Innovations

Stress Proteins as Molecular Chaperones: Implications for Toxicology

Although organisms encounter both natural and anthropogenic stress, until quite recently our knowledge of how they adapt to stress at the molecular and cellular levels has been limited. It is now becoming apparent that just as cells have evolved mechanisms to repair DNA, they have evolved an epigenetic repair and recycling system to maintain protein integrity. Protein denaturation and misfolding caused by weakening of polar bonds and exposure of hydrophobic groups are important targets of stressor-induced damage. A system that repairs this damage could provide valuable insights into the molecular mechanisms of toxicity, identification of target organs, and evaluation of physiological state.

The epigenetic repair system is called the cellular stress response or the heat-shock response. It is involved in protecting organisms from damage as a result of exposure to a wide variety of environmental stressors including elevated temperatures, ultraviolet light, trace metals, and xenobiotics. A major feature of the response is the rapid synthesis of "stress proteins" upon exposure to environmental stress. Stress proteins are highly conserved in evolution and play similar roles in organisms from bacteria to humans. Cell lines selected for survival at high temperatures constitutively synthesize the stress protein hsp70 at high levels, whereas temperature-sensitive mutants do not. A number of stress protein families, including stress90, stress70, chaperonin60, hsp40, the low molecular weight stress proteins, and ubiquitin, have been identified in diverse phyla. Studies comparing the stress protein response in closely related species that inhabit different environments have shown that differences in the response can be related to thermal resistance, suggesting that stress proteins help organisms adapt to harsh or unpredictable environments. Evidence is also mounting that the stress response is induced by chemical pollutants.

Maintenance of Protein Integrity

In the last few years scientists have gained a deeper understanding of how stress proteins provide protection. Under normal conditions, several of the major stress proteins are present at low levels and function as "molecular chaperones" to facilitate folding, assembly, and distribution of newly synthesized proteins. Under conditions of environmental stress, stress proteins are involved in protecting and repairing vulnerable protein targets. Stress proteins also play a role in the lysosomal and ubiquitin protein degradation pathways, by which damaged proteins are broken down. In essence, the cellular stress response entails the orchestrated induction of key proteins that form the basis for a cell's protein repair and recycling systems.

The remarkable way in which stress proteins act as catalysts of protein folding and repair is best understood by examining two major heat-inducible protein families, stress70 and cpn60. Stress70 is a large, multigene family with members residing in a number of subcellular compartments including the cytoplasm, mitochondria, and endoplasmic reticulum. Normally, stress70 proteins prevent incorrect folding of newly synthesized peptides by binding to the growing peptide chain and maintaining it in a loosely folded state until synthesis is complete. Another stress protein, hsp40, is suspected to interact with stress70 and participate in this process. Stress70 disassociation, an ATP-dependent process, occurs as the protein proceeds down its folding pathway to reach its correct three-dimensional shape. Proteins that need to be distributed to other subcellular compartments are maintained in an unfolded state and escorted to that destination for translocation. Once inside the organelle, the target protein interacts with another member of the stress70 family which performs similar folding functions.

Additional folding functions and assembly are carried out by yet another group of proteins called chaperonins. This class of proteins includes the chaperonin60 (cpn60) family and is found in eubacteria, mitochondria, and plastids. Chaperonins assemble into large "double donut"-shaped complexes that direct the higher-level folding and the assembly of subunits into complexes. A functional homolog, TCP1, is present in the cytoplasm, where it facilitates folding of actin and tubulin, and perhaps other proteins. This chaperonin family appears to be weakly related to the cpn60 group, but it is not considered a stress protein because its synthesis is not induced by stress.

Under environmental stress, dramatic changes occur in the stress70 and cpn60 families, which suggest that they take on dual roles in protein protection and repair. The synthesis of stress70 increases, and it protects from stress-induced damage by binding to vulnerable proteins, preventing denaturation and the formation of insolubility.
Association of Stress Proteins with Vulnerable Cellular Targets

Early studies by Hightower, Voellmy, and others on the regulation of the cellular stress response supported the notion that the synthesis of stress proteins is related to protein damage. Heat-inducible genes include a conserved sequence referred to as the "heat-shock element" in their upstream regulatory region. The gene is activated by a protein called the heat-shock factor, which binds to the heat-shock element. Although scientists do not precisely know how heat shock or other adverse environmental conditions activate the heat-shock factor, a number of studies suggest that stressors that cause an increase in damaged or abnormal proteins activate this process. It has been suggested that stress70 plays a role in this relationship and acts as a "cellular thermometer." Injection of denatured, but not native, protein results in transcription of heat shock genes in Xenopus oocytes. Amino acid analogues that create abnormal proteins induce synthesis of stress protein. Biochemical conditions that alter protein conformation also affect expression of the stress response in a predictable manner. However, the most intriguing aspect of the regulation of induction of the stress proteins is that denatured proteins are both the signal that activates transcription of the stress protein genes and the substrate for the proteins themselves.

The role of stress70 and cpn60 in repair is further substantiated by their cellular localization and distribution in response to stress. Lindquist's group was the first to show that cytoplasmic stress70 moves into the nucleus in response to heat shock, where it interacts with structures such as the nucleolus, the site of ribosomal assembly, and then it returns to the cytoplasm during recovery. Hattori and colleagues showed that hsp40 responds to heat shock in a similar manner. Recently Sanders and co-workers demonstrated that cpn60 also localizes in the nucleus in response to heat shock. The nuclear localization of stress70 and cpn60 in cells subjected to a heat-shock treatment occurs in a sequential fashion: stress70 is normally present at low levels, but two hours after heat-shock treatment it increases in the cytoplasm and associates with the nucleolus, and disperses throughout the nucleus two hours later. Within 24 hours, levels and distribution of stress70 return to normal.

While cpn60 is also normally present at low levels, in the mitochondria its abundance increases from two to eight hours after heat shock. Then, 12–24 hours after heat shock, cpn60 can be seen in the nucleus associating with the nucleolus and as discrete foci. These foci are strikingly similar to the distribution of the snRNP-rich organelles called "coiled bodies," which are believed to be involved in RNA processing. The fact that these two stress proteins interact differently with complexes in the nucleus suggests that they have distinct roles in facilitating repair of different nuclear structures. Further, although both proteins interact with the nucleolus, the nucleolar association of cpn60 occurs well after stress70 has migrated from the nucleolus. This sequential interaction is suggestive of their respective roles in folding in the mitochondria. This association of stress proteins with protein complexes particularly sensitive to stress-induced...
damage may prove helpful in identifying subcellular sites of toxicity for various chemicals and other stressors.

**Tissue Specificity and Target Organ Toxicity**

Induction of stress protein synthesis is highly tissue specific. Both the temperature range of induction and the extent of induction of each stress protein appears to depend on tissue type. This tissue specificity is probably a result of two different mechanisms: differences in gene expression among specialized cell types and the extent of tissue damage. Given the current understanding of the regulation and function of the stress response, the intensity and relative concentrations of stress70 and cpn60 should be greatest in tissues that are most vulnerable to damage caused by a particular environmental stressor. For chemical stressors these factors would include the distribution of the chemical among tissues; the ability of each tissue to detoxify the contaminant and minimize cellular damage; and the chemical’s molecular mechanisms of toxicity. Due to the interplay among the many physiological processes involved, the concentrations of stress proteins among tissues could differ significantly with the stressor. Such differences in stress protein accumulation might be useful in identifying target tissues and evaluating the extent of damage.

Studies in fish, mammals, and invertebrates lend support to this premise. In fathead minnows, the route of exposure dictates tissue-level responses to arsenite, a hydrophilic protein denaturant that can damage all tissues. The gill, which is in direct contact with the arsenite, is more sensitive than tissues not directly exposed. In contrast, the tissue-level response upon exposure to the pesticides diazinon and lindane is greatest in brain and muscle and lowest in gill. Because diazinon is a cholinesterase inhibitor and lindane inhibits GABA-activated chloride channels in neurons, tissues with greatest innervation would be the primary targets. The gill, a primary target organ for copper accumulation and toxicity in mussels exposed to elevated copper in seawater, also accumulates higher levels of stress proteins than other tissues. Stress protein synthesis is also target-organ specific in rats exposed to mercuric chloride, where synthesis occurs in the kidney before overt renal injury can be detected.

**Stress Proteins as Integrative Biomarkers of Physiological State**

Organisms encounter many stressors in their natural environment but they only have a finite capacity to adapt to these stressors. Yet toxicologists usually look at only one stressor among the multitude of environmental stressors with which an organism must cope. Since the synthesis of stress proteins is a broad response, induced regardless of the nature of the stressor, the accumulation of these proteins has the potential to provide information on the overall impact of multiple stressors on physiological state. Furthermore, since stress proteins are part of the cell’s protective strategy, this accumulation should be closely coupled with the organism’s physiological state, providing an early warning of impairment at the organismal level.

**Suggested Reading**

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Studies with aquatic organisms and soil invertebrates suggest that the accumulation of stress protein in wild populations from contaminated environments may be useful for quantifying protein damage from environmental factors. Other studies have shown exposure-response relationships between accumulation of stress70 and cpn60 and exposure concentration. This relationship occurs at contaminant levels that are lower than levels at which other measures of physiological impairment can be observed. The induction of stress proteins could provide the added benefit of evaluating the extent to which an organism is stressed.

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