How the ER Stays in Shape

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Understanding the molecular mechanisms that control the architecture of organelles is an area of intense study. In this issue of *Cell*, Voeltz et al. (2006) report that two membrane proteins, Rtn4a/NogoA and DP1/Yop1p, are responsible for the generation of tubular morphology in the endoplasmic reticulum (ER). The unusual membrane topology of these proteins may directly contribute to ER curvature.

The endoplasmic reticulum (ER) is an organelle enclosed in a continuous membrane, with a common lumenal space and several morphologically distinct areas. Around the nucleus, the ER takes the form of membrane sheets, which are interrupted by nuclear pores and stabilized by connections to the underlying lamina. Closer to the cell periphery the ER exists in a continuous network of membrane tubules with 3-way junctions that extend throughout the entire cytoplasm. In this issue of *Cell*, Rapoport and coworkers (Voeltz et al., 2006) describe two proteins, Rnt4a and DP1, that contribute to the formation and maintenance of ER tubules.

The starting point for their work was an assay that the Rapoport lab had developed to follow ER network formation using vesicles purified from *Xenopus* oocytes. After much hard work and heavy-duty biochemical analysis, Voeltz et al. (2006) discovered that the small vesicles coalesce and become transformed into an extensive tubular network in a reaction that requires only physiological concentrations of salt, GTP, and other components that are tightly associated with the ER vesicles. However, the investigators faced a difficult problem because the membrane vesicles both contain the factors responsible for tubulogenesis and are themselves the substrates upon which these factors act. Thus, the classical biochemical approach of purification and reconstitution was impossible. To bypass this roadblock, the investigators used a pharmacological strategy, first finding a drug that inhibits tubulogenesis and then identifying the drug's target. The authors found that extremely low levels of the sulfhydryl modifying reagent NEM inhibited network formation without impacting the vesicle fusion step. The cysteine residues targeted by NEM were also accessible to modified versions of NEM. The investigators took advantage of this, using a subtractive purification strategy to identify the target protein, a reticulon called Rtn4a. Rtn4a, also known as NogoA, has previously been identified as a factor involved in the inhibition of neurite outgrowth (Woolf, 2003). As one might predict for a protein involved in ER tubular network formation, Rtn4a is specifically located in the tubular subcompartments of the ER. Moreover, overexpression of Rtn4a enhances the formation of ER tubules at the expense of sheets, and anti-Rtn4a antibodies inhibit the formation of tubules in vitro (Voeltz et al., 2006).

Reticulons are ubiquitously expressed proteins, although in humans some

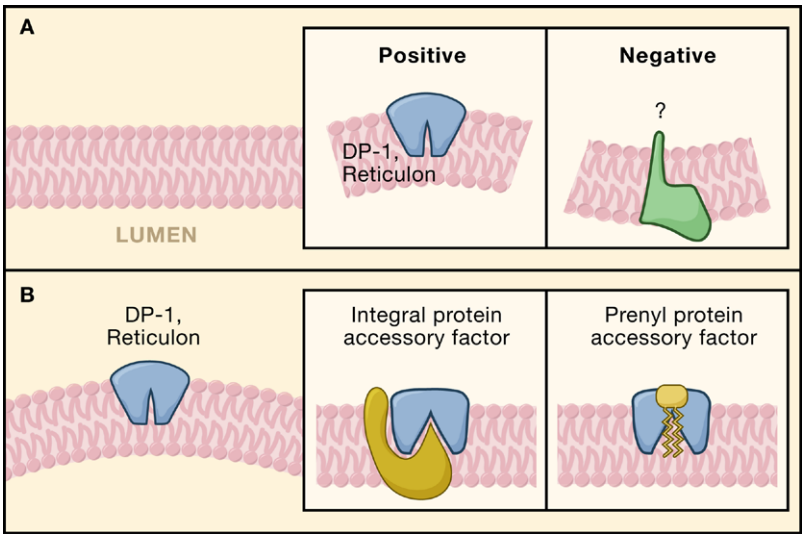

Figure 1. Protein Topology and the Control of Membrane Curvature
(A) The work of Voeltz et al. (2006) suggests that the insertion of a reticulon protein or DP1 into the endoplasmic reticulum membrane leads to the stabilization of positive membrane curvature. Proteins with topologies that are the opposite of reticulons or DP1, where the bulk of the membrane-embedded portion of the protein resides in the inner leaflet of the lipid bilayer, may instead stabilize negative curvature. Some membrane deformations in the endomembrane system, for example vesicle budding, require the generation of both positive and negative curvature.
(B) Depicted is a hypothetical model in which an accessory integral membrane protein changes the shape of reticulon/DP1 to influence membrane curvature. An alternative possibility is that the prenyl moieties of peripheral membrane proteins could also influence reticulon/DP1 proteins.

splice variants of the four genes may have distributions that are tissue specific. Given the evolutionary conservation of the reticulon, Rtn4a, it is possible to determine whether reticulons are required for the formation of ER networks in the model eukaryote, the budding yeast *Saccharomyces cerevisiae*. *S. cerevisiae* contains two reticulon genes (*RTN1* and *RTN2*), and surprisingly, when both of these are deleted, the morphology of the peripheral ER network is unaffected. At this stage, faced with such definitive negative data, many investigators would have abandoned the model. Instead, Voeltz et al. (2006) sought to determine if there were other factors, associating with Rtn4a, whose elimination would also be required before an impact on ER tubular formation would be observed in vivo. The investigators returned to the original purification experiment, reduced the stringency of the washing conditions, and also determined if any proteins could be copurified by immunoprecipitation with Rtn4a. Both approaches yielded the membrane protein DP1 (Deleted in Polyposis Locus Protein 1), an evolutionarily conserved protein whose *S. cerevisiae* counterpart is Yop1p. Like reticulons, Yop1p localizes to the peripheral ER and, importantly, when both *YOP1* and *RTN1* are deleted in yeast, there is a dramatic disruption of ER tubular morphology. The investigators suggest that the effect of combining *RTN1*, *RTN2*, and *YOP1* deletions results from a threshold effect; it is the relative abundance of the protein that dictates its impact on tubular morphology in vivo. Disruptions in the tubular network are only observed when the number of reticulon and Yop1 molecules dips below a critical point.

What is the mechanism by which reticulons and DP1/Yop1p influence tubule formation? Voeltz et al. (2006) propose that the answer involves protein topology. Both reticulon proteins and DP1 contain large hydrophobic segments that are longer than conventional α-helical transmembrane domains. Voeltz et al. (2006) provide data suggesting that these domains adopt a hairpin insertion into the lipid bilayer, reminiscent of caveolin. This topology results in the bulk of the hydrophobic portion of the protein being preferentially located in the outer leaflet of the lipid bilayer. The net result is that the protein has a "wedge-shaped" envelope and its insertion into a lipid bilayer might create membrane curvature by providing more bulk in the outer leaflet (Figure 1A). This model would account for the differential sensitivity of the in vitro and in vivo models to the loss of the various components and provides a clear starting point for future mechanistic analyses of the function of these proteins.

This beautiful study is a textbook case of intellectual courage and experimental discovery in molecular cell biology. The parallel approaches of biochemical purification, cell biological analysis, and phenotypic and genetic functional assays together argue convincingly for the role of reticulons and associated proteins in the creation of tubular networks and organelle morphology. We now have a new protein component that is able to directly control membrane curvature in the endomembrane system to add to those already known (Lee et al., 2005; Peter et al., 2004; Takei et al., 1995).

This study opens up new areas for discovery. Now that we know how reticulons and DP1/Yop1p function at a molecular level, we can begin to address how they work in cell and organismal physiology. For instance, how might ER tubular network formation via Rtn4a/NogoA relate to neurite outgrowth? Voeltz et al. (2006) demonstrate that the overexpression of Rtn4a leads to long processes in a cultured fibroblast-like cell line. Could these cells be a model for nerve growth cones and, if so, what is the relationship between the ER and the plasma membrane extensions? Are there functional connections with other processes involving subcellular tubule formation, such as the initial events of vesicle fusion that lead to tracheal development in the fruit fly *Drosophila*? Perhaps reticulons and DP1 are especially important for the development and maintenance of the sarcoplasmic reticulum in muscle cells, which have a particularly dramatic ER morphology. DP1 proteins have also been shown to facilitate the expression and transport of odorant receptors (Saito et al., 2004). How might the shape of DP1 and its role in tubule formation influence G protein-coupled receptor expression? Reticulon proteins are also known to be posttranslationally modified by phosphorylation. Reticulons could conceivably play a role in the breakdown and subsequent reassembly of the ER that is observed during mitosis in higher eukaryotes.

At a more basic level, this study suggests new ways of thinking about the evolution of membrane curvature in the endomembrane system. In future work, we will want to learn more about the proteins themselves, such as how regions other than the conserved reticulon family domain modulate reticulon function. It is also unclear how these proteins, in the absence of a signal sequence, become incorporated into the ER membrane. Another unknown is what factor underlies the requirement for GTP hydrolysis in network formation.

Small vesicles, the starting material for the in vitro assay used by Voeltz et al. (2006), have the same degree of membrane curvature as the tubules that are the product of the assay, raising the question as to what drives network formation. The homo- and hetero-oligomerization properties of reticulons and DP1 may influence membrane tubule formation and diameter. In addition, reticulons and DP1/Yop1p have also been reported to interact with other proteins. Accessory factors may associate with the reticulon/DP1 proteins to regulate their shape and ability to influence membrane curvature. For example, the yeast Rtn1p is known to interact with Yip3p (Geng et al., 2005), a membrane receptor for prenylated Rab proteins (Sivars et al., 2003) that cycles between the ER and Golgi (Otte et al., 2001). By associating with reticulons, these factors may allosterically regulate or alter the shape of the reticulon/DP1 proteins, thereby blocking their ability to influence membrane curvature. Tubule formation might proceed as these blocking factors are released (Figure 1B).

Elucidating the molecular mechanisms that control organelle shape, as exemplified by the work of Voeltz et al.

(2006), provides fertile ground for new hypotheses about how organelles are formed and function. Future work that unravels the biological roles of reticulons and DP1/Yop1p promises to yield more fascinating insights.

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Inflammation and Sex Steroid Receptors: A Motif for Change

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Homeostasis in reproductive tissues requires integration of hormonal and inflammatory signals. In this issue of *Cell*, Zhu et al. (2006) discover that proinflammatory signals switch repressed steroid hormone receptors into transcriptional activators by targeting TAB2, an adaptor protein that tethers corepressors. These findings have implications for the treatment of endocrine-resistant cancers.

Inflammation plays a crucial role in the protective response against infections as well as in tissue remodeling in many physiological processes, such as reproduction. The precise control of inflammation is essential for the prevention of chronic inflammatory disorders as well as for inhibiting the exacerbation or progression of diseases such as atherosclerosis and cancer. Natural and synthetic steroids can target distinct steps in the inflammatory process, and considerable progress has been made in elucidating the molecular mechanisms involved. Conversely, relatively little is known about the impact of inflammatory molecules on steroid hormone action. In this issue of *Cell*, Zhu et al. (2006) report that the proinflammatory cytokine interleukin-1β (IL-1β) reverses the inhibitory effects of sex hormone receptor antagonists. This surprising finding may have profound implications for the treatment of certain hormone-dependent cancers. In fact, their observations may be of broader physiological significance as the authors also show that IL-1β reverses the inhibitory effects of natural steroids, more specifically 17-β-estradiol (E_2), in MCF7 breast cancer cells.

The sex steroids (estrogens, androgens, and progestins) are not only indispensable for reproduction but also control many diverse physiological functions in other tissues. They are also implicated in the initiation and progression of many benign and malignant disease processes, perhaps most prominently in breast and prostate cancer. Their action is mediated by specific receptors that are members of the nuclear receptor family of ligand-dependent transcription factors. Consequently selective antagonists for the estrogen receptor and the androgen receptor are widely used for the prevention and treatment of breast and prostate cancers, respectively. Activation or repression of gene transcription by nuclear receptors is dependent on the recruitment of coactivators or corepressors that form scaffolds for the assembly of chromatin remodeling enzyme complexes. The ability of steroid hormones to control the transcription of distinct gene networks in target cells seems to reflect, in part, the recruitment of different chromatin remodeling complexes. The generation of diverse physiological responses, however, requires further