Editorial

Introduction to the special issue: Comparative biology of cellular stress responses in animals

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1. Introduction

This special issue of JEZ-A highlights the importance of cellular stress responses (CSR) for animal biology. The first goal of this volume is to define the CSR and show how it differs from cellular homeostasis responses (CHR) and the more complex integrative, systemic stress responses that are more commonly studied by comparative animal biologists. The second goal is to provide readers with an overview of the key mechanisms that constitute the CSR and show how their graded (hierarchical) nature permits tuning the magnitude of the CSR to the extent of stress the animal has faced. The third broad goal is to outline the non-specific nature of the CSR core elements, which can be elicited by perturbation from a wide variety of physical and chemical stressors that have the common effect of causing macromolecular damage. Thus, whether one is examining stress from temperature, hydrostatic pressure, osmotic conditions, or some other stressor that perturbs macromolecular structure, a common set of mechanisms—the core elements of the CSR—will be recruited to redress the stressor-induced perturbation to the cell.

The past few decades have seen an explosive growth of literature on the CSR. Most studies focus on "model" study systems such as yeast or mammalian cell lines, where the availability of genomic information has often allowed detailed description of the mechanisms of the different components of the CSR. However, comparative studies on many animal species have also blossomed, in large part because of growing concern about the effects of anthropogenic pollutants and global change on the biosphere. The CSR thus has gained relevance in contexts outside of biomedicine per se. The growing wealth of data from these many studies of the CSR is something of a mixed blessing, however. Although we are increasingly able to describe in fine detail the components of the CSR and the mechanisms by which their functions are regulated, it can be challenging to see the "forest, " i.e., the overarching common properties of the CSR, due to the large number of "trees," i.e., the plethora of minute details, that are the focus of many contemporary studies (Kültz, 2005).

The papers in this special issue attempt to redress this issue by providing a clear summary of the central characteristics of the CSR, the "forest," as it were. Thus, examples of general CSR principles are elucidated for a wide array of aquatic animals, to highlight the evolutionary conservation of these key ubiquitous elements of the CSR. The articles have been organized according to different types of common environmental stresses, including temperature (Somero, 2020), hydrostatic pressure (Yancey, 2020), salinity (Evans and Kültz, 2020), xenobiotic-induced oxidative stress (Silvestre, 2020), and pH (Tresguerres et al., 2020). This multi-stressor organization not only places the general non-specific nature of the CSR into sharp relief, but also permits analysis of finer-grained stress-specific aspects of the CSR. Although the list of environmental stresses considered is not exhaustive and could be expanded (e.g. to UV radiation, hypoxia/ anoxia, etc.), due to the common core elements of the CSR, the main principles of the CSR apply to all of these different stresses.

Whereas the core elements of the CSR have now been described for multiple stressors in many animal species, the depth of understanding of the CSR differs among types of stress. For some types of stress, notably temperature, much knowledge of the CSR in many aquatic species has been accumulated, while for other stressors (e.g. pH) the comparative literature is still very sparse. Thus, in addition to providing an overview of the state of this field, it is our hope that this special issue will point out gaps in our knowledge and suggest to readers exciting directions for future CSR research that are ripe for investigation by comparative animal physiologists.

2. Stress as a quantitative phenomenon that is counterbalanced by a graded CSR

When considering responses of biological systems to stress, it is first necessary to clearly define the meaning of the term "stress" in that context. There is considerable confusion and vagueness surrounding the use of this term and the corresponding term "stress response" in biology. For this

reason, a perspective article in this special issue derives a rigorous definition of biological stress from first principles that apply to biology as well as physics (Kültz, 2020a). Likewise, a clear definition of the CSR is important to distinguish it from less evolutionarily conserved stress responses, e.g. the neuroendocrine stress response (NESR), which is much more commonly studied by comparative animal physiologists. One critical feature of the CSR that has been emphasized by multiple articles in this special issue is its graded (Somero, 2020), hierarchical (Silvestre, 2020), or qualitatively successive (Kültz, 2020b) nature. There are, in fact, five tiers in the overall CSR, whose recruitment reflects the severity of stress and the likelihood that cellular damage from stress can be corrected.

To illustrate the tiered, stress intensity-dependent nature of the CSR, consider a stress that causes damage to proteins. The initial task of the CSR is to perform its sensory function: detect and quantify macromolecular damage (see section 3). Whereas there is always some macromolecular damage in cells, for example, some protein molecules may be partially unfolded and dysfunctional, if the damage exceeds normal levels, then the initial tier of the CSR, activation of damage repair mechanisms, occurs. In the case of protein damage, molecular chaperones, commonly known as heat-shock proteins (HSPs), help refold proteins into their functional states. These chaperone activities are commonly referred to as the heat-shock response, but any stressor that induces protein damage is likely to lead to increased chaperone activity. If activation of repair mechanisms does not rapidly remove excess damage from cells, then a second layer of the CSR is activated, in addition to the first layer, to provide more time for protein repair. This second layer consists of cell cycle arrest and redirection of chemical energy and reducing equivalents from cellular "housekeeping" functions (e.g. cell growth) to CSR functions. This second CSR layer has the added benefit of minimizing the mitotic propagation of cells harboring damaged macromolecules. If protein repair still fails, then a third layer of the CSR is activated to degrade and remove terminally damaged proteins from cells. This aspect of the CSR prevents non-specific protein aggregation, macromolecular crowding by denatured proteins and affords recycling of building

blocks (amino acids) for the synthesis of new proteins. If these three layers of the CSR are still not sufficient to restore proper cellular function and if the amount of damage exceeds cellular coping ability and repair capacity, then programmed cell death (PCD), the orderly removal of terminally damaged cells, is initiated as a fourth layer of the CSR. PCD serves several important functions. It removes terminally damaged cells from multicellular animals, it prevents the mitotic propagation of damaged genomes, and it leads to availability of cellular building blocks, energy, and reducing equivalents to support the repair and stabilization of other (neighboring) cells. In addition to PCD, recent studies have identified a fifth CSR layer, one that consists of an evolutionary bet-hedging strategy that may represent an ancient evolutionary mechanism conserved in all cells (Horne et al., 2014). This bet-hedging strategy has originally been termed stress-induced mutagenesis (SIM) in bacteria, but it is known under multiple additional acronyms for eukaryotes (Kültz, 2020b). SIM results in genomic variability that is thought to have the benefit of generating somatic cell clones that are better adapted to the stressful context and which can proliferate to maintain tissue and organ function during extreme and chronic stress. SIM and PCD appear to complement each other during the CSR. While PCD removes terminally damaged, maladaptive cells from the organism, SIM generates cells with an adaptive advantage that continue to proliferate and replace dead cells while taking advantage of the building blocks, energy, and reducing equivalents liberated by PCD. It is possible that one of the selective drivers for the evolution of PCD in multicellular animals is to minimize pathological outcomes of SIM (e.g. cancer) and that these two strategies are balanced carefully to maximize the survival of organisms and their reproduction for maintaining populations. In that sense, SIM represents the final layer of the CSR, a high-risk/ high-reward mechanism that selection has continued to favor in multicellular animals, not because it prolongs their life but, instead, affords time for reproduction. In fact, SIM may be an evolutionary driver for the origin of sexual reproduction, i.e. the origin of specialized, genetically stable, and well protected germ cell lineages that are minimally susceptible to mutagenesis and thus able to sustain their stabilities

over long periods of severe environmental stress. Even if life history stress causes mutagenesis of gamete genomes, recombining gametes from two different organisms during sexual reproduction increases the likelihood that any given part of the genome is preserved and fit as a proper homologous recombination template in at least one gamete. Comparative model organisms chosen according to the August Krogh principle (Krebs, 1975) should provide fascinating answers to the many questions surrounding the role of SIM for animal physiology.

3. Macromolecular damage as both stress-induced hazard and sensor

Stress causes strain on biological systems, which triggers damage to macromolecules in cells (Kültz, 2020a). Macromolecular damage is hazardous to cells because it impairs normal cell structure and function. However, the effects of such damage are not exclusively negative. Cells utilize molecular surveillance mechanisms to monitor macromolecular integrity and harness damaging effects on macromolecules to sense and quantify cellular stress. This, in turn, ensures an appropriate CSR that reflects the extent of stress and includes only those graded layers of the CSR (see section 2) that are necessary to restore normal cell and tissue physiology. Stress inflicts damage on all macromolecules including proteins, DNA, RNAs and lipids (membranes). In principle, any of these macromolecules could be CSR sensors and orchestrate feedback regulation of the CSR. Specific examples are discussed in several articles of this special issue, including RNAs as “cellular thermometers” (Somero, 2018, 2020), proteins with structures that are sensitive to inorganic ions (Evans and Kültz, 2020), and lipids for which the packing order in membranes is sensitive to hydrostatic pressure (Yancey, 2020). Importantly, these effects of stress are non-specific: “RNA thermometers” also sense osmotic and other stresses, the folding of ion-sensitive proteins is also influenced by temperature and other stresses, and the packing order of membrane lipids is also influenced by temperature. Nevertheless, the degree to which the structure of these macromolecular CSR sensors is disturbed depends on the nature of the stress and combinatorial signaling input from a variety of these sensors may enable cells to infer not only the

severity of stress but also the type of stress, which could reinforce stressor-specific cellular homeostasis responses (CHR) in addition to the CSR. Evolution may have favored selection for low "marginal stability" of select macromolecules (e.g. certain cytoskeletal proteins), which would increase the sensitivity range for monitoring cellular stress, promote evolvability, and accelerate the onset of the first CSR layer (macromolecular stabilization/ repair). Thus, vulnerability of macromolecules to stress-induced perturbation not only serves as a raison d'être for the CSR but also assists in the regulation of this multi-tiered process (Somero, 2020).

4. Cellular chaperones: macromolecules and micromolecules

The CSR utilizes a highly conserved and relatively small set of high molecular weight compounds (mostly proteins but also phospholipids) and low molecular weight metabolites for stabilization and repair of damaged cellular macromolecules. These extrinsic mechanisms of macromolecular stabilization are distinct from intrinsic mechanisms of stabilization that rely on the primary sequence and covalent modifications of the macromolecule itself (Somero, 2020; Yancey, 2020). Proteins involved in the CSR are conserved in all forms of life and constitute the cellular stress proteome (Kültz, 2003). They include, among others, molecular chaperones, antioxidant enzymes, and DNA repair proteins. Likewise, cytoprotective metabolites constitute a small, highly conserved set of "micromolecules" (Yancey et al., 1982; Somero et al., 2017) that are known under a variety of names including organic osmolytes, compatible osmolytes, counteracting osmolytes, chemical chaperones, piezolytes, micromolecular cosolutes, micromolecular cosolvents, compatible solutes, chemical chaperones, cytoprotective metabolites, and small antioxidants (Yancey et al., 1982; Evans and Kültz, 2020; Kültz, 2020b; Silvestre, 2020; Somero, 2020). Whereas a variety of classes of small organic molecules are cytoprotective, including carbohydrates, amino acids, and methylammonium and methylsulfonium compounds, their efficacies as protein stabilizers differ greatly. The articles in this special issue emphasize that an exceptionally powerful protein stabilizer, the methylammonium compound trimethylamine N-oxide

(TMAO), is one of the key cytoprotective metabolites used in the CSR of aquatic organisms exposed to a wide range of stresses. In addition, low molecular weight antioxidants such as vitamins C and E, carotenoids, and glutathione (GSH) play key roles in the CSR to counteract oxidative stress arising as a secondary consequence of many other stresses (Kültz, 2020b; Silvestre, 2020). Cytoprotective metabolites act in concert with the cellular stress proteome to afford extrinsic stabilization and facilitate repair of cellular macromolecules as one of the first and evolutionarily most highly conserved lines of defense against stress.

5. Cellular memory and the transient nature of the CSR

Stress results from changes in the environment that can be acute or chronic. Acute stress is a classical trigger of the CSR, but gradual, chronic stress can also lead to CSR activation. Nevertheless, cells cannot sustain elevated levels of macromolecular repair and protection mechanisms indefinitely because their activation and operation require reallocation of much of the cell's chemical energy resources at the expense of normal cell physiology. Therefore, the CSR is typically a highly transient phenomenon. However, the final layers of the CSR (PCD, SIM, see section 2) are an exception. PCD and SIM, unlike the initial stages of the CSR, lead to irreversible changes to cells and the tissues within which they occur. Such irreversible changes may constitute a form of cellular memory that captures life history experiences and constrains the physiological scope of responses to future environmental challenges (Kültz et al., 2013).

Life history represents the sequence of environmental exposures during development and the remaining course of a cell's and an organism's life. At the cellular level, this record of exposures is manifested by biochemical changes such as posttranslational protein modifications, epigenetic marks, and mutations that constitutes cellular memory, which constrains (informs) cellular responses to future challenges (Kültz et al., 2013). Cellular memory contributes to considerable phenotypic plasticity of

many CSR components, but not all forms of cellular memory are permanent. For example, transient cellular memory includes the timing and intensity of expression of many components of the CSR, e.g. HSPs, which, while elevated, protect cells during subsequent exposure to stress (Somero, 2020). These phenomena are generally referred to as cross-tolerance, whereby exposure to one type of stress increases tolerance to another type of stress, and stress hardening, which refers to increasing stress tolerance as a result of prior exposure to a stress that robustly activates the CSR but is well below the cell's tolerance limit. Cross-tolerance has been well documented for various combinations of stresses, e.g. thermal stress and hydrostatic pressure stress (Yancey, 2020), pH stress and thermal stress (Tresguerres et al., 2020; Somero, 2020), and thermal stress and osmotic stress (Evans and Kültz, 2020; Somero, 2020).

6. Anticipatory quality of the CSR

A fascinating aspect of the CSR is that it can have an anticipatory quality in a diverse array of organisms and stresses. Migratory species often anticipate stresses encountered en route. In these cases, the stress is not caused by an environmental change in a fixed location that animals are confined to (e.g. sessile invertebrates) but rather the environmental change encountered by the animals is a result of movement between different habitats. In these cases, certain aspects of the CSR are constitutively elevated as in cross-tolerance and stress hardening. The main difference is that elevation of CSR components does not require prior exposure to stress. For example, fish that migrate vertically on a diel, seasonal, or ontogenetic basis to feed, reproduce, or avoid predators are prepared to counteract hydrostatic pressure stress (Yancey, 2020). Preparative elevation of some CSR components like HSPs may be permanent and, perhaps, even evolutionarily fixed. For example, the standing-stock (constitutive) levels of heat shock proteins may be highest in intertidal invertebrates (relatively sessile limpets) that encounter extreme yet unpredictable periods of thermal stress (Dong et al., 2008). The anticipation of stress and the preemptive elevation of CSR components is energy- and resource-

expensive and represents a functional tradeoff, which has been optimized by natural selection (Evans and Kültz, 2020). Preparative adaptive changes of this sort add another dimension to the CSR, which must be carefully accounted for when studying the regulation of CSR components in aquatic animals. In the case of the limpet congeners studied by Dong et al. (2008), for example, the species with high constitutive levels of HSPs did not elevate synthesis of these proteins when given laboratory heat stress; congeners with lower heat exposure under field conditions and which possessed lower constitutive expression of HSPs did significantly increase HSP production. Thus, the observation that a species "fails" to produce elevated levels of HSPs in response to high temperature stress should not be taken as a sign of shortcomings in the animal's ability to mount a CSR, but instead may mean that key components of the CSR are maintained at high levels on a continuous basis, to allow survival under extreme but unpredictable bouts of heat stress.

7. Conclusion

This special issue of JEZ-A provides a brief update on studies of the CSR with focus on aquatic animals. The goal was to provide a synthetic overview of the CSR and to highlight emerging ideas and concepts that represent intriguing areas of future research by comparative animal physiologists. The breadth of this topic necessitated being highly selective in illustrating the major concepts and how they apply to aquatic animals and other non-canonical model species. The articles in this special issue outline stress as a non-specific phenomenon that is counterbalanced by a CSR framework consisting of graded (hierarchical) layers that qualitatively and quantitatively capture and compensate for effects of stress on cells. The key to cellular stress perception (sensing) is macromolecular damage, which represents the cellular strain resulting from stress. Macromolecular damage has a dual nature as both hazard to cells and sensor for triggering a non-specific CSR, which is distinct from cellular homeostasis responses (CHR). Stress sensors utilize the "marginal stability" of macromolecules to induce macromolecular repair and stabilization and the other layers of the CSR. Stabilization of macromolecules is achieved by extrinsic

mechanisms based on the actions of specialized macromolecules, e.g. molecular chaperones, and micromolecules (cytoprotective metabolites, cosolutes). In addition, intrinsic mechanisms that are based on the primary sequence and covalent modifications protect macromolecules to sustain their proper function during stress.

The articles in this special issue also point out several caveats that need to be considered more carefully in future studies of the CSR. These include (but are not limited to) cellular memory of stress that results in considerable phenotypic plasticity of the CSR, the anticipatory quality that contributes to the regulation of certain CSR components in some animals, and the graded nature of the CSR, which is exquisitely time-sensitive. A one-time snapshot of cellular transcriptomes (or proteomes) is often insufficient and can even be misleading when interpreting the CSR. Many attempts to use transcripts or proteins of CSR components as bioindicators of various stresses have failed because of the non-specific nature of the CSR and the multitude of CSR triggers and their combined occurrence in natural habitats. The transitory nature of most elements of the CSR also makes a one-time snapshot approach inadequate; maximal expression of CSR elements, e.g., HSPs, may not be captured if the time of sampling is well before or after the peak of expression. From consideration of such shortcomings of many studies of the CSR, it has become clear that more integrative systems approaches are needed, which consider the CSR, CHR, genomes, transcriptomes, and proteomes as dynamic entities. It is our hope that this special issue of JEZ-A will contribute to fueling such much needed studies in a larger variety of taxa that have evolved under diverse environmental conditions.

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