Division of labor in the growth cone by DSCR1

Timothy S. Catlett[1,2] and Timothy M. Gomez[1,2]

[1]Department of Neuroscience and [2]Cell and Molecular Biology Training Program, University of Wisconsin, Madison, WI 53706

Local protein synthesis directs growth cone turning of nascent axons, but mechanisms governing this process within compact, largely autonomous microenvironments remain poorly understood. In this issue, Wang et al. (2016. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201510107) demonstrate that the calcineurin regulator Down syndrome critical region 1 protein modulates both basal neurite outgrowth and growth cone turning.

Connectivity and function of a mature nervous system requires precise wiring between neurons and with target cells throughout embryonic development. Severe to mild errors in connectivity lead to neurodevelopmental disorders ranging from profound intellectual deficits to subtle behavioral abnormalities (Van Battum et al., 2015). For example, evidence from many groups has shown defective neuronal connections in several autism spectrum disorders, such as Fragile X syndrome and tuberous sclerosis complex, as well as in motor deficits and degenerative muscle diseases (Nie et al., 2010; Doers et al., 2014; Bakos et al., 2015). However, most disease etiologies cannot be unilaterally attributed to abnormal axon guidance, as many of the relevant proteins are functional in other important neurological processes, such as dendritic spine maturation and function (Hoeffer and Klann, 2010; Holt and Schuman, 2013).

One key player involved in morphogenesis and innervation of developing neurons is the nerve growth cone, the dynamic and motile sensory tip of growing axons and dendrites. Growth cones function with a large degree of autonomy from the cell soma, as they transduce contacted soluble and substratum-bound ligands into signals that coordinate cytoskeletal changes to regulate the rate and direction of axon outgrowth (Lowery and Van Vactor, 2009). Both growth-promoting and -inhibiting molecules are expressed along the pathways of developing axons and local discontinuities (e.g., gradients and borders) of extracellular cues are amplified into local biochemical changes within growth cones. Although many groups have given us insight into these processes over the past few decades, crucial mechanisms underlying guidance are still poorly understood (Goodhill, 2016). In this issue, Wang et al. demonstrate that Down syndrome critical region 1 protein (DSCR1) has two distinct roles in growth cones to control neurite outgrowth and guidance.

Importantly, biochemical changes within growth cones have been shown to both directly modulate the cytoskeleton and indirectly affect motility by regulating local synthesis of new proteins (Holt and Schuman, 2013). Despite significant advances, it is still unclear why and how growth cones use protein synthesis–independent and –dependent mechanisms to regulate motility. Precise spatiotemporal control of translation likely provides additional levels of cellular regulation. For example, local protein synthesis is controlled by numerous mRNA binding and trafficking proteins, which may be regulated by classic second messengers (Akiyama and Kamiguchi, 2015). Many proteins synthesized at the growth cone are ubiquitinated at a higher rate than those trafficked from the cell body, and protein degradation is regulated by axon guidance cues (Deglincerti et al., 2015). Distinct pathways could also be activated by newly synthesized proteins via their relative lack of post-translational modifications. Finally, new protein synthesis may sensitize growth cones to different types and concentrations of ligands. Interestingly, in vitro experiments have shown that basal axon outgrowth is independent of local protein synthesis, whereas local protein synthesis is necessary for guidance. For example, Nie et al. (2010) found that in a mouse model of tuberous sclerosis complex, which displays a defect in the regulation of the mTOR complex (a translational hub in the growth cone), Ephrin A–dependent local protein synthesis was required for proper retinogeniculate mapping, but no defects in retinal axon growth were observed (Nie et al., 2010). It is also interesting to note that several inherited autism spectrum disorders exhibit misregulation of protein synthesis (Van Battum et al., 2015), suggesting these mechanisms have important roles in human central nervous system assembly.

Down syndrome, or trisomy 21, affects human development and is caused, in part, by elevated expression of genes encoded by chromosome 21, resulting in intellectual disabilities. One particular protein implicated is DSCR1 (Fuentes et al., 2000), also known as regulator of calcineurin (RCAN1). One established function of DSCR1 is to inhibit calcineurin (CaN), which is a calcium- and calmodulin-dependent serine/threonine protein phosphatase. DSCR1 binds and inhibits CaN, whereas phosphorylation of DSCR1 releases CaN, which may actively or passively lead to CaN activation. DSCR1 and CaN are highly expressed in developing neurons (Fuentes et al., 2000), where they may cooperate to control morphological differentiation. DSCR1 also interacts with Fragile X mental retardation protein (FMRP), which is lost in Fragile X syndrome (Verkerk et al., 1991; Wang et al., 2012). FMRP is an mRNA binding protein that regulates local protein synthesis in dendritic spines and neuronal growth cones (Ashley et al., 1993; Sidorov et al., 2013). Moreover, previous results from Chang et al. (2013) suggest that DSCR1 and FMRP1 may participate in common

Correspondence to Timothy M. Gomez: tmgomez@wisc.edu

The Rockefeller University Press $30.00
J. Cell Biol. Vol. 213 No. 4 407–409
www.jcb.org/cgi/doi/10.1083/jcb.201605012

biological pathways leading to intellectual disability, including the maturation of dendritic spines.

In their most recent work, Wang et al. (2016) demonstrate that DSCR1 serves a dual function in growth cones to regulate both neurite outgrowth and guidance. Gain and loss of function of DSCR1 leads to increased and decreased axon extension in developing mouse hippocampal neurons, respectively. Consistent with the role of DSCR1 as a CaN inhibitor, DSCR1$^{-/-}$ growth cones have elevated CaN activity as indicated by reduced phosphorylated cofilin (nonphosphorylated cofilin is the active form of this actin depolymerizing factor), which leads to loss of F-actin and short axons. In contrast, DSCR1 transgenic neurons, which express 1.5-fold excess DSCR1 compared with wild-type neurons, display elevated phosphorylated cofilin (the inactive form) and increased F-actin in their growth cones, increasing neurite extension.

In chemotropic turning assays, DSCR1$^{-/-}$ neurons fail to orient toward brain-derived neurotrophic factor (BDNF), whereas transgenic DSCR1 neurons exhibit enhanced turning. Interestingly, Wang et al. (2016) find that although activation of CaN and cofilin caused by loss of DSCR1 function reduces axon extension, misregulation of CaN is not responsible for defective chemotropic turning toward BDNF by DSCR1$^{-/-}$ neurons. This result suggests that a different DSCR1-dependent target regulates axon turning. Here, Wang et al. (2016) find that local protein synthesis in response to BDNF depends on DSCR1 and Fmr1. They show that enhanced protein synthesis in growth cones and axon turning in DSCR1 transgenic neurons is abrogated in Fmr1 knockdown neurons. Together these results support a model where DSCR1 functions as an important regulatory switch to direct distinct aspects of axon growth and guidance machinery.

Wang et al. (2016) illustrate for the first time divergent activities of DSCR1 in the regulation of axon outgrowth and guidance, but many open questions remain. For example, there appears to be an important difference between the regulation of DSCR1 in axon guidance versus dendritic spine morphogenesis. Previous work by Wang et al. (2012) showed that Fmr1 is dephosphorylated by CaN in response to BDNF, which promotes protein translation. However, in their current work, inhibition of CaN does not prevent chemotropic turning toward a BDNF, which depends on DSCR1, Fmr1 and protein synthesis. Therefore, the role of CaN-mediated dephosphorylation of Fmr1 downstream of BDNF and DSCR1 is unclear in growth cone turning. It is also interesting to note that Wang et al. (2016) observe changes in total cofilin levels in growth cones from DSCR1 transgenic and knockout neurons, as well as after inhibition of CaN, suggesting that there may be homeostatic aspects of regulation of these pathways yet to be explored. Given the complexities of cofilin regulation in actin polymerization and evidence that cofilin can be locally translated in axons (Piper et al., 2006), it is clear that additional work is necessary to understand these pathways more completely.

Finally, how DSCR1 dysfunction contributes specifically to neural developmental disorders associated with Down syndrome is not known. DSCR1 gain-of-function experiments may most closely match chromosomal triplication conditions in developing trisomy 21 neurons. Under these conditions, Wang et al. (2016) find increased axon extension, as well as enhanced turning toward BDNF by mouse hippocampal neurons. However, it is not clear how elevated DSCR1 expression will affect CaN-cofilin signaling and protein synthesis downstream of the wide range of growth promoting and inhibiting guidance cues that use these signals (Gomez and Letourneau, 2014). It is also not clear how DSCR1 overexpression will affect human neurons under similar conditions. To address this question, specific classes of excitatory and inhibitory neurons differentiated from human embryonic stem cells and induced pluripotent stem cells (hiPSCs) should be tested. For example, in these in vitro systems, Crispr-Cas–mediated correction of specific genes of hiPSCs from Down syndrome patients or selected triplication of chromosome 21 target genes of unaffected hiPSCs could be used to test the necessity and sufficiency of specific genes on cellular phenotypes observed in vitro. If specific requirements for DSCR1 or other candidate genes can be identified by assaying neuronal morphogenesis of human cells in vitro, these findings would provide an excellent platform for therapeutic drug screening of treatments that rescue these cellular phenotypes.

Acknowledgments

We would like to thank Dr. Anita Bhattacharyya for comments on the manuscript.

This work was supported by National Institutes of Health R56NS41564 and R21NS88477 to T.M. Gomez and T32GM007215 to the Medical biosciences training grant.

The authors declare no competing financial interests.

Submitted: 5 May 2016
Accepted: 5 May 2016

References

Akiyama, H., and H. Kamiguchi. 2015. Second messenger networks for accurate growth cone guidance. *Dev. Neurobiol.* 75:411–422. http://dx.doi.org/10.1002/dneu.22157

Ashley, C.T. Jr., K.D. Wilkinson, D. Reines, and S.T. Warren. 1993. FMR1 protein: conserved RNP family domains and selective RNA binding. *Science.* 262:563–566. http://dx.doi.org/10.1126/science.7692601

Bakos, J., Z. Bacova, S.G. Grant, A.M. Castejon, and D. Ostatnikova. 2015. Are molecules involved in neuritogenesis and axon guidance related to autism pathogenesis? *Neuromolecular Med.* 17:297–304. http://dx.doi.org/10.1007/s12017-015-8357-7

Chang, K.T., H. Ro, W. Wang, and K.T. Min. 2013. Meeting at the crossroads: common mechanisms in Fragile X and Down syndrome. *Trends Neurosci.* 36:685–694. http://dx.doi.org/10.1016/j.tins.2013.08.007

Deglincerti, A., Y. Liu, D. Colak, U. Hengst, G. Xu, and S.R. Jaffrey. 2015. Coupled local translation and degradation regulate growth cone collapse. *Nat. Commun.* 6:6888. http://dx.doi.org/10.1038/ncomms7888

Doers, M.E., M.T. Musser, R. Nichol, E.R. Berndt, M. Baker, T.M. Gomez, S.C. Zhang, L. Abbeduto, and A. Bhattacharyya. 2014. iPSC-derived forebrain neurons from FXS individuals show defects in initial neurite outgrowth. *Stem Cells Dev.* 23:1777–1787. http://dx.doi.org/10.1089/scd.2014.0030

Fuentes, J.J., L. Genescà, T.J. Kingsbury, K.W. Cunningham, M. Pérez-Riba, X. Estivill, and S. de la Luna. 2000. DSCR1, overexpressed in Down syndrome, is an inhibitor of calcineurin-mediated signaling pathways. *Hum. Mol. Genet.* 9:1681–1690. http://dx.doi.org/10.1093/hmg/9.11.1681

Gomez, T.M., and P.C. Letourneau. 2014. Actin dynamics in growth cone motility and navigation. *J. Neurochem.* 129:221–234. http://dx.doi.org/10.1111/jnc.12506

Goodhill, G.J. 2016. Can molecular gradients wire the brain? *Trends Neurosci.* 39:202–211. http://dx.doi.org/10.1016/j.tins.2016.01.009

Hoeffer, C.A., and E. Klann. 2010. mTOR signaling: at the crossroads of plasticity, memory and disease. *Trends Neurosci.* 33:67–75. http://dx.doi.org/10.1016/j.tins.2009.11.003

Holt, C.E., and E.M. Schuman. 2013. The central dogma decentralized: new perspectives on RNA function and local translation in neurons. *Neuron.* 80:648–657. http://dx.doi.org/10.1016/j.neuron.2013.10.036

Lowery, L.A., and D. Van Vactor. 2009. The trip of the tip: understanding the growth cone machinery. *Nat. Rev. Mol. Cell Biol.* 10:332–343. http://dx.doi.org/10.1038/nrm2679

Nie, D., A. Di Nardo, J.M. Han, H. Baharanyi, I. Kramvis, T. Huynh, S. Dabora, S. Codeluppi, P.P. Pandolfi, E.B. Pasquale, and M. Sahin. 2010. Tsc2-Rheb signaling regulates EphA-mediated axon guidance. *Nat. Neurosci.* 13:163–172. http://dx.doi.org/10.1038/nn.2477

Piper, M., R. Anderson, A. Dwivedy, C. Weinl, F. van Horck, K.M. Leung, E. Cogill, and C. Holt. 2006. Signaling mechanisms underlying Slit2-induced collapse of *Xenopus* retinal growth cones. *Neuron.* 49:215–228. http://dx.doi.org/10.1016/j.neuron.2005.12.008

Sidorov, M.S., B.D. Auerbach, and M.F. Bear. 2013. Fragile X mental retardation protein and synaptic plasticity. *Mol. Brain.* 6:15. http://dx.doi.org/10.1186/1756-6606-6-15

Van Battum, E.Y., S. Brignani, and R.J. Pasterkamp. 2015. Axon guidance proteins in neurological disorders. *Lancet Neurol.* 14:532–546. http://dx.doi.org/10.1016/S1474-4422(14)70257-1

Verkerk, A.J.M.H., M. Pieretti, J.S. Sutcliffe, Y.H. Fu, D.P. Kuhl, A. Pizzuti, O. Reiner, S. Richards, M.F. Victoria, F. Zhang, et al. 1991. Identification of a gene (*FMR*-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell.* 65:905–914. http://dx.doi.org/10.1016/0092-8674(91)90397-H

Wang, W., J.Z. Zhu, K.T. Chang, and K.T. Min. 2012. DSCR1 interacts with FMRP and is required for spine morphogenesis and local protein synthesis. *EMBO J.* 31:3655–3666. http://dx.doi.org/10.1038/emboj.2012.190

Wang, W., A. Rai, E.M. Hur, Z. Smilansky, K. Chang, and K.T. Min. 2016. DSCR1 is required for both axonal growth cone extension and steering. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201510107.