caused by dominant SOD1 mutations. Like Gars\textsuperscript{Nmf249/+}
mice, mice expressing dominant human SOD1 mutants develop a length-dependent motor neuropathy that was not anticipated from clinical investigations. Because of the difficulties in distinguishing neuropathies from neuronopathies in humans, careful evaluation of animal models may reveal that axonal degeneration is the primary defect in a number of neurodegenerative diseases. Although many of these diseases are defined clinically owing to the particular populations of affected neurons, axonal disease may be a final common pathway that links them (Roy et al., 2005). A steady supply of new genetic causes of neuropathy and informative animal models will facilitate our understanding of the causes and treatments of neuropathy, both inherited and acquired, and perhaps even more complex disorders.

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Selected Reading


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Nervous Rac: DOCK7 Regulation of Axon Formation

Microtubules play an important role in neuronal polarity. In this issue of Neuron, Watabe-Uchida et al. link a novel Rac-mediated pathway that regulates microtubule dynamics to axon formation.

Polarization of most vertebrate neurons begins with the specification of one neurite as the axon while other neurites subsequently develop into dendrites. Although the molecular mechanisms that determine how a neuron specifies an axon and dendrites remain poorly understood, it has become clear that the establishment and maintenance of neuronal polarity depends upon the microtubule network. Many signaling cascades influence microtubule dynamics in the developing axon. Targets of these signaling pathways include microtubule motor proteins (Wiggin et al., 2005) as well as structural microtubule-associated proteins (MAPs) (Dehmelt and Halsey, 2004). When the function of these molecules is perturbed, neuronal polarity is disrupted. This type of disruption often results in neurons with multiple axons, multiple dendrites, or many long neurites that lack axonal or dendritic characteristics (Arnimura and Kaibuchi, 2005; Wiggin et al., 2005). The signaling pathways upstream of the MAPs, however, are not well delineated.

In this issue of Neuron, studies from the Van Aelst (Watabe-Uchida et al., 2006) group provide new insights into a signaling pathway upstream of a specific MAP that is mediated by a novel Rac-activating protein, DOCK7. In other cell types, Rac has been shown to influence the microtubule cytoskeleton (Wittmann et al., 2004). However, little is known about its effect on microtubule dynamics in neurons. Rac has been implicated in the regulation of neuronal polarity. Perturbation of Rac preferentially affects the outgrowth of axons but not dendrites in vivo (Luo et al., 1996). The Par-6/Par-3/aPKC polarity complex, which functions in axon specification, may directly influence Rac activation by regulating Rac-GEFs (guanine nucleotide exchange factors) (Nishimura et al., 2005). It is not known whether these Rac-associated signaling pathways eventually influence the microtubule cytoskeleton, and if so, through which MAPs.

The report by Watabe-Uchida et al. identifies a novel Rac GTPase activator, DOCK7, that plays a crucial role in axon formation. A member of the DOCK180-related superfamily, DOCK7 is an unconventional GEF, directly associating with Rac through its DHR2 domain. Although DOCK180-related family members have been shown to be regulators of polarization in different cell types (Meller et al., 2005), DOCK7 is the first member found to play a critical role in the early stages of axon formation in hippocampal neurons. Watabe-Uchida et al. observe that DOCK7 is concentrated in a single neurite after immature neurites have formed. DOCK7 is then selectively localized to the axon that forms. This observation suggests that DOCK7 is involved in the initial specification of the axon. Overexpression of DOCK7 disrupts polarity by promoting multiple axon formation; knockdown of DOCK7 expression blocks the development of polarity, preventing the formation of an axon. The investigators determine that regulation of Rac activity by DOCK7 seems to be important in its ability to promote axon formation.

It is interesting to note that other Rac-specific GEFs, Tiam1 and STEF, have also been implicated in axon formation. The implication suggests that the spatial and temporal activity of Rac is important in axon specification (Kunda et al., 2001; Nishimura et al., 2005). If neurons have Tiam1 and STEF, why do they need DOCK7? Perhaps different extracellular stimuli determine the type of GEFs that activate Rac. Alternatively, these different GEFs may affect different downstream effector molecules that Rac binds to and activates. It will be
interesting to understand the extent of overlap in the functions of these three GEFs.

It is presently unclear how Tiam1 and STEF contribute to axon formation. But what about the mechanisms by which DOCK7 contributes to axon formation?

Watabe-Uchida et al. take the story a step further. They highlight the role of the microtubule network in the regulation of axon formation and differentiation by identifying a novel pathway that links Rac-mediated signaling to stathmin/Op18, a microtubule-destabilizing protein (Figure 1). They determine that DOCK7 is required for laminin-dependent Op18 phosphorylation and that this occurs through activation of Rac by DOCK7. These data confirm previous work that demonstrates a role for Rac-mediated Op18 phosphorylation and promotion of microtubule growth in non-neuronal cells (Wittmann et al., 2004). In non-neuronal cells, p21-activated kinase (PAK) appears necessary for phosphorylation of Op18, but not sufficient for microtubule growth (Wittmann et al., 2004). Interestingly, the findings by the Van Aelst group imply that in neurons, unknown kinase(s) other than PAK are likely involved in mediating the effects of DOCK7 on Op18 phosphorylation and axon formation. Differences in the kinases that affect phosphorylation of Op18 in neurons and non-neuronal cells should be an interesting area of future study.

Op18 interacts with tubulin dimers and interferes with microtubule dynamics (Belmont and Mitchison, 1996). Local Op18 inactivation through DOCK7 provides a new mechanism by which axon formation is promoted in a microtubule-dependent manner. In fact, Watabe-Uchida et al. observe significant amounts of inactive Op18 in the developing axon compared to the future dendrites. Increased microtubule growth and stability are thought to facilitate axon elongation. Microtubules in the emerging axon show increased stability compared to those in neurites destined to become dendrites (Ferreira et al., 1989). Consistent with a role in axon differentiation, microtubules have also been shown to invade newly formed axons and become significantly longer in the neurite that becomes the axon than in the other immature neurites (Yu and Baas, 1994).

The Van Aelst group's observations raise several interesting questions. Although significant progress has been made in understanding the intracellular events that govern neuronal polarity via microtubules, we have yet to understand how the molecules involved in microtubule dynamics are spatially and temporally controlled. What regulates the asymmetric distribution of molecules like DOCK7 and how does this contribute to axon formation? Additionally, does DOCK7 affect the actin cytoskeleton either directly or indirectly through its effects on microtubules? Both the actin and microtubule cytoskeletons appear to be key determinants in axon development.

The Rho family of GTPases are well-characterized regulators of both actin and microtubules. The potential dual role for Rac is particularly interesting given the results in this paper. Evidence in non-neuronal cells suggests that key Rac effectors that regulate the actin cytoskeleton may also influence microtubule dynamics. PAK activation by Rac is thought to lead to the downstream activation of molecules that stabilize actin filaments and promote actin polymerization. Recent studies also implicate PAK proteins as regulators of microtubule dynamics through their ability to phosphorylate Op18 (Wittmann et al., 2004). Although PAK does not seem to be the key kinase in this study, a key challenge for future work is to identify kinases with dual roles in regulating both actin and microtubule dynamics in neuronal cells.

Axon formation and elongation involve coordinated changes between the actin cytoskeleton and the microtubule network. Actin filaments provide a means of generating force within a cell; microtubules are important for stabilization and maintenance of the future axon. The paper by Watabe-Uchida et al. provides new insights into the role of the microtubule network in axon formation. They are the first to show that a distinctive Rac-mediated pathway is implicated in the regulation of microtubule dynamics in neurons and therefore affects neuronal polarity.

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Selected Reading
The Synaptic Vesicle Cycle: Is Kissing Overrated?

In this issue of Neuron, Granseth et al. re-examine the mechanism of endocytosis at hippocampal synapses using a new optical reporter, syph. They conclude that only a single slow mode of endocytosis operates at this synapse and that retrieval after physiological stimuli is largely, if not solely, dominated by the clathrin-mediated pathway. These conclusions dispute previous assertions that “kiss-and-run” is a major mechanism of vesicle recycling at hippocampal synapses.

The cycling of synaptic vesicles through repetitive episodes of exocytosis and endocytosis is fundamental to synaptic transmission, but competing views of the underlying mechanisms are still hotly debated. On the one hand, considerable evidence suggests that synaptic vesicles fully incorporate into the plasma membrane as they release their neurotransmitter cargo (full fusion), followed by retrieval through clathrin-mediated endocytosis (see Figure 1, cycle A). On the other hand, other evidence supports a “kiss-and-run” cycle, in which fusing vesicles fully incorporate into the plasma membrane (see Figure 1, cycle B). It seems likely that both can occur, but the question is which is prevalent and under what conditions (reviewed by Matthews, 2004). In this issue of Neuron, Granseth et al. (2006) present evidence challenging the prevailing view that kiss-and-run is the dominant cycle at small synaptic boutons of cultured hippocampal neurons. Instead, they found that endocytosis was almost exclusively mediated by a relatively slow ($\tau = 15$ s at $23^\circ$ C) clathrin-dependent pathway, consistent with full fusion.

To monitor exocytosis and subsequent endocytosis, Granseth et al. made use of pHluorin, which is a pH-sensitive GFP variant that can be targeted to synaptic vesicles by fusing it to the intravesicular domain of vesicle membrane proteins. Because the interior of synaptic vesicles is acidic, pHluorin facing the vesicle lumen is protonated and its fluorescence is low in the resting state (Figure 1). Upon exocytosis, vesicles lose their protons, and the fluorescence of pHluorin increases. The fluorescence decreases again when vesicles reacidify after being retrieved by endocytosis. When fused to the lumenal end of the vesicle SNARE protein synaptobrevin/VAMP, pHluorin forms the widely used reporter synaptopHluorin (Miesenbock et al., 1998). For instance, synaptopHluorin was used by Gandhi and Stevens (2003) to conclude that an exocytic event at hippocampal synapses may be followed by one of three distinct modes of vesicle retrieval, whose relative prominence depends on the release probability of the synapse. Their measurements indicated a fast kiss-and-run mode lasting $\leq 900$ ms, a slower “compensatory” mode lasting 8–21 s, and a “stranded” mode where vesicles are caught at the plasma membrane to await retrieval triggered by subsequent stimuli.

Measurements using synaptopHluorin are complicated by the fact that it is expressed substantially in the plasma membrane as well as in synaptic vesicles, which introduces considerable background fluorescence. Also, synaptopHluorin molecules that appear in the plasma membrane after vesicle fusion are mobile and can move out of synaptic active zones into the surrounding axon after exocytosis (Sankaranarayanan and Ryan, 2000). Indeed, Granseth et al. (2006) concluded that a fast component of “endocytosis” reported by synaptopHluorin in their experiments is most likely an artifact produced by such lateral diffusion, leaving only a relatively slow decline in fluorescence ($\tau = 20$ s) attributable to true endocytosis. To overcome the problems introduced by diffusion of synaptopHluorin and to lessen background fluorescence, Granseth et al. designed an improved pHluorin fusion protein. Abbreviated syph, the new optical reporter was generated by fusing pH-sensitive GFP to the synaptic vesicle protein synaptodynamin. This proved to be more specific to synaptic vesicles and more confined to active zones after exocytosis than synaptopHluorin. Using syph, only a slow component of fluorescence decrease was detected after a single action potential, yielding an estimated time constant of $\sim 15$ s for endocytosis after correction for the measured time course of reacidification ($\tau = 4$ s; similar to the rate of vesicle acidification estimated previously by Atluri and Ryan, 2006). There was no indication of a rapid component of internalization of syph like that expected for kiss-and-run, in agreement with previous findings based on acid-quenching of synaptopHluorin fluorescence in hippocampal synapses (Atluri and Ryan, 2006). The results were identical at synapses with the lowest and highest release probability and were not dependent on the amount of stimulation, in contrast to previously reported evidence suggesting dependence of the prevalence of kiss-and-run endocytosis on release probability (Gandhi and Stevens, 2003) or stimulus frequency (Harata et al., 2006).

What is the molecular mechanism underlying the single component of endocytosis detected at hippocampal synapses by Granseth et al. (2006)? It is known that the machinery required for clathrin-mediated endocytosis is enriched at CNS synapses. To explore the role of clathrin at hippocampal synapses, Granseth et al. employed two complementary methods: overexpression of a dominant-negative form of the clathrin-adaptor protein AP180, and RNAi knockdown of the clathrin heavy chain. Both approaches led to a complete block of endocytosis in response to a weak stimulus. The results provide strong evidence that the principal mode of retrieval in hippocampal boutons is clathrin-dependent. Therefore, Granseth et al. concluded that cycle A of Figure 1 dominates at synapses of cultured hippocampal