The dynamic ER: experimental approaches and current questions
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The endoplasmic reticulum (ER) is an extremely plastic and dynamic organelle. Its size and shape can undergo drastic changes to meet changing demands for ER-related functions, or as a response to drugs or pathogens. Because of the ER’s key functions in protein and lipid synthesis, this organelle is a hotbed of detailed molecular analysis.

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Introduction
There are many instances in biology documenting drastic changes in the size of the ER compartment. Some of the most dramatic cases occur when cells differentiate into types that make extensive use of the ER, for example as a secretory apparatus [1], a lipid synthetic platform [2,3] or a calcium repository [4,5]. Outside influences — such as hepatitis C virus [6], or treatment with drugs such as statins [7,8] or phenobarbital [9] — can also cause or promote the expression of proteins that alter ER structure. Despite the large number of cases where the ER undergoes dramatic changes, little is yet known about the molecular mechanisms that underlie these observations.

Perturbations that cause ER expansion range from over-expression of a single membrane protein to the activation of coordinated developmental programs that drive cells down professional secretory lineages, as is the case with the plasma B cell. One challenge is to ascertain if common mechanisms are at play in these distinct ways of inducing changes in structure and size. Another is to integrate the findings from the extremely active study of signaling pathways that monitor and control ER status, such as the unfolded protein response (UPR) [10], with the wide variety of situations that cause changes in ER size and function.

A tale of two proliferations
We will focus on two extremes in the range of dynamic ER changes: the acquisition of professional secretory status, and proliferation of the smooth ER. These examples demarcate the wide territory of ER dynamics, a territory that needs to be covered by a unified model of ER plasticity.

Plasma B cells and other professional secretory cells
When B lymphocytes differentiate into plasma B cells, they must undergo changes that allow the synthesis and secretion of prodigious amounts of immunoglobin: ~200–1000 IgM multimers per second, or roughly their own weight in protein per day! Striking EMs (Figure 1) show that the resulting cell has a massive, rough ER that appears to fill most of the cytoplasm. This transition can be recapitulated in culture by treating B cell lymphoma line L29*-+- with LPS (lipopolysaccharide), causing the cells’ terminal differentiation into immunoglobulin-secreting plasma cells [11*]. Several ‘professional’ secre- tory cells exist, and, although they undergo different routes to reach their final state, they all, to differing degrees, have expanded ERs for high-capacity secretion of their appropriate proteins. The transition to the plasma B cell is marked by the expansion of the entire ER compartment, including extension of the membrane and increased numbers of the luminal and membrane proteins that dictate the functions and identity of this compartment. The resulting ER is thus the classic ribosome-studded ‘rough ER’ that is capable of guiding new protein translocation, folding, assembly and packaging in vesicles, as would be expected. What developmental program controls this expansion? How is ER expansion coordinated, to what extent are the processes that cause smooth ER expansions (see below) involved, and how do known or novel signaling pathways regulate this dramatic functional change?

Crystalloids and other expansions of the smooth ER
There are many examples of cells that undergo equally dramatic expansions of the ER as they become proficient to synthesize lipids or detoxify drugs, both functions that occur at the ER surface. Cells of the adrenal cortex or Leydig cells of the testes that synthesize large amounts of sterols have dramatic proliferations of the ER known as crystalloid ER, so called because of the ordered appearance of the resulting membranes [2,3] (Figure 1, right). Another classic example of smooth ER proliferation is observed in the liver cells of animals treated with phenobarbital [9]. Exposures lasting only a few days result in
hepatocytes with impressive increases in their smooth ER, where the membrane-integral drug-detoxifying cytochrome P450 enzymes reside.

Unlike with plasma B-cell formation, expansion of the smooth ER can be caused by sufficiently high expression of single proteins. For example, cultured cells expressing high levels of HMG-CoA reductase (HMGR), the ER-resident rate-limiting enzyme of sterol synthesis, generate a crystalloid ER that is identical in appearance to the membrane structures observed in cells synthesizing large amounts of sterols for hormone production. Importantly, these effects of HMGR are not due to increased sterol synthesis caused by the enzyme — in both mammalian and yeast cells, the same ER proliferation effects are caused by versions of HMGR that do not possess catalytic activity. Rather, it is purely an effect of the multi-spanning C-terminal membrane anchor of HMGR. Similarly, forced expression of cytochrome P450 by molecular biological means will cause proliferation of the smooth ER resembling that caused by P450-inducing drugs. This response of the ER is broadly conserved, and is observed in both budding and fission yeast [12,13]; in all likelihood, it reflects an ancient response to the increased demand for membranes imposed by integral membrane proteins such as HMGR and cytP450. In fact, examples of membrane proteins inducing proliferation of bacterial inner membranes have also been noted [14,15], implying that this ‘capacity control’ response may pre-date the split between prokaryotes and eukaryotes. Important questions remain: what is the signal that the various structurally distinct membrane proteins send to trigger ER proliferation? What are the molecular events that underlie the expansion of the ER caused by single-protein proliferants? Are there known or novel ER signaling pathways that play a role in smooth ER proliferation? Does this ancient response play a role in the more elaborate expansion of the ER seen in secretory cells?

The role of the unfolded protein response in ER proliferation

The unfolded protein response (UPR) is a conserved signaling pathway that measures unfolded protein levels in the ER and adjusts the production of ER chaperones and degradation factors to keep the levels of misfolded proteins in the lumen acceptably low [10,16]. The range of genes controlled by the UPR includes several enzymes involved in phospholipid synthesis. Thus, it is reasonable to wonder if the UPR participates in any of the cases of ER proliferation.

UPR in the formation of a professional secretory cell

Testing the role of the UPR in the formation of these specialized cells is a complex task, since the resulting increase in secretory protein production would be expected induce the unfolded protein response, making it hard to separate cause and effect. Which comes first, UPR or expanded ER? So far, the amassed evidence does not provide a simple answer. Support for a role for UPR in ER expansion comes from several observations. The XPB1 gene, which is induced and spliced into an active form as part of the mammalian UPR, is required for successful differentiation to a plasma cell identity [17]. Similarly, mimicking the UPR by simple overexpression
of the spliced form of XPB1 causes increased phosphatidylcholine production and the appearance of intracellular membranes in mammalian cells [18]. A time-series proteomic analysis of the LPS-induced transition of B cell lymphoma L29/C15 into plasma cells indicates that the UPR might play a role in the later stages of this process [11**]. When this cell line goes down the plasma cell pathway, a variety of ER components are strongly induced significantly before the upregulation of UPR occurs. This implies that UPR-independent early events conspire to expand the ER. However, it is important to remember that the UPR in mammals has three distinct branches, numerous effectors and may involve elaborate dynamics. When sufficient tools are available, it will be informative to overdrive all three mammalian UPR branches simultaneously by molecular biological means to directly examine the effect on the size and shape of the ER compartment.

**UPR in smooth ER proliferation**

The involvement of UPR in the simpler case of smooth ER expansion caused by single proliferant proteins is not completely resolved either. However, one thing is clear. There are circumstances where smooth ER proliferation does not require the UPR. Proliferation of the yeast ER by overexpression of HMGR isozymes neither stimulates nor requires the UPR pathway [19]. Conversely, in both yeast and mammals, it has been reported that high expression levels of cytochrome P450 do cause induction of the UPR [20,21]. However, again, the UPR is not required for P450-induced proliferations in yeast. Thus, it is clear that there are UPR-independent pathways involved in the response to single-protein proliferants, at least in yeast. Whether these operate in mammalian smooth ER proliferation or in the synthesis of new ER in secretory cells is still unclear.

**Other pathways involved in ER proliferation**

Although the above results are complex, the case can be made that there are undiscovered pathways that participate in ER expansion. A separate ER-localized signaling system has been described and termed the ‘ER overload response’ or EOR. It was discovered as a response to the high-level expression of viral membrane proteins that often accompanies infection [22]. When the burden of certain ER membrane protein expression is sufficiently high, the broad-action transcription factor NF-κB is activated. In the expression profiling study mentioned above, expression of cytochrome P450 at ER-inducing levels in mammalian cells caused induction of genes characteristic of both UPR and EOR, lending some credence to the idea that EOR may be involved in some types of ER proliferation [21]. Neither the role of EOR nor the need for NF-κB in the more complex case of ER expansion during secretogenesis has yet been studied. The ability of membrane proteins to trigger this signaling pathway leads to some interesting models integrating NF-κB and membrane dynamics; however, it is important to remember that there are some types of membrane proliferation that are conserved far more broadly than the NF-κB pathway.

More open-ended genetic and genomic analyses have not revealed a concrete pathway, but the number of attempts has not been exhaustive. The Wright lab conducted a traditional genetic screen to identify mutants deficient in formation of nuclear-associated, stacked ER called ‘karmellae’ using the yeast HMGR isozyme Hmg1p. A number of genes required for trafficking to vacuole (the yeast lysosome) were identified, but surprisingly no ER-resident factors [23]. Whether this reflects a mechanistic interplay between the ER and the vacuole or an indirect physiological effect of vacuolar deficiency on ER proliferation is not yet clear. A search for genes needed for cell fitness during expression of proliferation-inducing levels of HMGR was performed by the same group. Using null-gene bar coding and microarray analysis, they identified the ER-bound ubiquitin conjugating enzyme Ubc7p as one such factor. This is interesting in that degradation of ER proteins is strongly dependent on this ubiquitin E2 in at least two ER degradation pathways [24], but how this connects to ER expansion is not clear. It was also shown that the ubc7 null mutant drastically affected the ER proliferations caused by HMGR, implying that the E2 is somehow needed for the proliferative response to Hmg1p.

**Proliferation or rearrangement: lipid status in ER expansion**

The many images of ER proliferation in the literature are quite striking (see for example Figure 1). Both in the acquisition of secretory capacity and in the simpler proliferation of smooth ER, it would appear that additional lipids are synthesized or otherwise amassed as part of the process, but little experimental attention has been directed towards this idea. Early *in vivo* studies of ER proliferation in liver indicate that phospholipids synthesis does increase in a manner consistent with increased lipid mass associated with ER expansion [25,26]. Similarly, in yeast, bulk phospholipids increase ~1.3-fold upon elevated expression of ER-proliferating cytochrome P450, without an apparent change in lipid composition [20]. Also, as mentioned above, overexpression of the UPR-induced XPB1 in mammalian cells increases the rate of phosphatidylcholine synthesis [18**].

Increased synthesis (or decreased degradation) of phospholipids could occur by a variety of mechanisms. It may be that changes in enzyme expression alter lipid levels. However, the complete list of genes induced by ER-proliferating levels of cytochrome P450 in mammalian cells does not include rate-limiting enzymes of phospholipid metabolism. Of course, this does not take into account post-transcriptional mechanisms of enzyme induction. Alternatively, lipid-synthetic enzymes might
be activated allosterically in response to membrane protein overexpression or by increased secretory cargo synthesis. Since many of the key enzymes of lipid synthesis are localized to the ER membrane, they would be correctly situated to sense an impending protein burden. Such a mechanism could either be directly affected by increased flux of proteins into the ER, or be harnessed by the regulated production of a ‘trigger protein’ that activates lipid synthesis as part of a signaling pathway, as is the case during differentiation of a professional secretory cell. The latter model would explain the role of XBP-1 in the activation of enzymes involved in phospholipid biosynthesis. Whatever the mechanism of ER proliferation, it will be important to measure the normalized amounts and types of lipids in the various examples of cells with proliferated ER, as has been done in yeast with cytochrome P450 [20], in order to ascertain if and how lipid quantities are being altered by these fairly simple perturbations. This sort of analysis would also allow more detailed comparison of the various descriptive cases of ER proliferation at a more molecular level.

**Determinants of ER proliferation**

What are the features of single proteins that cause ER proliferation? Perusal of the literature reveals a bewildering array of different features. Usually proliferants are membrane proteins, but certainly not all membrane proteins can trigger expansion of the ER. A variety of ER-destined proteins might cause proliferation, if it is a general response to increased membrane occupancy. However, it appears that cytosolic portions of proteins can in some cases be critical as well. The formation of organized stacks of ER (OSER) in mammalian cells depends on weak interactions involving both the transmembrane domain and the cytosolic fusion domain of the protein [27]. Similarly, the formation of karmellae (Hmg1p-induced ER stacks) also requires a multimerizing activity in the C-terminal cytosolic domain of the Hmg1p molecule [28]. It is unclear how multimerization triggers the biochemical changes that lead to increases in ER lipids in these special cases. From the diverse array of proteins that can alter ER structure, it seems likely that common biophysical features of proliferation may determine this capacity. What these features are and whether they are harnessed in the more elaborate expansion of the ER observed in secretory lineages is not known.

**Reversal of ER proliferation**

Very little is known about the turnover or destruction of the proliferated ER. What happens when a signal for increased ER proliferation is eliminated, or when the serum level of a proliferant drug drops? One might imagine that organelle expansion is a permanent adaptation. However, early experiments indicate that the proliferated ER is a dynamic entity that can be disassembled. The mammalian cell line UT-1 overexpresses HMGCR to such an extent that the smooth ER is highly proliferated, creating a crystalloid ER almost identical to those observed in sterol-synthesizing cells (e.g. Figure 1). When these cells are treated with sterols, HMGCR synthesis is drastically curtailed and its degradation increased, leading to a rapid decline in HMGCR levels [29,30]. Remarkably, this is associated with rapid disassembly of the proliferated ER membranes, indicating that the proliferant provides a continuous signal needed to maintain the expanded ER.

What happens to the proliferated ER? There are hints that autophagy, by which intracellular contents and whole organelles are engulfed and delivered to the lysosome, may provide a route for its destruction. Early studies on the reversal of phenobarbital-induced smooth ER in rat livers showed a drop in smooth ER levels and an increase in autophagic bodies when the drug was withdrawn [31]. Recently, Oshumi’s group has demonstrated that the yeast ER can be degraded by the autophagic pathway [32], indicating this may be a broadly conserved pathway for turnover of the expanded ER. It will be interesting to test directly the role of autophagy in yeast that have a proliferated ER. It seems that a similar mechanism may also apply in the more complex case of secretory cells undergoing downsizing of the ER. Many studies have described a process called ‘crinophagy’, in which secretory vesicles and rough ER components are delivered to the lysosome in secretory cells [33,34], and this process may be used to adjust the size of the ER in less active secretory cells [35]. With our increased knowledge of molecules that mediate autophagic processes, it will be important to evaluate crinophagy to see if it overlaps with the known autophagic pathways conserved between yeast and man.

**Conclusions**

Though the phenomenon of ER proliferation is widely observed, there are still many questions that remain to be resolved about the molecular mechanisms that control these membrane dynamics. Some of these include: the nature and composition of the various ‘brands’ of proliferations, the signaling pathways that cause them, the physiological consequences of having a proliferated ER, the overlap of proliferation with mechanisms of ER quality control, the evolutionary connections to prokaryotic membrane expansion, and the mechanism(s) of ER destruction that allow removal of these structures. These and related mysteries all demand detailed and creative investigations from membranologists of all stripes.

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**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as.
of special interest


This work indicates that the now well-defined autophagic pathway may be able to participate in reversal of ER proliferation, as indicated by the classic studies in [29,31]. This sort of analysis needs to be applied to actual cases of proliferated ER.

