Membrane biogenesis and the unfolded protein response

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In addition to serving as the entry point for newly translated polypeptides making their way through the secretory pathway, the endoplasmic reticulum (ER) also synthesizes many lipid components of the entire endomembrane system. A report published in this issue implicates a signaling pathway known to respond to ER unfolded protein load in the control of phospholipid biosynthesis by the organelle (Sriburi et al., 2004). The reasonable notion that demand for ER membrane is integrated with protein processing capacity was initially suggested by genetic analysis of yeast. The new data lend direct support for this idea and imply interesting mechanistic possibilities for how this coupling develops.

The lumen and membranes of the ER contain hundreds of different resident proteins that deal with the influx of newly synthesized, unfolded, and unassembled ER client proteins. This apparatus of resident proteins and membranes promotes early steps in the biogenesis of soluble and membrane-bound client proteins, which in their mature form function extracellularly and throughout the endomembrane system. These steps include translocation across the ER membrane, signal sequence cleavage, specific posttranslational modification, chaperone-assisted folding and complex assembly, quality control and degradation of malfolded protein, and ER-to-Golgi trafficking. Although the aforementioned apparatus is present in all eukaryotic cells, the extent to which it is present varies considerably among different cell types and is influenced by the cell's physiological state (Nunnari and Walter, 1996). Transcriptional control accounts for some of this variation, as cells with an elaborate ER have higher steady-state levels of mRNAs that encode resident proteins performing the functions listed above. Furthermore, it is clear that a developed ER and endomembrane system also require a greater lipid bilayer mass, implying that there is some level of coordination between expansion of the protein machinery and the lipids in which it is embedded.

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Abbreviations used in this paper: UPR, unfolded protein response; XBP1, X-box binding protein 1.

Identification of the unfolded protein response (UPR) provided the first clues as to how such a circuitry might function; it was noted that pharmacological and genetic manipulations that increased misfolded client protein load on the ER machinery (causing so-called ER stress) led to transcriptional up-regulation of some of its components (Kozutsumi et al., 1988). Since that finding, many details of the signaling pathways of this UPR have been worked out, first in yeast and later in mammalian cells (for reviews see Kaufman, 1999; Patil and Walter, 2001). In mammals, three ER-localized upstream stress transducers respond to perturbations in the lumen of the ER and transmit the signal across the ER membrane to downstream effectors, culminating in changes in gene expression in the nucleus and translation in the cytoplasm (Mori, 2000). The current view is that the signaling action of each transducer is repressed by free ER chaperones (i.e., chaperones unengaged by client proteins), allowing these signaling devices to measure the functional capacity of the organelle by gauging its chaperone reserve (for review see Patil and Walter, 2001). This aspect of the UPR, resembling the phylogenetically older heat shock response (Bertolotti et al., 2000), fits well with current thinking about quality control in the ER; client proteins that have passed quality control dissociate from chaperones (Ellgaard et al., 1999), rendering average chaperone occupancy a suitably inclusive measure of the organelle's functional reserve. The gene expression program activated by the UPR has also proven to be appropriately broad. Exposure of cells to agents that impair protein folding in the ER up-regulates hundreds of genes encoding components that function at all levels of the endomembrane system (Travers et al., 2000; Murray et al., 2004). These observations suggest that cells monitor client protein load at the entry point and use that information to remodel the entire endomembrane system.

The new work by Sriburi et al. (2004) focuses on the most conserved arm of the UPR, that mediated by IRE1. IRE1 is an ER-localized transmembrane kinase-endoribonuclease conserved in all known eukaryotes. Genetic studies in yeast showed that ER stress–activated IRE1 specifically cleaves the mRNA encoding its effector, the transcription factor Hac1p, leading to a noncanonical splicing event, which in turn alters mRNA structure and the coding region, ultimately promoting Hac1p expression and UPR signaling (for review see Patil and Walter, 2001). The transcription factor X-box binding protein 1

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(XBP1) proved to be the metazoan homologue of HAC1, and ER stress–mediated IRE1-dependent splicing of the *XBP1* mRNA is required for XBP1 protein expression (Yoshida et al., 2001; Calfon et al., 2002). This finding was especially interesting in light of an observation reported a few months earlier, whereby XBP1 is required in B cells for their differentiation to plasma cells (Reimold et al., 2001). Acquisition of an elaborate ER, required for high capacity immunoglobulin secretion, is one of the striking aspects of the transition from B cells to plasma cells, and is blocked in the XBP1 mutants. Because XBP1 function is absolutely subordinate to activation by IRE1, these observations led to the speculation that client protein load plays an important role in remodeling and expanding the ER, through activation of an IRE1–XBP1 signaling pathway (Yoshida et al., 2001; Calfon et al., 2002).

A key role for XBP1 in promoting ER expansion in developing B cells is now supported by the observation that enforced, retroviral expression of the active, spliced form of the *XBP1* mRNA in a B cell line was sufficient to cause expansion of the endomembrane system (Shaffer et al., 2004). Sriburi et al. (2004) have independently arrived at the same conclusion, and have gone on to show that enforced, retroviral expression of active XBP1 also led to increased activity of key enzymes involved in phospholipid biosynthesis, in some cases up to sixfold. This remarkable up-regulation of choline phosphotransferase activity in lysates of cells with deregulated XBP1 activity mimicked the increase in enzyme activity observed during normal B cell differentiation and was associated with a marked increase in cellular phospholipid content (normalized to protein content). This is the sort of biochemical effect that would be expected of a signaling pathway promoting membrane expansion of the endomembrane system.

The specific dependence on IRE1–XBP1 in this lipid response might fit into a recently proposed temporal model of XBP1 function (Kaneko and Nomura, 2003; Yoshida et al., 2003). It was noted that expression of key proteins for ER degradation is controlled by the slower IRE1–XBP1-dependent branch of the UPR, which allows cells to first fold abnormal proteins (by activities induced by the faster branches of the UPR) before inducing genes that promote a last-ditch destruction of recalcitrant malfolded client proteins (Lee et al., 2003; Yoshida et al., 2003). This model predicts that in mammals, the IRE1–XBP1 branch of the UPR is biased toward gene expression that is needed to adapt to long-term or chronic ER stress. In the same manner, it would make sense to reserve expansion of the entire ER for situations in which the burden of client proteins heralds a long-term commitment to increased ER function, such as in plasma cells and, perhaps, other professional secretory cells.

The mechanistic aspects of IRE1–XBP1-stimulated endomembrane expansion remain poorly understood. Interfering with phospholipid biosynthesis activates the UPR in both yeast and mammalian cells (Cox et al., 1997; van der Sanden et al., 2003). However, it is not clear whether UPR signaling during phospholipid limitation is triggered indirectly by a primary perturbation to ER function that secondarily affects client protein folding and activates ER stress sensors, such as IRE1, by the canonical mechanisms, or whether a change in the lipid environment modulates these sensor's activities more directly. It is noteworthy, in this regard, that all three upstream ER stress sensors have transmembrane domains that could be influenced by changes in the lipid environment. On the downstream side, we are faced with the conundrum that in cultured mammalian cells, XBP1 contributes modestly to the overall gene expression program of the UPR (Lee et al., 2003; Yoshida et al., 2003; Shaffer et al., 2004); IRE1 makes but a similar modest contribution (suggesting the absence of other downstream IRE1 effectors; unpublished data). Furthermore, the known XBP1 target genes do not immediately explain the increased phospholipid biosynthesis and membrane biogenesis. Given the important role of posttranslational regulation in controlling choline phosphotransferase activity, the effects of XBP1 might be rather indirect.

In yeast, components of the UPR are required for cell survival when inositol, a precursor in phospholipid biosynthesis, is limited. And the UPR plays an ill-defined but important role in the sustained up-regulation of genes involved in inositol biosynthesis (Cox et al., 1997). However, it is unclear whether the inositol auxotrophy of *ire1* and *hac1* yeast reflects inadequate phospholipid biosynthesis or whether it reflects some other problem. Both IRE1 and XBP1 are essential in mammals (knockout mice die at early embryonic stages), and XBP1 is required for plasma cell development; however, the contribution of defective phospholipid biosynthesis, or defective membrane biogenesis, to these phenotypes is also not known.

It is interesting to consider how this link between the UPR and membrane biogenesis is integrated with other pathways controlling lipid biosynthesis. It is noteworthy that deregulated XBP1 did not affect cellular cholesterol levels (Sriburi et al., 2004). Thus, the selective increase in phospholipid content is consistent with the XBP1-dependent membrane expansion predominantly affecting the cholesterol-deficient ER membranes. This increase is also consistent with separate control of sterol and phospholipid biosynthesis: the former, by end product inhibition; the latter, by physiological stress signaling the need for more ER. However, intriguing connections abound. The activation of the UPR transducer ATF6 proceeds by precisely the same transport-regulated mechanism that affects cholesterol-regulated activation of SREBPs, the central transcription factors of cholesterol homeostasis (Ye et al., 2000). Furthermore, activation of the UPR is now thought to contribute to the cellular toxicity of cholesterol that is associated with certain clinical conditions (Feng et al., 2003). Although the mechanistic ties between ER biogenesis and cholesterol physiology remain to be determined, they too will be interesting and likely important.

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