Stress Biology
A Challenging Area in Integrated Biology

by Subhash C. Lakhotia
Cytogenetics laboratory, Department of Zoology, Banaras Hindu University,
Varanasi 221 005, India (e-mail: *lakhotia@banaras.ernet.in*)

Introduction

Biology has been and is being studied at various levels ranging from taxas to populations to individuals to its organ systems, tissues, cells and molecules. With the advent of biochemical and molecular approaches and the consequent excitement of being able to unravel the molecular details of a given life processes, an integrated approach to Biology was sometimes lost. Fortunately, as the tools available to Biologists have become more precise to allow deeper probing into the mysteries of living world, the wheel has now fully turned and there has been a resurgence of an integrated approach in Biology. Such an integrated approach has permitted correlation of very specific molecular events within a cell with individual or even population level macro events.

Living systems are continuously interacting with their environment and many a factors in the environment are not kind to the organisms. The entire history of living organisms 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. Since the environment is ever-changing, the living organisms cannot remain static entities. 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. 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 be harmful to the biological organization and therefore, even the most primitive organisms have evolved means to protect themselves from such damaging stresses.

The discoveries of appearance of new puffs in polytene chromosomes of salivary glands of *Drosophila* larvae that were briefly exposed to elevated temperature or to certain other conditions that disrupt oxidative metabolism in the cell during the sixties and of induced synthesis of certain new polypeptides in heat shocked cells of *Drosophila* in early 70s laid the foundation of cell stress studies. One of the most exciting finding of the 1970s was that a variety of chemical and physical factors evoke a highly conserved common cellular response in all organisms from the primitive prokaryotes to the most highly evolved eukaryotes. This common stress response is more widely known as "heat shock response".

During the past 10 years or so a large number of studies have focused their attention on the biological significance of the stress response, particularly functions of the stress proteins. Once again, a common theme has emerged that nearly all of the stress-

activated gene products (and their cognates synthesized in a developmentally regulated or constitutive manner in unstressed cells) function as molecular chaperones and are primarily involved in preventing mis-folding and aggregation of polypeptides and in translocation of proteins across the membranes separating different compartments in a cell. The concept of chaperone function has revolutionized our understanding of many of the basic problems or issues that have been confronting biologists.

Heat Shock Response:
A General Paradigm for Diverse Stress Responses

The seminal 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. Historically, this response was termed "heat shock response" because heat shock was the most common inducer used; however, it was soon obvious that a variety of stress conditions also elicit a similar response. Therefore, the term "stress response" has also been often used. The heat shock 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.

A variety of chemical agents induce synthesis of some or all the heat shock proteins in different organisms: these include oxidizing agents and drugs affecting energy metabolism, transition series metals, sulfhydryl reagents, chelating drugs, amino acid analogues, certain inhibitors of transcription and translation, steroid hormones, glucose deprivation (including treatment with 2-D-glucose, glucosamine, tunicamycin etc.), ionophores, teratogens, ethanol etc.; in addition, recovery from anoxia, wounding and several viral infections are also associated with induction of Hsps. A common response to such diverse stresses highlights the importance of these proteins in biological systems and this is reflected in growing interest in these proteins, their functions and regulation.

The heat shock response is a cellular response comprising of transient but complex reprogramming of gene expression that takes place when cells or organisms are subjected to a wide variety of physical or chemical stresses. This reprogramming, in most cases, includes depression of ongoing chromosomal transcription, induction of transcription of the heat shock genes, inhibition of RNA processing, inhibition of translation of general mRNAs and a preferential translation of the heat shock mRNAs. These coordinated changes in transcriptional and translational activities result in a massive accumulation of the newly synthesized heat shock proteins in the stressed cells. The stress response is an adaptive mechanism that enables cells to survive environmental insults that may otherwise be damaging to cell or be even lethal. The adaptive value of the heat shock response is obvious from the known thermotolerance acquired by exposure to a prior milder stress and from the fact that if RNA or protein

synthesis is inhibited during the period of stress, the cells are unable to recover from stress-induced damage.
The different major heat shock proteins are grouped, on the basis of their apparent molecular sizes (in kilodaltons), into different families, viz., Hsp100, Hsp90, Hsp70, Hsp60 and the small heat shock proteins family. Many of these stress proteins or their cognates (e.g. Hsc70) are also expressed constitutively and remain localized to different compartments within the cell. The stress proteins have important roles in a multitude of protein biosynthetic events, including protein folding, assembly and transport of proteins across membranes.

While the induced synthesis of Hsps in different tissues of a given organisms is generally similar, some studies have revealed significant differences in induction of Hsps in relation to developmental conditions and specific tissue types (Singh and Lakhotia, 1988; Lakhotia and Singh, 1989; Krebs and Feder, 1997). Such results emphasize the need to re-examine stress response of a wide variety of organisms under their natural habitats.

Although the expression of heat shock proteins in response to physical and chemical stresses is well known, recent evidence shows that neuro-hormonal stress also leads to synthesis of heat shock proteins. This suggests that in addition to the cell level functions, the heat shock response may also have important homeostatic roles at individual level in higher organisms in coping with the stresses of everyday life.

The increased radio-sensitivity of cancerous cells under conditions of hyperthermia may also be related in some ways to heat shock proteins. The basis of this phenomenon remains to be well understood. A pre-exposure to mild heat shock has been reported to reduce the cell-killing by UV in *Dictyostelium* although a post exposure to hyperthermia can enhance cell death by UV. The fact that low dose ionizing radiation evokes an adaptive response, analogous to that of the heat shock response, suggests some commonality in these adaptive response pathways. Although DNA damage by itself may not induce Hsps, several DNA damaging agents, like alkylating agents, nitrosoureas etc., are known to induce some of the Hsps probably through other direct pathways.

Besides the heat shock, many other stresses (like cold shock, oxidative stress, salinity stress, metal stress, starvation stress etc.) are now known to evoke analogous cellular responses in the form of induction of synthesis of specific set/s of new proteins. Many of these "stress proteins" are common to a variety of stresses while others are uniquely induced by a specific stress.

Many recent studies have also revealed intriguing relationship of heat shock proteins with the immune response in higher organisms, including man. In addition, heat shock protein have also been shown to provide protection against ischaemic damages to human organs. The heat shock response has, therefore, become very important from a clinical viewpoint as well.

In view of the ubiquitous nature of the stress responses and their importance for the day-to-day life of any living organism, it is not surprising that the number of "stress biologists" has been on the increase. The literature in this field is very extensive and

now even specialized journals (e.g. *Cell Stress & Chaperones*) that deal with this area are also available. Some of the recent books, reviews etc. that provide details of the various aspects of Stress Biology are: Nover and Scharf (1997), Lakhotia (1996), Michaud *et al* (1997), Morimoto *et al* (1994), Feige *et al* (1996), Mogk *et al* (1997), Csermely (1998), Csermely *et al* (1997), Srivastava (1994); Minowada and Welch (1995); de Pomerei (1997). In view of the ever growing importance of this field, several conferences (at international and national levels) are organized every year to discuss new developments in the field of Stress Biology.

International Workshop on Molecular Biology of Stress Responses

An international Workshop on "Molecular Biology of Stress Responses" was organized by S. C. Lakhotia (India) and W. Schumann (Germany) at the Banaras Hindu University (October 14-17, 1997) to bring together specialists from different countries studying responses of cells of diverse organisms (bacteria, plants and animals) to a variety of stresses (thermal, oxidative, starvation, osmotic etc.) and their regulations. This meeting, which was also sponsored by the IUBS, provided a good opportunity for cross-talks between scientists from different countries and working in different areas of "Stress Biology" and utilizing different organisms.

Approximately 100 scientists from 12 different countries assembled at the ancient city of Varanasi to discuss latest developments in molecular aspects of stress-research. A summary of the scientific discussions at this meeting is given in the following (for more details, see Csermely and Lakhotia, 1997) to illustrate some of the current trends in stress research.

Heat Shock Response in Eubacteria. T. Yura (HSP Research Institute, Kyoto, Japan) discussed the strategies of transcriptional and translational regulation of the heat shock response during evolution of gram-negative bacteria and the g proteobacteria. W. Schumann (Univ. Bayeuth, Bayreuth, Germany) presented evidence for the GroE chaperonin machine as the major regulator of heat hock response in *Bacillus subtilis*. U. Voelker (Phillips-University, Marburg, Germany) discussed regulation of the general stress response in *Bacillus subtilis*.

Salt Stress and Other Stresses in Bacteria. D. Le Rudulier (Univ. Nice-Sophia, Nice Cedex, France) discussed the various osmoprotection devices used in various bacteria. E. Bremer (Univ. Marburg, Marburg, Germany) presented his group's data on synthesis and uptake of glycine-betaine by *B. subtilis*. A. Tripathi (Banaras Hindu University, Varanasi, India), also the Convenor of the meeting, presented data on functions of the periplasmic glycine-betaine binding protein in *Azospirillum*. A. Strom (Norwegian Univ. Science & Technology, Nordheim, Norway) discussed the roles of glycine-betaine and trehalose in osmoprotection in *E. coli*. C. K. K. Nair (Bhabha Atomic Res. Ctr., Mumbai, India) reviewed the organization of *dnaK* locus in haloarchaea.

S. K. Apte (Bhabha Atomic Research Ctr., Mumbai, India) reviewed stress responses of the nitrogen fixing cyanobacteria *Anabaena*. M. Bazzicalupo (Univ. Florence,

Florence, Italy) discussed the role of heat shock proteases in stress response in *Azospirilllum*. V. Braun (Univ. Tubingen, Tubingen, Germany) summarized the iron-uptake mechanism in bacteria while S. Ohtake (Hiroshima Univ., Hiroshima, Japan) talked about adaptation of bacteria to phosphate starvation.

M. K. Ray (Ctr. Cell Molec. Biol., Hyderabad, India) and S. K. Mahajan (Bhabha Atomic Res. Ctr., Mumbai, India) discussed features of psychrotophic bacteria and adaptations of bacteria to cold temperatures, respectively. S. Srinivasan (Univ. New South Wales, Sydney, Australia) discussed survival of non-differentiating bacteria under conditions of starvation. R. Chowdhuri (Ind. Inst. Chem. Biology, Calcutta, India) discussed relation between stress response and virulence of *Vibrio cholerae*.

Regulation of Eukaryotic Stress Response and Heat Shock Factors. S. H. Satyal (Northwestern Univ., Evanston, USA) discussed the regulation of heat shock transcription factor 1 by *cis*-regulatory domains and *trans*-regulatory proteins. P. Sadhale (Ind. Inst. Science, Bangalore, India) raised the possibility of stress-induced changes in the RNA-polymerase II subunits as important regulators of transcriptional regulation of stress response in eukaryotes. K. -D. Scharf and L. Nover (Goethe Univ., Frankfurrt, Germany) detailed the roles of constitutive and inducible heat shock transcription factors in plants. U. K. Srinivas (Ctr. Cell. Molec. Biol., Hyderabad, India) presented her lab's finding that albumin is heat shock inducible in embryonic rat liver. E. S. Gonos (Inst. Biol. Res. Biotechnol., Athens, Greece) suggested that while constant large stresses accelerate, milder and repeated stresses can decelerate the cellular senescence clock and thus life expectancy.

Heat Shock Proteins, RNA and Molecular Chaperones. R. Tanguay (Univ. Laval, Quebec, Canada) showed a link between small heat shock proteins and a ubiquitin-conjugating protease in *D. melanogaster*. A. Richardson (Cent. Med. Univ., Geneva, Switzerland) described the structure and function of a T4 bacteriophage chaperonin.

Several papers at this meeting dealt with the Hsp90 family proteins which are fairly abundant even in unstressed cells. P. Csermely (Sommelweis Univ., Budapest, Hungary) discussed this protein's wide ranging roles in building and reorganizing the cellular architecture and suggested that its low affinity ATP/ADP-binding property plays a role in regulation of Hsp90-protein complexes. J. K. Pal (Univ. Poona, Pune, India) showed that interaction of Hsp90 with eIF-2a kinase in mammalian cells is important in translational regulation in normal and stressed cells. S. C. Lakhotia (Banaras Hindu University, Varanasi, India) presented his lab's data on genetic interactions between the non-protein coding, developmentally active as well as stress inducible *hsr-omega* gene of *D. melanogaster* and the *hsp83* and some Ras-signaling pathway genes, raising possibilities of novel roles for the non-translatable *hsr-omega* transcripts in normal and stressed cells. P. K. Srivastava (Univ. Connecticut, Farmington, USA) reviewed his pioneering studies on participation of heat shock protein-peptide complexes in MHC mediated antigen presentation and the possibilities of using this property for generating tumor-specific vaccines for human trials.

Stress Response in Plants. A. Grover and S. Singla (Univ. Delhi, N. Delhi, India) presented their lab's data on Hsp90 and Hsp100 family proteins in rice in response to heat, salt, desiccation, cold stress etc. M. V. Rajam (Univ. Delhi, N. Delhi, India)

reported genetic engineering of polyamine and carbohydrate metabolism to develop plants that can better tolerate osmotic stress. K. C. Upadhyaya (Jawaharlal Nehru Univ., N. Delhi, India) showed that *Arabidopsis ACaM5* calmodulin is a heat shock protein. Discussion on these talks brought out an important point that stress-resistant transgenic plants may not always be as attractive as they may appear since over-production of some of the stress proteins may also alter other desirable or essential properties of the plant.

Stress Proteins as Bio-markers. D. N. Deobagkar (Univ. Poona, Pune, India), D. de Pomerai (Univ. Nottingham, Nottingham, U.K) and D. Kar Chowdhuri (Industr. Toxilcological Res. Inst., Lucknow, India) discussed stress proteins in relation to environmental pollution and their roles as bio-indicators. Deobagkar showed that insecticides induce stress proteins in mosquitoes and this allows mosquitoes to carry higher loads of disease causing parasites. De Pomerai showed that microwave radiations in the range produced by domestic appliances and mobile phones can also induce cellular stress proteins.

Most of the presentations at this meeting will be published in a special issue of the Journal of Biosciences (Indian Academy of Sciences, Bangalore, India) by mid 1998.

Relevance of Stress Biology to the IUBS Program of "Integrated Biology"

Deliberations at the Varanasi meeting amply demonstrated the relevance of this field of study to the program of "Integrated Biology" which has recently been adopted by the IUBS. A group of leading participants in this International Workshop got together and decided to suggest to the IUBS that the field of Stress Biology should be integrated in the new program of "Integrated Biology". This group included Prof. S. C. Lakhotia (Department of Zoology, Banaras Hindu University, Varanasi, India), Prof. W. Schumann (Institute of Genetics, University of Bayreuth, Bayreuth, Germany), Prof. L. Nover (Department of Molec. Cell Biol., Goethe Univ. Frankfurt, Frankfurt, Germany), Prof. Peter Csermely (Department of Medical Chemistry, Sommelweis Univ., Budapest, Hungary), Prof. R. Tanguay (Lab. Cell. & Dev. Genet., Univ. Laval, Quebec, Canada) and Prof. P. Srivastava (Ctr. Immunotherapy Cancer & Infect. Diseases, Univ. Connecticut, Farmington, USA). The following has been compiled from inputs by these individuals to highlight some of the many areas in the field of Stress Biology that are of direct relevance to Integrated Biology and therefore, need to be actively pursued and supported internationally.

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 and, therefore, the relation between organism diversity, adaptation and stress responses has largely remained unexplored. Such studies will provide a very good bridge between the IUBS programs of **"Diversitas"** and **"Integrated Biology"**. Some possible areas are highlighted in the following:

Heat shock induced gene expression is mediated by a heat shock transcription factor (HSF) which is activated by the heat stress (oligomerisation and phosphorylation) and binds to highly conserved regulatory sequences in the promoter region of the stress inducible genes (the heat shock elements or HSEs). It is remarkable that the temperature at which cells of a given species begin to "feel" the stress is highly species specific although the HSEs are highly conserved and the HSFs are also moderately conserved. 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).

Bacterial Stress Response: Bacterial cells are relatively simple entities with small haploid genome. Despite their simplicity they are capable of coping with sudden and severe changes in environment. This adaptability enables bacteria to colonize a wide range of ecological niches. In their natural environment, bacteria spend most of their life in a starving or non-growing state because of different growth-limiting conditions. In addition, they may be exposed to different chemical and physical stress factors such as changes in temperature (heat or cold stress), changes in their external pH (acid or alkaline shock), osmotic or oxidative stress, pressure or UV irradiation. In order to adapt to these stressful situations, bacteria have developed a highly sophisticated regulatory network including sensors to monitor the stress factors (only a few are known) and a signal transduction pathway resulting in the transient induction of stress proteins which fall into two groups: general stress proteins (GSPs) and specific stress proteins (SSPs). While the GSPs most probably serve a general and non-specific protective function allowing adaptation to stress and starvation, regardless of the stress factor, the SSPs may exert a specific protective function against a unique stress factor. Comparative studies on different stress responses in bacterial species living in extremes of environmental conditions (high or very low temperatures, high salinity, high metal concentrations, anaerobic conditions, highly acidic or alkaline pH of their surrounding media either in the body of their host or soil or water etc.) are required to understand the basis of the success of these most ancient living group of organisms.

Complexity of the plant stress responses: The unfavourable environmental changes generally affect plants more than animals due to their local fixation. The usual situation for plant growth and development is best characterised by daily multistress challenges. As a result, a multiplicity of partially overlapping stress response systems has evolved. The tight and highly flexible stress network is characterised by a number of multivalent or even general stress metabolites and proteins. Hormones, in particular ethylene, abscisic acid and jasmonic acid, are not only frequently found as stress metabolites, but they are also part of systemic and developmental signalling systems. The increasing number of physical, chemical or biological stressors affecting plants may be linked to

each other by virtue of a 'natural coupling'. Some of the aspects of plant stress responses that deserve in depth studies are:
Heat shock and reduced supply of water are a natural pair of stressors with highest impact on plant productivity. Evaporation of water from the soil and the plants strongly increases with the environmental temperature, and the ensuing closure of the stomata results in increased leaf temperature. In addition to this, water deficiency may result from chilling or freezing and from osmotic or salt stress.

In many cases, biological stress by pathogen attack is dependent on or coincides with wounding or leads to local cell death. Many parts of these stress response systems are identical or very similar. They can be mimicked by application of plant- or pathogen-derived cell wall fragments (elicitors). Starting with the local cell death by apoptosis and probable elicitor formation, an unknown wounding signal is spread systemically inducing changes or resistance mechanisms in fairly distant parts of the plant.

Starvation or partial nutrient deficiency are frequently encountered, e.g. as a consequence of reduced photosynthetic activity due to low light intensities, damage of chloroplasts by photo-oxidation, stress-induced destruction of thylakoid membranes or closure of stomata because of drought or salt stress. On the other hand, unbalanced or poor nutrient uptake by the roots and inefficient distribution within the plant may result from nutrient deficits in the soil, salt stress, anaerobiosis, flooding, phytopathogen attack, poor transpiration or heavy metal intoxication.

In many plants, environmental factors like temperature, osmotic conditions etc. are important regulators of germination, growth, flowering and fruit development (Callahan *et al* 1997). The role of various "stress proteins" in these important developmental aspects of plants have been studied only to a limited extent in certain model systems. Such studies need to be extended to a wide variety of plant species thriving in different habitats, not only to gain basic information on biological systems but also to provide a rationale for biotechnological exploitations.

The well advanced analysis of stress interactions at the cellular level in the preceding years must now be complemented by investigations on the role of modulators and phytohormones, on enhancing or inhibitory effects between stressors and on the molecular basis of cross-tolerance. Studies on signal systems involved in the environmental integration and stress protection of plants will ultimately help to understand and possibly improve plant stress resistance.

Environmental factors in development, differentiation and thermotolerance in animals. Environmental factors, particularly temperature plays very important role in development and differentiation (including sex determination) of many **animal 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 (Pigliucci, 1997). In other instances, related species living in different ecological conditions (like temperate and tropical species of *Chironomus*) differ in their thermotolerance. Role of heat shock and other stress proteins in such adaptive phenotypes needs to be examined in much more wider groups of species.

Diversity in stress response in relation to tissue differentiation and habitat: A general dogma that has developed in relation to the heat shock response is that all cells of an organism respond nearly uniformly to a given stress. This dogma is based on studies in a limited set of laboratory animals and within them in the few easily manipulatable tissues/organs. Although there have been some indications of subtle but significant differences in the "stress response" of different cell types in some cases, 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. A systematic search for differences and similarities in stress responses in different tissues and different organisms will certainly be very rewarding from the point of view of adaptive significance of stress proteins in relation to organism diversity.

Gene Regulation, Protein Function and Stress Proteins

In view of the conditional and rapid response of specific set/s of genes to stresses at 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 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.

Stress Proteins and Biotechnology

The understanding of the genetic regulation of the stress network and of the function of the stress proteins is not only of basic interest, but the identification of stress genes and their function may have important impacts on Biotechnology. A few of the many possibilities are given in the following:

- Nitrogen-fixing bacteria living the rhizosphere of plants of agricultural interest can be manipulated by genetic engineering methods in such a way to withstand adverse conditions
- Bacterial stress genes can be transferred to cereal plants leading to salt and desiccation resistant plants to allow their growth in soils where growth of non-transgenic plants is not possible

- Enhanced production of molecular chaperones and protein folding catalysts may result in overproduction or over secretion of desired proteins
- Free radicals, generated during stress, are strongly oxidizing agents and have manifold effects on cellular macromolecules leading to their damage. Improving the cell's potential to overcome the oxidative stress and destroy these radicals will increase life span of the useful bacteria.

Knowledge gained from biology of stress proteins will thus be highly useful in Biotechnology since expression of proteins in most systems requires help in appropriate folding of the expressed proteins. This help can be provided by the addition or co-expression of stress proteins or chemical chaperones. Elucidating the molecular mechanism of how stress proteins help the folding of other proteins will also help us to design more folding-competent artificial proteins/enzymes. Stress proteins may also help in the correct folding of RNA which can be another area of widespread applications in biotechnology and related fields.

Clinical Applications of Stress Proteins

Recent studies have uncovered a broad role for stress or heat shock proteins in a variety of clinical applications:

- Hsps have been demonstrated to provide significant protection to brain, heart and other organs from ischaemic damages. This opens up a variety of newer applications in treatment and organ transplantation.
- Different heat shock proteins are involved in a number of auto-immune disorders.
- The pattern of heat shock proteins in mammalian brain, either synthesized in a developmentally regulated manner or in response to stress, is non-random. This has been related to specific functions of different parts of the brain, including role of Hsps, particularly the Hsp70, in short- and long-term memory and making different parts of the brain more or less susceptible to stress-induced injuries.
- A variety of pollutants induce detectable levels of stress proteins and therefore, relatively simple methods are being developed to use the stress-proteins as important bio-markers for environmental pollution. Such studies will be of significance in public health.
- Recent studies have shown significant roles for different Hsps in the immune response. A number of Hsps such as Hsp70, Hsp90 and Gp96/Grp94 have been shown to chaperone a broad array of peptides, derived from different cellular proteins, from the cytosol to the Major Histocompatibility Complex I molecules which in turn display the peptides on the cell surface. The Hsps therefore play a key role in antigen presentation. Hsp-peptide complexes, whether isolated from cells or reconstituted *in vitro*, have the ability to vaccinate against the complexed peptides. Such vaccination elicits powerful CD8+ cytotoxic T lymphocyte response. Hsps have thus become the first adjuvants of mammalian origin. Interaction of mammalian and microbial Hsps with phagocytic cells has been shown to result in secretion of a number of cytokines by the phagocytic cells. Hsps have been shown to stimulate proliferation of gamma/delta T cell receptor-bearing T lymphocytes. While the mechanistic details of this interaction are not yet clear, this observation is significant in providing a clue to the functions of gamma/delta lymphocytes. Hsps

have come to be recognized as major antigens in a variety of infections. Despite the significant cross-reactivity between the Hsps of hosts and infectious agents, Hsps of the infectious agents have been shown to elicit strong antibody responses, and in some cases T cell responses also, in a variety of hosts.

Collectively, these observations show that just as Hsps play a role in protection of individual cells from a variety of stresses, they also play a protective role in multicellular organisms through immunological mechanisms. Two types of roles are observed :

Role in innate immunity : The Hsp-phagocyte interaction results in release of cytokines. This response has no antigen-specific component and presumably plays a role in innate immunity in maintaining a particular type of immunological 'environment' within a particular niche (such as a lymph node, or a site of infection) within the host. This is presumably the more primitive immunological role of Hsps.

Role in adaptive immunity : The peptide-binding property of Hsps plays the key role in adaptive or antigen-specific immunity. Peptides provide the antigen-specificity lacking in the innate immunity, discussed above. This property is clearly a simple extension of the chaperoning function ascribed to a major sub-set of prokaryotic and eukaryotic Hsps and has been commandeered by the immunological functions expressed through MHC molecules and the T lymphocytes as they arose in evolution. In this view, the modern peptide-binding MHC molecules have been viewed as functional descendants of the primitive peptide-binding Hsp molecules.

These discoveries have opened the way for the use of Hsps in therapy of cancer and infectious diseases through the use of Hsp-peptide complexes isolated from cancer tissues and from infected cells, especially in cases where the protective antigenic epitopes are undefined or highly mutable, as vaccines to immunize patients against specific cancer or infection. These approaches are presently in the process of being tested in a number of clinical trials and have already become the cornerstones of a number of privately and publicly held biotechnology companies such as Antigenics in the USA and Europe and StressGen in Canada.

IUBS and Stress Biology

The above is not an exhaustive list of the various issues that are relevant to the general field of Stress Biology. Nevertheless, the various aspects as briefly discussed above reveal that *a concerted effort of the international scientific community is required to permit a comprehensive understanding of the different facets of Stress Biology and to be able to optimally exploit this knowledge for betterment of human and other lives.* **The IUBS has already prepared the ground for such studies by adopting programs like Diversitas and Integrated Biology.** The general field of Stress Biology easily integrates with both of them without any "stress"!

There are several possible ways through which the IUBS may facilitate and promote substantial progress in this important field of Integrated Biology:

- With its global network of affiliating institutions in nearly all countries, the IUBS may encourage this field to be considered as the priority or thrust area in individual countries' scientific programs.
- Since the IUBS also sponsors a large number of Workshops and Conferences, those dealing with this general area may be increasingly supported. It is suggested that instead of the developed countries hosting such workshops/conferences etc., these should be organized in different developing countries. Meetings of this kind in developing countries would promote the field of study in more widespread geographical areas so that more diverse groups of organisms are studied rather than the studies on stress response remaining confined to a few model organisms, as is more likely to happen in the developed countries. Holding of such meetings/workshops. in developing countries would also be more economical and at the same time promote wider international collaborations and concerted programs.
- The IUBS may help establish a Stress Forum as part of the BioMedNet network. This Forum would be a versatile medium for very meaningful communications.

Acknowledgments.

I gratefully acknowledge the inputs provided by Profs. Wolfgang Schumann, Lutz Nover, Peter Csermely, Robert Tanguay and Pramod Srivastava which made it possible to prepare this article. I also thank Dr. Talal Younès for encouraging me to write this paper and for his valuable suggestions.

References

Callahan, H. C., Pigluicci, M. and Schlichting, C. D. 1997. Developmental phenotypic plasticity: where ecology and evolution meet molecular biology. *Bioessays* 19: 519-525

Csermely, P. (ed.) 1998. Stress of life from molecules to man. *Ann. New York Acad. Sci.*. In Press

Csermely, P., Schnaider, T., Soti, C., Prohaszka, Z. and Nardai, G. 1997. The 90kDa molecular chaperone family: structure, function and clinical applications. A comprehensive review. *Pharmacol. & Therapeutics*. In press

Csermely, P. and Lakhotia, S. C. 1998. Molecular biology of stress response in India. *Cell Stress & Chaperones*. In press

de Pomerei, D. I. 1996. Heat shock proteins as biomarkers of pollution. *Human & Exp. Toxicol.* 15: 279-285

Krebs, R. A. and Feder, M. E. 1997. Tissue-specific variation in Hsp-70 expression and thermal damage in Drosophila melanogaster larvae. *J. Exp. Biol.* 200: 2007-2015

Fiege, U., Morimoto, R., Yahara, I., Polla, B. S. (eds.) 1996. *Stress-inducible cellular responses*. Birkhauser Verlag, Basel, Boston, Berlin

Lakhotia, S. C. (ed.) 1996. Special issue on Heat Shock Proteins. *Journal of Biosciences* 21, no. 2, Indian Academy of Science, Bangalore, India

Lakhotia, S. C. and Singh, A. K. 1989. A novel set of heat shock polypeptides in malpighian tubules of Drosophila melanogaster. *J. Genet.* 68: 129-137

Minowada, G. and Welch, W. J. 1995. Clinical implications of the stress responses. *J. Clin. Invest.* 95: 3-12

Mogk, A., Homuth, G., Scholz, C., Kim L., Schmid, F. X. and Schumann, W. 1996. The GroE chaperonin machine is a major modulator of the CIRCE heat shock regulon of Bacillus subtilis. *EMBO J.* 16: 4579-4590

Michaud S., Marin, R. And Tanguay, R. M. 1997. Regulation of heat shock induction and expression during development of Drosophila development. Cell Mol. Life Sci. 53: 104-113

Morimoto, R. I., Tissieres, A., Georgopolous, C. (eds.) 1994. *The biology of heat shock proteins and molecular chaperones*. Cold Spr. Harb. Press, Cold Spr. Harb. N.Y.

Nover, L. and Scharf, K.-D. 1997. Heat stress proteins and transcription factors. *Cell. Mol. Life Sci.* 53: 80-103

Pigliucci, M. 1997. Butterflies in the spotlight. *Bioessays* 19: 285-286

Ritoosta, F. 1962. A new puffing pattern induced by heat shock and DNP in Drosophila. *Experientia* 18: 571-573

Singh, A. K. and Lakhotia, S. C. 1988. Effect of low-temperature rearing on heat shock protein synthesis and heat sensitivity in Drosophila melanogaster. Dev. Genetics 9: 193-201

Srivastava, P. K. 1994. Heat shock proteins in immune response to cancer: the fourth paradigm. *Experientia* 50: 1054-60

Tissieres, A., Mitchell, H. K. And Tracy, U. M. 1974. Protein synthesis in salivary glands of D. melanogaster. Relation to chromosome puffs. *J. Moi. Biol.* 84: 389-398