Tyrosine Phosphorylation of IκBα

Beginning on page 753, Bui et al. present the first demonstration of ligand-induced tyrosine phosphorylation of IκBα, the well-known inhibitor of the transcription factor NFκB. In NFκB-activating pathways that have been studied previously, the transcription factor is activated by serine phosphorylation and proteasome-mediated degradation of IκBα. Bui et al. propose that the ability to activate NFκB through separate, non-redundant signal transduction pathways might be important in neuronal survival, as well as in inflammation and repair processes.

In the new work, Bui et al. set out to examine the effect of NGF on the expression of the antiapoptotic protein Bcl-xL. In rat PC12 cells, human neuroblastoma SH-SY5Y cells, and primary rat hippocampal neurons, NGF treatment increased NFκB activity and Bcl-xL expression. As expected, overexpressing IκBα in the cells inhibited these NGF-induced effects. However, the NGF-induced NFκB activation was not accompanied by significant IκBα degradation, and NFκB treatment caused tyrosine phosphorylation of IκBα, rather than the serine phosphorylation caused by TNF-α. Overexpressing a mutant form of IκBα lacking the tyrosine phosphorylation site specifically suppresses NGF-induced, but not TNF-α-induced, NFκB activation, further indicating that the two factors act through different signaling pathways. Although tyrosine phosphorylation of IκBα previously has been observed as a pathophysiological response in cells, the new study suggests that this mechanism of NFκB activation is also part of normal physiology.

Visualizing Autophagy

Although macroautophagy, a crucial mechanism for recycling long-lived proteins in cells, was discovered over 30 years ago, the mechanism of this process has remained poorly understood. Mizushima et al. (page 657) have now partially characterized the early steps of autophagy in mouse embryonic stem (ES) cells, providing new insights into the formation of isolation membranes and a description of an experimental system that should facilitate further studies on autophagy.

Macroautophagy involves the delivery of cytoplasmic components to autophagosomes, which are formed from cup-shaped isolation membranes. Mizushima et al.’s previous studies in yeast had shown that the ubiquitin-like Apg12–Apg5 conjugation system is required for this process, and that the Apg12–Apg5 system is conserved in mammals. In the new work, Mizushima et al. used GFP-tagged murine Apg5 to show that the cup-shaped membranes develop from small crescent-shaped compartments in ES cells. Apg5 preferentially associates with the outer side of the isolation membrane, indicating that the membrane is asymmetrical. Apg5–Apg12 conjugation is not required for Apg5 to localize to isolation membranes, but it is required for Apg5 to function in elongating the membranes. Apg5-deficient ES cells are defective in autophagosome formation. The results represent the first real-time visualization of autophagosome formation, and support a model in which the isolation membranes elongate and bend to form cup-shaped, and then spherical, autophagosomes.

MMPs as Inhibitors of Adipocyte Differentiation

While attempting to study the regulation of apoptosis during mammary gland involution, Alexander et al. (page 693) instead discovered a novel physiological role for matrix metalloproteinases: negative regulation of adipocyte metabolism and differentiation. Alexander et al. used both genetic and cellular approaches to examine the role of the matrix metalloproteinase MMP-3/stromelysin-1 (Str1) in mouse mammary involution, a model of induced apoptosis in which the mammary gland loses most of its lactating epithelial cells and repopulates the mammary fat pad with adipocytes. Previous work had shown that ectopically expressed Str1 can induce apoptosis.

In the new work, Alexander et al. studied mammary involution in Str1 knockout mice and transgenic mice overexpressing TIMP-1, a tissue inhibitor of metalloproteinases. Both systems exhibit accelerated differentiation and hypertrophy of adipocytes, but epithelial apoptosis is unaffected. In tissue culture, adipogenic 3T3-L1 cells exhibit increased transcription of several MMP and TIMP mRNAs, but only differentiated adipocytes express an activated MMP, and MMP inhibitors accelerate lipid accumulation as the cells differentiate. Based on their results, Alexander et al. conclude that Str1 and other MMPs do not induce epithelial cell death during mammary gland involution, but instead regulate adipocyte differentiation.
Degradation and Aggregation of an Amyloidogenic Protein

Dul et al. (page 705) show that two cellular mechanisms for coping with misfolded amyloidogenic light chain proteins, proteasome-mediated degradation and aggregation into an inclusion body, appear to compete with each other. In addition to illuminating an important aspect of the pathogenesis of light chain amyloidosis, the new work suggests that rationally designed peptides may be able to suppress aggregation and act as effective therapies for amyloid diseases.

Light chain amyloidosis is characterized by the misassembly of immunoglobulin light chains into fibrils that are deposited in tissues. Building on previous in vitro work, Dul et al. expressed a mutant light chain, or its wild-type counterpart, in COS cells. Whereas the wild-type protein is secreted, the mutant protein is translocated out of the ER into the cytosol, where it is either degraded by proteasomes or condensed into a large perinuclear aggresome. Overexpression of the chaperone Hsp70 decreases aggregation, but inhibition of the proteasome increases aggregation, indicating that the two processes are in competition.

Dul et al. also found that a light chain-derived peptide appears to facilitate the targeting of the misfolded protein to the proteasomal pathway, shifting the balance toward degradation and away from aggregation. If the extracellular aggregates observed in patients with light chain amyloidosis are formed by a similar mechanism, rationally designed peptides could have considerable therapeutic potential.

The Ins and Outs of Karyopherins

Yoshida and Blobel (page 729) report that the yeast Msn5p/Kap142p protein, a karyopherin that is known to export several factors from the nucleus, also mediates the nuclear import of a completely different set of proteins. The report is the first description of a transport receptor capable of acting as both an import and an export factor for different proteins.

Yoshida and Blobel purified a complex from the yeast cytosol that contained Kap142p and the trimeric replication protein A (RPA). RPA plays a variety of roles in DNA metabolism, and the three RPA proteins, all of which are essential for cell viability, are found primarily in the nucleus in wild-type cells. In a KAP142 deletion strain, RPA localizes to the cytoplasm, and the mutant cells display increased sensitivity to bleomycin, which induces double-strand breaks that are repaired primarily by an RPA-dependent process.

Although RPA is essential, the KAP142 deletion strain is viable, suggesting that an alternative nuclear import factor can partially compensate for the loss of Kap142p. Supporting this idea, Yoshida and Blobel found that in the deletion strain, RPA interacts with the karyopherin Kap95p. Additional work will be required to determine whether other karyopherins act as bidirectional transporters.