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MOLECULAR AND EVOLUTIONARY BASIS OF THE CELLULAR STRESS RESPONSE

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■ **Abstract** The cellular stress response is a universal mechanism of extraordinary physiological/pathophysiological significance. It represents a defense reaction of cells to damage that environmental forces inflict on macromolecules. Many aspects of the cellular stress response are not stressor specific because cells monitor stress based on macromolecular damage without regard to the type of stress that causes such damage. Cellular mechanisms activated by DNA damage and protein damage are interconnected and share common elements. Other cellular responses directed at re-establishing homeostasis are stressor specific and often activated in parallel to the cellular stress response. All organisms have stress proteins, and universally conserved stress proteins can be regarded as the minimal stress proteome. Functional analysis of the minimal stress proteome yields information about key aspects of the cellular stress response, including physiological mechanisms of sensing membrane lipid, protein, and DNA damage; redox sensing and regulation; cell cycle control; macromolecular stabilization/repair; and control of energy metabolism. In addition, cells can quantify stress and activate a death program (apoptosis) when tolerance limits are exceeded.

CELLULAR STRESS: WHAT IS THE THREAT AND HOW DO CELLS RESPOND?

The study of mechanisms of adaptation to stressful and extreme environments provides the basis for addressing environmental health problems, performing sound toxicological risk assessment, efficiently utilizing bioindication processes to monitor global environmental change, and clinically utilizing the inherent healing capacity of the adaptive response to stress. Detailed study of the cellular stress response (CSR) has revealed diverse molecular mechanisms too numerous to be considered comprehensively in this review. Highlighted here are evolutionarily conserved principles of the CSR that are critical for understanding the molecular mechanisms of cellular adaptation to stress.

Classical responses of animals to stress, the "fight-or-flight response" (1) or "general adaptation syndrome" (2), are controlled by hormones at the organismal

level (3). At the cellular level the CSR is a defense reaction to a strain imposed by environmental force(s) on macromolecules. Such strain commonly results in deformation of/damage to proteins, DNA, or other essential macromolecules (4). The CSR assesses and counteracts stress-induced damage, temporarily increases tolerance of such damage, and/or removes terminally damaged cells by programmed cell death (apoptosis). The capacity of the CSR depends on the proteome expressed in a cell at a particular time and is therefore species- and cell type-dependent.

THE MINIMAL STRESS PROTEOME OF ALL ORGANISMS

Functional Classification of Stress Proteins Conserved in All Cells

The CSR is characteristic of all cells. It targets a defined set of cellular functions, including cell cycle control, protein chaperoning and repair, DNA and chromatin stabilization and repair, removal of damaged proteins, and certain aspects of metabolism (4). Proteins involved in key aspects of the CSR are conserved in all organisms. They can be identified experimentally using proteomics approaches such as 2D electrophoresis or 2D chromatography in combination with mass spectrometry analysis. In addition, annotated proteomes of multiple organisms can be compared using bioinformatics approaches that identify evolutionarily conserved stress proteins. Such analysis of human (*Homo sapiens*), yeast (*Saccharomyces cerevisiae*), eubacterial (*Escherichia coli*), and archaeal (*Halobacterium spec.*) proteomes yields circa 300 proteins that are highly conserved in all (4). This protein set corresponds approximately to the size of a minimal gene set and includes tRNA synthetases for all essential amino acids, presumably inherited from the last universal common ancestor (LUCA) (5). Gene ontology and literature analysis of these 300 proteins have revealed 44 proteins with known functions in the CSR (Table 1).

Many more than the 44 proteins in Table 1 participate in the CSR. However, most stress proteins are not ubiquitously conserved in all three superkingdoms and are, therefore, not included in this minimal stress proteome of all organisms. Transcript levels for most universally conserved stress proteins (31 of 44) are up-regulated in response to diverse stresses in yeast (6). However, stress proteins are regulated not only at the mRNA level but also at other levels, e.g., by modulation of protein turnover or by posttranslational modification. Also, high constitutive expression of some conserved stress proteins confers increased cellular stress resistance. Cells with chronic stress exposure constitutively express several stress proteins at very high levels, including Hsp60, Hsp70, peroxiredoxin, and superoxide dismutase in mammalian renal inner medullary cells (7; N. Valkova & D. Kültz, manuscript submitted) and RecA/Rad51 in the extremophile archaeon *Pyrococcus furiosus* (8).

Functionally, the 44 stress proteins cluster into distinct categories that reflect different aspects of the CSR. They include redox-sensitive proteins as well as

TABLE 1 The minimal stress proteome of cellular organisms

Redox regulation	**DNA damage sensing/repair**	**Fatty acid/lipid metabolism**
Aldehyde reductase	MutS/MSH	Long-chain fatty acid ABC transporter
Glutathione reductase	MutL/MLH	Multifunctional beta oxidation protein
Thioredoxin	Topoisomerase I/III	Long-chain fatty acid CoA ligase
Peroxiredoxin	RecA/Rad51	
Superoxide dismutase		
MsrA/PMSR	**Molecular chaperones**	**Energy metabolism**
SelB	Petidyl-prolyl isomerase	Citrate synthase (Krebs cycle)
Proline oxidase[a]	DnaJ/HSP40	Ca^{2+}/Mg^{2+}-transporting ATPase[b]
Quinone oxidoreductase[c]	GrpE (HSP70 cofactor)	Ribosomal RNA methyltransferase[d]
NADP-dependent oxidoreductase YMN1[c]	HSP60 chaperonin[d]	Enolase (glycolysis)
Putative oxidoreductase YIM4[c]	DnaK/HSP70	Phosphoglucomutase
Aldehyde dehydrogenase[c]		
Isocitrate dehydrogenase[c]	**Protein degradation**	**Other functions**
Succinate semialdehyde dehydrogenase[c]	FtsH/proteasome-regulatory subunit[d]	Inositol monophosphatase[b]
6 phosphogluconate dehydrogenase[c]	Lon protease/protease La	Nucleoside diphosphate kinase[e]
Glycerol-3-phosphate dehydrogenase[c]	Serine protease	Hypothetical protein YKP1
2-hydroxyacid dehydrogenase[c]	Protease II/prolyl endopetidase	
Hydroxyacylglutathione hydrolase	Aromatic amino acid aminotransferase	
	Aminobutyrate aminotransferase	

[a]Proline oxidase degrades proline to pyrroline 5-carboxylate, hence it is also involved in amino acid degradation.

[b]Signaling functions (Ca^{2+}- and phosphoinositide-mediated).

[c]Many oxidoreductases are also important for energy metabolism.

[d]These proteins are also involved in cell cycle control.

[e]Involved in nucleotide synthesis (possible role in DNA repair).

proteins involved in sensing, repairing, and minimizing macromolecular damage, such as molecular chaperones and DNA repair enzymes. In addition, numerous enzymes (notably oxidoreductases) that are involved in energy metabolism and cellular redox regulation are part of the minimal stress proteome. Some conserved stress proteins also function in cell cycle control (HSP60, FtsH, and ribosomal RNA methyltransferase). Notably, not all aspects of the CSR, in particular signaling-related mechanisms, are based on ubiquitously conserved pathways and proteins. Eukaryotes and prokaryotes differ in the nature of phosphorylation-based signal transduction. Two-component systems based on His/Asp phosphorylation predominate in prokaryotes, whereas more complex eukaryotic signaling cascades are mainly based on Ser/Thr/Tyr phosphorylation. Second, in eukaryotes DNA is packaged into a nucleus, which is absent in prokaryotes, and the degree of packaging is higher because eukaryotic genomes are generally larger. Thus, chromatin organization is more complex and histones and other chromatin proteins have unique roles in eukaryotes. Consequently, eukaryotic mechanisms of transcriptional regulation and cell cycle control are more complex and depend on proteins that differ from those utilized for equivalent functions in bacteria. Exceptions include proteins that constitute the very basic transcription/replication machinery, such as DNA polymerases.

Two Cellular Responses to Environmental Change: Stress Response and Homeostasis Response

In 1974 Tissières and coworkers discovered that heat shock proteins (HSPs) are induced in salivary glands of *Drosophila melanogaster* during heat stress (9). More than a decade later the function of HSPs as molecular chaperones was elucidated. Today, we know that these proteins are induced and activated during many other types of stress as well. They share this responsiveness to diverse stresses with many other proteins, notably most of the proteins included in the conserved minimal stress proteome (6). In addition, diverse stresses activate or induce many more weakly conserved stress proteins (6). The low stressor specificity of stress proteins raises two questions: (*a*) Why are these proteins induced/activated by diverse stresses? (*b*) Where does specificity originate in cell responses to particular environmental perturbations?

Responsiveness to diverse stresses may arise from the most striking and common impact of stress: It deforms and damages macromolecules, mainly membrane lipids, proteins, and/or DNA (4). Some specificity may arise because the types of lesions and damage to proteins, DNA, and membranes vary somewhat depending on the type of stress. Another common feature of diverse stresses is the generation of oxidative stress and change in cellular redox potential (10), referred to as oxidative burst (11, 12). The molecular events that increase reactive oxygen species (ROS) during some types of stress, including exposure of cells to ionizing radiation or highly reactive chemicals, are a direct consequence of the stress. But the molecular basis for oxidative burst is poorly understood in, for example, osmotic

stress or heat shock. During many types of stress cellular oxidases such as the plasma membrane NADPH oxidase are very rapidly activated, which may explain increased ROS levels (see below). Different cellular oxidases occur in different compartments (mitochondria, plasma membrane, etc.) and compartment-specific regulation of redox potential may be important for the outcome of the CSR. ROS and cellular redox potential have long been regarded as key regulators of CSR signaling, with ubiquitous roles as second messengers in cells exposed to stress (10).

The molecular basis of stressor-specificity has been a subject of much debate. One way of achieving stressor-specificity with the same set of components (induced/activated stress proteins) is via stressor-specific interactions, posttranslational modifications, and compartmentation of stress proteins resulting from different relative levels of induction within a common set of stress proteins. In addition, every stress also disturbs cellular homeostasis and induces a second type of response distinct from the CSR (Figure 1). In contrast to the transient nature of the CSR, this second type of response, here called the cellular homeostasis response (CHR), is permanent until environmental conditions change again. Its aim is to restore cellular homeostasis with specific regard to the particular environmental variable that has changed. Unlike the CSR, CHR is triggered primarily not by macromolecular damage or oxidative burst but by stressor-specific sensors that monitor changes in particular environmental variables (4). For instance, during osmotic stress the Sln1 and Sho1 membrane proteins function as osmosensors in yeast (13). In mammalian cells a particular transcription factor, the tonicity response element binding protein (TonEBP/NFAT5), activates osmoprotective genes that serve to stably restore cellular ion homeostasis by adjusting the levels of compatible organic osmolytes during osmotic stress (14). Although CSR and CHR signaling pathways are linked and contain common elements, this review focuses only on the former.

Molecular Basis of Cross-Tolerance and Stress-Hardening

Environmental stress tolerance varies widely depending on the species (genome) and on cell type and differentiation state (proteome). The latter is a function of gene-environment interactions during development and of pre-exposure to stress during life history. Stress-hardening (increased tolerance of a stress after preconditioning at low doses of that stress) and cross-tolerance (increased tolerance of one stress after preconditioning by another) are common and significant. For instance, ischemic preconditioning and mild hyperthermia induce HSP70 and decrease reperfusion injury of human muscle and kidney (15). HSP70 induction is also associated with stress-hardening and cross-tolerance to heat and cold stress in the fruit fly *Drosophila melanogaster* (16). Additional stress proteins induce stress-hardening and cross-tolerance of temperature, salinity, ionizing radiation, pH, and chemical stressors in diverse eukaryotic and prokaryotic cells (e.g., 17–20).

The activation and induction of a common set of stress proteins is the molecular basis of both cross-tolerance and stress-hardening. After the initial stress, these

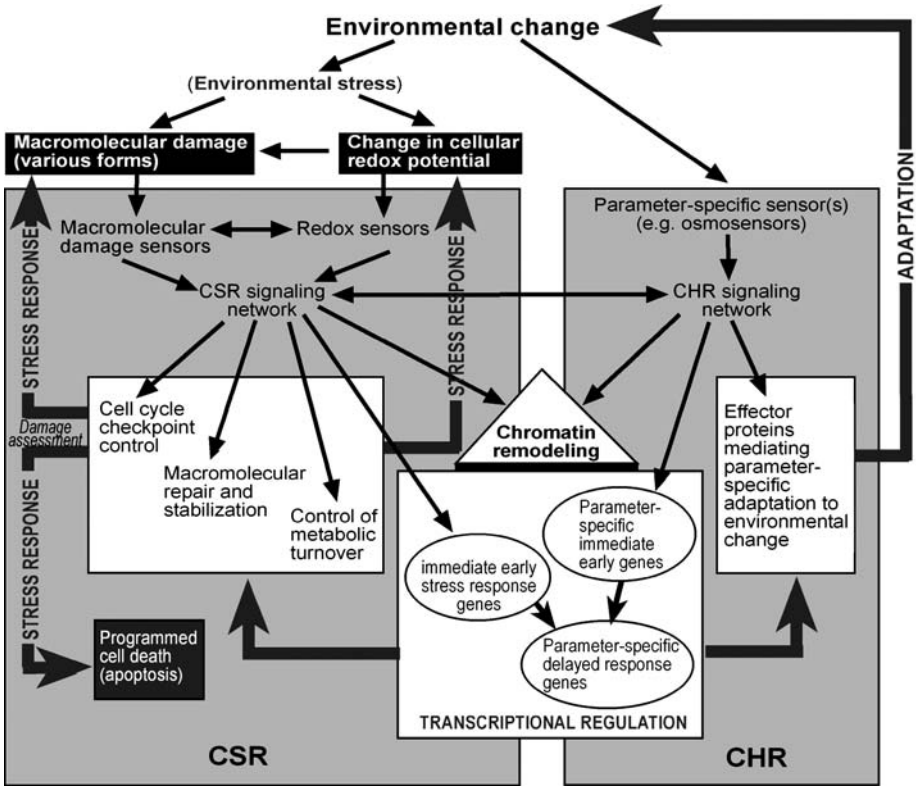

Figure 1 Schematic representation of key aspects of the cellular stress response (CSR) and its interaction with the cellular homeostasis response (CHR). The CSR serves to restore macromolecular integrity and redox potential that are disturbed as a result of stress. In contrast, the CHR serves to restore cellular homeostasis with regard to the particular environmental variable that has changed. Both types of cellular responses to environmental change are interconnected at numerous levels.

proteins remain active/elevated for a period that varies depending on species, cell-type, history of prior stress exposure, gene-environment interactions during development, and stress severity. During this period, activated/elevated stress proteins confer resistance to many different types of stress because of their involvement in general aspects of cellular protection such as protein stabilization, DNA repair, and free radical scavenging. In stark contrast to *Saccharomyces cerevisiae*, the yeast *Candida albicans* does not seem to induce a CSR via changes in gene transcription. Instead, it responds only by activation of the CHR, which correlates with a lack of cross-tolerance (21). This feature of *C. albicans* is exceptional and evolutionarily favored only in extraordinary stable environments (4).

Differences in constitutive levels of critical stress proteins are also responsible for cell type–specific variation in tolerance thresholds within multicellular

organisms. For instance, mammalian renal inner medullary cells tolerate many types of stress much better than do most other mammalian cell types, which correlates with increased constitutive levels of critical stress proteins in these cells (7, 22).

MACROMOLECULAR DAMAGE TRIGGERS THE CELLULAR STRESS RESPONSE

Cellular signal transduction networks commonly encompass three tiers: (*a*) sensors that perceive a signal; (*b*) transducers that carry, amplify, and integrate signals; and (*c*) effectors that adjust cell function corresponding to signals. In much of biology, extracellular signals are perceived by cell membrane receptors, and ligand-receptor interactions are highly specific. In addition, ligands are usually present at very low (nano- or micromolar) concentrations, and the affinity of the corresponding receptors is very high. Both paradigms apply poorly to the CSR. First, specific receptors are inconsistent with the lack of stress specificity in the CSR. Similarly, changes in environmental parameters are usually much more pronounced than minute changes in concentrations of specific ligands. For example, during osmotic stress total osmolyte concentrations can change by several hundreds of millimoles. Stress generally affects all cell compartments, whereas the cell membrane and other boundaries often exclude ligands from certain compartments. Finally, given the nature of stress-induced damage, stress sensors probably monitor the degree of macromolecular integrity in cells rather than an environmental signal per se. This mechanism would provide immediate feedback as to the effectiveness of the CSR once it has been activated. A second quasi-universal property characteristic of cells exposed to stress is an increase in ROS levels, which represents a critical second messenger for CSR signaling networks (23). Hence, sensors of membrane, protein, and DNA damage as well as redox sensors are key regulators of CSR signaling networks.

Lipid Membrane Damage Sensors

The cell membrane is the barrier to (and in direct contact with) the external environment and, therefore, well suited for sensing stress. In addition, secondary, calcium-mediated changes in properties of the mitochondrial membrane (mainly membrane potential and permeability) are important because they affect oxidative phosphorylation and redox potential directly, and thus may contribute to increases in ROS during stress (24, 25). Membrane and lipid damage occurs in all major groups of organisms in response to diverse stresses (e.g., 26–28). The extent of membrane damage and cellular tolerance limits during stress depend on lipid composition, fatty acid saturation, and membrane fluidity of the cell membrane (29, 30). Furthermore, the heat or salinity inducibility of a reporter gene that is driven by the CSR promoter element STRE (stress response element) is inversely correlated to the amount of unsaturated fatty acids in yeast, suggesting that induction of

the STRE pathway depends on membrane lipid composition (31). These authors also suggest that stress cross-tolerance may be (at least in part) a lipid-mediated phenomenon. Thus, the three universally conserved stress proteins involved in long-chain fatty acid (LCFA) metabolism and transport (Table 1) may contribute to changes in membrane lipid composition in response to stress. Moreover, the LCFA transporter mediates movement of LCFAs into peroxisomes, where they are metabolized by LCFA CoA ligase. This enzyme, present in multiple isoforms in many organisms, has been implicated in the metabolism of xenobiotics and reactive compounds generated during stress (32). In addition, fatty acyl-CoA esters produced by LCFA CoA ligase are emerging as physiological regulators of cell function, including transcriptional regulation (32).

Membrane damage from physical effects of environmental stress on cells is associated with altered membrane tension or stretch, permeability changes, lipid rearrangement, membrane protein rearrangement, changes in transmembrane potential, and formation of lipid peroxides and lipid adducts. Membrane lipid peroxidation, a common form of damage in response to stress, results from lipid auto-oxidation or catalysis by lipoxygenase (LOX) or the cytochrome P-450 system to yield highly reactive lipid peroxidation products (33, 34). Such products include isoprostanes from arachidonic, eicosapentaenoic, and docosahexaenoic acids; oxysterols from unesterified and esterified cholesterol; various other fatty acid hydroperoxides; and a wide spectrum of aldehydes (35, 36). Such membrane damage represents potential upstream signals for CSR signaling networks, and multiple mechanisms for translating nonspecific membrane damage into activation of CSR signaling pathways have been proposed (Figure 2).

First, nonspecific clustering of growth factor receptor tyrosine kinases and cytokine receptors during osmotic and UV-radiation stress can activate these receptors and the JNK cascade in mammalian cells (37). Activation of such cell surface receptors has other potential consequences, including the activation of PI-3-kinase, which catalyzes conversion of PIP_2 to PIP_3 (38). This activates the small GTP-binding protein Rac1, which, in turn, stimulates NADPH oxidase (38, 39). The NADP-dependent oxidoreductase contained in the minimal stress proteome may function in NADPH oxidase mode under such conditions. NADPH oxidase produces H_2O_2 (hydrogen peroxide), and, therefore, stress-stimulated nonspecific clustering of cell surface receptors provides a possible avenue for oxidative burst and activation of H_2O_2-induced signaling mechanisms during stress (Figure 2). A second potential avenue for oxidative burst and H_2O_2 generation during stress originates from lipid peroxidation (40). Lipid peroxidation products activate multiple signaling pathways, including MAP kinase pathways and the transcription factor AP-1 (activator protein 1), possibly via generation of H_2O_2 as a signaling intermediate (36, 40, 41). Third, processing of integral membrane proteins resulting in liberation of active signaling molecules is common and of extraordinary biological importance. For instance, phospholipase A_2 (PLA_2) activity depends on lipid packing density and membrane integrity and is elevated during stress (42). This enzyme catalyzes the hydrolysis of membrane glycerophospholipids, resulting in release

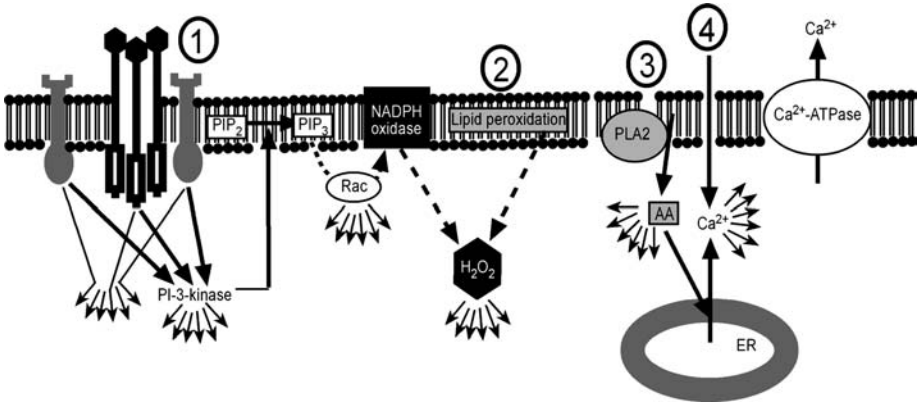

Figure 2 Potential stress sensing mechanisms that are based on lipid membrane damage/rearrangements. (1) Nonspecific clustering of growth factor and cytokine receptors due to membrane rearrangements leads to receptor activation. (2) Activation of NADPH oxidase resulting from receptor activation (1) and lipid auto-oxidation generate oxygen radicals that are converted to the second messenger hydrogen peroxide. (3) Changes in membrane tension or lipid rearrangement result in activation of phospholipase A_2, which leads to liberation of arachidonic acid from membranes. (4) Changes in membrane permeability lead to calcium influx into the cytosol. Multiple arrows emanating from several elements in the figure illustrate possibilities for further signal amplification. Please refer to the text for a discussion of these mechanisms.

of arachidonic acid (AA), an important signaling molecule in cells (43). Another example is the intramembrane proteolysis, liberation, and activation of the yeast transcription factor SPT23, a relative of mammalian NF-κB (nuclear factor kappa B). Because the proteasome-dependent processing of SPT23 is regulated by fatty acid pools, SPT23 may function in sensing membrane composition or fluidity (44). Fourth, changes in membrane permeability and the activity of mechanosensitive ion channels during stress promote calcium influx into the cytosol, an important signal for the CSR (45, 46). A Ca^{2+}-transporting ATPase is part of the minimal stress proteome and may be required to restore cytosolic calcium levels after the initial stress signal has been perceived. Although these mechanisms have not been extensively tested for their universal applicability to a broad spectrum of cells and stresses, they represent potential sensors of membrane lipid damage (Figure 2).

DNA Damage Sensors

Much work during the past decade has focused on DNA damage sensors, and consequently, we now know more about mechanisms of DNA damage sensing than those of membrane lipid damage sensors. Nonetheless, it is still nearly impossible to distinguish primary sensors from secondary transducers of DNA damage.

The problem lies in complex circuits of feedback regulation of proteins involved in sensing DNA damage. For example, many candidate sensor proteins are part of multiprotein complexes and, when activated, they become targets of further modification by their own substrates. Most studies on DNA damage sensors have focused on responses of cells to damage induced by ionizing radiation or highly reactive chemicals. However, recent work has demonstrated that during other types of stress, including osmotic stress and heat shock, DNA damage occurs and key mechanisms involved in eukaryotic DNA damage sensing, transduction, and repair are activated (47–49). These findings, in combination with extensive prior knowledge about ubiquitous effects of many types of stress on protein folding and stability, led to the hypothesis that the CSR represents a universal reaction to macromolecular damage (4).

Damage to DNA occurs in myriads of ways, ranging from common, i.e., certain base modifications such as 8-oxoguanine (8-oxo-7,8-dihydroguanine) formation, to more stressor specific, e.g., pyrimidine dimer formation during UV irradiation. However, despite numerous types of DNA adducts and base modifications, DNA damage can be grouped into a few major types, including DNA double-strand breaks (dsb), DNA nucleotide adduct formation and base modification, DNA base-pairing mismatches, and DNA single-strand breaks (ssb). Accordingly, the major classes of DNA repair are DNA dsb repair by homologous recombination (HR) or nonhomologous end-joining (NHEJ), nucleotide excision repair (NER), and nucleotide mismatch repair (MMR). DNA damage sensors probably recognize common intermediates of major types of DNA damage. Candidate intermediates are DNA ssb that occur during all types of DNA damage (50) and recognition motifs that are common to different base mismatches and modifications (51).

Much of the cellular machinery involved in DNA damage sensing is highly conserved in eukaryotes and prokaryotes but differs considerably between these two major forms of life. Nevertheless, some components of these complex networks are highly conserved in all three superkingdoms (Table 1), including MutS/MSH, MutL/MLH, RecA/RadA/Rad51, Top I/III, Mre11/Rad32, Rad50, and MutT/MTH. The latter two proteins show a lower degree of homology between prokaryotes and eukaryotes and do not meet the criteria for inclusion in the minimal stress proteome outlined above. However, they occur in all three superkingdoms (52) and are critical components of DNA damage sensing and signaling (see below). Mutations in all of these proteins cause defects in CSR signaling networks, resulting in diminished genomic integrity.

Bacterial MutS and eukaryotic MSH proteins recognize and bind to distortions produced by mismatches in DNA base pairing (53). In eukaryotes, the MMR proteins MSH2, MSH6, and MLH1 are part of the large BRCA1-associated genome surveillance supercomplex (BASC). BASC is important for recognizing and repairing base mismatches and in sensing other types of DNA damage in mammalian cells (54). After binding to sites of DNA damage, MutS/MSH proteins recruit MutL (in bacteria) or MLH (in eukaryotes) to those sites and initiate assembly of the MMR complex. The MutS gene in *E. coli* is induced by stress although it is

not considered part of the bacterial SOS response, the adaptive activation of stress proteins by the genetic regulator RecA (55). Thus MutS/MSH and MutL/MLH proteins are involved in sensing DNA base mismatches in all organisms, and this sensory capacity is increased by up-regulation of MutS/MSH during stress.

Another important DNA damage sensor in *E. coli* is the single-stranded (ss) DNA binding protein RecA, a recombinase also involved in DNA repair. The RecA protein represents a central part of the bacterial SOS response. RecA functions as a derepressor of LexA (which is a repressor of SOS genes) via its coprotease activity that degrades and inactivates LexA (56). It has been proposed that RecA is activated by recognizing and binding to ssDNA at sites of DNA base modifications or adducts and that this stimulates its coprotease activity (57). The functional homolog of RecA in archaea is the RadA protein, which increases when cells are exposed to stress (58). The eukaryotic homolog of RecA is Rad51, which is the key protein for homologous recombination-mediated repair of DNA dsb. Rad51 catalyzes the central step of homologous recombination, the DNA strand exchange reaction (59). Rad51 also binds ssDNA in eukaryotes (60) and Rad51 knockout mice are not viable, a finding illustrating that this protein is essential (61). Rad51 interacts with other proteins potentially involved in sensing DNA damage, including BRCA1, which is part of the same BASC supercomplex that also includes MSH and MLH proteins (see above; 62, 63).

Mre11 and Rad50 are also part of the mammalian BASC supercomplex (54). These proteins actually form a smaller complex with Nbs1 called the Mre11-Rad50-Nbs1 (MRN) complex, which interacts as a unit with other components of the BASC supercomplex. The MRN complex is required for both homologous recombination and nonhomologous end-joining (NHEJ) and is recruited to DNA dsb by another putative DNA damage sensor called MDC1 (mediator of DNA damage checkpoint 1) (64, 65). In *S. cerevisiae*, MRN complexes are targeted to sites of DNA dsb by direct association of Xrs2 (the yeast ortholog of mammalian Nbs1) with free DNA ends (66). In addition, mammalian Nbs1 regulates the kinetics and magnitude of ATM (ataxia telangiectasia mutated) serine-1981 autophosphorylation (67), and the MRN complex also stimulates ATM kinase activity (68). Thus, the MRN complex represents a critical element of rapid ATM activation resulting from autophosphorylation of serine-1981 and dimer dissociation in response to perturbation of chromatin structure (69). Consequently, it has been proposed that ATM is positioned downstream of the MRN complex and represents a secondary messenger rather than a primary DNA damage sensor (65, 70). ATM phosphorylates some of its own activators, including Nbs1 (70) and histone H2AX (71), both of which are involved in recruitment of the MRN complex to sites of DNA damage (72). Like ATM and its yeast ortholog Tel1, other PIKKs (PI-3-K like kinases) also seem to be early transducers of DNA damage signals rather than primary damage sensors. For instance, the catalytic subunit of mammalian DNA-PK (DNA-dependent protein kinase) is recruited to sites of DNA dsb by two other proteins, Ku70 and Ku80, which seem to be the sensors required for initiation of the NHEJ repair complex (65). Likewise, mammalian ATR (ATM-related)

and its yeast ortholog Mec1 are recruited to sites of DNA damage by association with the putative DNA damage sensor protein ATRIP (ATR interacting protein) in mammals and its ortholog Lcd1 in yeast, which results in activation of DNA damage-dependent cell cycle checkpoints (65, 73, 74).

The BASC supercomplex thus emerges as an important sensor of multiple types of DNA damage. The interaction of many highly evolutionarily conserved stress proteins, including MutS/MSH, MutL/MLH, RecA/Rad51, Mre11, and Rad50, with this DNA damage sensory supercomplex suggests that key aspects of DNA damage sensing mechanisms are highly conserved in all organisms. Additional support for the universal conservation of key aspects of genome integrity surveillance mechanisms comes from two other highly conserved proteins: MutT/MTH and topoisomerase. MutT/MTH is part of the nucleotide excision repair pathway that removes oxidized nucleotide precursors and prevents their incorporation into DNA during replication. Thus MutT/MTH is important for preventing replication-dependent oxidative DNA damage during stress-induced oxidative burst. The most stable and deleterious base modification caused by ROS is formation of 8-oxoguanine (8-oxo-7,8-dihydroguanine, 8-oxoG). 8-OxoG is produced not only in nucleotide pools of cells but also in DNA, where it mispairs with adenine and thus damages DNA. MutT/MTH protects cells from the mutagenic effects of 8-oxoG by degrading 8-oxo-dGTP to 8-oxo-dGMP (75).

Another potential DNA damage sensor that is part of the minimal stress proteome is topoisomerase. Cells have various isoforms of this enzyme that participate in different aspects of DNA metabolism. All topoisomerases alter DNA topology by introducing transient ssb into DNA during replication and NER. Because DNA ssb might represent a common intermediate recognized by DNA damage sensors (50), topoisomerase may be a critical element of DNA damage sensing. Indeed, topoisomerase I is involved in NER during the bacterial SOS response to stress-induced DNA damage (76, 77), and the homologous mammalian topoisomerase III is a sensor for the S phase DNA damage checkpoint (78). Additional highly conserved proteins involved in various aspects of DNA repair are candidate DNA damage sensors, notably the RecQ family of helicases that includes the Werner and Bloom syndrome helicases (79). New insights into how highly conserved stress proteins function during stress should further our understanding of DNA damage sensing mechanisms.

Protein Damage Sensors

Protein damage in cells exposed to stress occurs mainly as oxidative or structural (unfolding) damage. Some damaged proteins are repaired by enzymes that reverse oxidative damage or assist in protein refolding. But not all damaged proteins are repaired: Many terminally damaged proteins are removed by proteolytic degradation and regenerated by de novo synthesis. Thus, three processes are mainly responsible for removing protein damage: (*a*) repair of oxidative damage, (*b*) refolding of structurally damaged proteins, and (*c*) proteolysis. Methionine sulfoxide reductase

(MsrA/PMSR) and other conserved redox-regulatory stress proteins contribute to the recognition and repair of oxidative protein damage. The function of these proteins is discussed in the section Redox Sensors and Redox Regulation, below. In addition, five proteins of the minimal stress proteome are molecular chaperones. Because the key function of molecular chaperones pertains to protein maintenance and refolding, their role in the CSR is discussed under Maintenance of Macromolecular Integrity, below. Finally, six highly conserved stress proteins are involved in proteolysis (Table 1). These proteolytic enzymes help cells to monitor protein damage via mechanisms that are best illustrated using FtsH and Lon proteases as examples.

FtsH and Lon are regulatory protease subunits that are critical for removing damaged and abnormal proteins during stress and for controlling levels of key regulatory proteins with short half-lives. They are induced in response to many types of stress, including such unusual conditions as wine toxicity (80). FtsH and Lon both function as molecular chaperones by promoting the insertion of proteins into membranes and supporting the disassembly or oligomerization of protein complexes. FtsH is involved in stress resistance, membrane functions, cell cycle control, gene expression, translocation of secreted proteins, and degradation of some unstable and selected membrane and cytosolic proteins (81–83). Lon also contributes to the regulation of several important cellular functions, including stress resistance, cell division, cell morphology, proteolytic degradation of certain regulatory and abnormal proteins, and DNA maintenance (84–86). However, FtsH is the only membrane-integrated ATP-dependent protease that is universally conserved in all organisms (87). Moreover, in contrast to Lon, for which functionally redundant proteases such as Clp (caseinolytic) protease can substitute, FtsH is essential. But all of these proteases are part of protein complexes consisting of multiple subunits that have proteolytic core domains, regulatory domains with ATPase activity, and molecular chaperone domains (88).

FtsH lacks robust unfoldase activity and can only degrade proteins that are already damaged and partially unfolded. Moreover, FtsH uses the folding state of its protein substrates as a criterion for degradation (89). This feature delineates FtsH as a sensor of stresses that lead to protein unfolding. This property of FtsH further raises the possibility that its substrates are important components of CSR signaling. Indeed, FtsH displays high selectivity in protein degradation. It recognizes key signaling proteins by binding to specific motifs. Important FtsH substrates in bacteria include SecY (90), bacterial cell division protein FtsZ (91), and the heat shock transcription factor RpoH/σ^{32} (92, 93). FtsH is also involved in regulating the activity of σ^{54}-dependent promoters in bacteria (94), although it is not required for all σ^{54}-dependent promoters (95).

Lon also represents a potential sensor of protein damage because, like FtsH, it can recognize specific motifs in key signaling proteins (96–98). The recognition of such motifs depends on how these proteins are folded, which is affected by stress. For degradation of proteins containing specific Lon protease recognition motifs, no tagging (e.g., by ubiquitination) is required for protease activity. However, for recognition of additional, less specific substrates, Lon protease

cooperates with molecular chaperones that are also part of the minimal stress proteome, including DnaK/HSP70 (99) and DnaJ/HSP40 (98). A specific substrate of Lon protease in the bacterium *Streptomyces coelicolor* is the negative autoregulator HspR, which represses important CSR genes encoding DnaK/HSP70, Lon protease, and Clp protease when bound to free DnaK/HSP70. During heat stress, the levels of free DnaK/HSP70 decline as a result of increased binding to unfolded proteins, and HspR-mediated repression of DnaK/HSP70, Lon protease, and Clp protease is lifted leading to induction of those genes (100). Such feedback mechanisms illustrate the sensory role of cellular proteases during stress (101).

In eukaryotes, FtsH and Lon are mainly located in mitochondria and plant chloroplasts (102). Consequently, eukaryotic FtsH and Lon are involved in protein degradation in those cellular organelles. In addition to its recognition of protein substrates, Lon also binds to DNA (103) and shares sequence similarity with RecA, a putative DNA damage sensor discussed above (104). It may, therefore, also be involved in sensing damage to mitochondrial and chloroplast DNA, but this potential aspect of Lon function has received little attention. Alternatively, Lon might regulate mitochondrial DNA replication and/or transcription via degradation of DNA binding proteins (103). Recently, a specific isozyme of Lon has been detected in rat liver peroxisomes by an experimental proteomics approach (105). Moreover, FtsH is highly homologous to at least three ATPase regulatory subunits of the eukaryotic 26S proteasome, which is present in the cytosol and nucleus (93, 106). Therefore, functional homologs of Lon and FtsH occur in most compartments of eukaryotic cells.

The 26S proteasome is the only multisubunit ATP-hydrolyzing proteolytic complex in the cytosol and nucleus of eukaryotic cells (107). It is enormously large, consisting of about 50 subunits with a combined molecular mass of 2.4 MDa. It preferentially degrades proteins that are tagged by ubiquitination but also has the capacity to degrade some damaged proteins that lack a ubiquitin tag (108). Certain functional features of the 26S proteasome resemble those of Lon and FtsH. For instance, DnaJ/HSP40 and DnaK/HSP70 molecular chaperones cooperate with the 26S proteasome in protein degradation (107). In addition, recent work indicates that many important cell cycle regulators are targeted selectively for ubiquitination and subsequent degradation by the 26S proteasome (109).

The activation of protein degradation by the 26S proteasome has been studied in detail in plants, yeast, and mammalian cells, and many additional sensors that recognize protein damage/unfolding in the endoplasmic reticulum, cytosol, or plasma membrane have been identified. Such sensors, including BiP (110), ATF6 (111), IRE1 (112), SCF complexes targeting F-box proteins (113), and the COP signalosome (114), are exquisitely important for proteasome-dependent protein degradation. However, they are not as highly evolutionarily conserved as Lon and FtsH and are not considered here. With the exception of mechanisms related to 26S proteasome function, in particular the unfolded protein response (UPR) in the endoplasmic reticulum, protein damage sensors as potential upstream regulators

of CSR signaling networks have received less attention than have DNA damage sensing mechanisms. Protein damage sensors need further study and may well be key to understanding the diseases of misfolded protein accumulation, including Alzheimer's, Parkinson's, and Huntington's diseases, prion encephalopathies, cystic fibrosis, myeloma, and some cancers.

Redox Sensors and Redox Regulation

The CSR is intricately associated with free radical formation and changes in cellular redox state. Virtually every gene implicated in response to stress is also affected by changes in cellular redox state or free radical levels (115). Thus, alteration of cellular redox potential is a major trigger of the CSR. Curiously, life originated in an unstable and stressful archaic environment characterized by high ion and free radical density, high and fluctuating temperatures, and large pH gradients. While these extreme environmental conditions may have promoted the free radical reactions that could have led to the origin of life (116, 117), these highly reactive conditions are incompatible with cellular functions relying on homeostasis. This conundrum may have represented a major evolutionary driving force for selection of genes encoding redox-regulatory, free radical scavenging proteins in the last universal common ancestor (LUCA). Such genes have probably aided the transition from anaerobic to aerobic life by providing a means for minimizing oxygen toxicity in developing aerobic mechanisms (118).

All cells have free radical scavenging systems to minimize and repair oxidative damage, including compounds such as ascorbate, glutathione, thioredoxin, and various antioxidant enzymes. In addition, reactive oxygen and nitrogen species (ROS, RNS) are employed as second messengers that carry signals about alterations of cellular redox potential to activate the CSR and other physiological processes such as differentiation, aging, senescence, and pathogen defense (23, 119). In general, two types of free radical-mediated effects can be distinguished: (*a*) direct effects on signaling proteins and (*b*) indirect alteration of signaling pathways by specialized redox-sensitive proteins. Examples of direct alteration of signaling proteins include eukaryotic MAPKs (mitogen-activated protein kinases) and the transcription factors AP-1 and NF-κB (115, 120, 121). Indirect alteration of stress-responsive signaling pathways involves several redox-regulatory proteins that are universally conserved in all cellular life forms (Table 1).

Many oxidoreductases present in the minimal stress proteome are dehydrogenases. Some are elements of basic metabolic pathways, including glycolysis, pentose phosphate cycle, and the Krebs (citrate) cycle, and thus are essential even in the absence of stress. However, these dehydrogenases also influence cellular redox potential and oxidative damage repair by generating reducing equivalents for antioxidant enzymes that depend on NADPH as a cofactor, including thioredoxin reductase, glutathione reductase, and aldehyde reductase. Aldehyde dehydrogenase and aldehyde reductase are important for detoxification of aldehydes, which are common toxic intermediary metabolites during oxidative stress. ROS that are

generated during stress are neutralized by the action of antioxidant proteins, many of which are part of the minimal stress proteome.

Superoxide dismutase (SOD) converts superoxide radicals to H_2O_2, which is then further converted to water by peroxidases, including peroxiredoxin. Peroxiredoxin belongs to a family of antioxidative proteins that currently comprises six members in mammals (122). These enzymes are distributed in the cytosol, mitochondria, peroxisomes, and plasma membrane and have peroxidase activity that utilizes thioredoxin and/or glutathione as the electron donor. Peroxiredoxins also modulate cell proliferation, differentiation, and gene expression, probably in similar ways as thioredoxin. SOD and peroxidases such as peroxiredoxin must be coregulated during stress because an imbalance in the ratio of SOD and peroxidases in the presence of heavy metal ions causes conversion of H_2O_2 into noxious hydroxyl radicals via Fenton chemistry. Accumulation of hydroxyl radicals is highly deleterious to cells because they are very effective in causing damage to macromolecules such as DNA, protein, and lipids (123).

Oxidative damage to proteins occurs in multiple forms, most commonly cysteine oxidation (leading to formation of disulfide bonds) or methionine oxidation. The glutathione and thioredoxin systems repair such forms of oxidative protein damage. Glutathione (gamma-glutamyl-cysteinyl-glycine, GSH) is the most abundant low-molecular-weight thiol that is synthesized de novo in animal cells. In its reduced/oxidized forms (GSH/GSSG), it represents the major redox couple in animal cells. The main pathways for GSH metabolism are reduction of hydroperoxides by glutathione peroxidases and peroxiredoxins leading to generation of glutathione disulfide (GSSG). Glutathione-S-transferase catalyzes the conjugation of glutathione. Glutathione reductase catalyzes the NADPH-dependent reduction of GSSG to GSH. Because GSH is a universal free radical scavenger in cells, glutathione reductase is critical during stress, when levels of ROS increase. In contrast to GSSG, which is recycled to GSH by glutathione reductase, glutathione conjugates are excreted from cells (124).

Thioredoxin is a 12-kDa protein in which redox-active dithiol in the active site Cys-Gly-Pro-Cys constitutes a major thiol reducing system (125). The enzymes involved in repairing oxidative cysteine damage via the thioredoxin system are thioredoxin peroxidase and thioredoxin reductase (126). The function of thioredoxin reductase for repairing oxidative cysteine and methionine damage is to recycle oxidized thioredoxin back to its reduced state by using electrons from NADPH. In addition to its overall antioxidant properties, thioredoxin restores transcriptional activity of AP-1, NF-κB, p53, and PEBP2 (23, 128). It also interacts directly with other key signaling molecules such as ASK1 (apoptosis signal regulating kinase 1) (126). Thus, thioredoxin plays multiple roles in cellular processes like proliferation and apoptosis.

Peptide methionine sulfoxide reductase (MsrA) and thioredoxin reductase repair oxidative methionine damage (127, 128). Because of the benefits MsrA confers on oxidatively damaged proteins, it is an important repair enzyme during stress (129). In rats, MsrA was found in all tissues examined but was particularly

abundant in tissues routinely exposed to severe stress, such as the renal medulla and retinal epithelium (130). The recent identification of the small heat shock protein HSP21 as a physiological MsrA substrate suggests that heat shock protein activity is protected by MsrA during stress (131).

The seleno-cysteine-specific translation elongation factor SelB is also present in all three lines of organisms (Table 1). SelB, which is homologous to EF-Tu but has a unique C terminus (132), belongs to an ancient subfamily of GTPases (133). SelB is required for the synthesis of seleno-cysteine proteins such as glutathione peroxidases, thioredoxin reductases, and SelR, which has peptide methionine sulfoxide reductase (PMSR) catalytic activity similar to MsrA (134). Most of the other selenoproteins are also key enzymes functioning in antioxidant defense. These enzymes have seleno-cysteine in their active site, which increases their functionality because of the presence of more fully ionized seleno-cysteine compared with the thiol group of cysteine at physiological pH (135). The UGA stop codon encodes seleno-cysteine in archaea, eubacteria, and eukaryotes. Pyrrolysine is the other amino acid encoded by a stop codon (UAG) (136). SelB alters the translational machinery by recognizing a specific motif in mRNAs coding for seleno-cysteine proteins. In addition to SelB, seleno-cysteine tRNA (tRNA-SeC) is universally required for seleno-cysteine protein synthesis. Although SelB recognizes mRNAs encoding seleno-cysteine proteins by similar mechanisms and requires tRNA-SeC in all cases, the cofactors utilized by eubacteria differ from those in archaea and eukaryotes (137). In both cases, however, the incorporation of seleno-cysteine into protein requires several gene products in addition to SelB and tRNA-SeC and is based on the interaction of a C-terminal domain of SelB with a SECIS (seleno-cysteine insertion sequence) element present in mRNAs encoding seleno-cysteine proteins. This example illustrates yet again that large protein complexes functioning in the CSR are organized around universally conserved stress proteins. [For details of eubacterial and archaeal/eukaryotic mechanisms of seleno-cysteine protein synthesis, see (137, 138).]

The CSR utilizes ROS and RNS generated during stress as intracellular messengers. Thus, increased concentrations of free radicals are beneficial for cellular stress sensing and signaling while damaging to cellular macromolecules. The basis and relative importance of these contrasting roles of free radicals during stress merit further investigation.

KEY FUNCTIONS OF THE CELLULAR STRESS RESPONSE

Stress triggers diverse cellular mechanisms of macromolecular damage that are consequential. The focus here has been on outlining mechanisms that are organized around proteins belonging to the minimal stress proteome and represent early events associated with sensing and transducing common signals generated by stress. These conserved sensory mechanisms activate a very elaborate cellular

stress signaling network that involves different proteins in prokaryotes and eukaryotes. Prokaryotic stress response signaling mechanisms involve σ^{32}, σ^{54}, and σ^{S} transcription factors, but they also rely to a great extent on two-component signal transduction. Two-component systems often consist of a sensor kinase that autophosphorylates on histidine when certain variables in the environment change. Upon autophosphorylation, the sensor is activated as an aspartate kinase that phosphorylates a second protein, the response regulator, on aspartate (139). In some cases, a third protein serves as a mediator of phosphate transfer.The response regulator often functions as a DNA binding protein that modulates the expression of genes with adaptive value during stress.

In eukaryotes, CSR signaling networks are extraordinarily complex and involve numerous proteins. In particular, transcriptional regulation in eukaryotes is much more complex than in prokaryotes. It depends on stability, sequence-specific binding, and nuclear transport of numerous transcription factors and their regulators (140–142). In addition, transcription programs are controlled by chromatin rearrangements and many posttranslational histone modifications (143, 144). Thus for preventative or therapeutic purposes, identification of key elements of such networks that represent efficient targets for manipulating the stress tolerance of cells is critical. One method to do so is by using a comparative approach or, in other words, identifying proteins involved in cellular stress response signaling in many taxa. Examples of such proteins are MAPKs (145), 14–3-3 (146), Bcl-2 (147), ATM and ATR kinases (148), and insulin receptor-like tyrosine kinases (149). These are key regulators of the CSR in eukaryotes and can be regarded as hubs around which other signaling mechanisms are organized.

Recent cDNA microarray experiments have shown that the genome of *S. cerevisiae* is divided into genes preferentially targeted by the SAGA (Spt-Ada-GCN5-acetyltransferase) transcriptional complex (∼10% of the genome) and genes preferentially targeted by the TFIID transcriptional complex (∼90% of the genome) (150). Many SAGA-regulated genes are stress inducible, whereas most TFIID-regulated genes have housekeeping functions. This bimodal transcriptional regulation is indicative of a distinct stress-induced transcription program that mediates global and coordinated activation of yeast stress response genes (150). Similarly, the σ^{S} (RpoS) subunit of RNA polymerase, whose levels are controlled by proteolysis, is a master regulator of the general stress response in *E. coli* and other bacteria (151). Whether such global stress-induced transcriptional regulation is also utilized by multicellular organisms is presently unclear. Nonetheless, other global changes, such as chromatin organization, posttranslational histone modifications, and rearrangements of chromatin remodeling complexes occur in mammalian cells exposed to stress (152). [For a more detailed exploration of intracellular signaling mechanisms in response to stress, see (153–155).]

Growth Control and Cell Cycle Checkpoints

One universal effect of stress on cells is the impairment of growth and proliferation. Growth arrest represents an adaptive and integrated part of the CSR. It allows for

preservation of energy and reducing equivalents and redirects the utilization of these important metabolites toward macromolecular stabilization and repair. In addition, proliferating cells that actively undergo DNA replication and mitosis are more prone to suffer stress-induced damage to macromolecules than are cells in a resting state. In bacteria, the ability to resist stress is greater in stationary phase than in exponential phase, during which cells are rapidly dividing. Thus, rapidly dividing, metabolically active bacteria will experience growth arrest when exposed to stress (156). Similarly, eukaryotic cells also undergo growth arrest when exposed to stress (157). For these reasons, the activation of cell cycle checkpoints is a key aspect of the CSR. Cell cycle checkpoints monitor macromolecular integrity and the successful completion of cellular processes prior to initiating the next phase in the cell cycle (158).

Under extreme stress conditions many bacteria and fungi form stress-resistant spores. Sporulation can be regarded as the ultimate form of growth control and cell cycle regulation during stress. Growth arrest and the onset of sporulation in bacteria involve many proteins. When bacterial DNA replication is interrupted by stress, a component of the SOS response, the inhibitory factor SfiA, is induced and leads to transient inhibition of cell division (159). In addition, the universal stress protein UspA, which belongs to a family of proteins that is conserved in bacteria and many invertebrate eukaryotes, accumulates at very high levels in growth-arrested bacteria (160). The σ^S (RpoS)-driven transcription of stress response genes (see above) promotes growth arrest and counteracts proliferative activities that are primarily directed by σ^{70}. Counteraction of σ^{70} is mediated by an increase in the *E. coli* alarmone guanosine tetraphosphate (ppGpp), which shifts the equilibrium between σ^S and σ^{70} in favor of σ^S. The resulting change in relative competitiveness of these two subunits of the RNA polymerase complex leads to suppression of growth during stress (161).

The extraordinary significance of eukaryotic cell cycle checkpoints for proliferative disorders such as cancer has attracted much attention. Cell cycle regulatory proteins that control such checkpoints maintain the fidelity of DNA replication, repair, and cell division in normal as well as stressed cells. Checkpoints are built into every major transition in the cell cycle, including G1/S, intra-S phase, G2/M, mitotic spindle assembly, and cytokinesis. In mammalian cells such cell cycle checkpoints are controlled by a large number of proteins, of which ATM and ATR kinases, p53, GADD45 proteins, 14–3-3σ, CDC25, CDC2/cyclin B, p21, retinoblastoma protein (pRB), Chk1, Chk2, Polo kinases, and BRCA1 are key (157, 162–165).

One well-known mechanism of G2/M checkpoint induction is based on ATM and ATR kinase activation of Chk1 kinase, which phosphorylates the cell cycle phosphatase CDC25. Phosphorylation leads to binding of 14-3-3 protein on CDC25 and its subsequent sequestration in the cytosol. Cytosolic sequestration prevents CDC25-mediated dephosphorylation of CDC2, which is necessary for the promotion of mitosis by the CDC2/cyclin B complex (166). The p53 protein is involved in the G2/M checkpoint by inhibition of CDC2 via its transcriptional targets GADD45, p21, and 14–3-3σ (167). GADD45 levels increase during stress,

not only because of transcriptional regulation but also as a result of posttranscriptional mRNA stabilization (48, 157, 168). However, the mechanism by which GADD45 proteins induce G2 arrest is still elusive. A striking feature of eukaryotic cell cycle checkpoints as well as DNA damage repair pathways is the central role of ATM and ATR kinases (169). These kinases are critical intermediates between DNA damage sensors and effector protein complexes that control key features of the CSR, including cell cycle progression, DNA repair, and apoptosis.

Maintenance of Macromolecular Integrity

A hallmark of the CSR representing one of its first identified features is the induction of heat shock proteins, many of which function as molecular chaperones (9, 170, 171). In combination with the DNA repair machinery, molecular chaperones provide a rapid and direct mechanism of cellular defense against stress-induced damage. Chaperone proteins are required to recognize unfolded proteins and either target them for removal, deter their aggregation, or assist in their refolding into the native, functional state (172, 173). Five molecular chaperones, DnaK/HSP70, DnaJ/HSP40, GrpE, HSP60, and petidyl-prolyl isomerase (cylophilin), are part of the minimal stress proteome. These proteins illustrate the extraordinarily strong evolutionary conservation of this cellular function and the importance of molecular chaperones during stress. They are extensively utilized as bioindicators of environmental stress in many different types of organisms, and their study has extended well beyond laboratory-based analysis into the realm of field-based ecological physiology (174).

Many functional aspects of these five molecular chaperones are known in great detail. In archaea and eubacteria, the molecular chaperones DnaK/HSP70, DnaJ/HSP40, and GrpE are all transcribed from the same locus (175). Some species of archaea have apparently lost this locus, which is surprising and contrasts with the ubiquitous occurrence of these genes in eubacteria and eukarya with no known exception (175). The chaperone activity and induction of DnaK/HSP70, DnaJ/HSP40, and GrpE during stress are well established. Although these molecular chaperones are universally induced during stress, the mechanisms of induction seem to be more diverse. For example, as outlined above in *Streptomyces coelicolor* DnaK/HSP70 operon induction is mediated at the transcriptional level by the HspR repressor, which is degraded by proteolysis during stress (100). In *E. coli*, however, transcriptional induction of the DnaK/HSP70 operon is positively controlled by the RpoH/σ^{32} subunit of RNA polymerase (92, 176).

GrpE and DnaJ/HSP40 function as co-chaperone and nucleotide exchange factors for DnaK/HSP70. They control access of unfolded proteins to the substrate-binding domain of DnaK/HSP70 (177). GrpE is expressed in prokaryotes and eukaryotic mitochondria and plant chloroplasts but it is not present in eukaryotic cytosol, where a GrpE-like function is provided by the BAG1 protein (177). In higher eukaryotes the HSP70 family consists of numerous isoforms, some of which are stress inducible whereas others are constitutively expressed (178). Induction of mammalian HSPs is mediated by heat shock elements in the promoter

of their genes. These elements are binding sites for heat shock factors such as mammalian HSF1 that activate transcription of HSPs (179).

Cyclophilins, another universally conserved group of stress proteins, are well known as receptors of the immunosuppressive drug cyclosporin A. They are induced by many types of stress and have molecular chaperone activity (180). The molecular chaperone function of cyclophilins is mediated by their enzymatic peptidyl-prolyl isomerase (PPIase) activity, which has also been suggested to play a regulatory role for transcription and cellular differentiation (181, 182). Moreover, eukaryotic cyclophilin D is a mitochondrial matrix protein and an integral part of the mitochondrial permeability transition pore complex, which is intricately involved in the control of apoptosis (183).

Molecular chaperones have diverse impacts by interacting with proteins involved in other aspects of the CSR. For example, molecular chaperones protect processes for maintaining genomic integrity such as NER, which repairs oxidative nucleotide damage during stress. In a specific case, the UvrA protein in *E. coli* is stabilized and protected from heat inactivation by the DnaK/HSP70, DnaJ/HSP40, GrpE chaperone machinery (184). A related role in NER has recently been attributed to the eukaryotic 26S proteasome. This proteolytic supercomplex interacts with multiple NER proteins, including XPB, Rad4, and Rad23. The latter two proteins form a complex that binds to pyrimidine dimers generated during UV-radiation stress. The 26S proteasome may act as a molecular chaperone to promote disassembly of this NER complex (185). These examples suggest that molecular chaperones participate in NER by targeted protection of DNA repair proteins or disassembly of NER complexes after DNA repair is completed.

These examples also demonstrate that processes of protein and DNA maintenance/repair are co–regulated and share common elements during stress. [More detailed information concerning DNA repair modes is summarized in recent reviews that focus on mechanisms of NER (186–188), MMR (189, 190), and DNA dsb repair (191, 192).] The mechanisms by which molecular chaperones and the DNA repair machinery maintain protein and genomic integrity during stress are at the center of the CSR and remain a captivating subject of investigation.

Energy Metabolism

Another key aspect of the CSR is the modulation of major pathways of energy metabolism, which may be closely linked to the oxidative burst in cells exposed to stress. Minimal stress proteome enzymes such as glycerol-3-phosphate dehydrogenase (G3PDH), 6 phosphogluconate dehydrogenase (6PGDH), enolase, citrate synthase, and isocitrate dehydrogenase (IDH) contribute strongly to the control of key pathways of energy metabolism, including glycolysis, pentose phosphate pathway, and the Krebs (citrate) cycle. Induction of these enzymes during stress may be necessary for generating reducing equivalents (NADH, NADPH) that are needed for cellular antioxidant systems. For example, IDH is strongly elevated in macrophages exposed to pathogen-induced stress. Elevated IDH levels, in turn, lead to increased NADPH production and cellular protection from oxidative

damage caused by RNS and ROS (193). Another enzyme in this category, succinate semialdehyde dehydrogenase (SSADH), is also necessary for alleviating oxidative stress. This was demonstrated by constitutively elevated levels of reactive oxygen species in SSADH (−/−) mice (194).

Another potential reason for inducing such metabolic pathways lies in the energetic requirements of protein degradation, protein chaperoning, and DNA repair. Many steps in these adaptive processes depend on the hydrolysis of ATP, including the activity of proteolytic complexes (e.g., FtsH, Lon, 26S proteasome), chaperones (e.g., DnaK/HSP70, DnaJ/HSP40, GrpE), and DNA damage sensing/repair complexes (e.g., the BASC supercomplex). Thus, the induction of key enzymes of energy metabolism may provide the reducing and energy equivalents needed for stress-related cell functions. Furthermore, growth arrest results in redirection of NADPH/NADH and ATP utilization from proliferative processes to macromolecular stabilization and repair. Both processes, induction of energy metabolism and growth arrest, are closely coordinated with increased demands for reducing and energy equivalents during stress.

Some energy metabolism enzymes may have additional stress-related functions. For example, 6PGDH has been implicated in cell cycle control during osmotic stress in plants (195) and in increasing glutathione levels through stimulation of the pentose phosphate pathway during oxidative stress in mammalian cells (196).

Apoptosis

A universal response of severely stressed cells is to undergo cell death, but in two alternative ways: necrosis and apoptosis (programmed cell death, cell suicide program). Apoptosis is a common response of metazoan cells when stress exceeds cellular tolerance limits. It is also an important regulatory mechanism during development of multicellular organisms (197). Although mechanisms of programmed cell death that are similar to apoptosis have recently been identified in plants and bacteria (198, 199), apoptosis is best understood in metazoans.

Mammals have two major apoptotic pathways, intrinsic and extrinsic. The intrinsic apoptotic pathway depends on release of cytochrome c (Cyt c) and other apoptogenic factors from mitochondria. Once released, Cyt c binds to APAF-1 (apoptosis protease activating factor 1) and recruits procaspase 9, which is then processed by the apoptosome into active caspase 9. Caspase 9 triggers the caspase pathway by activation of caspase 3, with the final outcome being activation of caspase-activated DNAse (CAD) that digests chromosomal DNA (200). The extrinsic apoptotic pathway is triggered by cell surface receptors (e.g., TNFSF6) when activated by specific ligands. Upon activation, these receptors bind cytoplasmic adapter molecules such as FADD that, in turn, activate procaspases and start the caspase cascade (201).

Both apoptotic pathways are subject to extensive regulation by a complex array of pro- and antiapoptotic signals. The PI-3-K (phosphatidyl inositol 3 kinase)/AKT (V-Akt murine thymoma viral oncogene homolog) pathway is one of the critical pathways that generally suppresses apoptosis (202). Activated AKT kinase

phosphorylates several important targets, including the proapoptotic BCL-2 (B cell CLL/lymphoma 2) family member BAD (BCL-2 antagonist of death), forkhead transcription factors, and GADD45. These proteins are bound by 14-3-3 in their phosphorylated form, which results in cytosolic sequestration and inactivation similar to CDC25 inactivation discussed above (202). Molecular chaperones, including HSP60, HSP27, and HSP70, also have antiapoptotic effects. This property of major molecular chaperones is mainly the result of their binding to apoptosis regulating proteins such as AKT (200). The NF-κB pathway also has generally antiapoptotic effects. NF-κB is a transcription factor that induces antiapoptotic members of the BCL-2 family, including BCL-2 and BCL-xL (203). However, in certain cell types, NF-κB also induces proapoptotic genes, including BCL-xS and p53 (203). The p53 pathway promotes apoptosis mostly via p53 transactivation of proapoptotic members of the BCL-2 family, PTEN phosphatase (an inhibitor of the AKT pathway), and GADD45 (201). Like p53 itself, some of its targets such as GADD45 proteins modulate apoptosis (204) as well as cell cycle checkpoints (205). These and other proteins with similar roles in multiple stress response pathways likely are key to how cells encountering stress decide between induction of apoptosis versus cell cycle delay and repair. A mechanism of quantitative macromolecular damage assessment may contribute significantly to this important decision. However, how cells measure the amount of damage during stress and how they recognize whether such damage exceeds their tolerance limits is unclear. These are important unanswered questions that will drive future research on the cellular stress response.

CONCLUSIONS AND PERSPECTIVE

The CSR is a very complex mechanism that ensures survival of healthy (fit) cells and removal of damaged (unfit) cells during stressful environmental conditions. I have selectively summarized major physiological functions of the CSR based on the recent identification of a set of stress proteins that are highly conserved in all organisms. The minimal stress proteome provides an excellent starting point for obtaining insight into key functions of the CSR. This review also analyzes the common nature of stimuli that induce the CSR and discusses common mechanisms by which such stimuli are sensed and integrated into stress response networks by cells. These commonalities create a conceptual framework for further exploration and identification of key elements of the CSR.

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