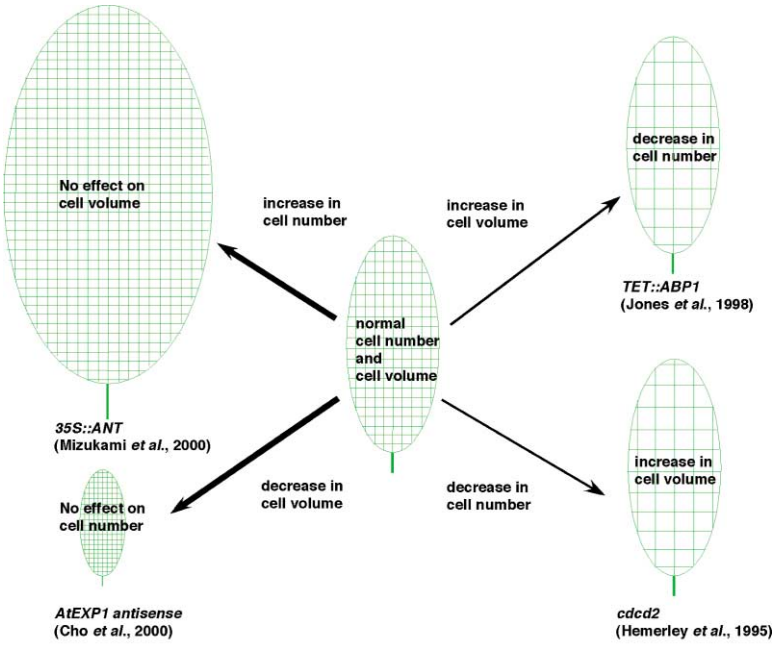

Figure 1. The Regulatory Relationship between Cell Number and Cell Volume

Leaves can compensate for increases in cell volumes by decreasing cell numbers and for decreases in cell numbers by increasing cell volumes. However, increased cell numbers are not compensated for, nor are decreases in cell volume. This suggests that compensation mechanisms that favor increased cell size are possible, while those that prevent this (by altering processes regulating cell size) or increased cell numbers are not possible in plants (or have yet to be discovered).

al., 1995; Jones et al., 1998). However, several examples suggest that compensation of increased cell proliferation by reduction of cell size or decreased proliferation by increasing cell sizes might not be always true (Figure 1; Cho and Cosgrove Daniel, 2000; Mizukami and Fischer Robert, 2000). Timing may be a critical "third dimension" that determines whether compensatory mechanisms will come into play or not. In addition to timing, these mechanisms are likely governed by multiple other components. These components need to be discovered in years ahead.

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All Roads Lead to ATF4

Multiple intracellular stress pathways converge on a single event—phosphorylation of the translation initiation factor eIF2α and subsequent translational activation of the transcription factor ATF4. Exploring the consequences of this event has highlighted the ways in which stress is sensed and responded to via many distinct pathways.

Cellular mechanisms for sensing and responding to stress underlie the ability of cells to withstand the many insults, both programmed and exogenous, that are encountered during development and differentiation. It is becoming increasingly evident that common pathways are shared in the responses to multiple, seemingly divergent stresses, yet the logic for this common response has been unclear. Now, a paper by Harding and colleagues (2003) in the March issue of *Molecular Cell* illustrates how the activation of one transcription factor by multiple stress pathways forms the basis of a generalized stress response.

Cells respond to stress, in part, through upregulation of genes that function specifically in mitigating that stress. For example, the accumulation of unfolded proteins in the endoplasmic reticulum (ER) is sensed by ER-resident transmembrane molecules, which then activate signaling cascades in the cytoplasm. One result of the activation of these pathways is the increased transcription of genes encoding chaperones, lipid synthesis machinery, and other proteins that function at multiple points within the secretory pathway (Travers et al., 2000).

In addition to transcriptional upregulation, many stress pathways converge on phosphorylation of eIF2α, which leads to a general inhibition of protein synthesis. Notably, four protein kinases—each of which appears to be specific to a particular stress response—can phosphorylate eIF2α. While phosphorylation of eIF2α leads to general inhibition of translation, it also results in translational upregulation of specific mRNAs. The mechanism behind this selective translation is best elucidated in the general amino acid control response in the yeast *S. cerevisiae*. Amino acid starvation in yeast leads to activation of the Gcn2p eIF2α kinase and inhibition of translation. However, the 5′ untranslated region of *GCN4* mRNA contains four short open reading frames. During normal physiologic conditions, scanning ribosomes synthesize these short peptides and dissociate from the mRNA prior to reaching the authentic GCN4 start codon. In contrast, phosphorylation of eIF2α inhibits efficient assembly of the 80S ribosome, and, so, a fraction of the scanning 40S subunits form active translational complexes only after the upstream ORFs have been bypassed, allowing initiation at the proper GCN4 start codon (Hinnebusch and Natarajan, 2002).

Higher eukaryotes have conserved a similar mechanism for translational upregulation during conditions of stress. mRNA encoding the metazoan transcription factor ATF4 also contains multiple upstream ORFs, leading to ATF4 protein expression when eIF2α is phosphorylated (Harding et al., 2000; Scheuner et al., 2001). A key difference between yeast and metazoans, however, is that only in the latter are multiple kinases known to phosphorylate eIF2α, suggesting that the targets of ATF4 are important in a generalized stress response.

What are the targets of ATF4? Using microarray analysis in cultured wild-type or ATF4 knockout fibroblasts exposed to ER stress (which activates the eIF2α kinase PERK), Harding and coworkers demonstrate that, among the genes whose expression is activated by ER stress, ATF4 controls many of those involved in amino acid metabolism and transport and in redox chemistry. Consequently, cells lacking ATF4 show increased susceptibility to ER and other stresses, including amino acid deprivation and oxidative stress.

What is the logic for activating antioxidant and amino acid metabolism pathways during ER stress? Harding et al. propose that the answer lies in the effects of protein secretion, which begins in the ER, on amino acid sufficiency and the redox state of the cell (Figure 1). Protein secretion can be seen as an irreversible loss of amino acids to the extracellular milieu; the greater the secretory burden, the more acute this problem. In addition, protein secretion also affects the net loss of reducing equivalents from the cell. Oxidative protein folding in the ER lumen is catalyzed via protein disulfide isomerase, with

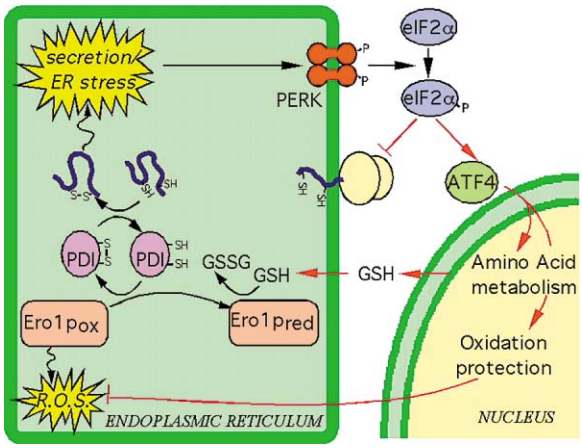

Figure 1. Phosphorylation of eIF2α Can Be Expected to Attenuate ER-Associated Amino Acid Stress and Oxidative Stress in Two Ways (Red Arrows)

First, inhibition of translation prevents the loss of amino acids and reducing equivalents by decreasing the load of proteins passing through the secretory pathway. Second, translational upregulation of ATF4 results in transcriptional activation of genes involved in amino acid metabolism (including glutathione biosynthesis) and protection against oxidative stress.

a transfer of electrons from the nascent protein to the FAD-dependent oxidoreductase Ero1p (Pollard et al., 1998; Frand and Kaiser, 1999). Ero1p, in turn, is oxidized by molecular oxygen, which acts as the terminal electron acceptor (Tu and Weissman, 2002). Thus, electrons removed from nascent proteins are apparently not recycled, but, instead, lost to oxygen. Just as in the case of amino acid supply, the greater the secretory burden on the cell, the greater the anticipated loss of reducing equivalents. ER stress, which can be seen as a secretory burden taken to its extreme, would thus be expected to promote both amino acid insufficiency and a loss of reducing equivalents. Indeed, this prediction is confirmed by showing that, in the absence of the eIF2α kinase PERK, the induction of ER stress leads to the rapid accumulation of reactive oxygen species, possibly due to the inability of PERK knockout cells to activate ATF4 and its downstream antioxidant effectors. ATF4 knockout and PERK knockout cells require supplementation with both extra amino acids and cysteine—the former presumably to replenish amino acids lost to secretion and the latter likely to stimulate the synthesis of glutathione, of which cysteine is a precursor. PERK provides a pivotal role in fine-tuning the requirement for oxidative protein folding in the ER by both regulating the rate of secretory protein synthesis and by activating long-term adaptive responses.

When ATF4 is absent and its downstream antioxidative targets are not upregulated, Ero1p might be expected to produce hyperoxidation within the ER. If so, then loss of Ero1p function should ameliorate the generation of reactive oxygen species in response to ER stress. Using RNAi to knock down Ero1p function in the nematode *C. elegans*, Harding et al. find such an effect. While loss of Ero1p function itself leads to ER stress, ER stress induced by this or other means occurs apparently unaccompanied by the generation of reactive oxygen

species, and ablating Ero1p function rescues premature lethality in worms with a defective UPR.

The work presented by Harding and colleagues highlights the way in which multiple stresses are interrelated and the conservation of a generalized stress response for adapting to these stresses. Questions, of course, remain. Are there other proteins that are upregulated specifically as a consequence of eIF2α phosphorylation, and what role would such proteins play in mediating adaptation to multiple stressors? What is the mechanism by which Ero1p generates reactive oxygen species? What fraction of cellular oxidative stress is attributed to ER function? Are there additional pathways through which stress in one location (e.g., the ER) is communicated to other organelles, enabling a cell-wide response to specific stressors? Unlocking the cellular response to stress may turn out to be a stressful exercise.

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Filling Gaps in Signaling to Actin Cytoskeletal Remodeling

A recent publication in the April 4 issue of *Cell* advances our understanding of stimulus response coupling leading to actin remodeling. It describes the identification of a novel membrane component Mig-2 that engages filamin A through a new intermediary, migfilin, to stimulate actin assembly and cell spreading on a substrate of extracellular matrix.

Diverse stimuli induce cells to remodel their actin cytoskeletons. Depending on the agonist and the signal intermediates it sets into motion, actin remodeling builds different structures. For example, TNFα activates the Rho GTPase Cdc42 to induce extension of linear actin bundles that project hair-like protrusions called filopodia. In contrast, extracellular matrices activate Rho GTPases, Rac and Rho, to cause circumferential actin assembly and cell spreading (Etienne-Manneville and Hall, 2002). Our challenge is to understand how hundreds of actin binding proteins that actually do the work of actin remodeling respond to upstream signals in a coordinated manner to shorten, lengthen, and organize the three-dimensional organization of actin filaments and control the reversible linkage of actin filaments to extracellular matrices (Pollard and Borisy, 2003).

From the starting point of cloning a previously uncharacterized gene, Mig-2, Tu et al. (2003) have added substantively to this understanding. They identified Mig-2 as a component of adhesion complexes where cells attach to the extracellular matrix. They then discovered a novel Mig-2 binding partner they named migfilin and demonstrated that Mig-2 recruits migfilin to adhesion complexes. Using RNA interference to downregulate protein expression, they showed that cell spreading requires both Mig-2 and migfilin. In addition, they identified filamin A (FLNa) as a ligand of migfilin and that the interaction of migfilin and FLNa is necessary for matrix- and Mig-2-induced cell spreading. The Fil-2, migfilin, and FLNa interaction is not merely a static chain of physical connections, because it promotes net actin assembly as cell spreading occurs.

Bringing FLNa into this story is a big step, because FLNa is the first-recognized non-muscle cell actin binding protein and has therefore been under investigation for over 25 years (Stossel et al., 2001). Originally defined as a potent actin filament gelation factor that promotes orthogonal branching and crosslinking of actin filaments, FLNa binds over 30 proteins of great functional diversity. These include membrane receptors for extracellular matrix components, receptors for various cell activating ligands, and even nuclear factors. Of greatest importance for signaling to actin remodeling, however, is the fact that FLNa binds intracellular signaling intermediates, including the Rho GTPases and Rho GTPase regulating proteins implicated in actin remodeling. Normal FLNa expression is a prerequisite for mammalian cellular locomotion.

FLNa is a large dimeric protein with filamentous subunits. Twenty-three repeating units of amino acid sequence and two short runs of unique sequence, termed "hinges," separate an amino-terminal actin binding domain from a carboxy-terminal repeat number 24, which is the dimerization site (Figure 1; Stossel et al., 2001).