Previous studies found that individual lateral line axons reliably re-establish connections with hair cells of the same orientation following repeated rounds of hair cell ablation and regeneration, indicating that afferents retain a 'memory' of hair cell polarity after denervation [5,6]. The current study suggests that this memory is at least partially maintained by inhibitory interactions between neighboring lateral line axons [3]. A similar strategy of "synaptic tiling" occurs between neighboring DA8 and DA9 motor neurons in *Caenorhabdities elegans* [12]. Much like lateral line afferents, DA8 and DA9 axons are closely associated with one another in the dorsal nerve cord, but segregate their synapses into adjacent, non-overlapping synaptic zones in an activity-independent manner. Additionally, the synaptic zones of both axons expanded in worms that had one axon genetically displaced from the dorsal nerve cord, suggesting that axons mutually inhibit the expansion of each other's synaptic territory. The marked similarities between these different species and neural systems raise the possibility that synaptic tiling may be a conserved mechanism for establishing and maintaining patterns of neural connectivity.

Although Pujol-Martí *et al.* [3] elegantly demonstrated that neighboring lateral line axons restrict each other's synaptic territories, the molecular signals underlying this regulation are unknown. Synaptic tiling between *C. elegans* motor axons requires plexin-1 and semaphorin-1 expression in DA9 motor neurons to segregate synapses [12]. Similarly, semaphorin signaling is required for restricting or eliminating synapses in the mouse spinal cord, hippocampus, and striatum [13–15]. In addition to negative regulators, positive synaptogenic interactions between hair cells and afferents are also likely required for establishing and maintaining lateral line circuitry. The molecules that mediate these interactions are also unknown, but lateral line axons with different orientation preferences could express different cell surface adhesion molecules (reviewed in [16,17]) that prefer binding partners differentially enriched in each hair cell population. Identifying the proteins mediating positive cellular interactions between lateral line neurons and hair cells, as well as negative interactions between neighboring afferent neurons with different preferences, will provide a molecular paradigm for the segregation and maintenance of neural circuits responding to distinct sensory stimuli.

References

1. Brann, J.H., and Firestein, S.J. (2014). A lifetime of neurogenesis in the olfactory system. Front. Neurosci. *8*, 182.
2. Feng, P., Huang, L., and Wang, H. (2014). Taste bud homeostasis in health, disease, and aging. Chem. Senses *39*, 3–16.
3. Pujol-Marti, J., Faucherre, A., Aziz-Bose, R., Asgharsharghi, A., Colombelli, J., Trapani, J.G., and Lopez-Schier, H. (2014). Converging axons collectively initiate and maintain synaptic selectivity in a constantly remodeling sensory organ. Curr. Biol. *24*, 2968–2974.
4. Pujol-Martí, J., and López-Schier, H. (2013). Developmental and architectural principles of the lateral-line neural map. Front. Neural Circuits *7*, 47.
5. Faucherre, A., Pujol-Martí, J., Kawakami, K., and López-Schier, H. (2009). Afferent neurons of the zebrafish lateral line are strict selectors of hair-cell orientation. PLoS One *4*, e4477.
6. Nagiel, A., Andor-Ardó, D., and Hudspeth, A.J. (2008). Specificity of afferent synapses onto plane-polarized hair cells in the posterior lateral line of the zebrafish. J. Neurosci. *28*, 8442–8453.
7. Obholzer, N., Wolfson, S., Trapani, J.G., Mo, W., Nechiporuk, A., Busch-Nentwich, E., Seiler, C., Sidi, S., Söllner, C., Duncan, R.N., *et al*. (2008). Vesicular glutamate transporter 3 is required for synaptic transmission in zebrafish hair cells. J. Neurosci. *28*, 2110–2118.
8. Walsh, M.K., and Lichtman, J.W. (2003). In vivo time-lapse imaging of synaptic takeover associated with naturally occurring synapse elimination. Neuron *37*, 67–73.
9. Darabid, H., Perez-Gonzalez, A.P., and Robitaille, R. (2014). Neuromuscular synaptogenesis: coordinating partners with multiple functions. Nat. Rev. Neurosci. *15*, 703–718.
10. Okawa, H., Hoon, M., Yoshimatsu, T., Della Santina, L., and Wong, R.O.L. (2014). Illuminating the multifaceted roles of neurotransmission in shaping neuronal circuitry. Neuron *83*, 1303–1318.
11. Nagiel, A., Patel, S.H., Andor-Ardó, D., and Hudspeth, A.J. (2009). Activity-independent specification of synaptic targets in the posterior lateral line of the larval zebrafish. Proc. Natl. Acad. Sci. USA *106*, 21948–21953.
12. Mizumoto, K., and Shen, K. (2013). Interaxonal interaction defines tiled presynaptic innervation in C. elegans. Neuron *77*, 655–666.
13. Pecho-Vrieseling, E., Sigrist, M., Yoshida, Y., Jessell, T.M., and Arber, S. (2009). Specificity of sensory-motor connections encoded by Sema3e-Plxnd1 recognition. Nature *459*, 842–846.
14. Liu, X.-B., Low, L.K., Jones, E.G., and Cheng, H.-J. (2005). Stereotyped axon pruning via plexin signaling is associated with synaptic complex elimination in the hippocampus. J. Neurosci. *25*, 9124–9134.
15. Ding, J.B., Oh, W.-J., Sabatini, B.L., and Gu, C. (2011). Semaphorin 3E–Plexin-D1 signaling controls pathway-specific synapse formation in the striatum. Nat. Neurosci. *15*, 215–223.
16. Williams, M.E., de Wit, J., and Ghosh, A. (2010). Molecular mechanisms of synaptic specificity in developing neural circuits. Neuron *68*, 9–18.
17. Margeta, M.A., and Shen, K. (2010). Molecular mechanisms of synaptic specificity. Mol. Cell. Neurosci. *43*, 261–267.

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ER Morphology: Sculpting with XendoU

Endoplasmic reticulum (ER) sheet membranes are covered with ribosomes and RNAs that are involved in protein synthesis. A new study reveals that a calcium-activated endoribonuclease of the EndoU protein family promotes the formation of tubular ER networks, contributing to dynamic shaping of the ER in cells.

Guohua Zhao and Craig Blackstone*

The endoplasmic reticulum (ER) is a continuous membrane system comprising the nuclear envelope, flat sheets often studded with ribosomes, and a polygonal network of mostly smooth tubules extending throughout the cell. Synthesis, modification, and transport of lipids and proteins as well as Ca^{2+} sequestration and protein quality control within the ER have been extensively investigated over many years, but mechanisms responsible for the distinctive morphology of the ER have only been uncovered more recently [1,2]. Several eukaryotic protein families, including reticulons and REEPs/DP1/Yop1p, harbor hydrophobic hairpin domains that partially insert into the lipid bilayer, shaping high-curvature ER tubules [3]. Members of the atlastin/RHD3/Sey1p family of large, membrane-bound GTPases

mediate the formation of three-way junctions via homotypic membrane fusion, generating the reticulated tubular ER network [4,5]. Additional classes of tubular ER proteins, including some REEPs and the ATPase M1 spastin (which severs microtubules), interact with the cytoskeleton [6,7]. Flat ER sheets possess a different cadre of proteins, such as p180, CLIMP-63 and kinectin, which have been implicated in shaping, cisternal stacking and cytoskeletal interactions, with reticulons shaping the high-curvature edges [8]. Other proteins, including members of the Lunapark, SNARE, and Rab protein families, have also been suggested to have a role in shaping the ER network [1]. In a recent issue of the *Journal of Cell Biology*, Schwarz and Blower [9] identify a new and unexpected member of the cellular ER-shaping team — the Ca^{2+}-activated ribonuclease XendoU (for *Xenopus* EndoU), previously studied mostly for its roles in processing intron-encoded small nucleolar RNAs and in viral replication [10–12].

Dramatic changes in intracellular organization and organelle structure occur during developmental differentiation, and this is certainly true for the ER network. Though published images of the ER tend to paint a static picture, the ER is in fact in constant motion, and numerous signaling pathways as well as interactions among cytoskeletal elements, the plasma membrane, and organelles cooperate to position and shape the ER dynamically. Striking morphological changes in the ER occur during cellular events, such as fertilization and cell division. For example, within minutes of fertilization, the ER in starfish eggs undergoes fragmentation, accompanied by Ca^{2+} release from internal stores [13]. Ca^{2+}-induced, reversible ER fragmentation has also been reported in cell lines and neurons [14,15], prefiguring key roles for signaling pathways in the regulation of ER morphology.

In the new work, Schwarz and Blower [9] set out to investigate the role of Ca^{2+}, which is increased upon fertilization, in the developmental transition from oocyte to embryo. Starting with metaphase-arrested *Xenopus* egg extracts, they added Ca^{2+} to mimic the cytoplasmic Ca^{2+} influx that occurs from both intracellular and extracellular stores at fertilization. They then purified a Ca^{2+}-dependent ribonuclease activity, identifying the protein as the XendoU ribonuclease. The authors further found that a subpopulation of XendoU is tightly membrane bound at the ER surface, where it functions in local RNA degradation. This Ca^{2+}-dependent degradation results in the removal of ribosomes, ribonuclear proteins (RNPs) and RNAs from the ER surface (Figure 1), favoring ER tubule formation and thus helping to regulate the dynamic balance between ER sheets and tubules. Depletion of XendoU caused expansion of sheets at the expense of tubules, an alteration that could be rescued by XendoU in a catalysis-dependent manner. The authors concluded that Ca^{2+}-dependent removal of RNA, ribosomes, and RNPs from the membrane by XendoU promotes ER remodeling and the formation of tubular ER [9].

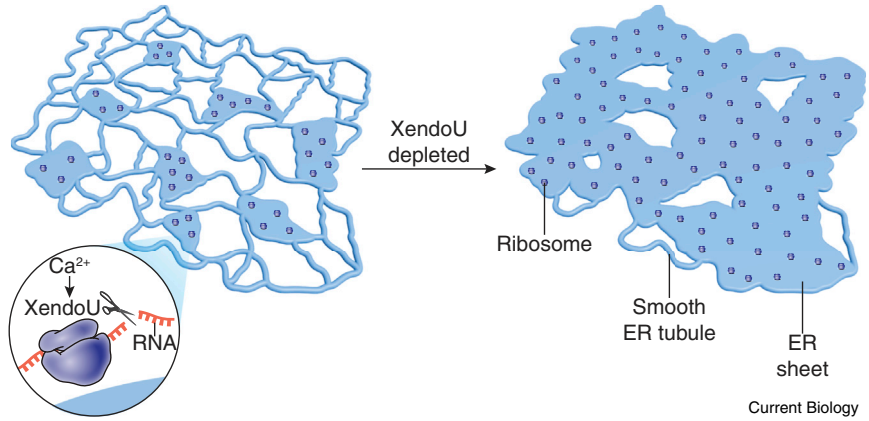

Figure 1. Schematic diagram of effects of XendoU on ER morphology.
Ca^{2+}-activated XendoU favors ER tubule formation, with its depletion or loss of catalytic activity resulting in expansion of ER sheets. (Image drawn by Ethan Tyler.)

Mechanistically, there are a number of possibilities for how XendoU functions in regulating ER morphology. First, there could be sheet stability conferred by the presence of ribosomes. Thus, removing them would destabilize sheets and help promote tubule formation. In this regard, ER network formation *in vitro* using purified *Xenopus* egg membranes was inhibited by specific antibodies against XendoU, emphasizing the direct role of the membrane-bound subpopulation of XendoU. Schwarz and Blower [9] postulate that oligomerization of atlastin GTPases mediates membrane fusion and subsequent Ca^{2+} release through Ca^{2+} channels on the membrane. XendoU would then be activated by Ca^{2+} and degrade RNA locally, resulting in the release of ribosomes, RNPs, and RNA [9]. Studies of *Xenopus*, human, and viral EndoU orthologs (including XendoU, PP11, and Nsp15) have demonstrated that these Ca^{2+}-dependent endonucleases are relatively nonspecific, cleaving RNAs *in vitro* after UU dinucleotides or a single U nucleotide. Interestingly, mild treatment of salt-washed vesicles with non-specific RNase A facilitated network formation, while an excess of RNAse A was very disruptive, emphasizing more generally the importance of RNA [9]. Similar findings were observed in mammalian HeLa cells, with effects on ER morphology also dependent on EndoU catalytic activity. Protein scaffolding could conceivably play a role, since a protein of the XendoU family produced by haemocytes of the moth *Heliothis virescens* can form large, amyloid-like fibrils at ER sheets, and these are released upon immune challenge [16].

Ca^{2+} is a ubiquitous signaling molecule implicated in a plethora of cellular pathways involved in organelle changes, including many that affect the ER. For instance, B lymphocyte activation is accompanied by increased Ca^{2+} signaling and expansion of the ER, with downregulation of EndoU suppressing activation-induced cell death in these cells [17]. Another example is found in neurons, where prominent dynamic changes in ER morphology occur in neuronal dendrites [18]. In this latter case, proteins including synaptic glutamate

receptors rapidly diffuse within the continuous network of dendritic ER but are confined by increased ER complexity at branch points of dendrites and near dendritic spines. The spatial range of receptor mobility is rapidly restricted by phosphoinositide-linked metabotropic glutamate receptor signaling, which is linked to intracellular Ca^{2+} release via inositol (1,4,5) trisphophate (IP_3) receptor channels, through a mechanism involving protein kinase C and the ER sheet protein CLIMP63. The morphological changes in local zones of ER have the effect of compartmentalizing ER export and also correspond to sites of new dendritic branches [18]. It will be particularly important to assess any role for XendoU in such Ca^{2+}-dependent processes, and small-molecule inhibitors effective against XendoU in the low micromolar range provide additional tools for such studies [12].

In future studies, investigations of the function of EndoU proteins *in vivo*, particularly within cells in tissues such as the central nervous system, will be of particular interest. Though these proteins are known to be aberrantly expressed in human diseases, such as cancer, loss of function may similarly be related to disease. In fact, many proteins involved in shaping the ER network are mutated in neurological disorders, including hereditary spastic paraplegia and hereditary sensory neuropathy [19,20]. It will be interesting to probe any links of EndoU mutations to human neurological disease.

References

1. Goyal, U., and Blackstone, C. (2013). Untangling the web: mechanisms underlying ER network formation. Biochim. Biophys. Acta *1833*, 2492–2498.
2. English, A.R., and Voeltz, G.K. (2013). Endoplasmic reticulum structure and interconnections with other organelles. Cold Spring Harb. Perspect. Biol. *5*, a013227.
3. Voeltz, G.K., Prinz, W.A., Shibata, Y., Rist, J.M., and Rapoport, T.A. (2006). A class of membrane proteins shaping the tubular endoplasmic reticulum. Cell *124*, 573–586.
4. Hu, J., Shibata, Y., Zhu, P.-P., Voss, C., Rismanchi, N., Prinz, W.A., Rapoport, T.A., and Blackstone, C. (2009). A class of dynamin-like GTPases involved in the generation of the tubular ER network. Cell *138*, 549–561.
5. Orso, G., Pendin, D., Liu, S., Tosetto, J., Moss, T.J., Faust, J.E., Micaroni, M., Egorova, A., Martinuzzi, A., McNew, J.A., *et al*. (2009). Homotypic fusion of ER membranes requires the dynamin-like GTPase atlastin. Nature *460*, 978–983.
6. Park, S.H., Zhu, P.-P., Parker, R.L., and Blackstone, C. (2010). Hereditary spastic paraplegia proteins REEP1, spastin and atlastin-1 coordinate microtubule interactions with the tubular ER network. J. Clin. Invest. *120*, 1097–1110.
7. Schlaitz, A.-L., Thompson, J., Wong, C.C.L., Yates, J.R., 3rd, and Heald, R. (2013). REEP3/4 ensure endoplasmic reticulum clearance from metaphase chromatin and proper nuclear envelope architecture. Dev. Cell *26*, 313–323.
8. Shibata, Y., Shemesh, T., Prinz, W.A., Palazzo, A.F., Kozlov, M.M., and Rapoport, T.A. (2010). Mechanisms determining the morphology of the peripheral ER. Cell *143*, 774–788.
9. Schwarz, D.S., and Blower, M.D. (2014). The calcium-dependent ribonuclease XendoU promotes ER network formation through local RNA degradation. J. Cell Biol. *207*, 41–57.
10. Gioia, U., Laneve, P., Dlakić, M., Arceci, M., Bozzoni, I., and Caffarelli, E. (2005). Functional characterization of XendoU, the endoribonuclease involved in small nucleolar RNA biosynthesis. J. Biol. Chem. *280*, 18996–19002.
11. Ricagno, S., Engloff, M.P., Ulferts, R., Coutard, B., Nurizzo, D., Campanacci, V., Cambillau, C., Ziebuhr, J., and Canard, B. (2006). Crystal structure and molecular determinants of SARS coronavirus nonstructural protein 15 define an endoribonuclease family. Proc. Natl. Acad. Sci USA *103*, 11892–11897.
12. Ragno, R., Gioia, U., Laneve, P., Bozzoni, I., Mai, A., and Caffarelli, E. (2011). Identification of small-molecule inhibitors of the XendoU endoribonucleases family. Chem. Med. Chem. *6*, 1797–1805.
13. Terasaki, M., Jaffe, L.A., Hunnicutt, G.R., and Hammer, J.A., 3rd (1996). Structural change of the endoplasmic reticulum during fertilization: evidence for loss of membrane continuity using the green fluorescent protein. Dev. Biol. *179*, 320–328.
14. Subramanian, K., and Meyer, T. (1997). Calcium-induced restructuring of nuclear envelope and endoplasmic reticulum calcium stores. Cell *89*, 963–971.
15. Kucharz, K., Krogh, M., Ng, A.N., and Toresson, H. (2009). NMDA receptor stimulation induces reversible fission of the neuronal endoplasmic reticulum. PLoS One *4*, e5250.
16. Falabella, P., Riviello, L., Pascale, M., Di Lelio, I., Tettamanti, G., Grimaldi, A., Iannone, C., Monti, M., Pucci, P., Tamburro, A.M., *et al*. (2012). Functional amyloids in insect immune response. Insect Biochem. Mol. Biol. *42*, 203–211.
17. Poe, J.C., Kountikov, E.I., Lykken, J.M., Natarajan, A., Marchuk, D.A., and Tedder, T.F. (2014). EndoU is a novel regulator of AICD during peripheral B cell selection. J. Exp. Med. *211*, 57–69.
18. Cui-Wang, T., Hanus, C., Cui, T., Helton, T., Bourne, J., Watson, D., Harris, K.M., and Ehlers, M.D. (2012). Local zones of endoplasmic reticulum complexity confine cargo in neuronal dendrites. Cell *148*, 309–321.
19. Blackstone, C. (2012). Cellular pathways of hereditary spastic paraplegia. Annu. Rev. Neurosci. *35*, 25–47.
20. Hübner, C.A., and Kurth, I. (2014). Membrane-shaping disorders: a common pathway in axon degeneration. Brain. http://dx.doi.org/10.1093/brain/awu287.

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Symmetric Development: Transcriptional Regulation of Symmetry Transition in Plants

Symmetry breaking and re-establishment is an important developmental process that occurs during the development of multicellular organisms. A new report determines that transcription factors regulate a symmetry transition event in plants by modifying the direction of auxin transport. This provides one of the first mechanistic descriptions of a transition from bilateral to radial symmetry in plants.

Liam Dolan

Two flattened leaf-like structures fuse to form the bilaterally symmetrical carpel in the *Arabidopsis thaliana* flower. Early in development, the organ is bilaterally symmetrical along its entire length. Then, a symmetry-breaking event occurs in cells in the distal regions which become committed to radialization. These tissues in the distal region develop into the radially symmetric style, a specialized structure that develops a papillate surface (stigma) to which pollen adhere during reproduction. Thus, a symmetry transition event occurs during the formation of a key structure in the life cycle of a flowering plant.

The major discovery of Moubayidin and Østergaard, published recently in *Current Biology* [1], is that the basic helix-loop-helix proteins SPATULA (SPT) and INDEHISCENT (IND) are necessary and sufficient for the establishment of a radially symmetric style from bilaterally symmetric tissue in the distal region of the young