

Stress Response - Ecological and Developmental Connections

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Introduction

Living systems continuously interact with their environment, and many factors in the environment are not kind to the organisms. The entire biological history is a reflection of this incessant antagonism between the organism and its environment: the diversity of life forms is primarily the result of this dynamic conflict, which is perceived by the organisms or its cells as "stress." These stresses range from toxic and harmful chemicals generated from within or present in the environment, to physical factors, like various kinds of ionizing and non-ionizing radiations, unphysiological temperatures, to emotional or neural stresses. All stressful situations may affect the Darwinian fitness and therefore, even the most primitive organisms have evolved means to protect themselves from such damaging stresses. A most remarkable feature of this dynamic interaction between the genome and the environment is the apparent monotony of the manner in which the genomes of very diverse organisms respond to situations which individual cells perceive as some kind of stress.

Heat Shock Response: A General Paradigm for Cellular Responses to Diverse Stresses

The initial observation by F. Ritossa in 1962 of activation of a new set of "puffs" in polytene chromosomes in salivary glands of *Drosophila* larvae following a brief exposure to sudden elevated temperature (heat shock) or to chemical agents that disturb oxidative phosphorylation in cells and the subsequent finding of Tissieres *et al.* in 1974 of synthesis of a common set of new proteins (the heat shock proteins) in different cell types of *Drosophila* in response to heat shock, initiated new chapters in our understanding of gene regulation and of the way the different biological systems cope with a variety of stresses experienced in day to day life. The heat shock or stress response is one of the most conserved responses in biological systems, since all organisms ranging from bacteria to mammals and higher plants display induction of a remarkably homologous set of proteins in response to different stresses (Morimoto *et al.*, 1994; Fiege *et al.*, 1997; Nover and Scharf, 1997; Lakhotia, 1998; Feder and Hofman, 1999).

Organism Diversity, Adaptation and Stress Responses

The enormous variety of environmental conditions under which the diverse organisms live is well known. It is also well known that what is the optimal set of environmental conditions for one organism, can be stressful to other, even related, organisms. Therefore, in order to understand and appreciate the organism diversity, it is necessary to understand the genetic basis for the capability of related organisms to live in very different environmental conditions. Although the stress responses have been intensively studied, most of the studies have remained confined to a few model organisms in the laboratory and, therefore, the

relation between organism diversity, adaptation and stress responses has largely remained unexplored. Some possible areas are highlighted in the following:

Evolution and Structure of the Heat Shock Transcription Factors

Heat shock induced gene expression is mediated by a heat shock transcription factor (HSF) which is activated by the heat stress (oligomerisation and phosphorylation) so that it can bind to the highly conserved regulatory sequences in the promoter region of the stress inducible genes (the heat shock elements or HSEs). Yet, the temperature at which cells of a given species begin to "feel" the stress is highly species specific. This species-specificity of HSF activation is a challenging area of study for evolutionary biologists (evolution of the genes that code for HSF), structural biologists (amino acid sequence variations in HSFs of related species and their consequence on the 3-dimensional structure of the HSF in relation to its stress-sensing property), cell and molecular biologists (compartmentalization and other interactions of the HSF in cells).

Environment and Phenotypic Plasticity

In many organisms, environmental factors like temperature, osmotic conditions, etc. are important regulators of developmental events and the resulting phenotype. Environmental factors particularly temperature, plays very important role in development and differentiation (including sex determination) in many species. It is known that depending upon the ambient conditions, the developmental paths may dramatically vary in certain species. For example, many species of butterfly, moths etc. develop different pigment patterns in different climatic conditions of the year (Brakefield, 1997). In other instances, related species living in different ecological conditions differ in their thermotolerance (Nath and Lakhotia, 1988; Norris *et al.*, 1995). Roles of various "stress proteins" in these important developmental aspects have been studied only to a limited extent in certain model systems. Functions of heat shock and other stress proteins in such adaptive phenotypes need to be examined in much more wider groups of species. Stress proteins seem to have roles in the life cycles of parasites that alternate their life cycle between a cold-blooded and a warm-blooded host (Feder and Hofman, 1999). The host as well as the parasite appear to experience "stress" and each responds in a characteristic manner which may be basis of the pathological consequences. Evolution of tolerance would involve modulation of the stress-response of the host as well as of the parasite. Even viruses seem to be capable of responding to the stress of being inside the host cells (McFadden, 1998). The conventional stress proteins may not be involved in all these cases but these aspects need to be examined.

Diversity in Stress Response in Relation to Tissue Differentiation and Habitat

Early studies on heat shock response in a few model organisms showed that the pattern of the induced synthesis of Hsps was more or less comparable in different tissues of an organism. Although there have been some indications of subtle but significant differences in the "stress response" of different cell types in some cases (Singh and Lakhotia, 1988; Lakhotia and Singh, 1989), this aspect has not received the attention that is due. Just as we realize the diversity of different organisms in their ecological contexts, we need to appreciate tissue and cell diversity in the ecological contexts applicable within the body of an organism. Our initial studies (Singh and Lakhotia, 1999) in natural populations of certain species of insects revealed remarkable tissue- and developmental stage-specific differences in the pattern of heat shock induced protein synthesis. A systematic search for differences and similarities in

stress responses in different tissues of organisms adapted to different habitats is, therefore, necessary from the point of view of adaptive significance of stress proteins in relation to organism diversity.

Regulation and Functions of the Multiple Members of a Heat Shock Gene Family

The different heat shock proteins are grouped into distinct families on the basis of their molecular size and other distinctive properties. It is interesting that in most species, each family of the Hsps is represented by more than one gene. This is particularly true for the more abundant Hsps like the Hsp70, Hsp60 and the low molecular weight Hsps. The significance of such multigene families is still unresolved. An intuitive explanation for the multiple copies of nearly identical genes has been that these proteins are required in good quantity in a short time and, therefore, multiple copies are helpful (e.g., see Feder and Krebs, 1997). Is this the real explanation, or are there additional points that we have missed? Results of our recent studies on the *hsp70* genes of *D. melanogaster* illustrate the need to seek a better explanation for the existence of multigene families for the various heat shock proteins.

Besides the many genes for Hsp70 cognate proteins (Hsc70), *D. melanogaster* has at least 5 genes coding for the heat-inducible Hsp70: these five genes are typically arranged in two clusters, two at the 87A7 locus, and three at the 87C1 locus. The polypeptides coded by these 5 genes are nearly identical in their amino acid sequence, with less than 2% divergence. Their 5' upstream regulatory sequences are also nearly identical while the 3'-UTRs show greater divergence between the two clusters. It has generally been believed that all 5 copies are comparably induced by heat shock. However, recent studies in our lab (Prasanth, K. V. and Lakhotia, S. C., unpublished), using the 3'-UTR sequences derived from the 87A7 and 87C1 *hsp70* gene copies as probes, have revealed unexpected but very significant differences in heat shock induced transcription of these *hsp70* genes and the stability of the induced transcripts in a developmental stage- and tissue-specific manner. In addition, careful studies have further shown that the heat shock induced form of Hsp70 is specifically present in unstressed late gonial cells in testes from 2nd instar larval stage to adult. The functional significance of such specific differences in induction of nearly similar proteins remains to be examined. Likewise, the mechanisms that regulate the induction or developmental activation of the different *hsp70* genes need to be examined afresh. It is notable that the heat shock response and its regulation have been most extensively studied in the case of *D. melanogaster*, and yet such dramatic differences in induction of the different *hsp70* genes had remained unnoticed. It is, therefore, necessary that we proactively look for such differences, not only in model lab organisms but also in organisms that live under natural conditions. Detailed intensive studies in a wide range of organisms outside the constant environment of the laboratory are necessary, since if such tissue- and developmental-stage specific differences have any meaning, it has to be in relation to the micro-environmental differences of specific cell types. The consequences of the apparently small differences in the amino acid sequence of the Hsp70 family members in a given species upon their functional capabilities in different cellular compartments also need to be explored.

Integration of Molecular Approach with Organismic and Evolutionary Biology

Regarding the conditional and rapid response of specific set/s of genes to stresses at the cellular level, molecular biological studies on different stress responses have contributed significantly to our understanding of regulation of gene activity at transcriptional and post-transcriptional (RNA processing, transport and turnover) and translational levels. Likewise,

the elucidation of the role of stress proteins (and their normal developmentally expressed cognates) as molecular chaperones has been a significant achievement of recent years. We now know that correct folding of newly synthesized or damaged proteins in our cells depends upon a significant amount of help provided by the stress proteins. A great variety of stressful events, like heat shock, cold shock, salt stress, light stress, poisoning, injury, abrupt changes in hormonal concentrations, mental stress, etc., result in extensive protein damage. The increased amount of stress proteins protects the cells from such damages by helping to preserve the structure of various proteins, to re-fold the damaged proteins and finally to remove the irretrievably damaged proteins through specific proteolytic pathways. While these phenomena have now been established, their mechanistic details need to be worked out using genetic, molecular and biophysical approaches. Such studies in relation to the above noted issues in organism diversity and adaptations are typical examples of an integrated approach in current Biology.

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