ER stress response: **Getting the UPR hand on misfolded proteins**

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Unfolded proteins are constantly delivered to the ER lumen, where they must be removed by folding or degradation. Recent studies show that the 'unfolded protein response' controls essentially all aspects of ER function, coordinating these two fates for misfolded proteins in a process necessary for normal cell life.

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The endoplasmic reticulum (ER) orchestrates the synthesis and processing of nearly all proteins that reside in, or pass through, the endomembrane system of a eukaryotic cell. This large group of soluble and membrane proteins is delivered to the ER as linear polypeptides that each acquire their final shape by the action of a battery of ER-resident enzymes and chaperones. The ER lumen thus has a large and varying concentration of unfolded proteins that are continuously in need of processing and folding. The importance of these reactions is underscored by the fact that many of the ER proteins serving this purpose are essential for life. The ER is also the site of a robust and broadly used degradation pathway that recognizes and destroys unfolded forms of both lumenal and integral membrane proteins, in a process called ER-associated degradation (ERAD).

Two different fates can thus befall an unfolded protein in the ER: folding or degradation. The new work featured in this dispatch [1], and related recent studies [2,3], show that a master ER surveillance system, called the 'unfolded protein response' (UPR) regulates essentially all aspects of ER function, and continuously coordinates the activity and participation of the processing and degradation pathways for unfolded proteins. The work is instructive in several ways, revealing a global view of ER physiology, and convincingly demonstrating the power of combining genomics and basic functional studies to understand integrated cellular functions.

UPR – knowin' when to fold 'em

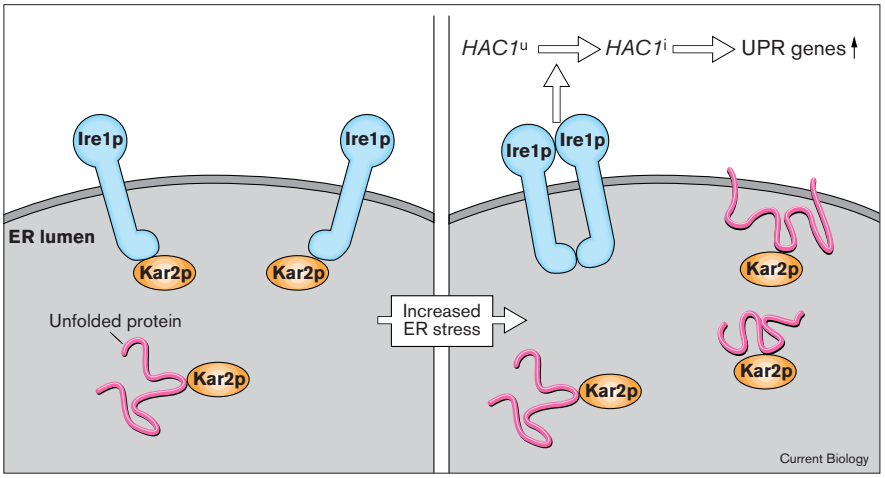
Many situations will increase levels of unfolded proteins in the ER lumen [4]. These include diminished protein glycosylation caused by starvation or treatment with drugs such as tunicamycin, increased production of misfolded mutant proteins or unassembled protein subunits, altered redox status of the lumen brought about by reducing agents such as DTT or genetic means, and altered lumenal ion content caused by ionophores or heavy metals. The common result of these treatments is a state of ER stress in which the burden of unfolded proteins exceeds the capacity of the ER machinery to deal with them. The ER unfolded protein burden can also vary widely between different normal cell types: professional secreting cells like plasma B lymphocytes produce and process secretory proteins to a far greater extent than cells with less extroverted occupations. So how does the cell adjust to changes in the ER unfolded protein burden brought about by these diverse contingencies? It turns out that the ER is not just a bag full of chaperones, but harbors a finely-tuned signaling pathway — the UPR (Figure 1), which continuously measures and responds to the ever-changing lumenal levels of unfolded proteins.

The UPR was first described over 10 years ago [5], with the observation that mammalian cells respond to ER stresses by increasing production of ER-resident proteins which assist protein folding, such as BiP, a member of the heat shock protein 70 (Hsp70) family of molecular chaperones. The subsequent demonstration that Kar2p, the yeast BiP homologue, is similarly regulated opened the genetic door to understanding conserved aspects of the UPR [6,7]. The resulting analysis of the UPR, performed by several laboratories but principally by Walter and colleagues, has been a genetic tour de force [8–12]. The details are the subject of numerous reviews (for example [4,13,14]), and so only receive brief treatment here, despite the temptation to retell a spectacular and surprising molecular tale.

The UPR starts with a transmembrane kinase called Ire1p, which has a cytosolic domain homologous to the ribonuclease RNaseL and a lumenal domain that somehow senses unfolded proteins. Earlier yeast studies and very recent work with mammalian cells support a model in which sensing is mediated by competition between the Ire1p lumenal domain and free unfolded proteins for binding to Kar2p [15,16]. Although the details have not been rigorously delineated, certainly something like this gets the UPR ball rolling. The current model rests on the idea that Kar2p binding inhibits Ire1p from dimerizing and so keeps it inactive. When levels of unfolded proteins increase, they more effectively compete for Kar2p, allowing the now-free Ire1p to dimerize and self-activate. Ire1p activation increases production of transcription factor Hac1p through non-canonical splicing of the *HAC1* mRNA, accomplished in part by a specific endonuclease activity of Ire1p cytosolic domain itself (hence the homology to RNaseL). The resulting increase in Hac1p then programs the transcription of numerous genes

Figure 1

The unfolded protein response (UPR): a model for this ER sensing and response pathway derived from the studies referenced in the text. When the burden of unfolded proteins is low, ER chaperone Kar2p binds to the lumenal domain of the Ire1p protein, thus limiting Ire1p self-association and activity of the protein. When the lumenal unfolded protein burden is increased as a result of pharmacological, genetic or developmental perturbation, the Kar2p molecules (and/or other chaperones) are 'distracted' from binding Ire1p, allowing self-association and activation of Ire1p. Active Ire1p participates in splicing of inactive *HAC1* mRNA, called *HAC1*^{u}, into a form, *HAC1*^{i}, that is efficiently translated, allowing production of Hac1p transcription factor and increased synthesis of myriad ER-related genes.



coding for ER folding and processing factors, including Kar2p itself. Elegant, effective and very novel.

As odd as this molecular mechanism seems, it is broadly conserved in eukaryotes [17], although the mammalian ER stress response is a more complicated affair, involving regulated cleavage of the Ire1p protein [18], an added branch to the UPR pathway employing a distinct transmembrane kinase (PERK) that functions to attenuate translation [19,20], and separate response pathways that measure ER stresses distinct from unfolded proteins [4].

UPR globalization – a block off the old chip

The UPR is thus a transcriptional response: when unfolded proteins in the ER increase, a transcription factor is activated to increase the expression of needed genes. Just how many genes does this pathway regulate? A broad role for the UPR in ER function was indicated by the previous genetic history of Ire1p. *IRE1* was originally isolated in an earlier screen for genes needed for the production of inositol — hence its name, derived from Inositol REquiring — a key precursor for an abundant group of ER membrane phospholipids [21]. This intriguing connection and subsequent studies indicated that the UPR regulates ER membrane structure as well as protein processing capacity [22]. Similarly, a new genetic screen by Ng *et al.* [2] beautifully complements the genomic analysis (see below), independently demonstrating a broad role for UPR in ER function.

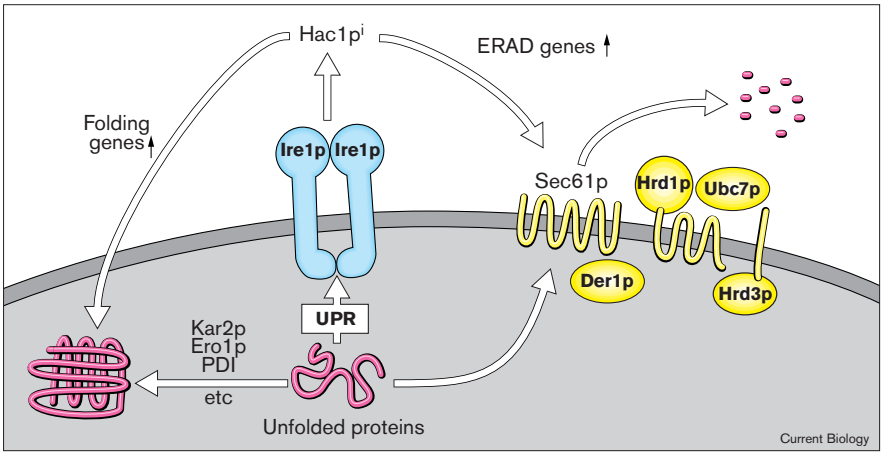
Traditional approaches to identifying transcriptional targets of the UPR would require a gene-by-gene query. Instead, Travers *et al.* [1] capitalized on the advanced state of yeast genomics to fully characterize the UPR. Like the genomes of a few other eukaryotes, that of the budding yeast *Saccharomyces cerevisiae* has been fully sequenced. The small size — a bit over 6000 genes — of the yeast genome and early completion of its sequence has spawned the development of several related methods for measuring the whole genome response to physiological or genetic perturbations (for one most relevant to this work, see [23]).

The basic strategy is now almost rote undergraduate midterm fare: 'prepare mRNA from two different conditions so that each batch is optically distinguishable, hybridize the mix to a microarray that will bind each message at a distinct position, and collect the results with optical detection for computer analysis'. Simple, no? The Travers *et al.* [1] paper is a remarkably instructive example of what is required to actually use this seemingly straightforward approach. A key to their successful genomic analysis was implementation of much previously harvested information about the UPR, obtained by gene-by-gene query, classical genetics and biochemistry, to extend understanding by the array approach.

In the array analysis performed by Travers *et al.* [1], the UPR was induced in yeast cells with either DTT or tunicamycin, drugs that work in very different ways to induce the UPR, for different times. Separate array analyses were done (drug versus control) with each drug at four induction times. Furthermore, two different UPR-deficient null mutants, *ire1Δ* or *hac1Δ*, were subject to similar treatments, so that the response of every yeast open reading frame to the two drugs was determined in the presence and absence of a functional UPR pathway. The responses of each open reading frame were next compared to those of a group of seven known UPR-regulated genes, and genes with a statistically significant, biologically similar response were ascertained.

The results of this impressive and biologically informed genomic analysis, which is available on the internet (http://www.cell.com/cgi/content/full/101/3/249/DC1) are

Figure 2



Two fates for unfolded ER proteins controlled by the UPR. Unfolded proteins in the ER lumen or the ER membrane can either be folded (left branch) or degraded by ER-associated degradation (ERAD), by the appropriate proteins dedicated to these functions. An increased level of unfolded proteins increases the 'tone' of the UPR, causing concomitant increases in activity of each process. Importantly, the studies with null mutants indicate that these two fates both operate continuously in normal cells, such that loss of capacity to perform either branch results in measurable cell stress.

satisfying and informative. First, it is clear that the UPR does indeed regulate a large battery of genes that are involved in many aspects of ER function. These include representatives of nearly all aspects of ER protein production and delivery. To be sure, there are also plenty of genes with high statistical significance and known non-ER-related functions. But within this constellation of UPR-regulated genes is a group required for ER-associated degradation (ERAD), including *HRD1/DER3*, *HRD3*, *DER1* and *UBC7*. These genes encode parts of a dedicated machinery for the destruction of misfolded lumenal and membrane proteins by the ubiquitin–proteasome pathway [24–28]. Thus it appears that the UPR includes coordinate upregulation of pathways that mediate the two fates for misfolded ER proteins: folding and export, or retrotranslocation and degradation (Figure 2).

The ER eats its young – destruction of proteins in the unstressed ER

The coordinate regulation of folding and degradation components divined from genomic analysis suggests a number of experiments examining the interplay of these processes [1–3]. The regulation of ERAD components by the UPR indicates that the 'tone' of this pathway could help determine the rates of ER protein degradation. Indeed, lowering Hac1p activity by loss of the UPR was found to diminish the degradation rates of ER proteins — Travers *et al.* [1] showed this specifically for the well-characterized ERAD substrate CPY*, a constitutively misfolded protein, and heterologously expressed class I major histocompatibility (MHC) proteins. Conversely, increasing *HAC1* expression with a regulated promoter was found to significantly hasten CPY* degradation. The UPR-regulation of ERAD may extend to previously unknown genes, as a null mutation of a novel UPR-regulated gene, *PER100*, identified in these studies does cause a detectable ERAD deficiency, although the modesty of the phenotype suggests it may be an ancillary or pleiotropic participant in protein destruction.

Further examination of the link between UPR and ERAD places the degradation of proteins in the center of normal ER physiology. If the ER deals with unfolded proteins by a branched pathway of either degradation or folding, then one might expect that simultaneous removal of these alternatives would have serious consequences. Indeed, strains with null mutations in both *IRE1* and any of several ERAD genes, such as *HRD1* or *HRD3*, grow poorly and die at elevated temperatures. This result also addresses the previously vexing viability of individual null mutants deficient in either UPR or ERAD. Using a sensitive green fluorescent protein (GFP) reporter for UPR activity, Travers *et al.* [1] further showed that loss of ERAD causes a constitutively higher level of UPR-dependant transcription, consistent with earlier observations on null mutants in *UBC7*, a key enzyme in the ubiquitination of ERAD substrates [29].

Taken together, these results show that ERAD is occurring all the time, and that its loss results in an elevation of unfolded proteins substantial enough to be felt by the ER scanning system. The lethality of the double mutants means that ERAD is a physiologically important alternative to the folding and processing pathways provided by UPR regulation. The synthetic lethality of the UPR- and ERAD-deficient null mutants also indicates that the two pathways must function independently to some extent: if ERAD were totally dependent on the UPR, then loss of ERAD components in addition to the UPR would be expected to have no additional phenotypes.

It has recently been shown that, in mammalian cells, loss of the translational attenuation mediated by the kinase PERK, which is on a UPR branch that is specific for metazoans, similarly has grave consequences for managing ER stress [20]. Thus, mammalian cells are also sensitive to an inability to handle newly made proteins, but they have the added tactic of diminishing their production as a stress-management tool. Although the interplay of folding

and degradation has not yet been tested in the mammalian UPR, the conservation of the signaling pathway, the folding machinery and various ERAD components makes it likely the URP and ERAD will turn out to be similarly intertwined across the eukaryotic board.

UPR genomics: the Utility of Previous Results

Besides the broad and useful insights into ER physiology that emerge from these studies, the work also provides an instructive and perhaps cautionary tale about the place and use of genomics in analysis of biological processes. One hears the most single-minded proponents of genomics — perhaps those with too much NASDAQ exposure — state the belief that this approach will herald in a 'new biology' where study of individual proteins, genes and phenotypes will be supplanted completely by analyses of large groups of genes with arrays and probably other future tools we have not yet imagined. Certainly, global analyses will change biology in big ways, and future biology will be more of an armchair affair. But what is clear from the successful study featured above [1] is that an understanding of the biology and genetics of UPR informed all aspects of the work.

The parallel work of Ng *et al.* [2], who took a more traditional genetic approach to studying the 'globality' of the UPR, turned up ER-functioning genes that interacted strongly with UPR mutations but were not regulated by this transcriptional axis, underscoring the importance of integrated approaches to studying big problems. These hallmark studies provide us with a more balanced view of where the genomic approach fits into current biology. It is a powerful way to extend our understanding of complex life processes, in which previous methods and understanding of molecular functions synergize, rather then compete, with this new avenue of inquiry.

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