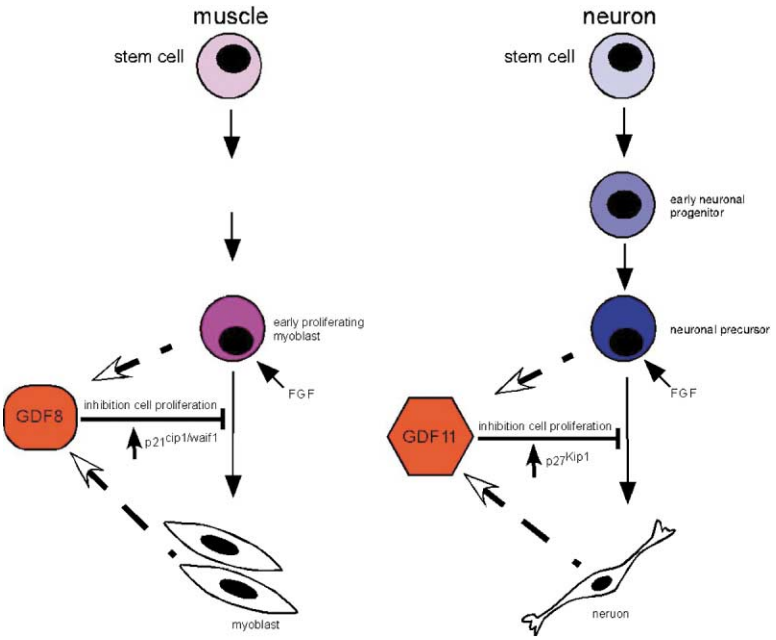


Regulation of Tissue Size by GDFs

During neurogenesis and myogenesis, the negative autoregulatory action of GDFs on cell proliferation sets a limit on the number of mature neurons and muscle cells, maintaining control of organ size (adapted from Wu et al., 2003).

Selected Reading

Bogdanovich, S., Krag, T.O.B., Barton, E.R., Morris, L.D., Whittemore, L., Ahima, R.S., and Khurana, T.S. (2002). Nature *420*, 418–421.

Bullough, W.S. (1965). Cancer Res. *25*, 1683–1727.

Daluiski, A., Engstrand, T., Bahamonde, M., Gamer, L.W., Agius, E., Stevenson, S.L., Cox, K., Rosen, V., and Lyons, K.M. (2001). Nat. Genet. *27*, 84–88.

Gamer, L.W., Wolfman, N.W., Celeste, A.J., Hattersley, G., Hewick, R., and Rosen, V. (1999). Dev. Biol. *208*, 222–232.

Gamer, L.W., Cox, K.A., Small, C., and Rosen, V. (2001). Dev. Biol. *229*, 407–420.

McPherron, A.C., Lawler, A.M., and Lee, S.J. (1999). Nat. Genet. *22*, 260–264.

McPherron, A.C., Lawler, A.M., and Lee, S.J. (1997). Nature *387*, 83–90.

Nakashima, M., Toyono, T., Akamine, A., and Joyner, A. (1999). Mech. Dev. *80*, 185–189.

Thomas, M., Langley, B., Berry, C., Sharma, M., Kirk, S., Bass, J., and Kambadur, R. (2000). J. Biol. Chem. *275*, 40235–40243.

Wu, H.H., Ivkovic, S., Murray, R.C., Jaramillo, S., Lyons, K.M., Johnson, J.E., and Calof, A.L. (2003). Neuron *37*, 197–207.

IRE1: A Role in UPREgulation of ER Degradation

The unfolded protein response entered the mechanistic realm with the discovery of IRE1 as the key signal transducer in yeast. Although also found in mammals, it appeared to function in assisting the work of other players. The featured studies indicate a separate role for IRE1, and highlight the flexibility that bigger eukaryotes possess in this critical pathway.

The lumen of the endoplasmic reticulum (ER) has an ever-present and varying burden of misfolded proteins. A signaling pathway couples lumenal levels of misfolded proteins to the production of factors needed for their refolding or destruction, allowing successful management of this potentially lethal stress. This signal-and-response system is collectively known as the unfolded protein response (UPR; Kaufman et al., 2002).

The yeast UPR is entirely dependent on the ER resident, transmembrane endoribonuclease Ire1p, which initiates nontraditional splicing of the *HAC1* message, allowing efficient production of Hac1p transcription factor and thus expression of HAC1-transcribed proteins required for lumenal folding and ER-associated degradation (ERAD; Patil and Walter, 2001).

The mechanisms of the mammalian UPR are more complex. Three separate signaling molecules, PERK, ATF6, and IRE1, reside in the ER membrane as dedicated heralds of lumenal chaos. PERK is a transmembrane kinase that phosphorylates eIF2α upon ER stress, thereby decreasing global translation to provide respite from continued production of nascent unfolded proteins, and influencing transcriptional regulation of UPR genes in ways that are less clear (Scheuner et al., 2001). ATF6 is a membrane-anchored bZIP transcription factor, and mammalian IRE1 functions analogously to its yeast cousin. Both IRE1 and ATF6 have α and β isozymes that appear to function similarly in different tissues. ER stress causes ATF6 cleavage and release of the soluble N-terminal bZIP domain that recognizes the ERSE in *cis* element found in many UPR-regulated genes. Mammalian IRE1's endonuclease activity mediates stress-induced splicing of the XBP1 message,

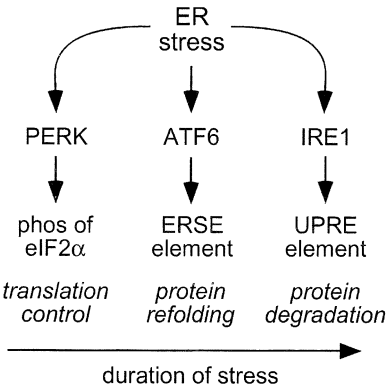
causing production of a variant protein with strong transcriptional activity. Active XBP1 is ATF6 regulated, and XBP1 itself (Lee et al., 2002; Yoshida et al., 2001) has high affinity for the ATF6-controlled ERSE.

Thus, mammalian IRE1 was posited to play a partner role in ATF6-dependent ERSE activation. In fact, mammalian IRE1 null cells induce chaperone genes in response to ER stress, whereas PERK action *is* required for UPR induction of many genes. Findings such as these moved the spotlight away from IRE1 in thinking about mammalian UPR. The work featured in this issue of *Developmental Cell* (Yoshida et al., 2003) brings IRE1 more to center stage, demonstrating an IRE1-mediated pathway with a key role in management of mammalian ER stress.

This new role for IRE1 emerged from vigorous attempts to unravel the mammalian UPR transcriptional axis. At least two separate transcription factors participate in the initial response to ER stress (ATF6 and XBP1), and secondary ones are induced by PERK (ATF4 and CHOP; see Figure). Furthermore, in vitro affinity screens revealed a second in *cis* element that binds to ATF6 (Wang et al., 2000), which was (ironically) named "the ATF6 site" to distinguish it from the previously known ATF6 binding ERSE site. Subsequent reporter gene studies indicated that ER stress-induced transcription from the "ATF6" site was not, after all, mediated by ATF6 at its normal genomic levels, but instead was dependent only on IRE1-mediated activation of XBP1 (Yoshida et al., 2001; Lee et al., 2002). Accordingly, the second control element was renamed UPRE, to distinguish it from the mammalian ERSE and highlight its connection to IRE1. Thus, the possibility of a distinct IRE1-dependent branch of the UPR loomed. The featured article directly examines this question.

To address the physiological significance of this IRE1-dependent branch, Yoshida et al. focused on the transcription of the gene encoding EDEM, a protein required for ER-associated degradation of lumenal glycoproteins (Hosokawa et al., 2001). The authors use cell lines derived from homozygous null mice for the ubiquitously expressed IRE1α (the β isoform is restricted to certain tissues). Upon ER stress, *IRE1*$^{-/-}$ null cells still undergo regulated cleavage of either ATF6 isoform, and upregulation of the ERSE-driven reporter gene, but show no induction of a UPRE reporter. Using two distinct ER stresses (thapsigargin or tunicamycin), they next show that the in situ EDEM gene is regulated in an entirely IRE1-dependent manner. In contrast, stress induction of the ATF6-regulated ER chaperone BiP or the PERK-regulated asparagine synthase gene is unaffected in the IRE1 nulls. This is the first case of a physiologically relevant gene regulated in an entirely IRE1-dependent manner

Due to the original connection between ATF6 and UPRE, the authors further explore the role of ATF6 in the UPRE pathway by overexpressing the transcriptionally active N terminus of ATF6, ATF6α(N), from a truncated coding region (Okada et al., 2002). Both EDEM and a UPRE reporter gene underwent increased transcription when ATF6α(N) was overexpressed. Considering that the UPRE was isolated as an in vitro ATF6 binding element, this effect is not that surprising. But then why is



A Highly Simplified Diagram Showing Proposed Functional and Temporal Details for the More Elaborate Mammalian UPR

The functional branches indicate necessary conditions, and they should not be interpreted as the only requirements for a given response. In fact, there is significant crosstalk between the branches. Nevertheless, induction of EDEM is absolutely dependent on IRE1 function, and this may be a general feature of ERAD regulation during ER stress.

UPRE-regulated EDEM only dependent on IRE1 in ER-stressed cells? The last experiment in this series resolves the confusion. When ATF6α(N) is induced to levels actually caused by ER stress, it is no longer capable of inducing EDEM, and only does so when expressed at high levels from a strong promoter. Thus, the specificity of UPRE regulation is real, but mostly due to restricted levels of ATF6 as opposed to a qualitatively distinct recognition. There may be physiological circumstances where endogenous ATF6 does have sufficient activity to act on UPREs. These studies do not address the separate question of whether ATF6 is necessary for EDEM regulation.

Because EDEM (Htm1p in yeast) is involved in destruction of misfolded glycoproteins, they directly test the effect of this class of stress on the ERSE and UPRE pathways. Using the secreted, normal α1-antitrypsin (A1AT) or a misfolded variant termed null Hong Kong (NHK), they demonstrate that expression of NHK protein stimulates both ERSE and UPRE reporters, and that the UPRE response is IRE dependent. Next, they employ NHK in its capacity as an ERAD substrate to examine the roles of IRE1 and EDEM in this process. NHK is degraded with the expected half-life ($\sim$2 hr), and added ER stress hastens NHK degradation, as expected (Hosokawa et al., 2001). In both cases, NHK degradation was completely dependent on functional IRE1, and as expected this defect was complemented by expression of exogenous IRE1 gene. However, the lack of NHK degradation in *IRE1*$^{-/-}$ cells was also completely restored by expression of the lone EDEM coding region, indicating that EDEM alone was rate limiting for degradation of this (and probably many) ERAD substrate.

These results demonstrate that IRE1 is required for a distinct branch of the UPR pathway in mammals, and indicate that ER degradation may be the bailiwick of IRE1, whereas chaperone expression is relegated to ATF6 (see Figure). The authors note that the IRE-dependent regulation of EDEM has a slower time course than

ATF6 regulation of chaperones. They posit the tempting model that this difference in timing is designed to optimize reversal of misfolding fortune: upon ER stress, the mammalian cell first produces chaperones in an attempt to correct the defects; if the signal persists too long, then the slower acting IRE1-dependent branch mediates production of factors that promote degradation of the recalcitrant misfoldees. Consistent with this model, human HRD1, an ER-associated ERAD ubiquitin ligase, is UPR induced with a significantly slower time course than PERK-regulated CHOP, and HRD1 induction is blocked by an IRE1 dominant negative (Kaneko et al., 2002). This sort of temporal subtlety is a benefit of the increased complexity of the mammalian UPR, and considering the number of ways the mammal appears to harness the ER stress response, it may be the tip of the functional iceberg.

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Selected Reading

Hosokawa, N., Wada, I., Hasegawa, K., Yorihuzi, T., Tremblay, L.O., Herscovics, A., and Nagata, K. (2001). EMBO Rep. *2*, 415–422.

Kaneko, M., Ishiguro, M., Niinuma, Y., Uesugi, M., and Nomura, Y. (2002). FEBS Lett. *532*, 147–152.

Kaufman, R.J., Scheuner, D., Schroder, M., Shen, X., Lee, K., Liu, C.Y., and Arnold, S.M. (2002). Nat. Rev. Mol. Cell Biol. *3*, 411–421.

Lee, K., Tirasophon, W., Shen, X., Michalak, M., Prywes, R., Okada, T., Yoshida, H., Mori, K., and Kaufman, R.J. (2002). Genes Dev. *16*, 452–466.

Okada, T., Yoshida, H., Akazawa, R., Negishi, M., and Mori, K. (2002). Biochem. J. *366*, 585–594.

Patil, C., and Walter, P. (2001). Curr. Opin. Cell Biol. *13*, 349–355.

Scheuner, D., Song, B., McEwen, E., Liu, C., Laybutt, R., Gillespie, P., Saunders, T., Bonner-Weir, S., and Kaufman, R.J. (2001). Mol. Cell *7*, 1165–1176.

Wang, Y., Shen, J., Arenzana, N., Tirasophon, W., Kaufman, R.J., and Prywes, R. (2000). J. Biol. Chem. *275*, 27013–27020.

Yoshida, H., Matsui, T., Yamamoto, A., Okada, T., and Mori, K. (2001). Cell *107*, 881–891.

Yoshida, H., Matsui, T., Hosokawa, N., Kaufman, R.J., Nagata, K., and Mori, K. (2003). Dev. Cell *4*, this issue, 265–271.

Neutrophils with a License to Kill: Permeabilized, Not Stirred

The neutrophil responds to invading microorganisms in part by assembling the NADPH oxidase complex and producing superoxide radicals. Relatively little is known about the intracellular assembly or activation of the oxidase, but Brown et al. in the January issue of *Molecular Cell* provide a useful strategy involving permeabilized neutrophils to tackle this question.

By all accounts, the neutrophil is a difficult cell to deal with. Short lived and essentially resistant to in vitro genetic manipulations, the prickly neutrophil undoubtedly blames its upbringing. In contrast to more friendly and outgoing cell types that willingly seek out the company of neighboring cells, neutrophils tend to live their entire, brief life in total isolation. Their sole purpose is to exist in an armed and dangerous state, roaming the body in a constant search for invading organisms. When trouble is found, neutrophils can turn from angry to downright nasty, releasing a biological cocktail of what might aptly be described as cellular weapons of mass destruction.

One of the primary weapons in the neutrophil arsenal is an enzyme complex that generates superoxide radicals. This multisubunit enzyme is termed the NADPH oxidase because it uses molecular oxygen and NADPH to generate superoxide anions. The oxidase is composed of two membrane-bound subunits, gp91phox and p22phox (referred together as cytochrome b558) as well as four cytosolic components, p47phox, p67phox, p40phox, and a small GTPase, Rac1 or Rac2. In addition to toxic free radicals, the neutrophil can also unleash a host of proteases, peroxidases, and biologically active molecules that are stored within at least five different types of intracellular granules. Activation of the neutrophil triggers assembly of the oxidase with the subsequent translocation of the cytosolic components to the membrane-bound cytochrome b558. Although those details are generally agreed upon, exactly where and how this assembly occurs has been the subject of considerable debate. Because the oxidase was classically viewed as an external generator of superoxide anions, the assembly of an active NADPH oxidase was long thought to be restricted to the plasma membrane. Nonetheless, although the oxidase is clearly found within the plasma membrane of activated neutrophils, the vast majority of the cytochrome b558 is contained within granule membranes, and there is evidence that the oxidase can assemble within this subcellular compartment. Perhaps more intriguing is the observation that the signaling pathway leading to plasma membrane NADPH oxidase assembly might differ considerably from the granule-based or intracellular assembly of the oxidase (Karlsson et al., 2000).

Although evidence has emerged that the intracellular assembly of the oxidase is important, few experimental systems have been available to study these events. Reconstitution systems in both established cell lines and cell-free systems have been employed to study NADPH oxidase activity; however, these systems lack the unique signaling and architecture of the neutrophil. To overcome these limitations, Yaffe and colleagues have employed a system in which neutrophils are gently permeabilized by the bacterial cytolysin, Streptolysin-O (Brown et al., 2003). This bacterial toxin produces pores in the plasma membrane of cells, allowing for the rapid efflux of cytosolic proteins while cytoskeletal-associated or membrane-bound proteins generally require