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Previews

BiP Binding Keeps ATF6 at Bay

A study by Shen et al. in this issue of *Developmental Cell* shows that transport to the Golgi complex and subsequent proteolytic activation of the stress-regulated transcription factor ATF6 is initiated by the dissociation of the ER chaperone BiP from ATF6. This demonstrates that BiP is a key element in sensing the folding capacity within the ER and provides mechanistic insights on how the activation of membrane-bound transcription factors can be regulated.

Because of their tendency to aggregate, unfolded proteins are highly toxic, and eukaryotic cells have therefore evolved elaborate systems to cope with them. For example, accumulation of aberrant proteins in the endoplasmic reticulum (ER) leads to a complex reaction known as the unfolded protein response (UPR). In mammalian cells, the UPR induces the transcriptional upregulation of specific genes, some of which encode ER chaperones and components of the ER-associated protein degradation system. A further reaction to unfolded proteins is attenuation of protein synthesis to reduce the load of newly synthesized proteins in the ER (reviewed in Mori 2000; Ma and Hendershot, 2001).

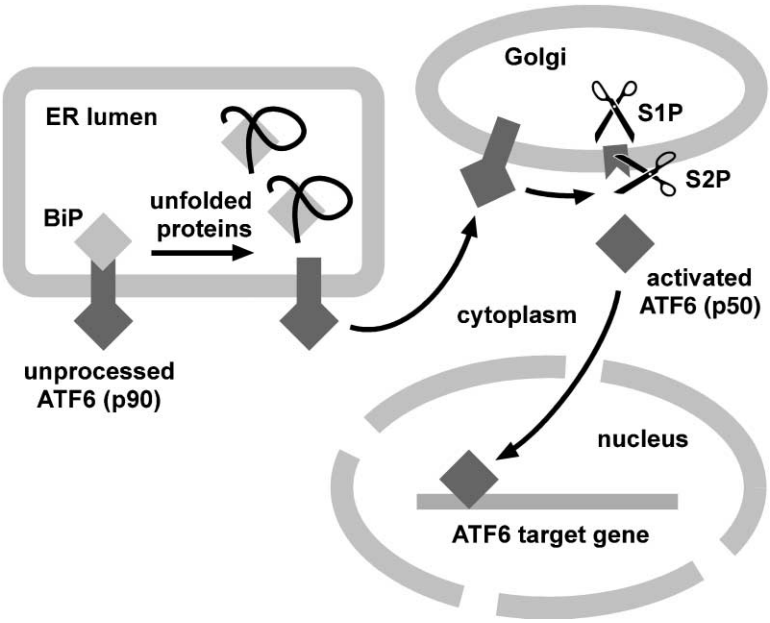
Two transcription factors, XBP1 and ATF6, and at least three ER membrane-bound signaling kinases are known to be involved in the UPR. The IRE1α and IRE1β kinases may have redundant functions, but the third kinase, PERK, is unique. UPR signaling is mediated by oligomerization and autophosphorylation of IRE1, which in turn causes the splicing of the XBP1 mRNA by an unconventional pathway. This allows translation of the potent UPR transcriptional activator XBP1 (Calfon et al., 2002). Signaling via PERK induces the phosphorylation of the translation initiation factor eIF-2α, which results in attenuation of protein synthesis. Both IRE1 and PERK "sense" the content of unfolded proteins in the ER with their lumenal tails (Bertolotti et al., 2000). In fact, these regions are interchangeable between IRE1 and PERK. In unstressed cells, both proteins bind to BIP, one of the major chaperones of the ER lumen. Changes in the ER that interfere with its folding capacity lead to dissociation of BiP, which causes activation of the cytosolic kinase domains of IRE1 and PERK.

Much less is known about the activation of the transcription factor ATF6. It is synthesized as a membrane-bound precursor and its maturation involves cleavage by two proteases, S1P and S2P, to liberate the soluble, active transcription factor (Ye et al., 2000). But how is the activity of these proteases toward ATF6 controlled? Since immature ATF6 is located in the ER membrane and S1P and S2P are in the Golgi compartment, it has been postulated that ATF6 has to be transported to the Golgi during the activation process (Ye et al., 2000). In addition, evidence has been presented that the lumenal domain of ATF6 is essential for sensing aberrant proteins in the ER and the transport to the Golgi (Chen et al., 2002). However, it remained unclear how transport and activation of ATF6 are linked.

Now, Shen et al. provide convincing evidence that BiP regulates the activity of ATF6 by controlling its release to the Golgi compartment. The lumenal domain of ATF6 contains BiP binding sites as well as regions that mediate transport to the Golgi. Under normal growth conditions, ATF6 is found as an ER membrane protein that is associated with BiP. When the ER folding machinery is stressed by treatment with drugs such as dithiothreitol (DTT), which prevent proper protein folding, BiP dissociates from ATF6, which is then transported to the Golgi and proteolytically processed (see Figure). The data presented by Shen et al. suggest that BiP binding inhibits the function of the Golgi localization signals and thereby retains ATF6 in the ER: deletion of the lumenal BiP binding sites causes constitutive transport of ATF6 to the Golgi. Vice versa, ATF6 mutants that lack the Golgi localization signals are unable to travel to the Golgi even when BiP interaction is diminished by DTT treatment. Overexpression of BiP causes an increased formation of the ATF6/BiP complex and delays the cleavage of ATF6 in response to ER stress. Finally, a mutant form of BiP, that is impaired in ATP hydrolysis, dissociates only slowly from ATF6 upon DTT treatment and thus abolishes ATF6 processing.

These important new findings emphasize the crucial role of BiP in measuring the content of unfolded proteins within the ER. The activity of all three membrane-bound UPR sensors, IRE1, PERK, and now also ATF6, are controlled by BiP binding and release. Moreover, the work from Shen et al. increases our knowledge on how membrane-bound transcription factors are activated by regulated intramembrane proteolysis (RIP) in response to specific stimuli. The Golgi localized proteases S1P and S2P have previously been shown to catalyze the proteolytic activation of a group of transcription factors, termed sterol regulatory binding proteins (SREBPs) (Brown and Goldstein, 1997). Like ATF6, the precursor forms of SREBPs constitute ER resident proteins. However, in this case another protein, termed *S*REBP *c*leavage *a*ctivating *p*rotein (SCAP), regulates the activity of SREBP processing. SCAP forms complexes with SREBPs and initiates their release from the ER to the Golgi to allow cleavage by S1P and S2P. Although activation of ATF6 seems to involve a mechanism similar to SREBP processing, it does not depend on SCAP action.

The experiments presented by Shen et al. raise important questions. For example, what is the molecular basis of the association between BiP and ATF6? The analysis of this interaction is important because it allows us to draw conclusions on how the release of BiP from the UPR sensor proteins in response to ER stress is accomplished. It is tempting to speculate that parts of the lumenal tails of ATF6, IRE1 and PERK may act as a "surrogate" substrate, which mimic an unfolded protein that is recognized by the peptide binding pocket of BiP. As a result, it is possible that a DnaJ homolog that helps to load BiP onto the UPR sensors is also involved. In support of such a model, Shen et al. show that dissociation of BiP from ATF6 in ER-stressed cells depends on conformational changes of BiP. These conformational



Schematic Model of ATF6 Activation

Upon accumulation of unfolded proteins in the ER, BiP dissociates from immature ATF6 (p90). As a consequence, Golgi localization signals in the lumenal domain of ATF6 are exposed and mediate exit from the ER. In the Golgi, two subsequent processing steps by the S1P and S2P proteases liberate the active ATF6 transcription factor (p50) from the membrane. The processed ATF6 is transported into the nucleus and upregulates the transcription of target genes.

changes are also necessary for the release of folding substrates, indicating that BiP may bind to ATF6 and unfolded proteins in a similar manner. If the BiP/UPR sensor complexes are highly dynamic, increasing amounts of unfolded proteins in the ER should efficiently compete with the sensors for BiP binding and reduce the amount of these complexes rapidly. On the other hand, a low off-rate of BiP from the sensor proteins would indicate a different mode of association and suggest that dissociation may be triggered by yet unknown modifications of either BiP or the UPR sensors.

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Selected Reading

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How to Grab a Microtubule on the Move

In migrating cells, Rho family GTPases and their effectors play a central role in polarizing and in organizing the actin and microtubule cytoskeletons. A study by Fukata et al. in the June 28th issue of *Cell* now shows that the Rac1/Cdc42 effector IQGAP1 captures microtubules by binding to CLIP170.

Many cellular functions require a defined polar organization of the cell body. Polarization is achieved through reorganization of the cytoskeleton. This leads to asymmetric distribution of organelles and key molecules, directs secretion, and provides forces to shape the cell.

Migrating cells are a particularly interesting system for studying the process of cell polarization. In these cells polarization is not simply a singular event, but, rather, an extremely dynamic process, whereby polarity is constantly reestablished, allowing cells flexibility to move and respond to various external stimuli. A plethora of data have revealed that small GTPases of the Rho family, particularly RhoA, Rac1, and Cdc42, are central to the process of polarization in all eukaryotic cells. In migrating fibroblasts, Rac1 and Cdc42 are active at the leading edge, where they organize small cell adhesions, lamellipodia, and filopodia. In contrast RhoA seems to be active all over the cell and is involved in the formation