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Review

Evolution of the cellular stress proteome: from monophyletic origin to ubiquitous function

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Summary

Cells respond to acute environmental change by activating a stress response that is widely studied. However, knowledge of this stress response is fragmentary, and a unifying concept explaining its universality for many different species and types of stress is lacking. The need for a holistic view emphasizing the key aspects of the stress response is addressed by the following hypothesis. The cellular stress response is a reaction to any form of macromolecular damage that exceeds a set threshold, independent of the underlying cause. It is aimed at temporarily increasing tolerance limits towards macromolecular damage by utilizing a phylogenetically conserved set of genes and pathways that mediate global macromolecular stabilization and repair to promote cellular and organismal integrity under suboptimal conditions. This mechanism affords time for a separate set of stressor-specific adaptations, designed to re-establish cellular homeostasis, to take action. Supporting evidence, emerging conclusions, and ways to test this hypothesis are presented.

Key words: evolution, cellular stress response, DNA damage response, apoptosis, cell cycle checkpoint, molecular chaperone, environmental stress.

Introduction

The cellular stress response can be defined as a reaction to the threat of macromolecular damage. It comprises an evolutionarily highly conserved mechanism that protects cells from sudden environmental change or frequent fluctuations in environmental factors. Environmental change has accompanied and influenced the evolution of life in many ways and will continue to do so at an accelerated pace owing to human impact on natural ecosystems. Therefore, it is critical to better understand how cells and organisms respond to environmental stress.

The cellular stress response is associated with essential aspects of protein and DNA processing and stability in all three super-kingdoms, the archaea (Macario et al., 1999), the eubacteria (Hecker and Volker, 2001) and eukaryotes (Feder and Hofmann, 1999; Pearce and Humphrey, 2001). Our current knowledge of the stress proteome, i.e. all the proteins that are involved in realizing the cellular stress response through induction, post-translational modification, or protein–protein/DNA interaction, is still fragmentary. Nevertheless, we know that common sets of homologous stress proteins, including molecular chaperones, cell cycle regulators, proteasome regulators and DNA repair proteins are induced by stress in archaea, eubacteria and eukaryotes.

Many of these proteins are among the most highly conserved proteins in all organisms (Table 1). In fact, stress response genes of humans account for 67 (18%) of the 368 phylogenetically most highly conserved proteins (Table 1). They are associated with the most basic constitutive functions of all cells, in addition to their roles for stress adaptation (Fig. 1). Because such functions are evolutionarily ancient it is likely that a core stress proteome appeared early in cellular evolution, helping cells to survive stressful fluctuations in the earth's archaic environment. Thus, the very first organisms and cells may have been eury-tolerant, i.e. they probably had high tolerance limits towards environmental change. Other stress proteins could have originated by gain-of-function mutations or adaptive radiation of genes involved in these basic cell functions at various times during the course of evolution (Fig. 2).

Despite their common origin, some stress proteins in contemporary species are less well conserved than the examples noted in Table 1. Two obvious reasons account for such apparent disparity. First, some fairly basic cellular structures and metabolic processes in bacteria have diverged significantly from bacteria during evolution and consequently the proteins involved in these functions have also diverged. Examples include, among other features, the development of a nucleus, and differences in the organization of the cell membrane, the nature of signaling systems (e.g. two-component systems *versus* Ser-, Thr-, Tyr-phospho-protein systems) and chromatin organization. Second, genes encoding the stress proteome in steno-tolerant species adapted to stable environments

Table 1. *The most highly conserved stress response proteins*

Gene (*Homo sapiens*)	Stress response function	*Drosophila melanogaster* (%identity/%similarity)	*Halobacterium* sp. (%identity/%similarity)	*Escherichia coli* (%identity/%similarity)
HSP70/dnaK (P08107)	Molecular chaperone	P11147 (83/91)	Q9HRY2 (47/63)	P04475 (50/64)
HSP40/dnaJ (O60884)	Molecular chaperone	Q9VFV9 (50/65)	Q9HRY3 (31/47)	P08622 (34/ 49)
PRS1/ftsH (P35998)	Proteasome pathway, Cell cycle checkpoint	Q9V478 (91/95)	Q9HNP9 (47/67)	P28691 (42/63)
PRS2/ftsH (Q9Y4W6)	Proteasome pathway, Cell cycle checkpoint	Q9VVE6 (63/75)	Q9HRW6 (40/60)	P28691 (49/67)
SelB (Q9BX10)	Selenocysteine-specific elongation factor, Free radical scavenging	AAF51935 (50/68)	Q9HPE4 (30/49)	P14081 (24/39)
MSH/mutS (P43246)	DNA repair	AAF53392 (44/64)	Q9HSM2 (30/48)	P23909 (32/50)
Lon protease (P36776)	Stress response protease	Q9VW20 (63/76)	Q9HSC3 (32/49)	P08177 (40/61)
HSP60/Cpn60 (P10809)	Molecular chaperone, Cell cycle regulation	O02649 (73/85)	Q9HNI0 (24/42)	P06139 (50/72)
DNA topoisomerase III/(I) (Q13472)	Chromosome maintenance, DNA repair	Q9NG98 (58/70)	Q9HS90 (27/44)	P06612 (25/40)
Glutathione reductase (Q16881)	Free radical scavenging	AAN09228 (55/68)	Q9HN74 (25/44)	P06715 (36/53)
MLH/mutL (P40692)	DNA repair	Q9V380 (45/61)	Q9HSM6 (33/50)	P23367 (35/57)
Peptide methionine sulfoxide reductase (Q9UJ68)	Free radical scavenging, Protein repair	AAF4963 (40/55)	Q9HQG0 (42/59)	P27110 (60/72)

Gene products with one or more known function(s) in the cellular stress response that are most highly conserved in all three super-kingdoms of life are listed.

The data were acquired by BLAST sequence comparison of whole proteomes of *Drosophila melanogaster*, *Halobacterium* sp. (strain NRC1), and *Escherichia coli* (K12) against the whole human proteome (expectation value=10^{-10}, matrix=BLOSUM62). Of 33,633 human proteins compared to the other three proteomes, 368 proteins representing the most highly conserved in all three super-kingdoms were identified using PyMood software (Allometra, Inc., http://allometra.com/). 12 of these 368 proteins with known roles in the cellular stress response are identified by name and Swiss-Prot/TrEMBL accession number in the table.

The degree of identity and similarity to the respective orthologous human sequence are also shown.

Multiple paralogous genes encoding these stress response proteins in humans (only one is shown in the table) account for 67 (18%) of the 368 phylogenetically most highly conserved proteins.

Proteasome regulatory subunits 1 and 2 (PRS1, 2) are encoded by the same gene in *E. coli*.

(organisms with low tolerance limits towards environmental change) were subject to modification by natural selection of mutations that decreased their functionality for the stress response. The apparent lack of an HSP70 gene in some archaea is an extreme example in this regard (Macario and de Macario, 1999). At the same time, new contingencies evolving around modified stress response genes would have favored specialization and improved organization. This process must have provided a selective advantage to steno-tolerant organisms by increasing their fitness and competitiveness in stable environments. For example, in vertebrates (particularly mammals), a large number of stress response genes have been recruited into signaling contingencies that are associated with the immune response, to accommodate the proteomic basis necessary for the ever more complex nature of the immune system (Moseley, 2000; Lutz, 2000).

Nonetheless, it is well documented that most basic aspects of the cell stress response are conserved in many species and across a wide spectrum of diverse stresses. This high degree of conservation provides the foundation for analyzing the common nature of the cell stress response and for tracing its evolution and molecular design. To undertake such an analysis, the molecular nature of the threat that induces the cell stress response must first be identified.

Cellular stress can be defined as the threat of damage to macromolecules

In this brief article I contend that the main essence of the cellular stress response consists of protection of macromolecules during the initial phase of exposure to any adverse environmental condition that significantly perturbs cellular homeostasis. The cellular stress response has been associated most clearly with protective effects during conditions that perturb both protein and DNA integrity. Many types of environmental stress have been shown to cause deleterious changes in protein conformation, including osmotic stress (Hochachka and Somero, 2002), thermal stress (Hochachka and Somero, 2002), heavy metal stress (Farrer and Pecoraro, 2002), ionizing radiation (Kempner, 1993), baric stress (Somero, 1992),

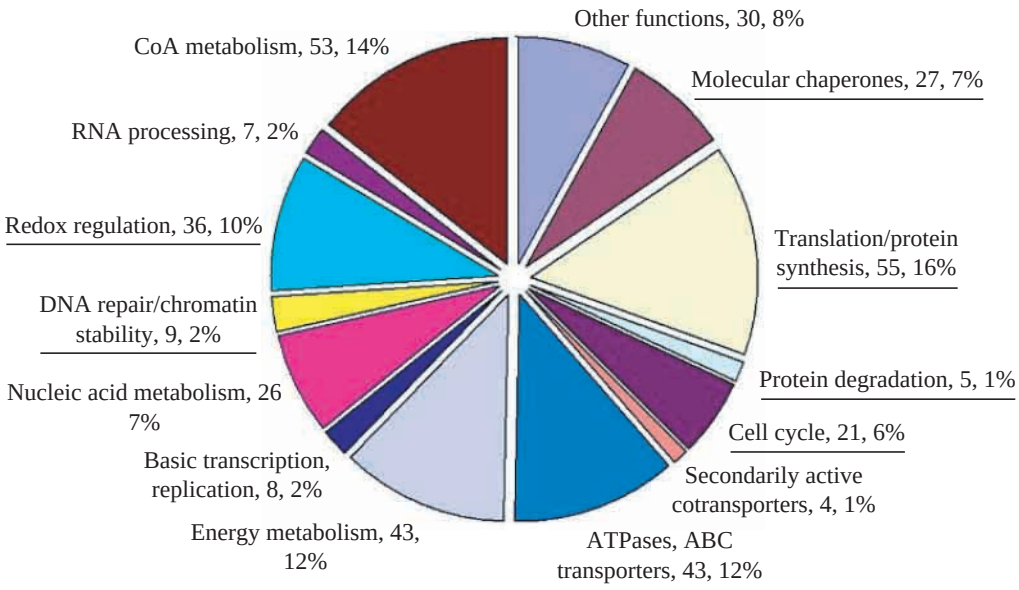

Fig. 1. Functional classification of 368 gene products that are most highly conserved in all three super-kingdoms (based on data obtained by whole proteome sequence comparisons of *Homo sapiens*, *Drosophila melanogaster*, *Halobacterium halobium* and *Escherichia coli*). Each protein was assigned one function when a major or well-understood function has been widely reported in the literature. Proteins with unknown or poorly characterized functions are contained in the set denoted 'Other functions'. The number of proteins and % of total assigned to each function are listed. Basic biological functions that are closely tied to fundamental aspects of the cellular stress response are underlined. The extraordinary conservation of such functions is a strong argument for the monophyletic origin of a core stress proteome at an early stage of cellular evolution.

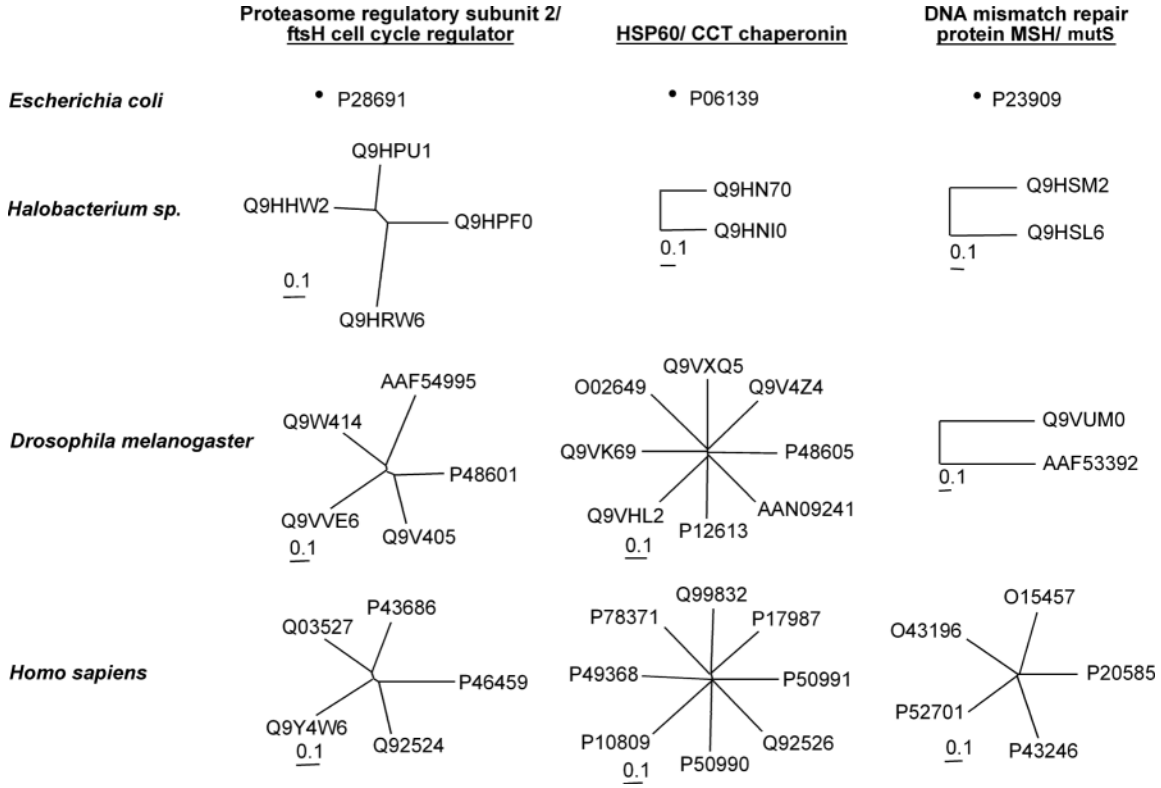

Fig. 2. Radial phylogenetic trees for selected stress proteins of four species from three super-kingdoms. Sequence comparisons were made with ClustalW (http://www.ebi.ac.uk/clustalw) and phylogenetic trees visualized using TreeView 1.6 (http://taxonomy.zoology.gla.ac.uk/rod/rod.html). Each sequence is labeled with their GenBank accession number. The length of the lines connecting individual sequences represents evolutionary distance, which is based on the degree of sequence similarity between paralogues. The examples shown illustrate that genes encoding stress proteins were subject to adaptive radiation at different times during the evolution of life, which presumably reflects their increasing role for multiple important cell functions. Other very important stress response genes such as mitogen-activated protein kinases (MAPKs) and 14-3-3 proteins display similar patterns of late adaptive radiation in eukaryotes as shown for the DNA mismatch repair factor MSH/mutS. In contrast to the latter, however, MAPKs and 14-3-3 proteins have originated in eukaryotes and are entirely absent from prokaryotes, whose phosphorylation-based signaling systems differ greatly from those in eukaryotes.

oxidative stress (Kasprzak, 2002) and hypoxia/ischemia (Borkan and Gullans, 2002). Likewise, many of these various stresses are also known to cause DNA damage (Kültz and Chakravarty, 2001b; Galloway et al., 1987; Kasprzak, 2002; Rydberg, 2001; Liu, 2001). Moreover, the cellular stress response may play roles as yet poorly known for the stabilization of other macromolecules, such as lipid structures (membranes) and RNA. Thus, it is feasible to define the cellular stress response as a reaction to the threat of macromolecular damage (independent of the means by which such damage occurs).

Its purpose and adaptive significance arises from temporarily increasing cellular tolerance limits towards such a threat. Because of this universal property, the cellular stress response consists of adaptations that maximize the stabilization, protection and repair of macromolecular structure and function. Such benefit carries the price of transiently decreasing the cells' capacity for most of its normal functions by draining metabolic energy and reducing the conformational flexibility of proteins and DNA. Reduced conformational flexibility decreases the efficacy of enzymes by slowing the rate at which structural changes occur in the active site during catalysis (Hochachka and Somero, 2002). Through similar kinetic effects, conformational flexibility is also rate-limiting for functions of other macromolecules.

Despite these disadvantages, the cellular stress response shelters the ultimate cell function during adverse environmental conditions – the survival of healthy cells. The core stress proteome involved in achieving this task must have evolved in the very first primordial cells because it is intimately associated not only with the cellular stress response but also with basic cellular house-keeping functions (Fig. 1). For instance, HSP70 is involved in such functions as protein maturation in the endoplasmic reticulum (Hartman and Gething, 1996) and mitochondrial biogenesis (Voos and Rottgers, 2002). Another example is MSH/mutS mismatch repair (MMR) proteins, which are not only involved in MMR and the repair of other types of DNA damage (Kolodner and Marsischky, 1999) but also in constitutive proof-reading activity during DNA replication (Marti et al., 2002).

Interestingly, the molecular mechanism of damage to DNA and proteins may be mediated in many cases by stress-induced radical formation and changes in cellular redox state. This has been demonstrated directly for heavy metal stress (Schutzendubel and Polle, 2002), ionizing radiation (Wallace, 1998), chemical genotoxin stress (Zeiger, 1993), osmotic stress (Borsani et al., 2001; Gueta-Dahan et al., 1997), mechanical injury stress (Hall and Braughler, 1993) and pathogen invasion stress (Splettstoesser and Schuff-Werner, 2002), in addition to direct oxidative stress. Thus, critical parts of a universal stress-sensing mechanism may include (1) macromolecular damage assessment and (2) monitoring of cellular redox state as a ubiquitous stress indicator.

It needs to be emphasized at this point that environmental stress often also leads to induction of a second set of adaptive responses in addition to the cellular stress response. This second set of responses differs from the cellular stress response in that it is stressor-/environmental factor-specific, has a slower onset, and is directed at re-establishing cellular homeostasis with regard to the particular environmental factor that is perturbed. Such homeostatic adaptations are only practical when healthy cells survive the initial period of stress by means of the cellular stress response.

The cellular response to environmental stress is highly conserved

The cellular stress response is a mechanism of extraordinary significance for many areas of biology and medicine. Consequently, responses of cells to various types of stress have been studied widely. For practical reasons, most individual studies have focused on cellular responses to perturbations in only a single parameter or a combination of very few environmental factors. The enormous number of detailed studies concerning cellular responses to many different types of stress has led to the discovery of a seemingly bewildering variety of molecular mechanisms by which cells respond to stress. Nevertheless, when attempting to view this body of literature from a global perspective, several common themes emerge. (1) The cellular stress response is a reaction to changes or fluctuations of extracellular parameters that damage the structure and function of macromolecules (see previous paragraph). (2) A conserved core set of homologous proteins is part of the stress proteome in all but a few organisms independent of the type of stress (Table 1). (3) Cellular responses to multiple stresses are synergistic, and pre-exposure to one form of stress induces transient stress-hardening or cross-tolerance to other forms of stress. (4) A transient and rapid stress response is required to facilitate additional adaptations that are stressor-specific and aimed at re-establishing cellular homeostasis. (5) Cells respond to all types of stress by activating four basic mechanisms, all of which are aimed at stabilizing macromolecular structure and function during adverse, abnormal or pathological conditions, and at conserving metabolic energy for homeostatic adaptations.

These four mechanisms and their transient activation can be regarded as the cornerstones of the cellular stress response. They consist of: (1) cell cycle checkpoint control leading to growth arrest – cell cycle checkpoints induced during stress in eukaryotic cells include the G_1/S checkpoint (Bartek and Lukas, 2001), the G_2/M checkpoint (Bulavin et al., 2002) and translational control mechanisms (Brostrom and Brostrom, 1998); (2) induction of molecular chaperones (HSPs) and protein stabilizers – molecular chaperones are commonly activated either by induction (Feder and Hofmann, 1999) or by post-translational modification, e.g. phosphorylation of HSP28 *via* the p38 MAP kinase signaling pathway (Kato et al., 2001); (3) activation of mechanisms for nucleic acid and chromatin stabilization and repair – for instance, eukaryotic pathways involved in DNA repair and chromatin stabilization include the p53 pathway (Harkin and Hall, 2000) and the NF-kappaB pathway (Vermeulen et al., 2002); (4) removal of macromolecular debris generated by stress – this aspect of the

cellular stress response is exemplified by the ubiquitin/proteasome pathway (Fuchs et al., 1998).

All of these mechanisms seem to be interconnected *via* a common stress signaling network, and have the major purpose of maintaining genomic and macromolecular integrity during stress. This can only be achieved at the expense of other cell functions, which explains the transient nature of the cell stress response and the need for re-establishing cellular homeostasis with regard to the perturbed parameter(s). For instance, hypertonic stress causes protein instability (Hochachka and Somero, 2002) and DNA damage (Kültz and Chakravarty, 2001a), which rapidly and transiently induce the cellular stress response, including cell cycle checkpoints leading to growth arrest (Kültz et al., 1998), increased DNA repair (Kültz and Chakravarty, 2001a), the ubiquitin/proteasome pathway (Pan et al., 2002), molecular chaperones (Rauchman et al., 1997), and in severe cases, programmed cell death (Michea et al., 2000). In addition to the transient cellular stress response, cells activate a second set of adaptations that are specific for re-establishing homeostasis perturbed by hypertonic stress. These adaptations are slower, permanent (until conditions change again), and exemplified by the activation of transporters and enzymes that catalyze the accumulation of compatible organic osmolytes (Hochachka and Somero, 2002). From an evolutionary point of view, the cellular stress response represents a great example for the inherent flexibility and robustness of cellular organization. It renders cells transiently more tolerant towards temporary damage-inflicting environmental extremes and allows for slower, stressor-specific adaptations to materialise.

In multicellular eukaryotes programmed cell death (often called apoptosis) represents an additional common stress response when the dose of stress exceeds the cell's capacity for maintaining genomic and macromolecular integrity. This process serves to avoid tumorigenesis and genetic instability of organisms. Hence, cells have the ability to monitor the severity/degree of stress or stress-induced damage. The monitoring systems must be integral parts of the cellular stress response and are likely to be composed of proteins that function in constitutive DNA repair and protein degradation pathways, as well as cellular redox regulation. Many genes involved in the cellular stress response have been identified, but immense gaps remain to be addressed with regard to their exact functions and interaction with other components of stress pathways.

Future directions and evolutionary perspectives on the cellular stress response

Our knowledge of the molecular basis of the cellular stress response has increased exponentially during the past decade. This response involves an elaborate stress proteome that far exceeds the mere induction of heat shock proteins. An important task for the future is the elucidation of the molecular identity of this stress proteome. Moreover, since many proteins and signaling pathways contribute to the cellular stress response we need to identify the key players that are situated at major nodes within the stress response network. A powerful way of identifying such master regulators of the stress proteome is a comparative functional genomics approach. Genes and proteins contributing to the cellular stress response in many different phyla as well as in response to many different stresses are likely to be most critical for stress adaptation. Identification of such a highly functionally conserved set of genes should provide us with powerful tools for assessing and manipulating the stress-tolerance of cells. Developing our ability to do so is crucial for environmental risk assessment of toxic compounds and for clinically utilizing the inherent healing capacity of the cellular stress response.

An interesting question from an evolutionary perspective pertains to the above-mentioned hypothesis that primordial cells and organisms were originally eury-tolerant. This hypothesis can be tested by comparing the degree of sequence conservation of key stress response genes in eury-tolerant *versus* steno-tolerant species. For many species high tolerance limits towards fluctuations in a particular environmental factor are indicative of high tolerance limits towards changes in other environmental factors as well. Thus, if ancestral cells were eury-tolerant (stress tolerant) we would expect key stress response genes to be more highly conserved in contemporary eury-tolerant species than in steno-tolerant species, in which these genes have been evolutionarily optimized for other functions (see above). More comparative data are needed to address this hypothesis.

A complicating factor in such a conceptual framework is the possibility that some species have secondarily acquired or 're-invented' eury-tolerance, perhaps by recruiting a few novel genes to reconstitute the cellular stress response network. This might principally be the case for organisms that consist mainly of cells with low tolerance limits towards stress, but also contain particular highly specialized tissues capable of withstanding extreme stress. Renal inner medullary cells of mammals that are able to tolerate many forms of extreme environmental stress provide a good example (Woo and Kwon, 2002; Borkan and Gullans, 2002), which also illustrates that in highly organized metazoans, critical parts of the stress proteome have to be constitutively expressed for cells to be able to display a high stress tolerance (Santos et al., 2003). The low osmotic stress tolerance of most non-renal mammalian cell types clearly indicates that it is not sufficient to hold a stress proteome blue-print encoded by the genome.

Further questions arise when analyzing the cellular stress response in the context of organismal plasticity towards environmental change. Does the expression of a highly functional stress proteome confer increased stress tolerance at the cost of decreased fitness in stable environments? The history of life on earth is that of periodic extinctions, e.g. in the Silurian, Permian, and late Jurassic periods, followed by explosive adaptive radiation of surviving species. Mass extinctions are commonly attributed to sudden and severe environmental change. One of the many factors that would favor survival during such stressful periods is a high capacity of eury-tolerant species to tolerate such environmental change. The extraordinary conservation of critical elements of the stress proteome, in combination with other adaptive features in

such species, may contribute towards enhancing their potential for surviving catastrophic events such as asteroid impacts. Following mass extinctions, lack of competition probably resulted in promoting rapid adaptive radiation of surviving species into suddenly open ecological niches.

Steno-tolerant species, although more complex and highly organized, are more susceptible to sudden changes in the earth's climate, possibly because critical stress response genes were subject to more extensive modification during evolution to accommodate the higher complexity and organization resulting in increased fitness in stable environments. On a vast time scale one could view the evolutionary process of life on earth as a succession of periods of stability favoring adaptive radiation of steno-tolerant species, interspersed with periods of sudden, severe and global environmental change favoring natural selection of eury-tolerant species. Although many factors are important for surviving mass extinctions, the phenomenon of maintenance and natural selection of eury-tolerance may be one of the critical elements. This phenomenon could be described as Mega-Evolution, and may explain the abundance of eury-tolerant species despite the enormous selection pressure towards ever greater specialization over successive cycles of mass extinctions and adaptive radiations. The underlying evolutionary driving force for such Mega-Evolution merits further study, but it seems plausible that a high capacity for a cellular stress response is one of the crucial pre-requisites for such a process. I hope that this paper helps to invigorate projects tackling the physiological and evolutionary significance of the cellular stress response.

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