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Rough Sheets and Smooth Tubules

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The endoplasmic reticulum (ER) has distinct morphological domains composed of sheets and tubules, which differ in their characteristic membrane curvature. Key proteins may drive the formation of these structural morphologies, which in turn could generate the rough and smooth functional domains of the ER.

The endoplasmic reticulum (ER) is among the most architecturally striking of all eukaryotic organelles. It is composed of the nuclear envelope, sheet-like cisternae, and a polygonal array of tubules connected by three-way junctions (Figure 1A). The relative amounts of these different domains vary greatly depending on the cell type. Invariably, however, they are all part of a single interconnected membrane system that contains a common luminal space and often extends to the farthest reaches of the cell.

ER sheets are relatively flat areas where the membrane extends for many microns with little membrane curvature. Although the nuclear envelope is spherical, the nucleus is so large that its surface can also be considered as a flat ER sheet. In contrast, ER tubules are long cylindrical units with high membrane curvature in cross-section (reviewed in Voeltz et al., 2002). Both sheet and tubular domains are present in all eukaryotes, from plants to humans (reviewed in Staehelin, 1997), but they have various organizations in different cells and species. In yeast, the peripheral ER (that is, any ER other than the nuclear envelope) is located close to the cell cortex and has only a few tubules connecting it to the nuclear envelope; in higher eukaryotes, the peripheral ER extends throughout the entire volume of the cytoplasm. Regardless of the cell type or subcellular location, the thickness of a sheet and the diameter of a tubule is typically 60–100 nm. This conserved structural regularity of the domains suggests that both sheets and tubules are being shaped actively.

The morphology of the ER was originally classified by electron microscopy into rough (RER) or smooth (SER) membrane domains (Figures 1B and 1C). The RER is defined by the presence of membrane bound ribosomes and performs all functions associated with the biosynthesis of membrane and secretory proteins, including their proper folding and modification. Conversely, the SER is simply defined by the absence of membrane bound ribosomes. The SER includes the ribosome-free areas of transitional ER, where vesicle budding and fusion take place, as well as zones of contact with other organellar membranes, possibly for the purpose of delivering lipids to them. Larger and more homogeneous forms of the SER are found in specialized cells—in the adrenal cells that secrete large amounts of steroids, in muscle cells with their sarcoplasmic reticulum that modulates Ca^{2+} levels to control muscle contraction, and in liver cells that make large amounts of enzymes for detoxification (reviewed in Baumann and Walz, 2001). Although the RER and SER are continuous membrane domains, they are spatially sepa-

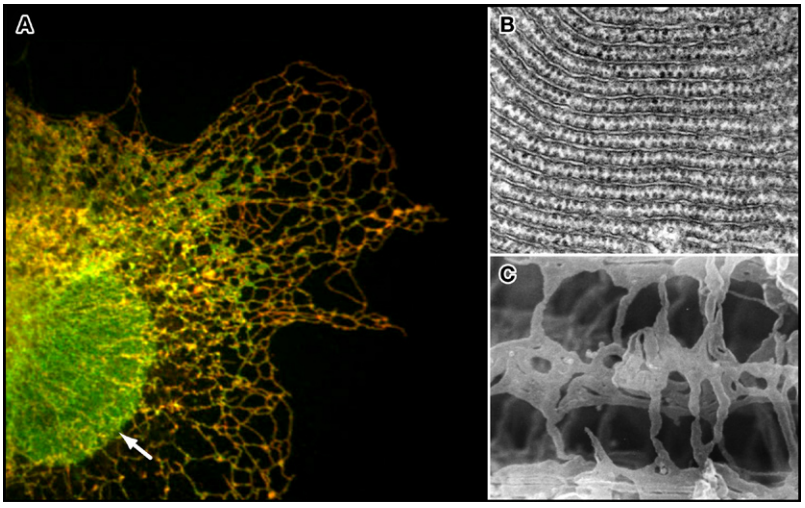

Figure 1. Different Structural Subdomains of the Endoplasmic Reticulum
(A) A cultured cell line coexpressing Rtn4c (red) and Sec61β (green) shows the low-curvature domains of the nuclear envelope (arrow) and the cisternal sheets (green), as well as the high-curvature domains of the peripheral tubules (red).
(B) Thin-section electron micrograph of the RER in secretory cells from the silk glands of the silkworm. Image courtesy of Takao Senda.
(C) Scanning electron micrograph of the SER in the sarcoplasmic reticulum of rat white skeletal muscle fibers. Image courtesy of Takuro Ogata. Note that the RER in (B) appears as sheets, whereas the SER in (C) is tubular.

rated. This is exemplified in neurons, where the RER predominates in cell bodies and the proximal portions of dendrites, whereas the SER is primarily found in axons and the distal tips of all neurites.

What might be the connection between the morphological distinction of sheets and tubules on the one hand, and RER and SER on the other? How can a continuous membrane system have segregated sheet and tubule structures or discrete areas with or without bound ribosomes? Here, we discuss how certain proteins may drive formation of membrane domains of low or high curvature—the sheets and tubules—and how the curvature itself may dictate the partitioning of other proteins, creating distinct functional domains. Differences in membrane curvature would thus ultimately differentiate the rough and smooth subdomains of the ER.

Setting up Tubules: Curving a Membrane

The frequent proximity of ER tubules with cytoskeletal elements suggests that the cytoskeleton might play a role in tubule structure. ER tubules can be pulled out from a membrane reservoir by molecular motors as they move along microtubules or actin filaments, or by the tips of microtubules or actin filaments as they grow by polymerization (reviewed in Du et al., 2004). However, although the cytoskeleton is required for the extension of the ER network, the alignment of membrane tubules with the cytoskeleton is not perfect, and the network does not rapidly collapse upon depolymerization of microtubules or actin filaments (Terasaki et al., 1986). In addition, ER networks can be formed in vitro from small vesicles where an intact microtubule or actin network is not required (Dreier and Rapoport, 2000). Thus, the cytoskeleton is most likely unnecessary for determining or maintaining the shape of ER tubules.

The most plausible models for shaping tubules are based on mechanisms that generate or stabilize high curvature in membranes. Although several different mechanisms can be envisioned, recent results from our group demonstrate that a class of integral membrane proteins, the reticulon and DP1 protein families, are responsible for generating and maintaining ER tubules (Voeltz et al., 2006). The reticulons and DP1 are ubiquitous proteins found in most, if not all, eukaryotic cells. These proteins are localized exclusively to ER tubules, avoiding the nuclear envelope and the sheets of the peripheral ER. Their overexpression generates long unbranched tubules, whereas their deletion leads to loss of tubular ER in yeast (Voeltz et al., 2006).

There is no primary sequence similarity between the reticulon and DP1 families; however, each family has two hydrophobic domains that are highly conserved, both of which seem to form a hairpin within the phospholipid bilayer without exposing a significant portion of itself on the luminal side of the membrane. We envision that these proteins may stabilize the high curvature of ER tubules by "wedging" themselves into the outer membrane leaflet, creating the necessary increase in local surface area needed for high membrane curvature (Voeltz et al., 2006). Similar models have been proposed for the generation of membrane curvature in other systems (reviewed in McMahon and Gallop, 2005; Zimmerberg and Kozlov, 2006). The total area of the outer membrane leaflet relative to the inner membrane leaflet must be increased by 10% or more along the entire length of the tubule for this mechanism to be effective (Zimmerberg and Kozlov, 2006). This indicates that any proteins involved in tubule formation must be highly abundant. In yeast one of the reticulons (Rtn1p) and the DP1 homolog Yop1p appear to be two of the most plentiful membrane proteins found in the ER.

An interesting aspect of tubule formation is that the high membrane curvature of a tubule occurs along only one dimension. The reticulons and DP1/Yop1p form extensive homo- and hetero-oligomers, and we propose that these proteins may generate a scaffold within the membrane along the tubule's length to create this anisotropy.

Sheet Formation: Flattening a Membrane

Creating the sheet-like domains of the nuclear envelope and the peripheral cisternae confronts the opposite problem from tubule formation: overall curvature between two membrane bilayers must be reduced and "flattened" out. In addition, the two membrane sheets must maintain a constant separation over a large area. Could sheet-like domains simply be caused by an absence of tubule-forming proteins? Some type of "tug-of-war" between sheet and tubule formation is suggested by the observation that overexpression of the reticulon isoform Rtn4a in mammalian cells leads to the proliferation of bundled ER tubules, whereas peripheral sheets are reduced. Likewise, when the reticulons and Yop1p are deleted in yeast, the tubules disappear and ER sheets predominate (Voeltz et al., 2006).

Considering the regularity of the lumen's width, however, it is more likely that structural elements are also needed to stabilize the nuclear envelope and the sheet-like peripheral cisternae. It has been proposed that ER luminal chaperones form a matrix that serves as a scaffold for membrane proteins, but data indicate that any such scaffold is highly dynamic (Nehls et al., 2000). Perhaps a more attractive model is that the luminal domains of abundant membrane proteins associate between the two membranes, forming a bridge so that the membranes maintain a constant distance from each other.

Interactions between membrane proteins on the cytoplasmic side have been known to generate broad planar membrane-membrane interactions. The Golgi apparatus is thought to use such a mechanism to stack its cisternae—members of the golgin family of proteins appear to interact with each other through their long cytoplasmic coiled-coil domains to bring individual cisternae together (reviewed in Short et al., 2005). Large stacks of smooth ER sheets (called

organized smooth ER; OSER) can form when certain membrane proteins that interact weakly through their cytoplasmic domains are overexpressed (Snapp et al., 2003). Similarly, canine p180, an RER protein with an extensive cytosolic coiled-coil domain, induces stacking of RER cisternae when overexpressed in yeast (Becker et al., 1999).

It is quite likely that an analogous mechanism exists in which protein-protein interactions on the luminal side generate ER sheets. Deep-etch electron microscopy indicates that, in both invertebrate and vertebrate cells, the nuclear envelope and the peripheral sheets are filled with proteinaceous bridges that span the width of the entire lumen (Senda and Yoshinaga-Hirabayashi, 1998). It is possible, however, that the nuclear envelope and the peripheral sheets employ different protein components to achieve sheet formation.

The best candidates to flatten the nuclear envelope may be the SUN proteins—membrane proteins specific to the inner nuclear membrane. SUN proteins span a major portion of the nuclear envelope lumen and are thought to interact with the nesprins, large proteins that sit in the outer nuclear membrane and connect the nucleus with the actin cytoskeleton (Crisp et al., 2006). Notably, the width of the luminal domain of the nuclear envelope becomes irregular upon downregulation of SUN protein expression (Crisp et al., 2006). The nuclear pore complex (NPC) likewise spans the entire diameter of the nuclear envelope and also may help in flattening the nuclear envelope, but it is unclear how important a role it plays. NPCs are absent from some regions of the yeast nuclear envelope (Jordan et al., 1977), and the number of NPCs varies widely between different cell types, with some dormant cells containing only a handful (Hetzer et al., 2005).

What stabilizes the sheets of the peripheral ER is even less clear. One candidate may be Climp-63 (cytoskeleton linking membrane protein), an RER membrane protein that is not present in the nuclear envelope (Klopfenstein et al., 2001). This protein has a large coiled-coil luminal domain that aids in extensive homo-oligomerization. The purified luminal domain forms 91 nm long rods, which are long enough to span the ER lumen (Klopfenstein et al., 2001). In addition, overexpression of Climp-63 in mammalian cells leads to proliferation of ER sheets (Y.S., G.K.V., T.A.R, unpublished data). This protein, however, seems to be present only in vertebrates, so other proteins that aid in peripheral sheet formation must also exist.

Links between ER Morphologies

The simplest idea is that sheets correspond to RER and tubules to SER. Classic electron microscopy depicts the RER ultrastructure as sheets of ribosome-covered cisternae stacked on top of each other. The SER, on the other hand, usually appears as vesicular-tubular structures (Fawcett, 1981). Moreover, one sees a proliferation of either sheets or tubules in certain cell types where the ER has become specialized for either RER or SER functions, respectively. Plasma B cells, which are antibody-secreting "factories," are filled almost completely with RER arranged in regular stacked sheets, whereas in adrenocortical and muscle cells, the ER appears as an abundant tubular network (Fawcett, 1981; Ogata and Yamasaki, 1997).

One may argue that ribosomes are known to reside on tubules, and that overexpression of certain SER proteins leads to arrays of smooth ER sheets as OSER formations (Baumann and Walz, 2001). However, it is unclear whether the ribosomes on tubules are part of active polysomes, or whether smooth ER sheets are caused by overexpression of membrane proteins.

Light microscopy is perhaps the most appropriate technique to localize individual ER proteins in sheets or tubules. Unfortunately, most of the studies done to date either have not preserved the ultrastructure of the ER or involve overexpression analysis, which generally leads to the proteins overflowing into both sheets and tubules. Nevertheless, several endogenous RER proteins do appear to become localized exclusively to the sheet domains in tissue culture cells (Y.S., G.K.V., T.A.R., unpublished data).

Segregation of Rough from Smooth ER Proteins

If the distinction between RER and SER indeed correlates with sheets and tubules, respectively, this raises the possibility that the different membrane curvatures of these two domains may be responsible for segregating RER and SER proteins. SER proteins most likely are not actively sorted away from the RER, however, because classic cell fractionation experiments show that although RER proteins are generally found only in dense ribosome-containing membrane fractions, most SER proteins are found in both dense and light fractions. RER proteins expressed in neurons of the worm *Caenorhabditis elegans* are also confined largely to the cell body, whereas SER proteins become localized to both the cell body and neurites (Rolls et al., 2002).

These observations lead to the assumption that most SER proteins are in both rough and smooth membrane domains, and that they have no preference for low-curvature sheets or high-curvature tubules. Of course, there may be notable exceptions where some SER proteins may have wedge shapes or physically associate with the reticulons or DP1/Yop1p, allowing them to partition preferentially into tubules. For RER proteins, however, we hypothesize that an active sorting mechanism restricts them to low-curvature sheets. These proteins may either have shapes that make it energetically unfavorable for them to sit in a curved membrane, or more likely, they may form large complexes that do not easily partition into tubules.

One possibility for how RER proteins segregate into sheets is that protein translocation complexes are bound together by linker proteins, perhaps even by the cytoskeleton, into large arrays. Alternatively, large

complexes may form as a result of the binding of ribosomes. Kreibich and colleagues have used FRAP (fluorescence recovery after photobleaching) to show that active translocons diffuse slowly within the membrane, consistent with the existence of large oligomers of protein translocation complexes (Nikonov et al., 2002). The association of RER proteins with ribosomes may also explain why these proteins are confined to cell bodies in *C. elegans* neurons, whereas SER proteins can freely diffuse into neurites (Rolls et al., 2002).

The high curvature of tubules may not be an obstacle for binding of nontranslating ribosomes or small polysomes because the protein translocation channel occupies only ~5%–7% of the 60 nm tubular cross-section. In addition, rough microsomes, which are small vesicles with a similar degree of curvature to ER tubules, can bind to translating ribosomes in vitro. Large polysomes, however, contain a dozen or more ribosomes on a single mRNA and are arranged on the ER in distinct and conserved shapes that adopt hairpin or spiral configurations, where the spiral configuration may be due to the polysome's intrinsic tendency to bend (Christensen and Bourne, 1999). It is difficult to imagine how such large and conserved polysomal shapes could sit comfortably on the highly curved surface of an ER tubule. All of these considerations suggest that polysomes segregate RER proteins into sheets.

Although the high curvature of tubules may generate membrane areas that are devoid of membrane bound polysomes, other SER areas are probably maintained in a different manner. For example, the transitional ER that "bulges" out of RER sheets and the nuclear envelope has a larger diameter than the cross-section of a normal tubule (Palade, 1975). It is therefore likely that mechanisms other than membrane curvature clear the transitional ER surface of ribosomes. In the case of crystalline ER composed of sinusoidal membrane arrays with cubic symmetry that occur in some physiological and pathological conditions (reviewed in Borgese et al., 2006), RER proteins may be excluded by the tight packing of abundant membrane proteins.

Functional Advantage of Segregating Sheets from Tubules

Although all cells contain a flat ER, at least in the form of the nuclear envelope, it is unclear whether tubules are absolutely essential because yeast can grow, albeit at a slower rate, when most of the tubular ER is converted into sheets by depletion of the reticulon proteins and Yop1p (Voeltz et al., 2006). Nevertheless, the conservation of both sheets and tubules indicates that they have general functional significance.

How does the differentiation into sheets and tubules actually benefit rough and smooth ER functions? As discussed above, sheets may be the best way to accommodate large polysomes on a membrane surface. In addition, the large luminal space and the possibility of two-dimensional diffusion in sheets may facilitate the processing, folding, and sorting of nascent proteins.

Although sheets may be advantageous for RER functions, the dense coverage of the membrane surface with bound ribosomes may impede SER functions. Enzymes that synthesize lipids and steroids or that act as detoxifiers, or components involved in vesicle budding and fusion, need access to the lipid surface—such access may be effectively prevented by bound ribosomes. Tubules would provide these SER proteins with a "protected" membrane domain that is inaccessible to RER proteins. Tubules have the added advantage of a higher surface-to-volume ratio compared with ER sheets. This feature would maximize the access of cytosolic phospholipid-interacting proteins and allow a higher packing density of proteins, both of which might increase the efficiency of lipid synthesis and Ca^{2+} release from the ER lumen.

Future Perspectives

Our discussion about the morphological structure of the ER touches on the broader, unresolved problem in cell biology of how the characteristic shapes of organelles are brought about and maintained. The past several decades have seen great progress in understanding how proteins are targeted to different organelles. It is now time to tackle the next level of complexity by addressing how the shape of biological organelles and their arrangement within the cell are generated and what consequences this has for organelle function. This level of complexity is inherently difficult to study, but recent advances suggest that classical problems in cell biology, such as the differences between RER and SER, will soon be solved. Membrane curvature may well hold the key for functionally defining distinct subdomains of the ER and other cellular organelles.

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