Signal transduction: **Splicing together the unfolded-protein response**

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Recent work has identified a transcription factor, Hac1p, in the yeast *Saccharomyces cerevisiae*, as a component of a pathway that signals to the nucleus the presence of unfolded proteins in the endoplasmic reticulum and has shown that Hac1p expression is regulated by a novel RNA splicing pathway.

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Electronic identifier: **0960-9822-007-R0067**

Current Biology 1997, 7:R67–R70

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Cells respond to the accumulation of unfolded proteins in the endoplasmic reticulum (ER) by increasing the production of ER-resident chaperones, such as BiP and protein disulfide isomerase (PDI), that expedite protein folding and assembly in the ER lumen. In organisms as diverse as yeast and humans, this is accomplished by increasing the transcription of the genes that encode these chaperones. The response, which involves a signal-transduction cascade from the ER to the nucleus, is called the unfolded-protein response (UPR).

Much progress in understanding the UPR has been made by studying the pathway in the budding yeast *Saccharomyces cerevisiae* [1]. The transcriptional element that mediates the response is the unfolded protein response element (UPRE), a 22 base-pair sequence found in the promoters of yeast genes induced by the UPR. The UPRE is both necessary and sufficient for transcriptional activation in response to the accumulation of unfolded proteins in the ER [2,3]. A second component of the pathway is Ire1p, a transmembrane serine/threonine kinase [4,5]. Ire1p is a type I transmembrane protein oriented with its amino terminus in the ER lumen and its kinase domain in either the cytoplasm or the nucleus (as the nuclear envelope is continuous with the ER membrane, these two possibilities are topologically identical).

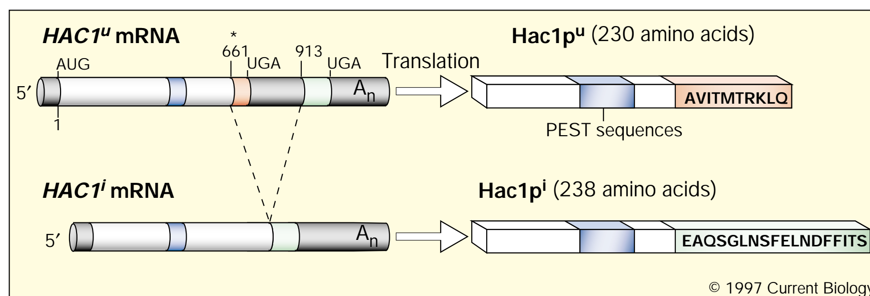
Ire1p detects the accumulation of unfolded proteins in the ER by an unknown mechanism. Ire1p then oligomerizes and is transphosphorylated by neighboring Ire1p molecules, thereby transducing the UPR signal across the ER membrane to downstream components of the pathway [6]. Both the Ire1p kinase domain and carboxy-terminal 133 amino acid tail are required for the UPR [5,6]. Thus, Ire1p appears to act in the UPR in the same way that receptor transmembrane kinases act to transduce growth factor signals across the plasma membrane. This analogy suggests that Ire1p might activate downstream factors by phosphorylating them directly and/or by providing a structural scaffold onto which active signaling complexes assemble. However, while the factors that bind and activate many of the growth factor receptor transmembrane kinases are known, the ligands for Ire1p have not been identified.

Another interesting and unresolved issue is the route taken by the unfolded protein signal on its way to the nucleus. Is the Ire1p kinase domain in the cytoplasm, signaling to cytoplasmic factors that enter the nucleus through the nuclear pore? Or is the kinase in the nucleus, signaling directly across the part of the ER membrane that is continuous with the inner nuclear membrane? The subcellular localization of Ire1p, which might distinguish between these possibilities, has not yet been determined.

To establish the route of the UPR and to elucidate details of the signaling cascade, Cox and Walter [7] recently carried out a genetic screen for factors that act downstream of Ire1p. They screened for genes that, when overexpressed in cells lacking Ire1p, activate the UPR constitutively. In this way, they found *HAC1*, a gene encoding a member of the bZIP family of transcription factors [8]. By several criteria, Hac1p appears to be the transcription factor that controls the UPR in yeast. Gel-shift experiments have demonstrated that Hac1p binds specifically to the UPRE sequence. Stable expression of Hac1p (see below) is sufficient to induce transcription of genes normally induced during the UPR. Finally, the phenotype of cells deleted for *HAC1* is identical to that of cells deleted for *IRE1*. Under normal growth conditions, neither *IRE1* nor *HAC1* is essential, and cells deleted for either gene cannot induce the UPR. Cells do, however, require *IRE1* and *HAC1* to survive when unfolded proteins are induced to accumulate in the ER ([4,5] and R. Chapman, personal communication).

How is the Hac1p transcription factor activated during the UPR? Cox and Walter [7] found that Hac1p accumulates only in cells carrying out the UPR and that this accumulation requires *IRE1*. While looking at *HAC1* mRNA levels under UPR-inducing and non-inducing conditions, Cox and Walter identified two *HAC1* mRNAs. *HAC1*^{u} mRNA is the longer of the two, and is present in cells whether or not the UPR is induced. The second, shorter mRNA, *HAC1*^{i}, appears only when the UPR is induced; its appearance requires the presence of functional Ire1p. The analysis of cDNAs made from *HAC1* mRNA shows that the only difference between the *HAC1*^{i} and *HAC1*^{u} mRNAs

Figure 1



HAC1 mRNA is spliced. Two different forms of *HAC1* mRNA, shown on the left, are found in cells carrying out the unfolded-protein response. When unfolded proteins accumulate in the ER, a 252-base intron (indicated by the dotted lines) is excised from *HAC1^u* to form *HAC1^i*. The asterisk at nucleotide 661 in *HAC1^u* indicates the position of the stop codon inserted in *HAC1^Δtail* mRNA. The translation products of the mRNAs are depicted on the right. The carboxy-terminal amino acids unique to each protein are spelled out in single-letter code (not drawn to scale), and color coded to correspond to the sequences in the mRNAs. See text for details.

lies near their 3′ ends: *HAC1^i* mRNA lacks a 252-base intron (or intervening sequence) that is present in *HAC1^u* mRNA (Fig. 1).

Both transcripts have the same transcription start site, so the proteins they encode differ only at their carboxyl termini. *HAC1^u* encodes a 230 amino acid protein. The excision of the 252-base intron causes the last 10 amino acids of Hac1p^u (the protein made from *HAC1^u* mRNA) to be omitted, and a different stretch of 18 carboxy-terminal amino acids to be added instead, generating Hac1p^i (Fig. 1). Western blot experiments with antibodies directed against the Hac1p^i tail showed that Hac1p^i is the only form of the transcription factor that is ever detected in yeast cells. Moreover, cells expressing ectopic *HAC1^i* induce the UPR in an Ire1p-independent fashion, demonstrating that expression of Hac1p^i is sufficient to induce the UPR. Thus, the primary role of Ire1p seems to be to stabilize the expression of the Hac1p transcription factor by promoting *HAC1* mRNA splicing. This is the first time that mRNA splicing has been shown to directly regulate a step in a signal transduction pathway.

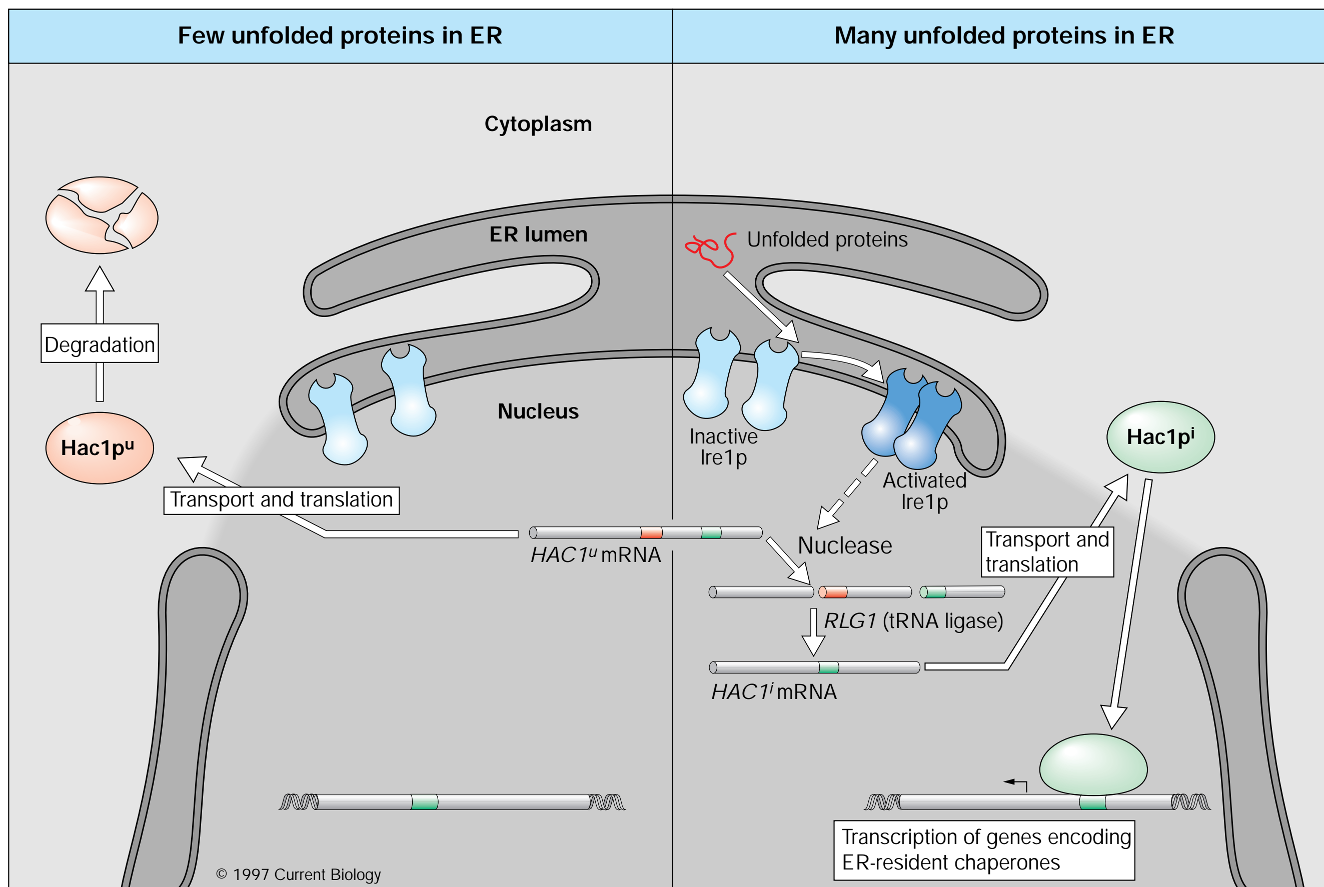
Why does the splicing of *HAC1* mRNA lead to increased levels of Hac1p transcription factor? Both the *HAC1^u* and *HAC1^i* transcripts have similar half-lives and both are translated, as judged by their recruitment into polyribosomes. Thus, it appears that Hac1p^u does not accumulate because it is an unstable protein. Consistent with this, there are clusters of 'PEST' sequences in the carboxy-terminal half of Hac1p (Fig. 1). PEST sequences destabilize proteins by targeting them for ubiquitin-mediated degradation. Indeed, the UPR is induced constitutively in yeast cells carrying mutations in ubiquitin-conjugating enzymes. This constitutive induction still requires *HAC1* but does not require *IRE1* [7].

Identical PEST sequences are present in Hac1p^u and Hac1p^i, so their presence alone cannot explain the difference in stability between the two proteins. This is not surprising, because it has been shown that PEST sequences in the yeast protein Cln2p are required for Cln2p degradation but, taken out of the context of Cln2p, the PEST sequences are not sufficient to target other proteins for proteolysis [9]. Cox and Walter [7] speculated that it is the combination of the PEST sequences and the carboxy-terminal tail of Hac1p^u that makes the protein unstable. To test this possibility, they inserted a stop codon just before the *HAC1^u* intron, generating a truncated protein that lacks either the Hac1p^u or the Hac1p^i carboxyl terminus (Hac1p$^{\Delta tail}$, Fig. 1). Cells expressing the *HAC1^Δtail* mRNA induced the UPR constitutively, to the same levels as cells expressing *HAC1^i*. Thus, the carboxy-terminal 10 amino acids unique to Hac1p^u appear to make the protein unstable. The removal of this sequence, but not the addition of the 18 amino acids unique to Hac1p^i, are required for induction of the UPR.

Most mRNA splicing in yeast, as in other eukaryotes, is carried out by the spliceosome. However, the splice junctions in *HAC1^u* mRNA do not resemble consensus splice junctions, and *HAC1* mRNA splicing is not affected by several mutations that inhibit spliceosome function [10]. Although it is still possible that some spliceosome components might play a role, these results suggest that a different splicing pathway is involved in the splicing of *HAC1*. Unexpectedly, yeast tRNA ligase has been identified as a factor required for the splicing of *HAC1^u* mRNA (to yield *HAC1^i* mRNA) [10]. tRNA ligase, encoded in *S. cerevisiae* by the essential gene *RLG1*, is one component of the protein complex that splices the small introns from pre-tRNAs [11]. As a product of a genetic screen, Sidrauski *et al.* [10] identified a special allele of *RLG1*, *rlg1-100*, that inhibits the UPR. In the *rlg1-100* mutant, a protein that differs from wild-type tRNA ligase by only one amino acid (tyrosine instead of histidine at position 148) is produced. This single change is sufficient to inactivate the UPR without affecting tRNA splicing.

Could the function of tRNA ligase in the UPR be the ligation of *HAC1^i* mRNA? Three observations provide clues that this unusual idea may, indeed, be correct. First,

Figure 2



A speculative model for the unfolded protein response pathway in yeast. The transmembrane kinase Ire1p is activated when unfolded proteins accumulate in the ER. Activation of Ire1p leads to activation of an as-yet-unidentified nuclease that cleaves *HAC1^u* mRNA. The tRNA ligase encoded by the *RLG1* gene is required to finish the splicing reaction to produce *HAC1^i* mRNA. Hac1p^i is made and induces transcription of genes encoding ER-resident chaperones. Note that the localization of Ire1p has not been determined; however, as depicted here, it is possible that it lies near the nuclear pore. See text for details.

inducing the accumulation of unfolded proteins in the ER of *rlg1-100* cells results in the disappearance of all *HAC1* mRNA, possibly because the first step of splicing (cleavage) occurs in the absence of the second step (ligation), and the cleavage products are degraded. Second, in *rlg1-100* cells that also lack *IRE1*, *HAC1^u* mRNA is present but *HAC1*^{i} mRNA is never produced. This is consistent with the idea that the splicing process has never been initiated. Third, constitutive *HAC1^i* mRNA expression induces the UPR in *rlg1-100* mutants, suggesting that tRNA ligase is upstream of, or necessary for, the production of *HAC1^i*.

These observations lead to the model for the *S. cerevisiae* UPR pathway diagrammed in Figure 2. In response to the accumulation of unfolded proteins in the ER, the Ire1p kinase is activated and induces the cleavage of *HAC1^u* mRNA. tRNA ligase is required to finish the splicing reaction to produce *HAC1^i* mRNA. Finally, Hac1p^i, the stable form of the transcription factor, is expressed and transcription of the ER chaperone genes is induced. As tRNA splicing is completely normal in *rlg1-100* mutants, Sidrauski *et al.* [10] speculate that splicing of *HAC1* mRNA represents a specialized function of tRNA ligase, the nature of which remains to be determined.

The details of *HAC1* mRNA splicing are still unknown but it is clear that it proceeds by a novel mechanism. The next step is to identify the factors that splice *HAC1* mRNA, particularly the nuclease that acts on *HAC1^u*. This nuclease might be tRNA endonuclease. *HAC1* mRNA does not, however, display any obvious homology to pre-tRNAs, and the *HAC1* intron is much larger than tRNA introns [11]. Nonetheless, this possibility can be tested once the genes encoding the *S. cerevisiae* tRNA endonuclease are cloned. One nuclease candidate that can be evaluated immediately is Ire1p. The carboxy-terminal tail of Ire1p is 29 % identical to the presumed endonuclease domain of mammalian 2-5A-dependent RNase [12]. 2-5A-dependent

RNase, also known as RNase L, is an interferon-induced enzyme that is activated by 5′-phosphorylated, 2′,5′-linked oligoadenylates and may be involved in the inhibition of viral replication and in tumor suppression. Interestingly, Ire1p and 2-5A-dependent RNase share other features as well. 2-5A-dependent RNase also has a kinase domain preceding its putative endonuclease domain, and it oligomerizes when activated [12,13]. Thus, Ire1p may turn out to be a remarkable signaling molecule — an ER-localized transmembrane kinase with endonuclease activity.

In addition to identifying an unusual splicing pathway, the new components of the UPR shed light on the route taken by the unfolded protein signal on its way from the ER to the nucleus. Splicing of pre-mRNAs generally occurs in the nucleus, and tRNA ligase, as well as other proteins involved in pre-tRNA splicing, have been localized near nuclear pores [14,15]. Sidrauski *et al.* [10] therefore propose that activated Ire1p lies in the inner nuclear membrane, possibly at the nuclear pore, where it controls splicing of *HAC1* mRNA as it is exported to the cytoplasm to be translated. Thus, the UPR may travel a path more circuitous than previously expected — from the ER to the nucleus, to the cytoplasm, and back to the nucleus — before transcription is activated.

This recent work on the *S. cerevisiae* UPR has provided a basic outline of the pathway as it extends between the ER and the nucleus. Several important issues remain, however. How is the accumulation of unfolded proteins in the ER detected by Ire1p? Why is the pathway so complex — does Hac1p^{u} have a physiological function? How closely does the mammalian UPR resemble the UPR in yeast? Given the unprecedented types and combinations of signaling components found in the pathway so far, the smart scientist would not place bets on how the rest will turn out.

Acknowledgements
I thank Peter Sorger, Maho Niwa, Susanna Lee, Rowan Chapman and Ted Powers for comments on the manuscript, and acknowledge the support of a postdoctoral fellowship from the Jane Coffin Childs Memorial Fund for Medical Research.

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