Neuron
Previews

Controlled Demolition: Smurf1 Regulates Neuronal Polarity by Substrate Switching

Michael Stiess[1] and Frank Bradke[1,*]
[1]Axonal Growth and Regeneration Group, Max Planck Institute of Neurobiology, Am Klopferspitz 18, 82152 Martinsried, Germany
*Correspondence: fbradke@neuro.mpg.de
DOI 10.1016/j.neuron.2011.01.007

During axon specification, growth promoting proteins localize selectively to the growing axon. In this issue of *Neuron*, Cheng et al. report how selective protein degradation, controlled by a substrate switch of the ubiquitin ligase Smurf1, specifies Par6 and RhoA localization and thereby regulates neuronal polarity.

Although psychological experts tell us to avoid becoming too compartmentalized in our thinking, compartmentalization is a key feature of neurons. Generation of an axonal and a somatodendritic domain is a prerequisite for the directed flow of information in the nervous system. Therefore, the establishment of the complex neuronal morphology with one axon and several dendrites is a critical step during neuronal differentiation. The underlying mechanisms that regulate the formation of neuronal polarity are currently under intense investigation.

In culture, hippocampal neurons start off as round, unpolarized cells that transform into a multipolar cell with several short neurites that all have the potential to become an axon. Only one of these neurites will grow quickly and turn into an axon while the other neurites only start to grow later and become dendrites. Stable microtubules in the axon shaft and a dynamic actin network in the axon growth cone are instructive for axon growth (Stiess and Bradke, 2010). However, so far, it has remained unclear how a neuron coordinates intracellular changes that could lead to the growth of the axon and the simultaneous halt of the other neurites.

The reported restriction of growth permissive proteins, including the partitioning-defective (Par) proteins Par3 and Par6 (Shi et al., 2003) and Rap1B (Schwamborn et al., 2007), to the nascent axon may present a hallmark of neuronal polarity. The asymmetric localization of axon determinants can be achieved by transport into one process (Bradke and Dotti, 1997), e.g., along selectively stabilized microtubules in the growing axon (Stiess and Bradke, 2010). In addition, the selective stabilization of the proteins in the future axon might lead to the asymmetric localization of polarizing proteins. Indeed, the small GTPase Rap1B in its inactive form becomes ubiquitinated and thus targeted for proteasomal degradation by the E3 Ligase Smurf2 in the minor neurites (Schwamborn et al., 2007). The resulting axonal localization specifies the future axon and is required for neuronal polarization.

In this issue of *Neuron*, Cheng et al. (2011) report another sophisticated example of selective protein degradation promoting axon growth and simultaneously inhibiting growth of the minor neurites, by showing that the E3 ligase Smurf1 regulates axon formation by switching its substrate preference from the axon determinant Par6 to the growth inhibitory small GTPase RhoA. This axon-specific switch primes RhoA for degradation in the axon while Par6 becomes stabilized (Figure 1). The substrate switch of Smurf1 can be induced extracellularly via a protein kinase A (PKA)-dependent pathway.

Whereas neuronal polarization happens spontaneously in vitro and is based on cell-intrinsic mechanisms, extracellular cues can regulate axon specification and play an important role in vivo. Previous studies have shown that the localized exposure of extracellular polarizing factors to one neurite can transform this neurite into an axon. These factors include transforming growth factor β (TGFβ) (Yi et al., 2010), brain-derived neurotrophic factor (BDNF) or cAMP (Shelly et al., 2007). As previously shown, neurons, plated on the border of stripes coated with BDNF or cAMP, preferentially initiate their axons toward the cAMP or BDNF stripe (Shelly et al., 2007). Cheng and colleagues (2011) provide now evidence that the extracellularly stimulated polarization involves selective degradation via the ubiquitin/proteasome system (UPS). Preferential polarization through BDNF/cAMP was blocked by global inhibition of the UPS. Moreover, local inhibition of the UPS in only one neurite using stripes coated with proteasome inhibitors triggered axon formation mimicking BDNF or cAMP exposure.

The authors then examined whether these cues differentially regulate ubiquitination and degradation of candidate polarity regulators. Importantly, they found that BDNF and the cell-permeable db-cAMP increased the stability of the polarity regulators Par6 and LKB1, whereas the growth inhibitory molecule RhoA was degraded. Consistently, db-cAMP stimulation decreased the ubiquitination of Par6 and LKB1, but enhanced RhoA ubiquitination.

To better understand the pathways in this process, the authors performed a screen to find the E3 ligases responsible for the ubiquitination of the axonal proteins. They found that Par6 is a direct substrate of the E3 ligase Smurf1 and that only Smurf1 targets Par6 for proteasomal degradation, but not other E3 ligases, including Smurf2. Consistently, downregulation of Smurf1 or overexpression of a ligase-deficient Smurf1 mutant increased Par6 and RhoA protein levels. The most intriguing observation is the converse ubiquitination of Par6 and RhoA by Smurf1 upon BDNF/cAMP stimulation. How is this opposite ubiquitination of the two substrates achieved? Are the substrates differently primed for their ubiquitination or is the substrate specificity regulated by the ligase itself?

BDNF activates PKA (Shelly et al., 2007). The observed stabilization of Par6 and LKB1 as well as the degradation of RhoA was diminished by inhibiting PKA-dependent phosphorylation. Interestingly, upon BDNF/cAMP treatment, PKA did not phosphorylate the substrates themselves, but the ligase Smurf1. Elegant experiments with phosphorylation-resistant and phosphorylation-mimicking Smurf1 mutants further showed that this phosphorylation affects a change in substrate specificity of the E3 ligase. While overexpression of the phosphomimetic Smurf1 decreased Par6 ubiquitination and increased RhoA ubiquitination, the overexpression of the nonphosphorylatable Smurf1 caused the opposite effects. This switch of substrate specificity was due to a higher binding affinity of phosphorylated Smurf1 to RhoA than to Par6. Therefore, PKA-dependent phosphorylation of Smurf1 switches its substrate preference from Par6 to RhoA causing the stabilization of Par6 and proteasomal degradation of RhoA.

How does this switch determine axon specification? The local exposure of BDNF to one neurite led to a localized accumulation of phosphorylated Smurf1 in the neurite tip. Consistent with the fact that such a local exposure of BDNF can induce axon growth, increased phosphorylated Smurf1 levels were also detected in the future axons of spontaneously polarizing neurons. Indeed, overexpressing the phosphomimetic Smurf1 mutant increased the formation of multiple axons, while Smurf1 knockdown by shRNA or overexpression of nonphosphorylatable Smurf1 inhibited axon formation. Together, with the observation that RhoA was reduced in the growth cone of future axons and the rescue of the Smurf1 knockdown with Par6 overexpression, these results indicate that increasing the Par6/RhoA ratio is necessary and sufficient for axon formation.

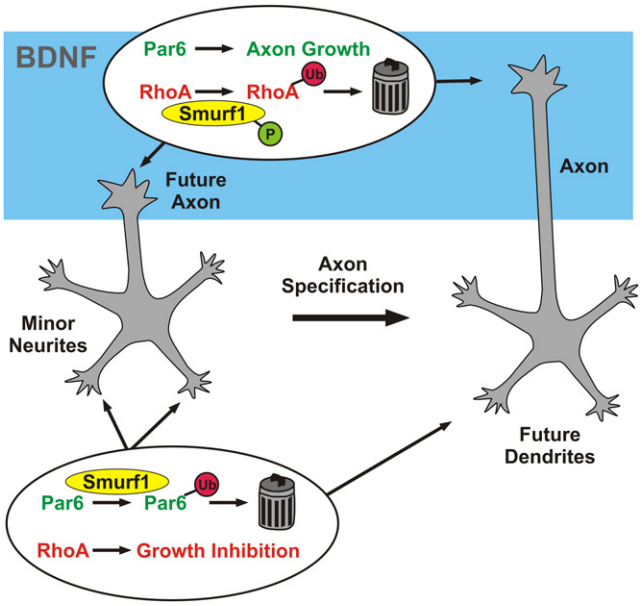

Figure 1. BDNF-Induced Phosphorylation of the E3 Ligase Smurf1 Induces a Substrate Switch and Thereby Specifies Axon Formation
Hippocampal neurons initiate their axon toward brain-derived neurotrophic factor (BDNF) exposure. Cheng et al. (2011) show that BDNF induces the phosphorylation of the E3 Ligase Smurf1. Without phosphorylation, Smurf1 ubiquitinates Par6 but not RhoA, leading to growth inhibition. In the future axon, Smurf1 is phosphorylated and targets RhoA for proteasomal degradation, while Par6 becomes stabilized and promotes axonal growth.

Why is the Par6/RhoA ratio so important for axon specification? Par6 and its binding partner Par3 localize specifically to the nascent axon (Shi et al., 2003), where they modulate the small GTPases Cdc42 and Rac1. Cdc42 and Rac1 are known to promote axon growth (Garvalov et al., 2007; Tahirovic et al., 2010), and thus, increasing the Par6 levels in the future axon could trigger axon formation. Simultaneous RhoA degradation would be also beneficial for axon specification, as RhoA is known to inhibit axon growth by modulating the actin cytoskeleton via Rho kinase (ROCK) (Da Silva et al., 2003). Indeed, local ROCK inhibition transformed a neurite into an axon and a constitutively active form of RhoA abolished neurite formation completely, indicating that RhoA inhibits axonal growth in the minor neurites. In addition, a Smurf1-resistant, nondegradable mutant of RhoA inhibited spontaneous as well as BDNF-induced axon growth. Therefore, these data suggest that both BDNF-induced and spontaneous axon formation are based on the degradation of RhoA via the UPS. Loss of RhoA in turn causes reduced ROCK activity and may change the actin cytoskeleton in the axonal growth cone into a growth permissive state.

Interestingly, the Smurf1 knockout mouse has no distinct neuronal phenotype and only the double knockout of Smurf1 and Smurf2 leads to very severe defects in neuronal development (Narimatsu et al., 2009). Future studies will be needed to address how the data in the current paper relate to these in vivo results (which may be due to compensatory mechanisms of other targets of the two E3 ligases). In addition, BDNF or BDNF receptor TrkB knockout mice show no defects in axon formation (Klein, 1994). This raises the question whether other extracellular factors could regulate Smurf1 dependent selective degradation. In epithelial cells, Par6 phosphorylation by the activated TGFβ receptor TβR2 induces the ubiquitination of RhoA by Smurf1 (Ozdamar et al., 2005). As TGFβ plays an important role in axon specification in vivo (Yi et al., 2010), the Smurf1 dependent RhoA degradation could be also activated by TβR2 in the nascent axon.

In summary, Cheng et al. (2011) show convincingly how PKA-dependent Smurf1 phosphorylation upon BDNF stimulation triggers Par6 accumulation and RhoA degradation in the future axon (Figure 1). The increased Par6/RhoA ratio may also support a proposed positive feedback loop promoting axon specification. In this feedback loop, it is proposed that increased Par6 activity signals back to the Par complex via Rac, PI3 Kinase, and Cdc42 and thereby increasingly promotes axon growth (Arimura and Kaibuchi, 2007). Loss of RhoA would further promote this feedback loop, as RhoA was shown to disrupt the Par complex via ROCK (Nakayama et al., 2008). However, it is worth noting that so far, there is still no genetic loss-of-function data verifying the role of the Par complex as well as RhoA in neuronal polarization in the developing mammalian

cortex and future studies will be needed to show whether these pathways are required for axon specification in vivo and whether such a feedback loop may also be the driving force of neuronal polarization.

REFERENCES

Arimura, N., and Kaibuchi, K. (2007). Nat. Rev. Neurosci. *8*, 194–205.

Bradke, F., and Dotti, C.G. (1997). Neuron *19*, 1175–1186.

Cheng, P.-l., Lu, H., Shelly, M., Gao, H., and Poo, M.-m. (2011). Neuron *69*, this issue, 231–243.

Da Silva, J.S., Medina, M., Zuliani, C., Di Nardo, A., Witke, W., and Dotti, C.G. (2003). J. Cell Biol. *162*, 1267–1279.

Garvalov, B.K., Flynn, K.C., Neukirchen, D., Meyn, L., Teusch, N., Wu, X., Brakebusch, C., Bamburg, J.R., and Bradke, F. (2007). J. Neurosci. *27*, 13117–13129.

Klein, R. (1994). FASEB J. *8*, 738–744.

Nakayama, M., Goto, T.M., Sugimoto, M., Nishimura, T., Shinagawa, T., Ohno, S., Amano, M., and Kaibuchi, K. (2008). Dev. Cell *14*, 205–215.

Narimatsu, M., Bose, R., Pye, M., Zhang, L., Miller, B., Ching, P., Sakuma, R., Luga, V., Roncari, L., Attisano, L., and Wrana, J.L. (2009). Cell *137*, 295–307.

Ozdamar, B., Bose, R., Barrios-Rodiles, M., Wang, H.R., Zhang, Y., and Wrana, J.L. (2005). Science *307*, 1603–1609.

Schwamborn, J.C., Müller, M., Becker, A.H., and Püschel, A.W. (2007). EMBO J. *26*, 1410–1422.

Shelly, M., Cancedda, L., Heilshorn, S., Sumbre, G., and Poo, M.M. (2007). Cell *129*, 565–577.

Shi, S.H., Jan, L.Y., and Jan, Y.N. (2003). Cell *112*, 63–75.

Stiess, M., and Bradke, F. (2010). Dev. Neurobiol., in press. Published online December 7, 2010. 10.1002/dneu.20849.

Tahirovic, S., Hellal, F., Neukirchen, D., Hindges, R., Garvalov, B.K., Flynn, K.C., Stradal, T.E., Chrostek-Grashoff, A., Brakebusch, C., and Bradke, F. (2010). J. Neurosci. *30*, 6930–6943.

Yi, J.J., Barnes, A.P., Hand, R., Polleux, F., and Ehlers, M.D. (2010). Cell *142*, 144–157.

The Multiple Faces of RIM

Alejandro Pernía-Andrade[1] and Peter Jonas[1,*]
[1]Institute of Science and Technology (IST) Austria, Am Campus 1, A-3400 Klosterneuburg, Austria
*Correspondence: peter.jonas@ist.ac.at
DOI 10.1016/j.neuron.2011.01.010

Rab3 interacting molecules (RIMs) are highly enriched in the active zones of presynaptic terminals. It is generally thought that they operate as effectors of the small G protein Rab3. Three recent papers, by Han et al. (this issue of *Neuron*), Deng et al. (this issue of *Neuron*), and Kaeser et al. (a recent issue of *Cell*), shed new light on the functional role of RIM in presynaptic terminals. First, RIM tethers Ca^{2+} channels to active zones. Second, RIM contributes to priming of synaptic vesicles by interacting with another presynaptic protein, Munc13.

A hallmark of synaptic transmission is speed. Although synaptic transmission involves two chemical messengers, Ca^{2+} and the transmitter, the entire signaling process takes place within less than a millisecond under physiological conditions. To minimize delays generated by the diffusion, an ideal synapse would have to be constructed as a point-to-point device, in which the relevant molecules are tightly packed on the nanometer scale at both sides of the synaptic cleft. While a lot of information is available about the molecular composition of postsynaptic densities, little is known about the organization of presynaptic active zones.

Active zones are composed of several different proteins, including Munc13s, Rab3 binding proteins (RIMs), RIM-binding proteins (RIM-BPs), ELKSs, and many others (Wojcik and Brose, 2007; Müller et al., 2010). Among these proteins, RIMs have received particular attention as binding partners of Rab3, a highly abundant protein in synaptic vesicles (Castillo et al., 2002; Takamori et al., 2006). RIMs are multidomain proteins, comprised of a Rab3 binding domain at the N terminus, a Zn^{2+} finger domain, a putative protein kinase A (PKA) phosphorylation site, a PDZ domain, a C2 domain, a proline-rich domain, and another C2 domain at the C terminus (Wojcik and Brose, 2007). The functional significance of these multiple domains, however, is largely unclear. It is generally thought that RIMs operate as Rab3 effectors. Furthermore, RIMs are substrates of PKA and are thought to play important roles in presynaptic forms of synaptic plasticity (Wang et al., 1997; Castillo et al., 2002).

Three recent papers (Kaeser et al., 2011; Han et al., 2011, Deng et al., 2011; the latter two of which can be found in this issue of *Neuron*) shed new light on the function of RIMs, approaching the problem by genetic elimination (knockout). RIM proteins in mammals are highly diverse. They are encoded by four genes (*Rim1–4*) that drive the expression of seven known RIM isoforms: RIM1α and 1β; RIM2α, 2β, and 2γ; RIM3γ; and RIM4γ. Unfortunately, RIM1α and RIM2α double knockout mice die immediately after birth (Schoch et al., 2006), preventing a systematic analysis of the function of RIMs in synaptic transmission. The Südhof group (Kaeser et al., 2011) has now solved this problem by generating