembrane proteins that associate with TAG-1 in a cis manner and another involving Src family kinases (SFKs) localized to lipid rafts (163). Previous studies identified several transmembrane proteins, including L1, NrCAM, and contactin-associated protein-like 2 (CASPR2), as cis-associating proteins for TAG-1. Although these molecules have been shown to be involved in neuronal migration (175, 264, 342), they seem not to be involved in the TAG-1-mediated MP-to-BP transition or axon specification in vivo (237). TAG-1 colocalizes with SFKs, including Src, Fyn, and Lyn, in lipid rafts (164, 165, 270). Among these SFKs, Lyn is a downstream kinase of TAG-1 in cultured neurons (164, 165) and in vivo (237), and it regulates neuronal polarization (237). Given that Lyn can activate Rac1 (58), the TAG-1-Lyn signaling pathway might activate Rac1 in neurons to induce polarization (237) [FIGURE 3]. In conclusion, TAG-1-dependent cell-to-cell interactions between MP cells and pioneering axons activate Lyn and Rac1 and promote neuronal polarization (237).

II) N-cadherin. N-cadherin is a member of the classical cadherin family that mediates Ca2+-dependent adhesion (130). In the embryonic cerebral cortex, N-cadherin is expressed in both radial glial cells and neurons and mediates the interaction between these two cell types (81, 91, 120, 158, 168). N-cadherin-mediated cell-to-cell interactions are important for radial glia-guided neuronal migration. A recent study demonstrated that this type of migration is precisely regulated by N-cadherin trafficking in neurons (168). Inhibition of the endocytic or recycling process via transfection with Rab5 or Rab11 dominant-negative mutant, respectively, reduces N-cadherin surface expression in neurons, thereby affecting neuronal migration. Although the surface expression of N-cadherin has been reported to be regulated by Reelin signaling via Rap1, as described above (81, 158), the mechanism by which Reelin signaling regulates Rab5- and Rab11-dependent trafficking remains unknown. Therefore, the functional interactions between Rap1 and Rab5 or Rab11 must be elucidated.

N-cadherin is also involved in the MP-to-BP transition in cultured neurons (92). Nonpolarized stage 1 neurons generate their initial neurites from the N-cadherin-contacting site. In the embryonic cerebral cortex, N-cadherin has been shown to regulate the orientation of the leading process of BP cells (92). In addition, inhibiting N-cadherin function using a dominant-negative mutant impairs the MP-to-BP transition (81, 91, 158, 168). Taken together, these results indicate that N-cadherin regulates leading process formation during the MP-to-BP transi-
tion and subsequently mediates radial glial cell-dependent neuronal migration.

2. Regulation of gene expression

A) TRANSCRIPTION FACTORS. Proneural transcription factors are known to be important regulators of neural development (108). Conventionally, these molecules are primarily considered to be involved in cell-type specification. Recent studies have suggested that basic helix-loop-helix (bHLH) proneural transcription factors regulate not only neurogenic differentiation but also neuronal migration and the MP-to-BP transition (125, 258). In the embryonic cerebral cortex, neurogenin2 (Ng2) induces neuronal migration and the MP-to-BP transition by negatively regulating RhoA, presumably via two different mechanisms (95, 117, 241). Ng2 negatively regulates RhoA transcription. In contrast, Ng2 positively regulates Rnd2 transcription. The Rnd subfamily (Rnd1, Rnd2, and Rnd3/RhoE) represents a new branch of the Rho family of GTPases (78, 105, 247). Unlike other Rho family members, Rnd proteins lack GTPase activity. Thus they have been considered constitutively active (78, 105, 247), and they are thought to be regulated primarily at the level of their expression (46). Rnd2 activates p190RhoGAP, a GAP of RhoA, thereby inhibiting RhoA activity (364). Rnd2-deficient neurons display MP morphology with long processes, suggesting that Rnd2 is important for the MP-to-BP transition (126, 236). Rnd2 may be involved in the trafficking of membrane-bound molecules that regulate the MP-to-BP transition (126, 265). Rnd3 induces F-actin depolymerization, resulting in the stabilization and attachment of the leading process to a radial glial fiber via the regulation of RhoA activity (265). Furthermore, the zinc-finger transcriptional repressor RP58 and chicken ovalbumin upstream promoter-transcription factor 1 (COUP-TF1), an orphan nuclear receptor, also repress Rnd2 expression and regulate neuronal migration and the MP-to-BP transition (126, 251). Ng2 also positively regulates the expression of Dcx and p35 (95), both of which are known to be regulators of neuronal migration and the MP-to-BP transition. Interestingly, p35 activates CDK5 and then stabilizes the Ng2 protein via the CDK inhibitor p27 (Kip1) (241). Therefore, this positive feedback system represses RhoA activity and expression.

The members of the forkhead transcription factor FoxO family (FoxO1, 3 and 6) and FoxG1 regulate neuronal migration, the MP-to-BP transition, and axon-dendrite specification in cultured neurons and in vivo (57, 215). FoxO knockdown reduces the expression of Par6, Pak1, R-Ras, APC, and CRMP-2 (57). Among these genes, the Pak1 promoter contains putative FOXO-binding sites; in fact, Pak1 transcription is directly regulated by FoxO (57). FoxG1 knockout mice show altered expression of several genes that may be involved in neural development (215). The most strongly downregulated gene is deleted in autism 1 (DAB1), a key factor in Reelin signaling. The most strongly upregulated gene is deleted in autism 1 (DIA1, GoPro49). DIA1 is localized to the Golgi complex and is primarily expressed in mesenchymal and cartilaginous tissues but is also mildly expressed in the cortical hem of the cerebral cortex (332). Although this gene has been implicated in autism spectrum disorder (ASD), its function remains unclear (15, 226).

B) EPIGENETIC MODIFIERS. Studies of epigenetics have advanced remarkably during the past decade. Epigenetic mechanisms, such as DNA methylation, histone modification, and miRNA, are important for the expression of genes that regulate neural development (128). Most epigenetic studies have focused on progenitor cell differentiation and cell-type specification; however, several studies have demonstrated the epigenetic control of neuronal polarization. miRNAs are produced from pre-miRNA following processing mediated by the RNAse III enzyme Dicer. Neuronal differentiation, dendrite morphogenesis, and axon formation are affected in Dicer knockout mice (194, 356). In addition, cultured hippocampal neurons from Dicer knockout mice exhibit increased numbers of neurons displaying multiple axons (194). Various types of miRNAs regulate the expression of polarity-regulating genes. miR-219 regulates the expression of Par3 and aPKC in zebrafish larvae (147). miR-134 inhibits neuronal migration in the embryonic cerebral cortex by suppressing Dcx gene expression (94). miR-124 regulates the expression of the corepressor for element-1-silencing transcription factor (CoREST) in Xenopus retinal ganglion cells (28) and in mouse (356). miR-22 also regulates the expression of CoREST (356). CoREST forms a complex with REST and represses target gene expression by recruiting chromatin-binding proteins and histone modifiers (274). In the developing cerebral cortex, CoREST and REST regulate Dcx gene expression, thereby regulating neuronal migration (84, 202). Epigenetic regulation of polarity genes may fine-tune gene expression in a time- and space-dependent manner. Interestingly, miRNA can be transported to the extracellular space (321). Therefore, miRNA may act as a microenvironmental cue for neuronal development.

IV. THEORETICAL BASIS OF NEURONAL POLARIZATION

Various molecules have been identified as regulators of neuronal polarity, as described above. However, integrating and assembling these signaling molecules into one model might be important for understanding the entire picture of neuronal polarity. One suitable method is mathematical modeling (149, 239, 349). Under physiological conditions, neurons generally extend one axon, and other minor neurites never generate an axon. How is this winner-takes-all process accomplished? A local activation-global inhibition mechanism is a likely model that explains this winner-takes-all process (11, 149, 239).
This model contains three key components: a bistable switch, a negative feedback signal(s), and a positive feedback signal(s) (240, 344) [FIGURE 7]. The selective accumulation of positive factors (termed factor X), such as CRMP-2 (for microtubule polymerization and receptor trafficking), Par complex (for Rac1 activation), and HRas and Shootin1 (for PI3K activation), has been shown to facilitate the development of a neurite into an axon (148, 244, 345). This accumulation is regulated by the active anterograde transport, diffusion, and degradation of these positive factors. Because anterograde and retrograde transport are primarily mediated by motor proteins and by diffusion, respectively, positive factors might accumulate at the tips of the longest neurites. If the rate of motor protein-mediated transport is equivalent to or lower than that of diffusion, it is difficult for neurons to establish axons in the simulation (240, 344). Indeed, inhibition of Kinesin-1 function in neurons abolished axon formation (174). Then, the local accumulation of factor X activates signaling molecules (termed factor Y, e.g., PIP3, cAMP, and Ca2+) that induce neurite elongation via downstream pathways as described in section III.

The activity of factor Y depends on the concentration of factor X and the activity of factor Y act as a bistable switch. When the concentration of factor X exceeds the threshold, then factor Y is in the “on state.” Once factor Y is activated, its activity is sustained even if the concentration of factor X is reduced. Thus the neurite continuously elongates and ultimately develops into an axon. In contrast, because the concentration of factor X depends on the length of the neurite, the concentration of factor X in other neurites cannot increase, and these neurites fail to elongate. Notably, negative feedback signaling molecules are not necessary in this model. Whether the negative feedback signal is a retrograde propagation of the signaling molecules or a lower concentration of factor X in the soma and in other minor neurites remains controversial. Experimentally identifying the negative feedback signal is a critical issue regarding neuronal polarity. The possible candidates include second messengers. It has been proposed that cAMP and cGMP have antagonistic effects on each other (304). Locally induced cAMP elevation induces PKA activation and subsequent axon initiation. Notably, the cAMP level in the unstimulated neurites begins to decline just after the stimulation, and the cGMP level in these neurites is reciprocally increased. This result suggests that cAMP acts as a long-range negative feedback signal. However, it remains unclear whether cAMP directly transmits the negative signal from the stimulated neurite to other neurites. One of the axon-initiating extracellular molecules, neurotrophin, induces not only cAMP elevation but also IICR or Ras activation (233, 302), suggesting that these molecules potentially transmit the long-range negative signals. It has also been shown that cGMP stimulation does not reduce the cAMP level in other neurites (304). Taken together, these results suggest that the positive signal (cAMP) can generate a long-range negative signal for other neurites but that the negative signal (cGMP) cannot induce long-range positive signals. Therefore, positive stimulation (e.g., neurotrophin) seems to be more critical for the timing of neuronal polarization than negative stimulation (e.g., semaphorin).

Furthermore, the local accumulation and activation of factor X drive a positive feedback loop. Several studies have experimentally demonstrated that the Par complex-Rac1-Cdc42 and HRas-PI3K positive feedback loops induce axon formation (11). In addition, turning off the negative feedback loops might be a positive feedback signal. The GEF-H1-RhoA-Rho-kinase axis may act as a negative feedback loop. Once Rho-kinase is activated by GEF-H1 and RhoA, Rho-kinase phosphorylates and inactivates p190RhoGAP (224). This process might lead to sustained RhoA activation. Because suppression of GEF-H1 and Rho-kinase expression in cultured neurons induces the formation of multiple axons (49, 51, 53), this pathway plays critical roles in the “stochastic” neuronal polarization. PKA activates LKB1 and thereby induces...
axon initiation, as described above. At the same time, PKA can inhibit the activation of RhoA through the inactivation of GEF-H1 (209). Therefore, PKA activation drives neuronal polarization in two different ways: activation of a positive signal and inhibition of a negative feedback loop. Extracellular signals such as neurotrophin and cell adhesion might trigger the positive feedback loops.

**V. MAINTENANCE OF NEURONAL POLARITY**

After neurons are polarized, neurons must maintain their polarity to perform their function. Experimental manipulations can alter axon-dendrite polarity either in cultured neurons or in vivo (65, 101, 103, 122). Banker and colleagues (65, 103) demonstrated that axonal transection induces polarity changes in stage 3 neurons. When the axon is transected at its distal part, the damaged axon undergoes regrowth. In contrast, axonal transection at the proximal part induces axon development in one of the minor neurites. Similar phenomena were detected when axonal transection was performed on completely polarized stage 4 or 5 neurons (101). In completely polarized neurons, axonal transection at the proximal part causes dendrites to adopt axonal identities. These results suggest that the proximal part of the axon is crucial for maintaining axon identity. A specific subcellular structure, termed the AIS, is known to be localized to the most proximal region of the axon in polarized cells (259). The AIS is composed of the scaffold protein ankyrinG, βIV spectrin, actin filaments, cell adhesion molecules, and channels (30, 278, 327). The AIS performs three fundamental functions: regulating action potentials, selecting axon-directed cargo, and maintaining neuronal polarity (327). A series of studies revealed that disrupting the AIS in polarized neurons by knocking out ankyrinG causes axons to acquire characteristics of dendrites, such as the formation of spines and synapses (122, 314). In contrast, the loss of the AIS does not affect the establishment of neuronal polarity (89). One question remains unanswered: How does the AIS maintain neuronal polarity? One possible mechanism is the AIS-dependent regulation of transport. A previous study suggested that cargo selection occurs in the AIS (315). At the AIS, the transport efficiency of the axon-directed motor-cargo complex is higher than that of the dendrite-directed motor-cargo complex (315). Because F-actin is enriched at the AIS (315) and myosin is required for dendrite-directed transport (192, 315), the exclusion of dendrite-directed cargo from axons may occur at the AIS in a myosin-dependent manner (192). Because the AIS is required for maintaining the axon (122, 314), preserving neuronal polarity may require AIS-regulated selective cargo transport.

**VI. INVOLVEMENT OF NEURONAL POLARITY IN DISEASE AND TRAUMATIC BRAIN INJURY**

Several genes that regulate neuronal polarity have also been identified as causal genes of neurodevelopmental diseases. Of these diseases, we focus on lissencephaly and ASD. Lissencephaly is a malformation of cerebral cortical development and is categorized into group IIB (26). This malformation is due to generalized abnormal neuron migration. Because the neurons migrate improperly, the brain becomes smooth, lacking most of the typical sulci and gyri. Thus far, several gene mutations have been found in lissencephalic patients. Most of these mutations are associated with microtubule dynamics or Reelin signaling. In the early 1990s, Ledbetter and colleagues (65, 188) identified microdeletions in 17q13.3 of patients with Miller-Dieker syndrome, a type 1 lissencephaly (64, 188). Then, Reiner et al. (282) identified a causal gene, termed LIS1 (282). Other groups identified the DCX gene as a causal gene of X-linked subcortical laminar heterotopia (SCLH) (60, 98). Mutations in TUBA1A (previously referred to as TUBA3) were identified in a patient with lissencephaly without any mutations in the LIS1 or DCX gene (169, 182, 268). All of these genes, which correspond to tubulin or to microtubule modulators, have been experimentally demonstrated to regulate neuronal migration, as described above. Another causal gene of lissencephaly is associated with Reelin signaling. Mutations in RELN or VLDLR were found in patients with autosomal recessive lissencephaly (36, 137). Based on the finding that the corpus callosum is thin in these patients, Reelin signaling regulates not only neuronal migration but also axon formation during human brain development, which is identical to its role in rodents.

Although the etiology of ASD remains unknown, many researchers tend to understand the etiology of ASD in terms of neurodevelopmental disabilities (220). Several susceptibility genes of ASD are known to regulate neuronal migration and morphogenesis. In some ASD patients, several SNPs were identified in the 3’ untranslated region of MARK1, thereby increasing the expression level of MARK1 (207). Because precise regulation of MARK2 expression is important for neuronal migration and for the MP-to-BP transition in the mouse cerebral cortex (291), neuronal polarity might be affected in these ASD patients.

The fundamental mechanisms that regulate axon specification may contribute to axon regeneration. For example, microtubule stabilization induces axon regeneration after nerve injury (124, 299). Axon regeneration after optic nerve axotomy is enhanced in phosphatase and tensin homolog (PTEN) knockout mice, as PTEN exerts an antagonistic effect on PI3K (260). Axon regeneration in PTEN knockout mice is partially, but not completely,
inhibited by treatment with rapamycin, an inhibitor of mTOR. Therefore, other downstream signaling molecules, such as Akt and GSK-3β, might be involved in axon regeneration, suggesting that microtubule stabilization via GSK-3β inactivation may induce axon regeneration.

VII. NEW ERA OF NEURONAL POLARITY

In recent decades, many researchers have focused much effort on understanding neuronal polarity. As technologies have improved and developed, we have opened the door and entered new research fields. One of the major open questions has remained the same for 30 years: how do neurons generate only one axon? As described above, a local activation-global inhibition model likely explains this process; however, this model has not been experimentally verified. One reason for this lack of verification is the lack of suitable technologies. Given that many signaling molecules, such as Ca2+, cAMP, cGMP, and PIP3, have been shown to mediate neuronal polarization in a specific subcellular region (4, 233, 303), multiple and super resolution imaging will aid in the discovery of the spatiotemporal regulation of these signaling molecules. In addition, the mathematical prediction of “missing links” will assist in the discovery and understanding of key molecules or events. Integrating different fields may provide additional breakthroughs that could provide further insight into this process.

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