Developmental Cell, Vol. 8, June, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.devcel.2005.05.011

From Sensing Cellular Sterols to Assembling Sensory Structures

The genome of the nematode *C. elegans* is peppered with novel genes belonging to a superfamily whose members function in cellular cholesterol homeostasis (Niemann-Pick C1) and Hedgehog signal transduction (Patched) and biogenesis (Dispatched). In this issue of *Developmental Cell* and an analysis of a pair of Patched- and Dispatched-related proteins in *C. elegans* extends the superfamily's repertoire to include the formation of tubular organs.

Members of what could be called the eukaryotic RND-SSD superfamily (Figure 1) function in a surprising diversity of cellular and developmental processes. These proteins are hybrids of sorts, related to prokaryotic resistance-nodulation-division (RND) permeases, proton antiporters that catalyze the transmembrane efflux of numerous substrates. They also contain a conserved region of five membrane-spanning α helices—the sterol-sensing domain (SSD)—first identified in enzymes and regulatory proteins of the cholesterol metabolic pathway. The archetypal superfamily member is Patched (Ptc), the inhibitory receptor for cholesterol-linked Hedgehog (Hh) morphogens. Studies of Hh signaling spawned another member, Dispatched. The cholesterol moiety of Hh can stably anchor the protein to membranes, but Dispatched catalyzes Hh release during biogenesis for long-range signaling. The Niemann-Pick C1 protein (NPC1) functions in the redistribution of cholesterol and glycosphingolipids internalized through endocytosis, and its absence underlies a catastrophic lysosomal storage disorder marked by cholesterol accumulation. Because of their complexity, "RND" and "SSD" each could be the focus of an entire Preview. Here, I focus on the latter.

SCAP (Figure 1) has the most intensively studied SSD. With sterol deprivation, SCAP shuttles SREBPs (the transcription factors that control expression of cholesterol metabolic genes) from the endoplasmic reticulum (ER) to the Golgi, where they are activated by proteolysis. When cellular cholesterol rises, feedback inhibition is achieved when cholesterol binds the SCAP SSD directly, causing a conformational change that allows a third protein to retain SCAP and SREBPs in the ER (Radhakrishnan et al., 2004). Mutations of key residues in the SSD disable it and cause SCAP to shuttle SREBPs to the Golgi constitutively. These key residues are conserved in NPC1 and Ptc, and the NPC1 SSD probably also binds cholesterol directly (Ohgami et al., 2004). Analogous mutations in NPC1 cause intracellular cholesterol accumulation. NPC1 normally enters the complex internal membranes of late endosomes, but the SSD mutant is retained on the limiting membrane (Watari et al., 1999). The story for Ptc is not so straightforward. One analogous mutation in *Drosophila* Ptc results in a protein that fails to inhibit Hh signaling but still sequesters Hh in endocytic vesicles (Martín et al., 2001; Strutt et al., 2001). Similar SSD mutations do not affect the ability of mammalian Ptc-1 to inhibit Hh signaling (Taipale et al., 2002).

If you are not bewildered by these proteins already, you will be after considering findings from the *C. elegans* genome. *C. elegans* has nearly 30 genes for RND-SSD proteins related to Ptc, Disp, and NPC1 (Kuwabara et al., 2000). The majority of these genes are most like Ptc. Yet *C. elegans* has no Hh orthologs, and the potentially ligandless Ptc-1 ortholog functions in cytokinesis in the gonad (Kuwabara et al., 2000). Perens and Shaham (2005) now demonstrate novel functions for the Ptc-related protein DAF-6 and describe its interaction with the Disp-related protein CHE-14 (Michaux et al., 2000).

A pair of sensory structures called amphids comprises the simple "nose" of *C. elegans*. Each amphid is innervated by neurons that have terminal cilia extending through a channel formed by two glial cells. As the amphids sense multiple stimuli, mutations affecting their formation were uncovered through a variety of functional screens, such as chemotaxis (e.g., *che-14*) and dauer formation (e.g., *daf-6*). The *daf-6* and *che-14* genes nonetheless appear to have related cellular functions during morphogenesis of the amphid channel. Formation of the amphid channel and other tubular tissues and organs may ensue through the coalescence of cytoplasmic vesicles at an apical epithelial surface. Apical membrane biogenesis, vesicular transport, and a balance between endocytosis and exocytosis are probably all key to cellular tube formation. The lumen of the amphid channel is defective in both *daf-6* and *che-14* animals.

Although DAF-6 function is required for lumen formation, CHE-14 functions in channel maintenance. DAF-6 and CHE-14 proteins are coexpressed in amphid glia and epithelia of other tubular organs (e.g., intestine, vulva) and function in concert to regulate lumen formation in a variety of these contexts. Both proteins localized predominantly to apical membranes and luminal surfaces and, to a lesser extent, in punctate cytoplasmic structures (presumably vesicles). Neuronal mutants that fail to form cilia have amphid glia with morphology strikingly like *daf-6* mutants and caused DAF-6 to localize in a large cytoplasmic structure instead of the cell surface. Thus, neuronal cilia provide a cue that is required for amphid-channel lumen formation and affects the subcellular distribution of DAF-6. Because Hh also influences the subcellular localization of Ptc, this observation may imply interactions between DAF-6 and an unknown neuron-derived signal. What about the DAF-6 SSD? Two mutants had no change in function, although one caused a fraction of the protein to have a diffuse intracellular distribution reminiscent of the ER.

Because of the apical membrane defects in *daf-6* and *che-14* mutants and potential defects in membrane deposition/stabilization during cytokinesis in *C. elegans ptc-1* mutants, it has been proposed that these proteins

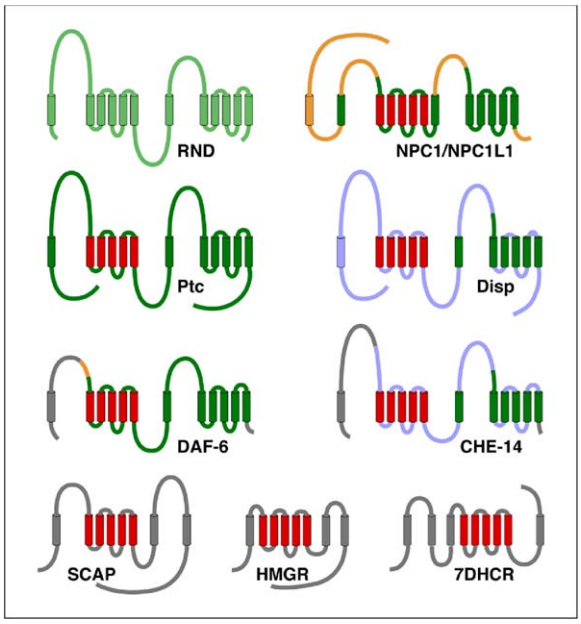

Figure 1. Schematic Structures of RND-SSD Superfamily Members and SSD-Only Proteins

Conserved regions among the most closely related members are color coded and are based on a combination of Clustal W and Blast analyses. Cylinders represent transmembrane (TM) segments with the five SSD segments in red; extramembrane loops are only roughly to scale. A generic representation of an RND family member (light green) is based on the *E. coli* AcrB protein. As the archetypal RND-SSD member, Ptc has weak homology to RND permeases throughout (dark green), including within the SSD. Regions with strong similarity to Ptc are shown in dark green in other superfamily members. Regions unique or strongly similar to NPC1 are in gold, and those unique or similar to Disp are in lavender. Regions with no significant similarities are in gray. Abbreviations not in the text are HMGR, 3-hydroxymethyl-3-glutaryl coenzyme A reductase; 7DHCR, 7-dehydrocholesterol reductase; and SCAP, sterol response element binding protein (SREBP)-cleavage activating protein.

may function in bulk membrane transport. Perens and Shaham favor a model in which DAF-6 and CHE-14 promote deposition of membrane as the lumen grows. However, the cell-surface localization observed for PTC-1, DAF-6, and CHE-14 suggests other models. NPC1 is exclusively endosomal, and although fly and vertebrate Ptc can be detected on the cell surface biochemically, standard imaging techniques reveal only vesicular signal. One situation where stable surface localization of Ptc was imaged was when the rate of Ptc internalization was slowed by a transmembrane form of Hh on an adjacent cell (Incardona et al., 2000). Could DAF-6 be stabilized at the cell surface as a receptor for the lumen-forming cue from neuronal cilia? What stabilizes *C. elegans* PTC-1 at the plasma membrane?

So many fundamental questions remain regarding these enigmatic proteins. Are the Ptc-related proteins orphan receptors in need of ligands or binding partners? Are they catalytic or structural? Are all SSDs really sterol or lipid sensors? Are they multimeric (like bona fide RND permeases)? Do they have small molecule substrates or regulators or even require a proton gradient for function? I could go on. Hopefully, study of the multiplicity of *C. elegans* RND-SSD genes will not complicate the situation further but perhaps help identify a unifying theme to this bewildering superfamily.

John P. Incardona
Environmental Conservation Division
Northwest Fisheries Science Center
National Oceanic and Atmospheric Administration
2725 Montlake Blvd E
Seattle, Washington 98112

Selected Reading

Incardona, J.P., Lee, J.H., Robertson, C.P., Enga, K., Kapur, R.P., and Roelink, H. (2000). Proc. Natl. Acad. Sci. USA *97*, 12044–12049.

Kuwabara, P.E., Lee, M.H., Schedl, T., and Jefferis, G.S. (2000). Genes Dev. *14*, 1933–1944.

Martín, V., Carrillo, G., Torroja, C., and Guerrero, I. (2001). Curr. Biol. *11*, 601–607.

Michaux, G., Gansmuller, A., Hindelang, C., and Labouesse, M. (2000). Curr. Biol. *10*, 1098–1107.

Ohgami, N., Ko, D.C., Thomas, M., Scott, M.P., Chang, C.C., and Chang, T.Y. (2004). Proc. Natl. Acad. Sci. USA *101*, 12473–12478.

Perens, E.A., and Shaham, S. (2005). Dev. Cell *8*, this issue, 893–906.

Radhakrishnan, A., Sun, L.P., Kwon, H.J., Brown, M.S., and Goldstein, J.L. (2004). Mol. Cell *15*, 259–268.

Strutt, H., Thomas, C., Nakano, Y., Stark, D., Neave, B., Taylor, A.M., and Ingham, P.W. (2001). Curr. Biol. *11*, 608–613.

Taipale, J., Cooper, M.K., Maiti, T., and Beachy, P.A. (2002). Nature *418*, 892–897.

Watari, H., Blanchette-Mackie, E.J., Dwyer, N.K., Watari, M., Neufeld, E.B., Patel, S., Pentchev, P.G., and Strauss, J.F.I. (1999). J. Biol. Chem. *274*, 21861–21866.