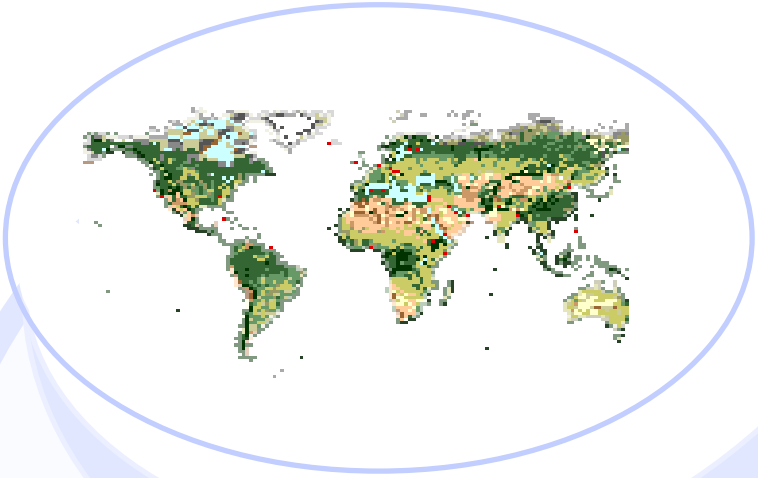
STRESS BIOLOGY

A Paradigm for Integrative Biology



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Editorial

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Stress is an inevitable part of the life of all organisms. The evolutionary process is, in a large measure, a reflection of the continuing conflict between the organisms and their environment. Since any stressful condition is potentially harmful to the cells, even the most primitive organisms are armed with cellular mechanisms that can safeguard against the potential damages due to stresses. Because of the ubiquitous nature of the diverse stress responses, it was pointed out earlier (Lakhotia, 1998) that studies in the field of stress biology provide a very good model for integrative biology. Accordingly, the IUBS mandated me to organize a special exploratory meeting within the framework of the 2nd International Workshop on Stress Biology, held on October 15-18, 1999, at Wuhan, China to review the present scenario in different areas of Stress Biology from the perspective of the "Towards An Integrative Biology (TAIB) Program" of the IUBS and to make recommendations for future directions which the IUBS may consider within the framework of the TAIB program.

The special session was held on October 16, 1999, with participation of the following leading Stress Biologists: Prof. Takhashi Yura, Japan; Prof. Peter Csermely, Hungary; Prof. Wolfgang Schumann, Germany; Prof. A. Patrick Arrigo, France; Prof. Robert Tanguay; Dr. Tangchun Wu, China; Dr. J. Gowrishankar, India; Prof. Larry E. Hightower, USA; Prof. Richard I. Morimoto, USA; Prof. Martin Feder, USA; Dr. Zihai Li, USA and Prof. S. C. Lakhotia, India (Convenor). A brief report of this meeting and its recommendations were published earlier (Lakhotia, 2000).

The group felt that the following aspects of Stress Biology are of special importance for future studies and their integrative studies will provide deep insights into the mechanisms that enable the diverse organisms to survive the variety of the omnipresent stresses.

1. Stress genes: evolution and roles of stress genes in adaptation and in generating bio-diversity
2. Functions of Stress Proteins: as molecular chaperones, in compartmentalization of molecules in cells and in maintenance of the cytoarchitecture
3. Regulation of Stress Responses:
   - 3.1. Evolution, structure and other roles of the Heat Shock Transcription Factor/s in different organisms
   - 3.2. Inter-individual variability in the stress response and its relation to genotypic variations and "fitness"
   - 3.3. Intra-individual tissue-specific variability in the stress response and its functional significance

4. Stress Proteins as Bio-indicators of pollution and to identify agents that may be stressful to cells
5. Stress proteins in Health and Disease
   - 5.1. Response of the host cells to parasite/pathogen
   - 5.2. Response of the parasite/pathogen to host's biological environment
   - 5.3. Involvement of stress proteins in pathological conditions involving triplet-repeat expansions in specific genes in man
   - 5.4. Role of stress proteins in humoral and cellular immune responses
   - 5.5. Stress proteins in cancer and apoptosis
   - 5.6. Hyperthermia and radio-sensitivity/radio-protection
6. Biotechnological applications: Transfer of stress genes to improve survival of crop plants under different stress conditions prevailing in the field.

The present collection of articles provides more detailed accounts of the current status of selected sub-fields of Stress Biology, future directions of studies and how they relate to Integrative Biology.

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References

Lakhotia, S. C. 1998. Stress biology: a challenging area in integrated biology. *Biology International*, 36: 18-30

Lakhotia, S. C. 2000. TAIB special exploratory meeting on molecular biology of stress responses. *Biology International*, 38: 9-10

CIRCE, HAIR and ROSE: Regulation of the Bacterial Heat Shock Response

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The ability to adapt rapidly to changes in their environment is essential for the survival of all microorganisms. Temperature is an important environmental factor. When it changes, it requires adaptive responses, designated the heat shock response. This adaptive response leads to the transiently enhanced expression of a subset of genes, the so-called heat shock genes, which encode heat shock proteins. Bacteria have evolved complex regulatory circuits to measure a sudden increase in temperature and to induce the heat shock genes. The principle function of heat shock proteins is to assist in protein folding, assembly, transport and degradation during normal growth and, in particular, under stress conditions.

Escherichia coli has long served as a paradigm for bacterial heat shock regulation. Regulation in this species is based on alternative sigma factors that direct RNA polymerase to specific promoters that differ from the housekeeping promoters. Two major and one minor alternative sigma factors have been described: sigma-32, sigma-E and sigma-54. While some 50 genes are induced by sigma-32 when denatured proteins accumulate within the cytoplasm, another about 10 genes exhibit transient increased expression when non-native proteins appear within the periplasm, and these genes are under the positive control of sigma-E. There is just one operon controlled by sigma-54, and this sigma factor seems to be activated by perturbations within the inner membrane (see recent reviews by Yura *et al*., 1993; Missiakas and Raina, 1998; Model *et al*., 1997). The activity of sigma-32, encoded by the *rpoH* gene, is post transcriptionally regulated and involves protein stability and translation efficiency. Under non-heat shock conditions, the amount of active sigma-32 is kept rather low. This is accomplished by its rather short half-life (less than one min) and the low translation rate of its mRNA which results from the sequestration of the Shine-Dalgarno sequence and the start codon within the secondary structure of the *rpoH* mRNA. After a sudden increase in temperature, this secondary structure is removed ensuing enhanced translation (Morita *et al*., 1999). Concomitantly, the half-life of sigma-32 increases from less than one minute to 4-5 minutes for several minutes to drop to about 20 seconds, resulting in shut off of the heat shock response (T. Yura, personal communication). The activity of sigma-E is modulated by an anti-sigma factor inserted into the inner membrane. Upon accumulation of denatured proteins within the periplasm, sigma-E is released to positively control expression of a subset of genes preceded by sigma-E-dependent promoters. Here, it is completely unclear how the appearance of non-native proteins is sensed and how this results in the release of sigma-E. Either, the anti-sigma factor may directly bind denatured proteins, or this might occur indirectly through one or more proteins which transduce the signal to the anti-sigma factor. Last but not least, the mechanisms resulting in the activation and deactivation of sigma-54 remain elusive.

As to the regulation of its heat shock genes, *E. coli* seems to be an exception in the sense that all major heat shock genes are part of the sigma-32 regulon. This is not the case for most other bacterial species, where the essential heat shock genes are part of several regulons. For example, in *Bacillus subtilis*, four classes of heat shock genes have been

identified so far (and there will be more). Class I comprise the *dnaK* and *groE* operon and are under the negative regulation of the HrcA repressor protein binding to the CIRCE operator. The activity of the HrcA repressor is modulated by the GroE chaperonin system (Mogk *et al*., 1997). This regulatory system is the most widespread within the bacterial kingdom and has been described in more than 40 different species so far (Hecker *et al*., 1996).

Class II genes constitute the sigma-B regulon- with about 100 members, by far the largest group (Hecker and Völker, 1998). The activity of the sigma-B factor is modulated by an anti-sigma factor. A second player is an anti-anti-sigma factor which is present in a phosphorylated form in the absence of stress. Stress and starvation enhance the level of non-phosphorylated anti-anti-sigma factor, which is then able to bind to the anti-sigma factor, resulting in the release of sigma-B.

Class III heat shock genes are controlled by another repressor, the CtsR protein, interacting with the CtsR box. Only the three operons *clpP, clpE* and *clpC* are controlled by this repressor (Derré *et al*., 1999). How the activity of this repressor is modulated after a sudden increase in temperature is unknown. There are some additional heat shock genes not regulated by any of the aforementioned mechanisms, among them: *ftsH, lon, htrA* and *htpG* (Homuth *et al*., 1999). There are hints that the *htpG* gene is under the control by a transcriptional activator (S. Versteeg and W. Schumann).

Two additional negative regulation systems have been reported. One has been identified in *Streptomyces* and consists of the HspR repressor and the HAIR operator. This regulatory system controls the *dnaK* operon and the *clpB* gene in *Streptomyces*, and is present in some additional bacterial species (Bucca *et al*., 1995; Grandvalet *et al*., 1999). The second system seems to be limited to *Bradyrhizobiae*. Here, a negative *cis*-acting element called ROSE has been described (Narberhaus *et al*., 1998). The ROSE element is mainly involved in controlling the regulation of heat shock genes that encode small heat shock proteins (Münchbach *et al*., 1999). The repressor protein interacting with ROSE remains elusive.

The important outcome of the research carried out during the last five years in several laboratories has revealed three important findings: (i) Besides alternative sigma factors, repressor proteins are used to regulate expression of subsets of heat shock genes; (ii) in most bacterial species, the major heat shock genes are distributed among several regulons; (iii) one heat shock operon can be subject to more than one regulation mechanism. Important future directions in the field of heat shock research are the following:

1. To check for additional regulatory systems.

2. To elucidate the mechanisms by which the transcriptional regulators are modulated. Since the heat shock response is always transient, they have to be inactivated shortly after a heat shock and reactivated later to ensure repression of the heat shock genes.

3. In many bacterial species, only the *groE* operons are subject to negative control by the HrcA/CIRCE control system. Here, stress factor(s) different from heat and still unknown, must exist to induce this operon selectively.

4. In bacterial species with more than one heat shock regulon, it must be asked whether there exists a superimposed regulatory network combining all the regulons into one super-regulon. Alternatively, the regulons might act independently of each other, which I regard as relatively unlikely.

References

Bucca, G., Ferina, G., Puglia, A. M., and Smith, C. P., 1995. The dnaK operon of *Streptomyces coelicolor* encodes a novel heat shock protein which binds to the promoter region of the operon *Mol. Microbiol*., 17: 663-674

Derré, I., Rapoport, G., Msadek, T., 1999. CtsR, a novel regulator of stress and heat shock response, controls *clp* and molecular chaperone gene expression in Gram-positive bacteria. *Mol. Microbiol*., 31: 117-131

Grandvalet, C., de Crecy-Lagard, V., and Mazodier, P., 1999. The ClpB ATPase *of Streptomyces albus* G belongs to the HspR heat shock regulon. *Mol.Microbiol*., 31:521-532

Hecker, M., Schumann, W. and Voelker, U., 1996. Heat-shock and general stress response in *Bacillus subtili*s. *Mol. Microbiol*.,19:417-428

Hecker, M., and Voelker, A., 1998. Non-specific, general and multiple stress resistance of growth-restricted *Bacillus subtilis* cells by the expression of the σB regulon. *Mol Microbiol*., 29:1129-1136

Homuth, G., Mogk, A. and Schumann, W., 1999. Post-transcriptional regulation of the *Bacillus subtilis dnaK* operon. *Mol. Microbiol*., 32:1183-1197

Missiakas, D. and Raina, S., 1998. The extracytoplasmic function of sigma factors: role and regulation. *Mol. Microbiol*., 28:1059-1066

Model, P., Jovanovic, G. and Dworkin, J., 1997. The *Escherichia coli* phage-shock-protein (psp) operon. *Mol. Microbiol*., 24:255-261

Mogk, A., Homuth, G., Scholz, C., Kim, L., Schmid, F. X. and Schumann, W., 1997. The GroE chaperonin machine is a major modulator of the CIRCE heat shock regulon of *Bacillus subtilis*. *EMBO J*., 16:4579-4590

Morita, M. T., Tanaka, Y., Kodama, T. S., Kyogoku, Y., Yanagi, H. and Yura, T., 1999, Translational induction of heat shock transcription factor σ32: evidence for a built-in RNA thermosensor. *Genes Dev*., 13:655-665

Münchbach, M., Nocker, A. and Narberhaus, F., 1999. Multiple small heat shock proteins in rhizobia. *J. Bacteriol*., 181:83-90

Narberhaus, F., Kaser, R., Nocker, A. and Hennecke, H., 1998. A novel DNA element that controls bacterial heat shock gene expression. *Mol. Microbiol*., 28:315-323

Yura, T., Nagai, H. and Mori, H., 1993. Regulation of the heat-shock response in bacteria. *Annu. Rev. Microbiol*., 47:321-350

Toward Integration of Bacterial Stress Responses

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Numerous works done during the past decades established the important and ubiquitous functions of heat shock proteins (HSP), including molecular chaperones, ATP-dependent proteases and folding catalysts in protein folding, assembly and repair under normal and stress conditions (Morimoto *et. al.,* 1994; Ellis and van der Vies, 1991; Hendrick and Hartl, 1993). Given the vital importance of HSP in various aspects of biology today, the regulatory mechanisms of the heat shock response have been the subject of great interest. Extensive work with *E. coli*, *B. subtilis* and a number of other bacteria provided basic information not only on the structure and function of HSP genes and their products, but also on some major features of the regulatory mechanisms (Yura *et. al.,* 1993; Gross *et. al.,* 1996; Yura and Nakahigashi, 1999; Narberhaus, 1999).

In bacteria as in eukaryotic organisms, heat shock induces the synthesis of HSP primarily at the level of transcription, reflecting increased cellular demands for HSP at higher temperature. The response is very rapid and transient: maximum induction is attained within several minutes, and gradually declines to reach a new steady-state level. Both positive control mediated by minor sigma factors and negative control mediated by repressors are employed, depending on bacterial species. Although the regulatory mechanism is basically transcriptional, various forms of post-transcriptional regulation play important roles in tightly controlling expression of HSPs to cope with sudden changes in temperature. In the case of *E. coli* studied in detail, the response primarily depends on transient increase in the amount of heat shock sigma factor, sigma32, which can be regulated at all levels. Whereas controls of transcription and translation of *rpoH* encoding sigma32 are important for maintaining steady-state levels of sigma32, controls of stability and activity of sigma32 play major roles in fine adjustment of the sigma32 level to deal with stress-induced unfolded/misfolded proteins (Yura *et. al.,* 1993; Gross *et. al.,* 1996; Yura and Nakahigashi, 1999).

Two distinct types of signalling pathways have been recognized that are of general interest. One is the chaperone (and protease)-mediated autogenous control of the activity or stability of the key regulatory factors such as sigma32 in *E. coli* (Yura *et. al.,* 1993; Gross *et. al.,* 1996; Yura and Nakahigashi, 1999) and HrcA in *B. subtilis* (Mogk *et. al.,* 1997), whose exact mechanisms have yet to be elucidated. The other is direct temperature control of key regulatory factors such as rpoH mRNA and sigma32 itself: temperature-dependent change in the rpoH mRNA secondary structure modulates efficiency of translation (Morita *et. al.,* 1999), whereas that in the conformation of sigma32 apparently modulates susceptibility to proteases that are themselves HSP (Kanemori *et. al.,* 1999). These elaborate mechanisms must be integrated to work cooperatively in adjusting the level and activity of HSP to meet the complex and changing cellular requirements. Further analysis of each of the regulatory circuits, signaling pathways, and the nature of their integration would be an exciting problem for the future. In addition, there are certain events known to be caused by temperature upshift that have escaped people's attention for years; for example, transient and coordinate decrease in the components of translational

apparatus. These events should be analysed to better understand the global picture of the heat shock response.

As to the integration and/or interconnection between the heat shock regulatory pathways and the pathways involved in other stress responses, very little is known despite the extensive work done with each of these responses. Future studies should therefore include systematic analyses of relationships between heat shock and other cellular stresses such as cold shock, oxidative stress, osmotic stress, and general stresses including nutritional starvation. This would be a very demanding but rewarding enterprise, because it would be essential for our eventual understanding of the ability of the bacterial cell to survive and adapt to various stressful environments as well as of the mechanisms of signal sensing and transduction under physiological and pathological conditions. Finally, such efforts should be assisted by genomic information coming from diverse bacteria, because they should certainly help formulating unique models with prokaryotes for similar attempts in future with eukaryotic systems. Finally, such studies would also provide useful information for biotechnological application of bacteria for human welfare.

References

Ellis, R. J., & van der Vies, S. M., 1991. Molecular chaperones. *Annu. Rev. Biochem*., 60, 321-347.

Gross, C. A., 1996. in "*Escherichia coli* and *Salmonella*: cellular and molecular biology", eds. Neidhardt, F. C., Curtiss III, R., Ingraham, J. L., Lin, E. C. C., Low, K. B., Magasanik, B., Reznikoff, W. S., Riley, M., Schaechter, M. & Umbarger, H. E. (ASM Press, Washington, D. C.), pp. 1389-1399.

Hendrick, J. P. & Hartl, F. U., 1993. Molecular chaperone functions of heat-shock proteins. *Annu. Rev. Biochem.* 62, 349-384.

Kanemori, M., Yanagi, H. & Yura, T., 1999, Marked instability of the sigma(32) heat shock transcription factor at high temperature. Implications for heat shock regulation. *J. Biol. Chem.* 274, 22002-22007

Mogk, A., Homuth, G., Scholz, C., Kim, L., Schmid, F. X. and Schumann, W., 1997. The GroE chaperonin machine is a major modulator of the CIRCE heat shock regulon of *Bacillus subtilis*. *EMBO J*., 16:4579-4590

Morimoto, R. I., Tissieres, A. and Georgopoulos, C. (eds.), 1994. *The Biology of Heat Shock Proteins and Molecular Chaperones* (Cold Spring Harbor Lab. Press, NY).

Morita, M. T., Tanaka, Y., Kodama, T. S., Kyogoku, Y., Yanagi, H. and Yura, T., 1999, Translational induction of heat shock transcription factor σ32: evidence for a built-in RNA thermosensor. *Genes Dev*., 13:655-665

Narberhaus, F., 1999. Negative regulation of bacterial heat shock genes. *Mol. Microbiol*. 31, 1-8.

Yura, T. and Nakahigashi, K., 1999, Regulation of the heat-shock response. *Curr. Opin. Microbiol.* 2, 159-165.

Yura, T., Nagai, H. and Mori, H., 1993. Regulation of the heat-shock response in bacteria. *Annu. Rev. Microbiol*., 47:321-350

Components and Cellular Mechanisms of Adaptation to Biological Water Stress

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Water is essential for the growth and survival of all forms of life, and its inadequate availability for the purpose is referred to as biological water stress. Water stress may be imposed in a variety of ways: some of these are obvious e.g., water deprivation (including drought) or excessive water loss (including desiccation, diarrhea or diabetes insipidous); and others less so e.g., osmotic effects consequent to the presence of dissolved solutes or to the formation of ice in the extracellular milieu (Yancey *et. al.*, 1982; Le Rudulier *et. al.*, 1984).

The mechanisms of adaptation to water stress may broadly be classified, for operational reasons, into cellular and supracellular mechanisms. Supracellular mechanisms act at the level of the whole multicellular organism and are designed to either increase water intake or reduce water loss; they include (for example) the thirst response or the antidiuretic hormone response in animals and leaf stomatal closure in plants. Cellular mechanisms of adaptation to water stress, on the other hand, act at the level of the individual cells: both in the free-living unicellular micro-organisms such as bacteria, yeasts, fungi, algae etc. (Csonka, 1995; Csonska and Hanson, 1991; Potts, 1994); and in cells of the tissues of plants and animals, particularly aquatic animals, (Yancey *et. al.*, 1982; Le Rudulier *et al.*, 1984).

The cellular mechanisms for adaptation to water stress appear to be remarkably similar in the diverse biological kingdoms (with the exception of the Archaea). A description of the cellular mechanisms of adaptation to biological water stress constitutes the subject matter of this article. It has been suggested that their apparent conservation across the spectrum of living things reflects a process of convergent evolution (that is, of having arisen on multiple independent occasions in different lineages during the course of biological evolution) rather than one of evolutionary selection for an essential ancient ancestral trait (Yancey *et. al.*, 1982).

In both the experimental situation as well as the real world, the mechanism of inhibition of growth of biota by water stress imposed by a dissolved solute such as NaCl can be subdivided into two components (Greenway and Munns, 1980; Wyn Jones, 1984). The first component is variously referred to as salinity stress or ionic stress or ion excess, and is related to the toxicity of the particular ionic species (eg., sodium or chloride stress) to the organism. Ionic stress is therefore specific to the type of chemical species of ions in solution. Tolerance to the stress of toxic ions is often achieved by processes that exclude the ions from the cytoplasmic compartment. The exclusion mechanisms might include a plasma membrane that intrinsically exhibits low permeability to the ionic species in question, and active transport systems that pump the ions out of the cytoplasm into either the extracellular fluid or a vacuolar compartment.

The second component of stress imposed by NaCl is associated with the fact that NaCl is an impermeable solute and therefore exerts an osmotic effect which draws water out of the cytoplasm; for this reason, the second component is also variously referred to as osmotic stress or turgor stress or water deficit. Because this component of NaCl stress is an osmotic effect, it is elicited to an equivalent extent by any other impermeable ionic or nonionic solute such as K2SO4, sucrose, raffinose, mannitol, polyethylene glycol etc. Growth inhibition associated with the second component is caused by the reduction in cytoplasmic volume and the loss of cell turgor (plasmolysis) consequent to the osmotic outflow of intracellular water (Yancey *et. al.,* 1982; Le Rudulier *et. al.,* 1984; Csonka, 1995; Csonska and Hanson, 1991).

The cellular mechanism of adaptation to the osmotic stress component of biological water stress is one that is shared, at least in principle, across most life forms. This shared principle for adaptation is directed towards the restoration of intracellular volume and cell turgor by increasing the content of impermeable dissolved solutes within the cells so as to match the high extracellular osmolarity. Consequently, there occurs an osmotic re-inflow of water into the cells (Yancey *et. al.,* 1982; Le Rudulier *et. al.,* 1984; Csonka, 1995; Csonska and Hanson, 1991; Potts, 1994; Greenway and Munns, 1980; Wyn Jones, 1984). Since the external osmotic load may be 0.7 M NaCl (approximately 1.2 Osm) or even higher, one is speaking of cytoplasmic solute accumulation to concentrations of several hundreds of millimolar in order to achieve osmotic balance across the plasma membrane. Most substances would themselves exert toxic effects on cellular metabolism at such high concentrations, and only a very small set of compounds would be expected to be inert under these conditions. It has therefore been argued that the mechanisms now witnessed in extant organisms are the results of striking convergent evolution, whereby a limited set of solutions to the problem have been selected for, on multiple occasions and in phylogenetically diverse organisms (Yancey *et. al.,* 1982). The substances that have been shown to accumulate within cells subjected to osmotic stress are all small-molecular-weight organic compounds and belong to the categories of polyols, amino acids and their quaternary amine derivatives (Yancey *et. al.,* 1982; Le Rudulier *et. al.,* 1984; Csonka, 1995; Csonska and Hanson, 1991). They have also been referred to as 'compatible solutes', for the reason that they are compatible with essential biochemical reactions even at high concentrations. Several eubacteria also accumulate K+ as an immediate response to osmotic stress (Csonka, 1995; Csonska and Hanson, 1991), but in the strict sense, K+ is not a compatible solute.

It has been stated that the selective advantages associated with the categories of compatible solutes are, first, a compatibility with macromolecular structure and function at high or variable osmolyte concentrations, and second, greatly reduced needs for modifying proteins to function in the concentrated intracellular solutions (Yancey *et. al.,* 1982). The compatible solutes are kosmotropes (water structure builders) that are excluded from the immediate vicinity of protein molecules, so that the latter are preferentially hydrated and retain biological activity despite the elevated osmolarity of the cytoplasm (Potts, 1994). Examples of compatible solutes of the different categories encountered in various organisms include the following (Yancey *et. al.,* 1982):

(i) Polyols : glycerol (in yeasts and algae where the plasma membrane is impermeable to this solute); glucosylglycerol; arabitol; mannitol; sorbitol; trehalose.

(ii) Amino acids and derivatives: glutamate, proline, g-amino butyric acid, glycine betaine, ectoine.

(iii) Combination of urea with methylamines such as trimethylamine-N-oxide.

The cytoplasmic accumulation of compatible solutes may occur following increased biosynthesis, increased uptake from the extracellular medium, or both. In many cases, the molecular processes involved in such accumulation have been reasonably well characterized, as has the osmotic regulation of these processes (Le Rudulier *et. al.*, 1984; Csonka, 1995; Csonska and Hanson, 1991). Even in mammals (including humans), where, as mentioned above, supracellular homeostatic mechanisms exist that strive to maintain the constancy of osmolarity of the internal milieu, the renal medulla represents a unique high-osmolarity niche, which has been shown to accumulate the compatible solutes sorbitol, glucosylglycerol and glycine betaine in order to compensate for the high osmolarity of urine and the extracellular fluids in this tissue (Uchida *et. al.*, 1989; Garcia-Perez and Burg, 1991).

The experimental distinction between the two components of water stress discussed above, salinity stress and osmotic stress, has been based on the test whether a particular effect or response elicited by NaCl is specific to this salt (salinity stress-effect) or is also elicited by other ionic and nonionic impermeable solutes (osmotic stress-effect). With the aid of such a distinction, the existence of distinct salinity stress effects and osmotic stress effects has been established in a variety of systems including plants, yeasts and the Gram-positive and Gram-negative bacteria (Uma Prasad and Gowrishankar, 1998, and refs. cited therein).

In studies on osmotic stress, several researchers have also employed what may be termed as an inclusive-exclusive definition in its delineation. That is, in addition to demonstrating that a particular osmotic stress-effect is elicited by a variety of impermeable solutes (the "inclusive" definition), these workers have also determined that the effect is not elicited by permeable solutes such as glycerol, urea, ethanol or ethylene glycol, which are not expected to draw water out of the cytoplasm (the "exclusive" definition). Examples of organisms where such an inclusive-exclusive definition has been employed to identify osmotic stress-effects include plants, mammals and the Gram-positive and Gram-negative bacteria (UmaPrasad and Gowrishankar, 1998, and refs. cited therein).

Recently, while testing particular water stress-effects in *Escherichia coli* by the criteria of the inclusive-exclusive definition above, we have identified certain phenomena which we believe indicate the existence of a third and novel component of water stress (UmaPrasad and Gowrishankar, 1998). We have coined the term anhydrotic stress for this third component. An anhydrotic stress phenomenon is one that is elicited to an equivalent extent by all varieties of solutes, ionic as well as nonionic, impermeable as well as freely permeable. The fact that it is elicited also by freely permeable solutes such as glycerol and ethylene glycol distinguishes anhydrotic stress from the other two components of water stress. Our hypothesis is that anhydrotic stress reflects that component of growth inhibition caused by the reduction in cytoplasmic water activity *per se*, and which is not expected to be altered by accumulation of the compatible solutes discussed above.

We have shown that, in *E. coli*, conditions that lead to an increased cytoplasmic concentration of L-ornithine are correlated with an increased sensitivity to anhydrotic stress, but the underlying mechanism remains to be identified (UmaPrasad and

Gowrishankar, 1998). Preliminary data indicate that an association between L-ornithine and anhydrotic stress sensitivity exists in other Gram-negative and Gram-positive bacteria. In other unpublished work, we have also identified new transposon insertion *E. coli* mutants that are sensitive to anhydrotic stress (unrelated to the L-ornithine effects described above), and their characterization is expected to throw additional light on the molecular mechanisms of cellular adaptation to anhydrotic stress in this organism.

In conclusion, it is reasonably clear that diverse organisms experience similar kinds of perturbations when exposed to environments of low water activity and that they respond by somewhat common mechanisms to at least two of the components of water stress, namely ionic stress and osmotic stress. Evidence has also been obtained recently for the existence of a third component of water stress, designated anhydrotic stress, but additional research is required to determine its universality as well as the mechanisms of adaptation thereto.

References

Csonka, L. N., 1989. Physiological and genetic responses of bacteria to osmotic stress. *Microbiol. Rev.*, 53: 121-147.

Csonka, L. N., and Hanson, A. D., 1991. Prokaryotic osmoregulation: genetics and physiology. *Annu. Rev. Microbiol*., 45: 569-606.

Garcia-Perez, A., and Burg, M. B., 1991. Renal medullary organic osmolytes. *Physiol. Rev*., 71: 1081-1115.

Greenway H., and Munns, R., 1980. Mechanisms of salt tolerance in nonhalophytes. *Annu. Rev. Plant Physiol*., 31: 149-190.

Le Rudulier, D., Strom, A. R., Dandekar, A. M., Smith, L. T., and Valentine, R. C., 1984. Molecular biology of osmoregulation. *Science*, 224: 1064-1068.

Potts, M., 1994. Desiccation tolerance of prokaryotes. *Microbiol. Rev*., 58: 755-805.

Uchida, S., Garcia-Perez, A., Murphy, H., and Burg, M. B., 1989. Signal for induction of aldose reductase in renal medullary cells by high external NaCl. *Am. J. Physiol*., 256: C614-C620.

UmaPrasad, G., and Gowrishankar, J., 1998. Evidence for the existence of a novel component of biological water stress (anhydrotic stress) in Escherichia coli. *J. Genetics*, 77: 1-11.

Wyn Jones, R.G., 1984. Phytochemical aspects of osmotic adaptation. *Recent Adv. Phytochem*., 18: 55-78.

Yancey, P. H., Clark, M. E., Hand, S. C., Bowlus, R. D., and Somero G. N., 1982. Living with water stress: evolution of osmolyte systems. *Science*, 217: 1214-1222.

Production of Stress-Tolerant Transgenic Plants

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The improvement of techniques involved in making transgenic plants constitutes one of the major developments which have taken place in plant science. The earliest transgenic plants produced were mostly those showing resistance to antibiotics or expressing reporter genes. In the subsequent years, transgenic plants showing enhanced resistance to herbicides, virus infestations, fungal diseases, insect attacks, and, for better genetic quality of the produce, altered levels of hormones or increased levels of secondary metabolites have been engineered. These developments have ushered plant molecular biology and biotechnology research into a highly exciting phase with respect not only to the fundamental science but also from the point of view of commercial applications. In spite of these developments, it is still a major challenge to genetically engineer crop plants showing improved performance against abiotic stresses. The term abiotic stress refers to factors such as sub- and supra-optimal temperatures, excess salt (primarily NaCl) levels, reduced water availability leading to dehydration stress, excess water resulting in flooding stress (which is associated with reduced oxygen supply leading to anaerobic stress as well) and oxidative stress (caused by low temperature stress, water stress, light stress, chemical stress etc.). Abiotic stresses adversely affect almost all major field-grown plants belonging to varied ecosystems. The severity of abiotic stresses is on the rise due to the practice of intensive cultivation in farming areas as well as due to environmental deterioration caused by the greenhouse effect. These stresses cause a great amount of loss, both in terms of biomass as well as economic returns, and the extent of this loss depends on the crop species, its location, growth stage and the intensity of the stress. A considerable proportion of the potential biomass of the crops remains untapped due to such stresses.

Transgenic plants tolerant to abiotic stresses

Transgenic plants showing tolerance to salt stress, water stress, oxidative stress, low temperature stress and high temperature stress have been produced by various means in the past 5 years. Tolerance to abiotic stresses has so far mainly been achieved through engineering for increased cellular levels of osmotically-active solutes (such as proline, glycinebetaine, mannitol, trehalose, fructans, etc.). Another noteworthy point is that increased levels of osmolytes have often enhanced tolerance for water stress, salt stress and cold stress at the same time, implying that genetic engineering by altering osmolytes is a fruitful approach for obtaining combined tolerance to different abiotic stresses. However, stress tolerance may not only be accounted for by osmotic adjustment. Importantly, genes encoding for antifreeze proteins (AFPs), unsaturase enzyme and superoxide dismutase (SOD) protein have proven useful in engineering tolerance to abiotic stresses.

It is well established that tolerance to abiotic stresses is mediated by a number of biochemical reactions/ physiological processes, which essentially means that it is a multi-genic trait. This is evidenced by the fact that: (a) more than one hundred transcripts are altered upon subjecting cells to salt stress, and (b) a large number of proteins are co-altered in response to different abiotic stresses. As number of cellular processes together appear to determine high-level tolerance to abiotic stresses, it is possible that stress tolerance through

single gene transfers obtained thus far can be augmented by pyramiding different stress-responsive genes. For the gene pyramiding to work in real terms, following inputs need to be critically investigated: (a) Are there vectors available which can carry large-sized DNA fragments containing more than one gene? (b) How much load of "foreign sequences" can be introduced in a given host system? (c) Are there sufficient numbers of promoters available to drive different transgenes in the same host, so as to avoid homology-based recombination and gene silencing? As answers to these queries are not yet satisfactorily obtained, more refinements in the tools and techniques of plant genetic engineering are needed for achieving gene pyramiding at the routine level. We must further admit that even if the new vectors and promoters are generated with specificities as intended above and the restrictions on the "load" of foreign sequences which can be transferred are overcome, how are we going to obtain the target genes for stress tolerance to be pyramided? The identification and isolation of stress-responsive genes thus emerges as the central theme in plant-stress studies.

Isolation of stress-responsive genes

In general, the obstructive step in developing modified plants through biotechnological means is the isolation of the relevant genes. Gene isolation and cloning through molecular biology research can be based on mRNA or protein expression, differential screening, differential display technique, DNA insertions such as transposon or T-DNA insertions, map-based cloning and methods of random cDNA sequencing and genome sequencing. Differential hybridisation technique has been exploited for isolation of a number of stress-responsive genes. A large number of genes, including those involved in stress responses, have recently been identified by random nucleotide sequencing of cDNA clones in rice and other plant species. There has been a great upsurge in genome technology in the recent years as evidenced by the rapid progress being made in the human genome project and in similar projects for several other organisms. In plants, efforts are being made to sequence the complete genomes of *A. thaliana* and rice. Making gene sequences available in this way will hopefully bring a major change in the isolation and characterization of new stress- responsive genes. Considerable progress has been made in mapping and tagging many agriculturally important genes with molecular markers. Construction of yeast artificial chromosome (YAC) and bacterial artificial chromosome (BAC) libraries for a number of plant species (*A. thaliana*, rice etc.) is a significant development in this regard.

Concomitant to induced stress tolerance, the protein metabolism of the cells undergoes pronounced changes in terms of acquiring specific stress proteins which are either not detected, or else present in low amounts, in un-induced cells. Excellent progress has been made towards understanding the structure, function and regulation of stress proteins which are expressed in response to heat shock, salt stress, water stress and anaerobic stress. The analysis of stress proteins and the corresponding genes has provided a wealth of literature on novel genes involved in plant-stress interactions.

Engineering the cascade of multiple genetic changes through single gene transfers

The above discussion has largely been on genes which have a role in imparting stress tolerance through their expression leading to physiological/biochemical action. It is obvious that for the activation of such genes, a distinctive set of transcription factor genes must be involved. The regulatory machinery involving transcription factors has emerged as

a new focal point for controlling expression of stress-responsive genes. Thus, by changing the expression of the transcription factor genes, it should be possible to alter levels of several target genes at the same time. For this approach to be applied extensively, there is a need to identify, clone and characterise more stress-responsive transcription factor genes. There is another level in the hierarchy of genetic control which may have important bearing in regulating stress responses. This level of control represents components of signal transduction. Physiological, genetic and biochemical approaches have yielded a great deal of information about several signal transduction pathways in plants. The thrust so far has been placed on identifying the biochemical nature of the components of photo-signal transduction and cloning of their genes. These studies have provided evidence for participation of G-proteins, cAMP, cGMP, calcium/ calmodulin, inositol phospholipids and kinases/ phosphatases in this regard. There are limited studies on signal transduction mechanisms in plants and abiotic stresses interactions.

Synthesis and perspective

Abiotic stresses are serious threats to sustainable food production. Notably, genetically improved tolerance to abiotic stress must prove to be a stable inheritable trait, unlike tolerance against biotic stress (as caused by fungal, bacterial or insect pathogens), where the tolerance breaks with the evolution of the pathogen. It is a challenge to plant genetic engineers to generate crop plants which can stand, reproduce and set seeds at least milder levels of abiotic stress, if not at extremes. There are more than a dozen recent reports in which increased level of resistance to abiotic stress has been achieved. The concern now is to consolidate these advancements in different crops and make further in-roads in raising the genetic level of stress tolerance.

The *Poeciliopsis* Heat Shock Model: The Integrative Strategies Behind the Data

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Herein, I have attempted an integrative approach to the discussion of future research directions in the field of Stress Biology and the programmatic emphasis of the IUBS on Integrative Biology. I will use as a case study one of the first units organized to develop integrative approaches in the cellular stress response field, The Marine/Freshwater Biomedical Sciences Center, established at the University of Connecticut in 1987 for the study of molecular, cellular and organismal responses to environmental stress. The name of the center derived from the program at the U.S. National Institute of Environmental Health Sciences (NIEHS) that provided initial funding, a program originally developed to take advantage of exaggerated physiologies of marine animals with relevance to human disease. The potential value of using multidisciplinary approaches in biological research is being recognized, and for our field in particular, a workshop sponsored by the U.S. National Science Foundation was held in 1996 with the theme "Molecular, functional, and evolutionary approaches to stress and inducible stress responses: bridging the gaps." Several of us gathered around this table (Feder, Hightower, and Morimoto) were participants at the NSF workshop as well. The case study that follows addresses several issues raised in Recommendation 4 of the workshop report: "Educate the community of prospective multidisciplinary scientists about the philosophy, attitute, and practice of multidisciplinary research. This should occur at every level of instruction. Some critical elements in implementing this education are the availability of funding for postdoctoral [fellows] and midcareer training/retraining. A crucial but unresolved issue is how to enhance cooperation of diverse administrative units both within and among academic institutions; analysis of this issue should receive priority." (Feder and Berenbaum 1996).

Integrative research incorporates different levels of biological organization and thus crosses one or more boundaries of biological organisation. Many biological disciplines remain narrowly defined and nested in one level of biological order; and therefore, integrative research is almost always multidisciplinary. The boundaries between disciplines continue to diminish in large part due to the broad application of molecular biological methods and the broad use of evolutionary theory as an organizing framework. For several closely related disciplines, it is difficult to walk into a laboratory and decide whether it belongs to a cell biologist, molecular geneticist, biochemist or biophysicist. New words have been coined to celebrate our new-found freedom, such as functional genomics and physiological genomics. Of course, there are still philosophical barriers and misunderstandings that derive from a low frequency of meaningful conversations among colleagues in different disciplines. An example that comes to mind is an amusing exchange that occurred between an ecologist and a molecular biologist during the NSF multidisciplinary workshop. The ecologist volunteered that a major difference between ecological research and molecular biology is that the former is hypothesis driven whereas the latter is problem solving. This came as a great shock to the molecular biologist who had been under the impression for at least several years that failure to state a testable

hypothesis in a grant proposal was a “kiss of death.” Another example will serve to launch the discussion of the *Poeciliopsis* model.

R. Jack Schultz, a member of the Ecology and Evolutionary Biology Department, had studied tropical and desert fish in the genus *Poeciliopsis* for 25 years from many different perspectives including thermal stress. Even though we were located in the same building, it took 12 years for us to have the conversation that I am about to relate. What finally brought us into the same room was the prospect of additional research funding. During an initial roundtable discussion among potential participants in the NIEHS proposal, Jack heard me describe our work on heat shock proteins (Hsp) and thermotolerance. Several days later, he was standing in my lab asking questions: "What is the most exciting thing about heat shock genes?" I responded with my best material, "Heat shock genes are among the most highly conserved genes known and they are involved in acquired or inducible thermotolerance". He responded, "Are you telling me that there is no diversity among hsp70 genes in a species? Because if you are, I am telling you that they have little to do with thermotolerance in natural populations." He explained that he had already found considerable genetic variation among individuals of even the same species in their ability to survive heat shock and that a reasonable hypothesis would be that the genes involved in acquisition of thermotolerance ought to contain variation that affects protein function. The next question was "Had anyone bothered to look?". The answer that emerged after some thought was no, not in a way that would have tested the hypothesis. That is, almost everyone was working with either a few cultured cell lines or inbred organisms. We found only one published study indicating that some mammalian and avian species have different Hsp70 isoforms (Anderson *et. al.,* 1982).

Jack provided a thirty year reservoir of information on the ecology and genetics of these fish into which I dipped frequently. We agreed upon a basic strategy employing Jack's fish colony. We would first compare Hsp patterns of closely related *Poeciliopsis* species from the Sonora Desert of Northwestern Mexico. Jack had developed inbred strains of about a half dozen of these species. Then we would search for within species diversity, taking advantage of a peculiarity of Poeciliopsis gene transmission known as hybridogenesis. An example of this is a mating between a wild *P. monacha* female and an inbred *P. lucida* male. This results in fertile, intraspecific hybrid fish called *P. monacha-lucida*. During oogenesis in a hybrid female, the entire paternal genome is discarded during the mitotic cell divisions preceding meiosis, so the paternal genome is not present to undergo reassortment or recombination with the maternal genome. The result is that natural combinations of alleles are preserved in the monacha genomes, which are passed along hemiclonally. When a standardized *P. lucida* genome is added back upon mating, the result is a clonal vertebrate. Due to a quirk of sex determination, the entire brood of hybrid fish is female. Thus, wild monacha genomes can be captured from a natural population and amplified clonally in the laboratory. Phenotypic difference among different hybrid fish can be ascribed to genetic differences in the monacha genomes. We further decided to search for protein polymorphisms, rather than attempting to clone and sequence all members of the Poecilopsis Hsp70 family. The use of high resolution two-dimensional polyacrylamide electrophoresis of proteins denatured with sodium dodecyl sulfate and mercaptoethanol would allow us to follow several protein families at once. Based on studies of other organisms, we assumed that the two heat shock protein families most frequently linked to acquired thermotolerance, Hsp70 and Hsp27-30, would be encoded in multigene families in Poeciliopsis. We surveyed the heat shock protein patterns of six

desert species using radioisotopic labeling of intact fish and primary liver cell cultures and silver staining of two dimensional gels (White *et al.,* 1994). Later, we benefited from the collecting skills of several colleagues who managed to obtain a permit from the Mexican government, and we added two tropical species of *Poeciliopsis* from southern Mexico to our survey (Norris *et al.,* 1995).

There were a large number of Hsp27-30. Every species had a unique isoform pattern and within *P. monacha*, there were several different patterns (Norris *et al.,* 1997). We also found polymorphisms in stress inducible Hsp70 but not constitutive Hsc70 and Grp78 both between and within species, in what is still the most comprehensive published survey of diversity in Hsps. No strategy is without its complications, and for this one, additional experiments were needed to rule out posttranslational modifications as a source of isoforms. Using short radioisotopic pulse-chase protocols, we were able to rule out phosphorylation and processing of precursors into products. We also did Southern blots using a probe specific for inducible Poeciliopsis Hsp70, and these results were consistent with the existence of a small multigene family. Obviously this is not as direct as analysis of nucleotide sequence data for this gene family, and this was the major compromise that we made. Even today with PCR cloning and more rapid sequencing, it is still not easy, outside of major sequencing projects to obtain complete sequence information on all members of a multigene family.

During the pre-funding phase of this project, which lasted almost two years, some projects were dropped and some investigators left the project. The core that persisted were those who found areas of common interest and who were able to form working relationships. Essential to this process is the willingness to be both student and teacher and the desire to think outside of the ‘box’ of one’s main discipline. In this era, many investigators will not take this risk out of concern that their colleagues and their granting agency will view them as having lost ‘focus.’ Layered on this tendency has been a further narrowing of biological investigation by emphasis upon only a small number of model organisms. The genome projects have exacerbated this tendency to the point where many molecular biologists will only work on the 'chosen organisms.' This is likely to be but a temporary constriction point in biological research. New methods of DNA sequencing are under development which, if successful, will allow any genome of interest to be sequenced rapidly and economically (Alper 1999). But for the present, it is still usually the case that one must chose organisms that have either a deeply understood organismal biology or a well-developed molecular biology. These prevailing conditions and attitudes are barriers to integrative research.

As in virtually all university-based research programs, graduate students and postdoctoral fellows played essential roles. Postdoctoral fellows were recruited to add expertise missing from the core group. We tended to attract fellows who were interested in broadening themselves through exposure to the multidisciplinary environment of the Center. We shared graduate students by participating in their thesis committees and by allowing them to move freely among labs in carrying out their experiments. They became the essential glue that held collaborative projects together on a daily basis. The structure of the graduate school at the University of Connecticut substantially aided us in sharing students. Here, students are accepted into the graduate school, not directly into departments. They are guided by advisory committees whose members can be drawn from different departments, and even schools, within the university. I have viewed this structure

at various times as both inspired and insane, but it certainly worked to our advantage in the Center, since there were no departmental boundaries with which to contend. Numerous undergraduates seeking research experiences were also attracted to the project and several completed senior honors theses. Our students were exposed to an eclectic mix that included protein biochemistry, evolutionary biology, fish physiology and genetics. It was an exciting time.

In a sense, heat shock genes and proteins were at the crossroads of the various research projects. In fact, I had approached the prospect of collaborating with the attitude that these genes were evolutionarily so highly conserved that any system under study in the Center would have a heat shock response that my lab was prepared to study at the molecular level. Our reward would be the opportunity to place this response in the context of tissue physiology and intact animals. We often hear integrative research promoted as a way to find new and unexpected information at the interface of disciplines. I will not detail all of the small advances, but I think that there were at least three findings that fulfilled this expectation. In collaboration with J. Larry Renfro, we showed that cytoprotection of transport systems of flounder kidney epithelium, induced *in vitro* by a sublethal pre-stress, was due to an increased transport capacity (Brown *et. al.*, 1992). When the lethal stress was applied, as much damage occurred to transporters in cytoprotected cells as in unprotected controls, but the extra capacity provided a buffer that kept the cytoprotected cells transporting at normal levels. The molecular aspect of the study was to show that flounder heat shock proteins accumulated in cytoprotected tissue. Few studies of cytoprotection have been carried out using tissue level function as an indicator. Previously, hypotheses for thermotolerance and other forms of cytoprotection invoked protection of cellular components from damage, so here the integrative approach yielded an entirely new concept.

Second, the demonstration of polymorphisms in the Hsp70 family, even within a population of the same species, was novel and has encouraged searches for diversity in other organisms. In the context of desert fish, we learned that Hsp70 polymorphisms probably had not arisen in the Sonora Desert. This is a relatively lush subtropical desert with a variety of distinct ecological niches. Our original hypothesis had been that processes of mutation and selection had occurred after ancestral fish colonized the desert streams as part of adaptation to different environments. The finding of all of the major Hsp70 desert isoforms in two species of tropical fish changed our thinking. Now, our hypothesis is that ancestral fishes, similar to modern tropical *P. gracilis*, were pre-adapted to high temperatures in the tropics and had Hsp70 polymorphisms prior to migrating north along the western coast of Central America to eventually reach the desert. This hypothesis fits with the biogeography of these fishes. Was this genetic diversity useful in adaptation to desert life? We do not know yet. As a first step, we asked whether or not Hsp70 levels induced in gill tissue by pre-heating correlated with amounts of acquired thermotolerance in individuals from a natural population of *P. gracilis*. Our use of two-dimensional gels allowed us to identify the Hsp70 isoform pattern of each fish in the study. We found that fish carrying isoform 3, the most frequently encountered Hsp70 major isoform in desert species, showed a significant positive correlation between levels of Hsp70 and thermotolerance, whereas fish without isoform 3 showed no significant correlation (Hightower *et. al.*, 1999). The literature contains numerous examples in different organisms of correlations between Hsp70 levels and acquired thermotolerance and also examples where no correlation was found.

For the third novel finding, our study reproduced both of these observations in one population of individuals of the same species. Is there something special about isoform 3? This remains for future work.

Even though the value of integrative approaches is being recognized, the tasks of assembling a group of investigators across departmental and sometimes institutional lines and of finding a receptive funding agency with which to work remain daunting. The NSF workshop report provides several suggestions for lowering these barriers, and this report should be made more broadly available on the web. And what about future directions for stress research in general? Using our experiences as a case study, we think integrative research deserves a place at the table. The prevailing views of the heat shock response and acquired thermotolerance are still based on a very narrow sampling of organisms, primarily inbred laboratory strains and dedifferentiated cultured cell lines. The latter materials should be supplemented with studies of natural populations as well as tissue level studies of differentiated function as an intermediate step in connecting the molecular biology of heat shock genes and proteins to responses in animals and plants. This will be particularly important to a more complete understanding of inducible states of protection. The evolutionarily conserved nature of several heat shock gene families has encouraged the attitude that this is a research area in which we can safely concentrate on a few model organisms and find essentially everything important. This notion underestimates the power of the natural forces that drive genetic diversification and adaptation.

References

Alper, J., 1999. From the bioweapons trenches, new tools for battling microbes: meeting report, Biotechnology Industry Organization. *Science*, 284: 1754-1755

Anderson, N. L., Giometti, C. S., Gemmell, M. A., Nance, S. L. and Anderson, N. G., 1982. A two-dimensional electrophoretic analysis of the heat-shock-induced proteins of human cells. *Clin. Chem.*, 28: 1084-1092

Brown, M., Upender, R., Hightower, L. and Renfro, J., 1992. Thermoprotection of a functional epithelium: heat stress effects on transepithelial transport by flounder renal tubule in primary monolayer culture. *Proc. Natl. Acad. Sci. USA*, 89: 3246-3250

Feder, M.E. and Berenbaum, M., 1996. Report of a workshop on multidisciplinarity in biological research and education. *National Science Foundation*

Hightower, L.E., Norris, C. E., di Iorio, P. J. and Fielding, E. 1999. Heat shock responses of closely related species of tropical and desert fish. *Am. Zool.*, in press

Norris, C. E., Brown, M.A., Hickey, E., Weber, L. A. and Hightower, L. E., 1997, Low-molecular-weight heat shock proteins in a desert fish (*Poeciliopsis lucida*): homologs of human Hsp27 and *Xenopus* Hsp30. *Mol. Biol. Evol.*, 14: 1050-1061

Norris, C. E., diIorio, P. J., Schultz, R. J. and Hightower, L. E., 1995, Variation in heat shock proteins within tropical and desert species of Poeciliid fishes. *Mol. Biol. Evol.*, 12: 1048-1062

White, C. N., Hightower, L. E. and Schultz, R. J., 1994, Variation in heat-shock proteins among species of desert fishes (Poeciliidae, Poeciliopsis). *Mol. Biol. Evol.*, 11: 106-119

Ecological and Evolutionary Functional Genomics of Molecular Chaperones

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To date, the major focus of research on molecular chaperones and Hsps has been on the central tendencies or generalities of their structure, function, regulation, encoding genes, and role in health and disease, as elucidated by laboratory study of the major model organisms (*E. coli*, yeast, Drosophila, mammalian cells in culture, etc.), or purified molecular chaperones in vitro. Interest in these topics has spawned a massive community of investigators and a correspondingly large body of discovery (now including more than 15,000 references). The clinical implications of this information have long been obvious and are now in the process of being realized in discrete therapeutic regimes. Here, however, I emphasize an alternative but complementary focus on how and why molecular chaperones and Hsps differ among diverse tissues, organs, individual organisms and higher taxa undergoing ecologically and evolutionarily relevant stresses. This focus has long received some attention, but it is now achieving critical mass due to the joint efforts of molecular chaperone investigators and researchers from outside this community.

Natural HSP-Inducing Stress

Because so much of the research on Hsps has been undertaken in the laboratory, the question arises as to whether and how frequently organisms in nature undergo Hsp-inducing stress. Whether organisms undergo such stress in the wild is no longer equivocal, having been demonstrated many times in diverse species and natural environments. Indeed, numerous investigators and commercial concerns have begun to exploit this feature to assess biological and anthropogenic stress. How frequently wild organisms undergo Hsp-inducing stress, by contrast, is largely unknown for several reasons. We do not know if natural exposure to stress and consequent gene expression is routine, frequent, or rare; nor whether the studied species are typical or deviant in their exposure to stress and gene expression. Such knowledge is needed, because it is fundamental in evaluating the effects of stress and their significance. Moreover, it requires a speciomic approach (i.e., a comprehensive survey of species or species sampling regime that is unbiased with respect to the variable under study, likelihood of stress).

Variation along Microgeographic, Geographic and Climatic Stress Gradients

If organisms in nature undergo variation in stress along gradients of latitude, altitude, season, rainfall, competitive intensity, etc., then a potential outcome is that such organisms may demonstrate corresponding differences in their stress-induced expression of molecular chaperones. Expression could vary in at least five non-exclusive ways, in the:

(a) magnitude of expression, with organisms from a high-stress environment expressing more Hsps than organisms from low-stress environments;

(b) threshold for expression, with organisms from a low-stress environment expressing Hsps at lower levels of stress than organisms from high stress environments;

(c) breadth of expression, with organisms from variable-stress environments expressing Hsps over a broader range of stresses than organisms from environments with constant levels of stress;

(d) kinetics of expression, with organisms from a high-stress environment expressing Hsps more rapidly and recovering more rapidly than organisms from a low-stress environment;

(e) efficacy of function, with the Hsps of organisms from high-stress environments being more effective chaperones than equimolar amounts of Hsps of organisms from low-stress environments, all else equal.

At present, (a), (b), and (c) are becoming increasingly well established (so much so that little additional work may be needed), but (d) and (e) have received little scrutiny. Also, systematic studies of Hsp expression and underlying stress across a species' ranges are lacking, as are rigorous comparisons of the stress response in widespread and narrowly distributed species. The prospect of global climate change further increases the importance of such studies.

Given that patterns of Hsp expression vary among species and along environmental gradients, a related issue is HOW evolution has engineered these changes; i.e., what genes have been modified to result in differing Hsp expression, and in what ways? Laboratory studies of Hsp expression and its regulation have elucidated a host of candidate mechanisms that could be modified: the coding regions of the hsp genes themselves, non-coding regions (promoter; 3'- and 5'-untranslated regions, which affect message stability), hsp gene copy number, trans-acting factors (e.g., heat-shock factors, heat-shock binding protein), and the stability of proteins whose denaturation triggers the stress response, among others. Ecological and evolutionary variation in these mechanisms is just beginning to receive scrutiny.

From Heat Shock Proteins to Fitness

While heat-shock proteins are often invoked as contributing to evolutionary fitness by enhancing stress tolerance and/or as putative adaptations, rigorous examinations of these claims are surprisingly few. A first step in establishing adaptation is demonstrating a direct cause-and-effect relationship between an individual Hsp or chaperone machine and enhanced stress tolerance. Our still-growing ability to manipulate Hsps genetically has now unequivocally established this link in numerous instances.

Although recent work clearly establishes that Hsps have diverse protective or restorative effects in tissues, organs, and whole organisms, how these effects arise is remarkably poorly understood. Presumably, stress-intolerant organisms have some especially vulnerable protein(s) or other cellular component(s) that fail under stress, and Hsps confer inducible stress tolerance by somehow repairing this damage or preventing it from occurring through their function as molecular chaperones. For example, recent work has implicated the digestive organs and development of Drosophila and the photosynthetic apparatus of plants as especially vulnerable targets with correspondingly distinctive patterns of Hsp expression, but the exact identity of their most vulnerable molecules (presumably proteins) remains to be discovered. Many mammalian diseases are thought to

be due to defects in chaperoning of essential proteins, but comparable explanations of Hsp-mediated protection of key structures under stress remain a goal for the future.

Increased expression of Hsps, however, is not uniformly beneficial. Increasing numbers of studies report deleterious effects of Hsps, attributable to at least two potential mechanisms. First, expression of Hsps, which can be massive, may consume so much biosynthetic substrate and occupy so much of the protein expression apparatus that not enough remains for other important biosynthesis. Second, presumably by binding other proteins too tenaciously, Hsps can be toxic. Although the generality and details of both mechanisms are yet to be established, the deleterious consequences themselves are clear and apparently trade off against the benefits of Hsps in an evolutionary sense, either constraining directional selection for increased Hsp expression or necessitating especially effective autoregulation of Hsp expression.

A second step in rigorously establishing the adaptive significance of an Hsp is showing how it affects fitness in the wild. In this regard, the demonstration that the subject organism undergoes stress in nature (see above) becomes critical, as does demonstrating that altered stress tolerance affects fitness in natural populations. The tolerance-fitness relationship, however, can be complex, indeed. Unlike in the laboratory, wild organisms may seldom encounter single stresses, and combinations of stresses may have unexpected impacts. The impact (and the ability of Hsps to ameliorate it) may be in terms other than survival vs. death of the affected organisms. For example, in natural populations of *Drosophila melanogaster*, natural heat shock of embryos, larvae, and pupae may induce severe morphological abnormalities in the adults that eclose from these stages. The affected flies are alive, but are likely to have greatly reduced fitness. In a sibling species, *Drosophila simulans*, natural heat shock and Hsp expression may well mediate release from a bacterially-mediated reproductive incompatibility. To date, even demonstrations of simple relationships between Hsps, thermotolerance and fitness are still extremely rare.

A final step in establishing the adaptive nature of an Hsp is in showing that it actually satisfies the other criteria for origin and maintenance by natural selection: interindividual variation and heritability. The conundrum here is that Hsps and their encoding genes are extremely ancient and highly conserved, which could override small-scale variation. A surprisingly large number of studies have now established both interindividual variation and heritability of Hsps and their encoding genes. In many of these cases, moreover, interindividual variation is correlated with stress tolerance.

Large Scale Evolutionary Variation in HSPs

The extraordinary conservation of hsp gene sequences and Hsp function make these genes and proteins superb subjects for historical studies of evolutionary change. Hsps are recognizable in all kingdoms of living things, and in species resembling those thought to have arisen early in the history of life on earth. Apparently, the need for molecular chaperoning of proteins is as old as proteins themselves. Comparative studies have now suggested how the extant hsp genes have arisen and diversified from ancestral genes, have taken on novel roles as chaperones, and have assumed non-chaperone functions (e.g., a modified Hsp (alpha-crystallin) is a major component of the lens of the eye). In turn, hsp genes can yield distinctive insights into the phylogenetic relationships and evolutionary origins of the major groups of extant organisms and their organelles.

Have heat-shock proteins themselves affected the course of evolution? One theme in evolutionary biology is that phenotypic plasticity (of which Hsps can be a significant component) buffers organisms against continuous evolution in response to routine environmental stress, leaving organisms especially vulnerable to large-scale changes in environmental stress. Alternatively, Hsps and stress together may actually potentiate evolution of novel traits. As Rutherford and Lindquist posit, in the absence of stress, Hsp90 enables developmentally defective proteins to function normally and so preserves their encoding genes, which selection would otherwise modify. Upon stress, the ensuing damage titrates Hsp90 away from these proteins, leaving them free to initiate abnormal development. Whereas the consequent abnormal development is usually harmful if not lethal, occasionally it may represent beneficial phenotypic novelty that can then be assimilated genetically. Future study will no doubt elucidate the importance of such phenomena for evolution in nature.

Ecological and Evolutionary Functional Genomics

A new multidisciplinary approach is now arising: ecological and evolutionary functional genomics refers to investigations of genomic/genetic variation that seek to understand the functional significance of such variation for organisms and populations in natural environments and the evolutionary processes that create and/or maintain such variation. It thus includes investigators who try to combine evolution, ecology, functional analysis (physiology, biochemistry, neurobiology, endocrinology, functional morphology, etc.) and genetics (description and manipulation of discrete genes and quantitative traits) in a single research program, out of the conviction that all of these components are necessary for a rigorous analysis of biological phenomena. While this new approach is applicable to diverse traits, investigations into molecular chaperones/heat-shock proteins have a remarkable potential to cultivate growth in ecological and evolutionary functional genomics.

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.

References

Brakefield, P. M., 1997. Phenotypic plasticity and fluctuating asymmmetry as responses to environmental stress in butterfly *Bicyclus anynana*. In: "*Environmental Stress, Adaptation and Evolution*" (ed. R. Bjilsma and V. Loeschcke), Birkhauser Verlag, Basel, pp 65-78

Brakefield, P. M., Kesbke, F., Koch, P.B., 1998, The regulation of phenotypic plasticity of eyespots in the butterfly *Bicyclus anynana*. *Amer. Nat*., 152: 853-860.

Feder, M. and Krebs, R. A. 1997. Ecological and evolutionary physiology of heat shock proteins and the stress response in *Drosophila*: complementary insights from genetic engineering and natural variation. In: "*Environmental Stress, Adaptation and Evolution*" (ed. R. Bjilsma and V. Loeschcke), Birkhauser Verlag, Basel, pp 155-173.

Feder, M. and Hofmann, G. E., 1999. Heat shock proteins, molecular chaperones and the stress response: evolutionary and ecological physiology. *Annu. Rev. Physiol*., 61: 243-282.

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

Lakhotia, S. C., 1998. Stress biology: a challenging area in integrated biology. *Biology International*, 36: 18-30

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

McFadden, G., 1998. Even viruses can learn to cope with stress. *Science*, 279: 40-41

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.

Nath, B. B., Lakhotia, S. C., 1989. Heat shock response in a tropical *Chironomus*: seasonal variation in response and the effect of developmental stage and tissue type on heat shock protein synthesis. *Genome*, 32: 676-686

Norris, C. E., Brown, M. A., Hickey, E., Weber, L. A. and Hightower, L. E., 1995. Variation in heat shock proteins within tropical and desert species of Poeciliid fishes. *Mol. Biol. Evol*., 12: 1048-1062.

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

Ritoossa, 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

Singh, A. K. and Lakhotia. S. C., 2000. Tissue-specific variations in induction of Hsp70 and Hsp64 by heat shock in insects. *Cell Stress & Chaperones*, 5: in press

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

Chaperones as possible elements of eukaryotic cytoarchitecture

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After the discovery of the need for extensive assistance in protein folding in the case of many nascent or damaged proteins, heat shock proteins and other stress-induced proteins have come to be regarded as molecular chaperones; thus their major cellular function is considered to be established. When protein folding is studied *in vitro*, the experimenter has to use rather diluted conditions to prevent unwanted aggregation. Dilution also helps to make the kinetical analysis easier, and conserves precious research materials. Contrary to these usual experimental conditions, the cellular environment is crowded (Zimmerman and Minton, 1993). Molecular crowding promotes protein aggregation and thus enhances the need for chaperone action. On the other hand, bona fide chaperones are not the only cellular solutions for aggregation-protection. Several "innocent bystanders," such as tubulin (Guha *et. al.,* 1998) or even small molecules (lipids, other amphiphyles, sugars, a class of compounds called as chemical chaperones, Welch and Brown, 1996) may assist folding and prevent aggregation albeit at higher concentrations than the efficient concentration of heat shock, or other stress-induced proteins. Though we have several important lines of evidence, which undoubtedly show the necessity of chaperones in folding of numerous protein kinases, receptors, actin, tubulin, etc. (Hartl, 1996), we do not really know how big is the segment of the life of an ordinary chaperone during which it “chaperones" unfolded or misfolded proteins in eukaryotic cells.

I should make it clear that with the above argumentation, I do not want to question the importance of chaperones in folding-assistance. Nevertheless, I would like to stress that there is enough room to think about other important functions of chaperones related, but not equal to their participation in protein folding. One of these possibilities is that peptide-binding chaperones are the "dustmen" of the cells. The proteasomal apparatus is most probably linked with oligo- and dipeptidases, and therefore the "leaking" peptide- end products of proteasomal degradation (Kisselev *et. al.,* 1998) are presumably cleaved further into single amino acids. However, direct evidence for this efficient degradation-completion is missing.

Released peptide segments may often contain elements of important binding sites and thus might efficiently interfere with signaling and, metabolic processes. If this happened, this would be a disaster for the cell. Peptides need to be eliminated, and safeguarding mechanisms must exist to correct the occasional "sloppiness" of degradative processes. Chaperones are excellent candidates for this purpose, and their role in collection of "peptide-rubbish" must be considered, besides their well-established function in peptide presentation for the immune system (Srivastava *et. al.,* 1998).

As yet another important, and non-conventional, aspect of chaperone action (from the many more possible) lies in their incredible stickiness. Chaperones often form dimers, and tend to associate to tetra-, hexa-, octamers and to even higher oligomers (Csermely *et. al.*, 1998, Trent *et. al.,* 1998, Benaroudj *et. al.,* 1996). Oligomerization usually affects only a few percent of the total protein; but addition of divalent cations, certain nucleotides, heat

treatment, etc enhances oligomer formation. It is important to note that oligomerization studies were usually performed under "normal," *in vitro* experimental conditions, using a few mg/ml of purified chaperone. The *in vivo* concentration of chaperones is estimated to be around a hundred-, or thousand-fold higher. This may significantly enhance the *in vivo* oligomerization tendencies of these proteins. Oligomer formation of chaperones might be further promoted by the large excluded volume effect of the "molecularly crowded" cytoplasm (Zimmerman and Minton, 1993).

Different chaperones associate with each other. The Hsp90-organized foldosome may contain almost a dozen independent chaperones, or co-chaperones. The stoichiometry and affinity of these associations dynamically varies, and the variations are affected by the folding state of the actual target (or targets), which associate with these extensive folding machinery (Csermely *et. al.,* 1998).

Besides binding to themselves, to their sibling-chaperones, and to their targets, many chaperones bind to actin filaments, tubulin, and other cellular filamentous structures, such as intermediate filaments. There is a chaperone complex associated with the centrosome (Wigley *et. al.,* 1999), and several chaperones, especially Hsp90 were considered to be involved in the direction of cytoplasmic traffic (Pratt, 1997).

The above model, which describes chaperones as a highly dynamic "appendix" of various, and often quite poorly identifiable, cytoplasmic filamentous structures, is reminiscent of the early view (Wolosewick and Porter, 1979; Schliwa *et al*., 1981) about the microtrabecular network of the cytoplasm. Although a rather energetic debate has developed about the validity of the electron microscopical evidence of the microtrabeculae, several independent findings support the existence of a cytoplasmic and nuclear mesh-like structure (Clegg, 1984; Jacobson and Wojcieszyn, 1984; Luby-Phelps *etal*., 1988; Penman and Penman, 1997; Hendzel *et al.,* 1999). The major cytoplasmic chaperones (TCP1/Hsp60 and Hsp90 and their associated proteins) may well form a part of this network in cells.

One of the major advances of the eukaryotic cell is probably centered around its superior compartmentalization and organization compared with that of the prokaryotic organisms. However, cellular order must be maintained and repaired. Chaperones may be important elements of this job in eukaryotes. Further studies to explore the details of this putative function may easily lead to exciting, novel aspects of chaperone action.

References

Benaroudj, N., Triniolles, F. and Ladjimi, M.M., 1996, Effect of nucleotides, peptides, and unfolded proteins on the self-association of the molecular chaperone, HSC70. *J. Biol. Chem*., 271: 18471-18476.

Clegg, J. S., 1984, Properties and metabolism of the aqueous cytoplasm and its boundaries. *Am. J. Physiol*., 246: R133-R151.

Csermely, P., Schnaider, T., S ti, Cs., Prohsszka, Z. and Nardai, G., 1998, The 90-kDa molecular chaperone family: structure, function and clinical applications. A comprehensive review. *Pharmacology and Therapeutics*, 79: 129-168.

Guha, S., Manna, T.K., Das, K.P. and Bhattacharya, B., 1998, Chaperone-like activity of tubulin. *J. Biol. Chem*., 273: 30077-30080.

Hartl, F.-U., 1996, Molecular chaperones in cellular protein folding. *Nature*, 381: 571-579.

Hendzel, M.J., Boisvert, F.-M. and Bazett-Jones, D.P., 1999, Direct visualization of a protein nuclear architecture. *Mol. Biol. Cell*, 10:2051-2062

Jacobson, K. and Wojcieszyn, J., 1984, The translational mobility ofsubstances within the cytoplasmic matrix. *Proc. Natl. Acad. Sci. USA*, 81: 6747-6751.

Kisselev, A.F., Akopian, T.N. and Goldberg, A.L., 1998, Range of sizes of peptide products generated during degradation of different proteins by archaeal proteasomes. *J. Biol. Chem*., 273: 1982-1989.

Luby-Phelps, K., Lanni, F. and Taylor, D. L., 1988, The submicroscopic properties of cytoplasm as a determinant of cellular function. *Annu. Rev. Biophys. Biophys. Chem*., 17: 369-396.

Penman, J. and Penman, S., 1997, Resinless section electron microscopy reveals the yeast cytoskeleton. *Proc. Natl. Acad. Sci. USA*, 94: 3732-3735.

Pratt, W. B., 1997, The role of the Hsp90-based chaperone system in signal transduction by nuclear receptors and receptors signaling via MAP kinase. *Annu. Rev. Pharmacol. Toxicol.*, 37: 297-326.

Schliwa, M., van Blerkom, J. and Porter, K. R., 1981, Stabilization of the cytoplasmic ground substance in detergent-opened cells and a structural and biochemical analysis of its composition. *Proc. Natl. Acad. Sci. USA*, 78: 4329-4333.

Srivastava, P.K., Menoret, A., Basu, S., Binder, R.J. and McQuade, K.L., 1998, Heat shock proteins come of age: primitive functions acquire new roles in an adaptive world. *Immunity*, 8: 657-665.

Trent, J. D., Kagawa, H. K., Yaoi, T., Olle, E. and Zaluzec, N. J., 1997, Chaperonin filaments: The archaeal cytoskeleton? *Proc. Natl. Acad. Sci. USA*, 94: 5383-5388.

Welch, W. J. and Brown, C. R., 1996, Influence of molecular and chemical chaperones on protein folding. *Cell Stress Chaperones*, 1: 109-115.

Wigley, W.C., Fabunmi, R.P., Lee, M.G., Marino, C.R., Muallem, S., DeMartino, G.N. and Thomas, P.J., 1999, Dynamic association of proteasomal machinery with the centrosome. *J. Cell Biol*., 145, 481-490.

Wolosewick, J. J. and Porter, K. R., 1979, Microtrabecular lattice of the cytoplasmic ground substance. Artifact or reality. *J. Cell Biol*., 82: 114-139.

Zimmerman, S. B. and Minton, A. P., 1993, Macromolecular crowding: biochemical, biophysical and physiological consequences. *Annu. Rev. Biophys. Biomol. Struct*., 22: 27-65.

Heat Shock Proteins as Peptide Chaperones and the Initiators of T Cell Immunity

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The expectations of wiping out all human malignancy with a simple "shot" of tumor vaccines are rooted deeply in one basic principle generated from a series of experimental tumor rejection studies. As early as in 1940s, it was shown that animals that are previously injected with or exposed to inactivated tumors are resistant to a subsequent challenge from the same but live tumors (Gross 1943, Baldwin 1955, Prehn and Main 1957, Klein *et. al.*, 1960, Old *et. al.,* 1962). When experiments were carefully controlled with regard to the genetic background of the animals, route of administration of the vaccines, and the number of tumor cells used for challenge, it was soon realized that this phenomenon is not restricted to any particular tumors or hosts. Therefore, every tumor cell must contain tumor specific rejection antigens that can trigger specific immune responses. It is the curiosity and effort to define the tumor rejection antigens molecularly that has brought Heat Shock Proteins (HSPs) into the spotlight of immunology. Through biochemical fractionation of Meth A fibrosarcoma and testing of each fraction for its ability to immunize BALB/c mice against Meth A challenge, a number of tumor rejection antigens were isolated, and each of them was found to be a HSP. They are HSP gp96, HSP90, HSP70 and calreticulin (reviewed by Li 1997 and Srivastava *et al.*, 1998, Basu and Srivastava, 1999).

The Hypothesis

HSPs as tumor rejection antigen are surprising in light of the fact that HSPs are ubiquitously present in life and conserved in structure and perhaps function along the phylogenic tree. They are believed to play an indispensable role in the conformational maturation of a nascent polypeptide chain in all its aspects: the folding and unfolding of polypeptide chain, the transport and targeting of proteins into the proper subcellular compartment, the assembly of multimolecular complexes, etc (see review by Parsell and Linquist 1993, Gething and Sambrook 1992). Through genetic and biochemistry work, it became clear that HSPs themselves are neither tumor-specific, nor antigenic (reviewed by Srivastava *et. al.,* 1998). Since tumor rejection after HSP vaccination is dependent on T cell responses, and the T cell recognizes a short stretch of peptides in the context of the Major Histocompatibility Complex (MHC) molecules, it was proposed that HSPs themselves are not immunogenic, but rather contain tightly associated peptides. It is the HSP-associated, non-covalently bound small peptides that are the true antigens, and HSPs are merely carriers of antigenic peptides (Srivastava and Maki 1991).

Evidence That HSPs are Chaperones for Antigenic Peptides

HSPs as chaperones for endogenous peptides are firstly confirmed with the endoplasmic reticulum HSP, gp96/grp94 (Li and Srivastava 1993). It was shown by High Performance Liquid Chromatography (HPLC) that peptides were present and could be dissociated from highly purified gp96 without proteolysis. Binding of HSP70 to peptides are suggested by *in vitro* binding experiment (Flynn *et. al.,* 1991) and confirmed by crystallographic study

(Zhu *et. al.,* 1998). The peptide as the basis of immunogenecity associated with HSP70 was shown nicely in the fibrosarcoma system. This became possible as the HSP70-peptide complex was found to dissociate in the presence of ATP. ATP-treated HSP70 loses its immunogenicity (Udono and Srivastava, 1993; Peng *et al.,* 1997).

Although HSPs as a carrier for antigenic peptides were firstly suggested in tumor rejection assay, this phenomenon has since been validated by multiple independent researchers in multiple antigen systems including viral, minor antigen and other model antigens**.** Using the vesicular stomatitis virus (VSV) system, van Bleek's group showed that purified gp96 preparation from VSV infected cells contains the VSV epitope. This was shown physically by HPLC and functionally by T cell recognition. Moreover, immunization with gp96 purified from VSV-infected cells can immunize mice to generate specific T cell response against VSV (Nieland *et. al.,* 1996). Similarly, it was shown by Rammensee's laboratory that the gp96 isolated from β-galactosidase (β-gal) expressing P815 cells elicits cytotoxic T lymphocytes (CTLs) specific for β-gal and minor H antigens expressed by these cells (Arnold *et. al.,* 1995).

Both HSP70 and gp96 preparations purified from the ovalbumin (OVA)-transfected cell line EG7 are associated with processed H-2K^b-binding peptides which contain the major H-2K^b-associated epitope SIINFEKL (OVA257-264) (Breloer *et. al.,* 1998). Nakayama's group has examined the peptide precursors bound to three major HSPs, gp96, HSP90 and HSP70. It was shown that that a L^d-restricted CTL epitope of a mouse leukemia Rlmale symbol1 and its precursors are associated with all the three chaperones. HSP70 was associated with only the final sized octamer, while HSP90 was found to associate with the octamer and two distinct precursor peptides. Gp96 was associated with the octamer and one of the two precursors. Thus, each of the HSPs bound a distinct set of peptides (Ishii *et. al.,* 1999). Recently, it was shown by Nicchita's group in an adoptive immunotherapy protocol, dendritic cells pulsed with calreticulin and gp96 isolated from B16/F10.9 murine melanoma, EG7-OVA, or EL4 thymoma tumors elicited a CTL response to as yet unknown tumor-derived antigens or the known OVA antigen (Nair *et. al.,* 1999).

Heat Shock Protein-Peptide Complexes are Effective Tumor Vaccines

The advantage of HSP vaccinations stems from two major principles. Since genetic mutations are a largely active, but random phenomenon during, or perhaps as the result of malignant transformation, the composition of the total pool of antigenic peptides would be expected to be different from tumor to tumor. Therefore, the only true vaccines have to be based upon antigens derived from the autologous cancer of the patients themselves. Autologous HSPs represent the best source, since they potentially carry the entire repertoire of antigenic peptides. Furthermore, HSP vaccinations induce not only measurable T cell responses, but also lead to tumor rejection.

The effectiveness of vaccination with HSPs is unprecedented, in that it is several orders of magnitude more potent than peptides alone, or peptides plus adjuvant, in inducing T cell responses (Blachere and Li *et. al.,* 1997). When tumor rejection is used as the only parameter for a successful vaccine, HSPs-peptide complexes are by far the most effective and consistent. Effective HSP vaccinations as a prophylaxis against subsequent live tumor challenges have been validated in more than 10 types of tumor model systems of different histologies, in different species (Table 1).

Table 1: Tumor-derived Heat Shock Proteins are Effective Vaccines against Cancer

Host	Tumor Model	Heat Shock Protein	Reference
Mouse	Meth A fibrosarcoma	gp96, HSP90, HSP70	Udono and Srivastava 1993
		CRT	Basu and Srivastava 1999
	UV-induced sarcoma	gp96, HSP70	Janetzki *et. al.,* 1998
			Tamura and Peng *et. al.,* 1997
	Colon cancer	gp96, HSP70	Tamura and Peng *et. al.,* 1997
	Thymoma	gp96, HSP70	Li *et. al.*, unpublished
	Melanoma	gp96, HSP70	Tamura and Peng *et. al.,* 1997
	Lung cancer	gp96, HSP70	Tamura and Peng *et. al.,* 1997
Rat	Prostate cancer	gp96	Yedavelli *et. al.,* 1999
	Hepatoma	gp96	Srivastava and Das, 1984
Dog	melanoma	gp96	Menoret, unpublished
Xenopous	lymphoma	gp96, HSP70	Robert *et. al.*, unpublished

The efficacy of HSPs in the therapy of preexisting primary and metastatic tumors was also demonstrated in multiple systems including metastatic murine 3LL Lewis lung carcinoma, murine colonic adenocarcinoma (CT26) and murine fibrosarcomas Meth A, B16 F10.2 melanoma and the UV induce spindle cell carcinoma UV6139 (Tamura and Peng *et. al.,* 1997). In each case, a pre-existing tumor can be eradicated solely by treatment with either gp96 or HSP70 purified from the respective primary tumors. This principle was validated independently in the B16/F10.9 melanoma model (Nicchitta 1998). It underscored that vaccinations with tumor-derived HSPs were effective, tumor-specific, and generalizable.

There are a total of 6 clinical trials at different phases worldwide on the role of gp96 in the treatment of human malignancy. Two trials has been completed, including the original pilot trial in Germany (Janetzki *et. al.,* 1996 and 1999), and the pancreatic cancer trial in Memorial Sloan-Kethering Cancer Institute, New York (Lewis *et. al.,* 1999). In the pilot trial in Germany, sixteen patients, with various advanced malignancies (mostly gastrointestinal, thyroid or breast origin) which had become refractory to established therapies, were treated with autologous tumor derived gp96 (Janetzki *et. al.,* 1999). Patients were injected subcutaneously with 25 μg gp96 for four times at weekly intervals. It was shown convincingly that immunization with gp96 elicited MHC class I-restricted, tumor-specific CD8+ T cell responses in 6/12 patients analyzed. A total of 42 patients were enrolled in the renal cell carcinoma trial, of which 38 patients are evaluable (Amato *et al.,* 1999). Significant clinical responses were achieved with the dosage of 25 μg per injection.

Heat Shock Proteins as the Signals of Danger and Initiators of Immunity

Although HSP-peptide complexes are effective tumor vaccines, the roles of HSPs in natural immune response are not immediately obvious. Since tumor immunity after HSP vaccination is dependent on CD8+ CTLs, it was reasoned that exogeneous HSP-peptide complexes must find a way to be represented to MHC class I. Indeed, representation to MHC class I can be shown nicely *in vitro,* in that macrophage-like Antigen-Presenting Cells (APC) pulsed with HSP-peptide complexes are killed by antigen specific CTLs in MHC-dependent manner. Interestingly representation can only occur with a subset of APCs, macrophage-like (Suto and Srivastava 1996), or dendritic cells (Nair *et. al.,* 1999). Such cells are essential for HSP vaccination, since functional depletion of phagocytic cells by carrageenan abolishes completely the immunogenecity elicited by gp96 (Udono and

Srivastava 1993) and HSP70 (Peng and Srivastava, unpublished). The interactions of HSPs with APCs, are directly confirmed by three independent groups, showing the presence of a receptor-type molecule on the surface of phagocytic cells (Wassenberg *et al.,* 1999, Arnold-Schild *et al.*, 1999, Binder *et al.*, 1999). Presently, the nature of this molecule is being pursued molecularly. Another surprising finding is that HSPs themselves, including HSP90, gp96 and HSP70 can alert and activate APCs in an antigen-independent manner (Basu and Srivastava, unpublished). Purified macrophages or dendritic cells can be activated and secrete IL-1β, TNF-α, GM-CSF and IL-12. Moreover, gp96 can even promote maturation of bone marrow-derived dendritic cells evidenced by up-regulation of surface MHC class II molecules, B7.1, B7.2 and CD11b. These effects on APCs are not dependent on LPS, a Gram negative bacterial product that may contaminate the buffers.

Taken together, the pivotal roles of HSPs in immune responses are emerging. The trigger for launching specific immune responses is the release of intracellular HSPs as a result of either stress (Booth and Koch 1989) or cell death (Melcher *et. al.,* 1998). Being the most abundant intracellular proteins, the extracellular presence of HSPs is perceived as the signal of danger to the host (Matzinger 1994). A cascade of events then ensues, with the activation of local APCs as the rate-limiting or critical step. Naïve T cells are finally activated and expanded through T cell receptor engagement and signals for co-stimulation as the result of APC activation. This model predicts several additional testable hypotheses. First, T cell education through positive and negative selection is dependent on the interaction of T cell receptors with peptides in the context of MHC molecules. Peptides that gain access to the thymus epithelium could come from HSP-peptide complexes released distally. Therefore, representation of HSP-peptide complexes to MHC class I might contribute significantly to the shaping up of T cell repertoires. Second, auto-immune diseases are the result of excessive stimulation of APCs from the excessive and chronic release of HSP-peptide complexes. The connection of auto-immunity with chronic infections such as that caused by hepatitis virus, C virus, *borrelia burgdorferi*, etc. support such a theory.

References

Amato, R. J., Murray, L., Wood, L., Cherylyn, S., Tomasovic, S. and Reitsma, D., 1999, Active specific immunotherapy in patients with renal cell carcinoma (RCC) using autologous tumor derived heat shock protein-peptide complex-gp96 (HSPPC-96) vaccine. In: *Proceedings of the 35th Annual ASCO Meeting*, Abstract #1278, page 332a

Arnold, D., Faath, S., Rammensee, H. G., Schild, H., 1995, Cross-priming of minor histocompatibility antigen-specific cytotoxic T cells upon immunization with the heat shock protein gp96. *J. Exp. Med.*, 182: 885-889

Arnold-Schild, D., Hanau, D., Spehner, D., Schmid, C., Rammensee, H. G., de la Salle, H., Schild, H., 1999, Cutting edge: receptor-mediated endocytosis of heat shock proteins by professional antigen-presenting cells. *J. Immunol.,* 162:3757-3760

Baldwin, R. W., 1955. Immunity to methylchalanthrene-induce tumors in inbred rats following atrophy and regression of the implanted tumors. *Br. J. Cancer*, 9:652-657

Basu, S. and Srivastava, P. K., 1999, Calreticulin, a peptide-binding chaperone of the endoplasmic reticulum, elicits tumor- and peptide-specific immunity. *J. Exp. Med*., 189: 797-802

Binder, R., Harris, M., Menoret, A. and Srivastava, P. K., 1999, Events in the presentation of heat shock protein chaperoned peptides. *Immunity* (submitted)

Blachere, N. E., Li, Z., Chandawarkar, R. Y., Suto, R., Jaikaria. N. S., Basu, S., Udono, H., Srivastava, P. K, 1997, Heat shock protein-peptide complexes, reconstituted *in vitro*, elicit

peptide-specific cytotoxic T lymphocyte response and tumor immunity. *J. Exp. Med*., 186:1315-1322

Blachere. N. E., Udono, H., Janetzki, S., Li, Z., Heike, M., Srivastava, P. K., 1993, Heat shock proteins vaccines against cancer. *J. Immunother*., 14:352

Booth, C., Koch, G. L., 1989, Perturbation of cellular calcium induces secretion of luminal ER proteins. *Cell*, 59: 729-737

Breloer, M., Marti, T., Fleischer, B., von Bonin, A., 1998, Isolation of processed, H-2Kb-binding ovalbumin-derived peptides associated with the stress proteins HSP70 and gp96. *Eur. J. Immunol*., 28: 1016-1021

Flynn, G. C., Pohl, J., Flocco, M. T. and Rothman, J.E., 1991. Peptide-binding specificity of the molecular chaperone Bip. *Nature*, 353:726-730

Gething, M. J. and Sambrook, J., 1992, Protein folding in the cell. *Nature*, 355: 33-45

Gross, L., 1943. Intradermal immunization of C3H mice against a sarcoma that originated in an animal of the same line. *Cancer Res*., 3:323-326

Ishii, T., Udono, H., Yamano, T., Ohta, H., Uenaka, A., Ono, T., Hizuta, A., Tanaka, N., Srivastava, P. K. and Nakayama, E., 1999. Isolation of MHC class I-restricted tumor antigen peptide and its precursors associated with heat shock proteins hsp70, hsp90, and gp96. *J. Immunol*., 162: 1303-1309

Janetzki, S., Blachere, N.E. and Srivastava, P. K., 1998. Generation of tumor-specific cytotoxic T lymphocytes and memory T cells by immunization with tumor-derived heat shock protein gp96. *J. Immunother*., 21: 269-276

Janetzki, S., Rosenhauer, V., Palla, D., Srivastava, P. K. and Lochs, H., 1996. Immunization of cancer patients with autologous cancer-derived heat shock protein-peptide complexes-a pilot study. In: *Cancer Vaccine*, Abstract T68, Cancer Research Institute, New York

Janetzki, S., Palla, D., Rosenhauer, V., Lochs, H., Lewis, J. and Srivastava, P. K., 1999. Immunization of cancer patients with autologous cancer-derived heat shock protein gp96 preparations: a pilot study. *submitted*

Klein, G., Sjogren, H. O., Klein, E. and Hellstrom, K. E., 1960. Demonstration of resistance against methylcholanthrene-induced sarcomas in the primary autochthonous host. *Cancer Res*., 20: 1561-1572

Lewis, J. L., Janetzki, S., Livingston, P. O., Desantis, D., Williams, D., Klimstra, D., Reitsma, D., Houghton, A. N., Srivastava, P. K. and Brennan, M. F., 1999. Pilot trial of vaccination with autologous tumor-derived gp96 heat shock protein-peptide complex (HSPPC-96) in patients with resected pancreatic adenocarcinoma. In: *Proceedings of the 35th Annual ASCO Meeting*, Abstract #1687, page 438a

Li, Z., 1997. Priming of T cells by heat shock protein-peptide complexes as the basis of tumor vaccines. *Semin. Immunol*., 9: 315-322

Li, Z. and Srivastava, P. K., 1993. Tumor rejection antigen gp96/grp94 is an ATPase: implications for protein folding and antigen presentation. *EMBO J*., 12: 314

Matzinger, P., 1994. Tolerance, danger, and the extended family. *Annu. Rev. Immunol*., 12: 991-1045

Melcher, A., Todryk, S., Hardwick, N., Ford, M., Jacobson, M. and Vile, R. G., 1998. Tumor immunogenicity is determined by the mechanism of cell death via induction of heat shock protein expression. *Nat. Med*., 4: 581-587

Nair, S., Wearsch, P. A., Mitchell, D. A., Wassenberg, J. J., Gilboa, E., Nicchitta, C. V., 1999. Calreticulin displays in vivo peptide-binding activity and can elicit CTL responses against bound peptides. *J. Immunol*., 162: 6426-32

Nieland, T. J., Tan, M. C., Monne-van, M. M., Koning, F., Kruisbeek, A. M. and van Bleek, G. M, 1996. Isolation of an immunodominant viral peptide that is endogeneously bound to the stress protein gp96/grp94. *Proc. Natl. Acad. Med. Sci. USA*, 93: 6135-6159

Old, L. J., Boyse, E. A., Clarke, D. A. and Carswell, E. A., 1962. Antigenic properties of chemically induced tumors. *Ann. N. Y. Acad. Sci*., 101:80-106

Parsell, D. A. and Linquist, S., 1993. The function of heat shock proteins in stress tolerance: degradation and reactivation of damaged proteins. *Annu. Rev. Genetics*, 27: 437-496

Peng, P., Menoret, A. and Srivastava, P. K., 1997. Purification of immunogenic heat shock protein 70-peptide complexes by ADP-affinity chromatography. *J. Immunol. Methods*, 204:13-21

Prehn, R. T. and Main, J. M., 1957. Immunity to methylcholanthrene-induced sarcomas. *J. Natl. Cancer. Inst*., 18: 769

Srivastava, P. K. and Das, M. R., 1984. The serologically unique cell surface antigen of Zajdela ascites hepatoma is also its tumor-associated transplantation antigen. *Int. J. Cancer*, 33:417-422

Srivastava, P. K. and Maki, R. G., 1991. Stress-induced proteins in immune response to cancer. *Curr. Top. Microbiol. Immunol*., 167:109-123

Srivastava, P. K., Menoret, A., Basu, S., Binder, R. J. and McQuade, K. L., 1998. Heat shock proteins come of age: primitive functions acquire new roles in an adaptive world. *Immunity* 8: 656-665

Suto, R. and Srivastava, P. K., 1995. A mechanism for the specific immunogenicity of heat shock protein-chaperoned peptides. *Science*, 269:1585-1588

Tamura, Y., Peng, P., Liu, K., Daou, M. and Srivastava, P. K., 1998. Immunotherapy of tumors with autologous tumor-derived heat shock protein preparations. *Science*, 2781: 17-20

Todryk, S., Melcher, A. A., Hardwick, N., Linardakis, E., Bateman, A., Colombo, M. P., Stoppacciaro, A. and Vile, R. G., 1999. Heat shock protein 70 induced during tumor cell killing induces Th1 cytokines and targets immature dendritic cell precursors to enhance antigen uptake. *J. Immunol*., 163: 1398-1408

Udono, H. and Srivastava, P. K., 1994. Comparison of tumor-specific immunogenicities of stress-induced proteins gp96, hsp90, and hsp70. *J. Immunol*., 152: 5398-5403

Udon.. H. and Srivastava, P. K., 1993. Heat shock protein 70-associated peptides elicit specific cancer immunity. *J. Exp. Med*., 178:1391-1396

Wassenberg, J. J., Dezfulian, C. and Nicchitta, C. V., 1999. Receptor mediated and fluid phase pathways for internalization of the ER hsp90 chaperone GRP94 in murine macrophages. *J. Cell. Sci*., 112: 2167-2175

Wells, A. D., Rai, S. K., Salvato, M. S., Band, H. and Malkovsky, M., 1998. Hsp72-mediated augmentation of MHC class I surface expression and endogenous antigen presentation. *Int. Immunol*., 10: 609-617

Yedavelli, S. P., Guo, L., Daou, M. E., Srivastava, P. K., Mittelman, A. and Tiwari, R. K., 1999. Preventive and therapeutic effect of tumor derived heat shock protein, gp96, in an experimental prostate cancer model. *Int. J. Mol. Med*., 4: 243-248

Zhu, X., Zhao, X., Burkholder, W. F., Gragerov, A., Ogata, C. M., Gottesman, M. E. and Hendrickson, 1996. Structural analysis of substrate binding by the molecular chaperone DnaK. *Science*, 272:1606-1614

IUBS News and Events

27th IUBS General Assembly
8-12 November, 2000, in Naples, Italy

The IUBS 27th General Assembly was held on November, 8-12, 2000, at the Hotel Royal Continental in Naples, Italy. The complete Proceedings of this Assembly will be published by IUBS in 2001. A brief summary of the GA, its activities and resolutions are excerpted below.

Executive Committee 1997-2000

The outgoing Executive Committee met on 7 November before the Assembly, chaired by President Jean-Claude Mounolou. Open to all, the Meeting was attended by a number of observers on behalf of IUBS Ordinary and Scientific Members. Following the admission, in 1999, of Tunisia - "*l'Association Tunisienne des Sciences Biologiques*" as a new IUBS Ordinary Member, the Executive Committee Meeting in Naples, agreed on the admission of Portugal as its 43rd Ordinary Member, adhering through its *Ordem dos Biologós*.

General Assembly

The General Assembly convened on 8 November, attended by 79 participants, representing 30 Ordinary Members and 24 Scientific Members.

Following the Welcome by the President, Reports of the Officers and Opening Address by the Executive Director, presentations were given on the IUBS Scientific Programmes: Towards and Integrative Biology (TAIB), DIVERSITAS, Systematics Agenda 2000 International, Reproductive Biology in Aquaculture (RBA), Bionomenclature, Bioethics and Biology Education (CBE).

Ad hoc Committees were established for: Admissions (Chair: P. Biró), Credentials (Chair: M.H. Wake), Finances (Chair: M. Hoshi), Nominations (Chair: D. Hawksworth), Resolutions (Chair: M. Boulter), Scientific Programmes Committee (Chair: P. Whittaker), Statutes (Chair: E. Beck), IUBS 28th General Assembly (Chair: H. Baijnath).

Reports and Resolutions

The following proposals were put to the General Assembly and approved on 11.11.2000:

Admissions

The International Congress of Zoology (ICZ) is re-admitted as a Scientific Member of IUBS.

Finances

The Treasurer's Report and its Proposals are adopted:
For the duration of the next triennium, Members shall be requested to increase their dues by 5% annually (2.5% to offset inflation; 2.5% to support intensified Union Programme activities. Dues shall be stated and collected in Euros as of January 2001.

A Long Range Planning and Finance Committee shall be established to develop and carry out an effective plan of fund raising for the IUBS and its programmes.

The Committee recommends the insertion of a separate line item for new initiatives in all IUBS budgets and financial statements. This item would contain funds for discretionary expenditures to support scientific activities not included within formally adopted scientific Programs of IUBS.

Nominations and Elections

The Officers and Members of the Executive Committe 2001-2003 are as follows: M.H. Wake (USA) President; J-C. Mounolou (France) Past President; M. Hoshi (Japan), J.G. Tundisi (Brazil) – Vice-Presidents; P. Whittaker (Ireland) Secretary General; O. Hänninen (Finland) Treasurer; H. Baijnath (South Africa), E. Beck (Germany), A.H. Bittles (Australia), M.C. Boulter (United Kingdom), E. Gomez (Philippines), S.C. Lakhotia (India), M. Loreau (Belgium/France), M. Marrakchi (Tunisia), D.S. Pavlov (Russia), D. Piñero (Mexico), C. Scheidegger (Switzerland), E. Szathmáry (Hungary), S. Uchmanski (Poland), S. Yan (China) – Executive Committee Members.

Resolutions

1. *The GA urges the Union to explore new ways to involve young scientists, especially from the developing world and regions with low representation of scientists and data.*
2. *The GA sees an urgent need for responsible publicity on biological issues and recommends active learning procedures in all its Programmes.*
3. *The GA believes that the diversity of the Union's membership should be reflected in its Scientific Programs.*
4. *The GA approves cooperation with other ICSU Unions, as in the programmes on Bionomenclature and Diversitas.*
5. *The GA continues to support the priority that biodiversity research receives in its programs. It encourages responsible, open exchange of scientific material.*
6. *The GA requests greater use of internet services for IUBS communications. Updating of the IUBS website must be ensured.*
7. *The GA requests a mid-term evaluation of targets and progress.*
8. *The GA requests the Executive Committee to devise and implement systems of appraisal and accountability for its officers and programme leaders.*

Scientific Programmes

9. General resolutions
    - *More young scientists must be involved in the activities of IUBS.*
    - *New ways must be found to communicate the activities of IUBS to biologists world-wide.*
    - *IUBS should ensure good channels of communication with its Scientific Members and seek to utilise their expertise wherever possible.*
    - *Opportunities for cooperation should be actively investigated, particularly with reference to TAIB, Bioethics and Biological Education, whose activities could complement more scientifically focused programs.*

For recommendations and resolutions concerning specific Scientific Programmes, please request the full Report of the ad hoc Scientific Programmes Committee.

28th General Assembly in 2003

Egypt's offer to host the Assembly is accepted with thanks. IUBS officers are to liaise with the local organising committee of the Egyptian Academy of Scientific Research and Technology regarding date, venue and theme of the Assembly.

Marvalee H. Wake, President
Talal Younès, Executive Director

ICSU STATEMENTS

PRINCIPLES FOR USE OF ANIMALS IN RESEARCH AND EDUCATION

The International Council of Scientific Unions (ICSU) recognizes the essential contribution of the use of animals in research and education aimed at improving the health and well-being of humans and animals. Animal experimentation remains critical in understanding the fundamental processes of life, including behaviour, and in developing treatments for injury and disease. Members of the constituent bodies of ICSU believe that the use of animals in research and education imposes a responsibility on the scientists concerned to provide for the proper care and humane treatment of such animals, in accordance with ethical codes of conduct. ICSU reaffirms the scientific community's responsibility to establish its own mechanisms to evaluate the necessity and conduct of animal experimentation. Further, ICSU affirms that all research on animals should be designed taking into consideration its relevance to the improvement of human and animal health and welfare, and to the advancement of knowledge for the good of society. ICSU recognises that alternative methods of experimentation, such as cell culture and related systems, and computer modelling, are important adjuncts to animal experimentation and should be utilized whenever possible; however, they cannot at present replace the responsible use of animals in research and education.

18 July 1996

GENE PATENTING

The International Council for Science• (ICSU) is an international non-governmental organization whose mandate includes the promotion of cooperation in the basic sciences, and the safeguarding of the principle of the universality of science and of the free flow of scientific knowledge.

The Council is aware of the tremendous potential benefit of genetic research for humanity and realizes that new ethical and social dimensions arise from this. Accordingly, ICSU strongly believes that efforts to patent genetic information should not jeopardize either progress in the basic sciences or access to the information which is necessary for such progress to continue.

ICSU asserts its view that information about nucleic acid sequences cannot be patented *per se*. Such sequences should be patentable solely within the context of their demonstrated significance and/or application (*e.g.* regulatory signals, antisense RNAs, probes, etc.) - and not of their **potential** products (*e.g.* proteins) - and provided that this can be shown to be "novel", "non-obvious" and "useful".

Under such circumstances, patenting of complementary DNA sequences (cDNAs) would distort the patent process, which is designed to protect applications, methods and products, on the basis of proven facts and not mere expectations, and normally serves society by stimulating the investments and developments necessary to provide useful products and services. Any deviation from such patenting principles would run counter to the best interests of science and hinder international collaboration in such endeavours. ICSU therefore cautions against decisions which may be irreversible, such as those possibly emerging as a result of the recent patent requests concerning complementary DNA (cDNA) sequences corresponding to portions of unknown messenger RNAs

• The name of the Council was changed from the International Council of Scientific Unions to the International Council for Science at an Extraordinary General Assembly held in 1998. The acronym, "ICSU", was retained.

(mRNA).

ICSU urges the relevant authorities, particularly in countries where patent applications in this field have been or are soon to be filed, to consider such applications taking due account of the possible implications and to ensure a strict application of established patenting principles, thereby setting an example for other countries in which similar cases may arise in the future.

ICSU would welcome a formal international agreement on this subject.

Paris, June 1992

International Training Workshop on Inland Fisheries Management and Aquaculture for Sustainable Development

Wuxi, Jiangsu, China
15 October – 10 November 2001 (tentative dates)

Background

Inland fishery and aquaculture are important components of the world fisheries industry. Due to the over-exploitation of marine fishery resources, the share of inland fish production has increased steadily over the past decades in the world. In 1997, the world inland fish production (from capture and aquaculture) reached 24.83 million metric tons, accounting for 20.35% of the total world fisheries production in that year. In comparison, inland fish production contributed only 14.91% in 1970. The inland fishery and aquaculture will continue to play a significant role in national development, improving animal protein food supply and livelihood in rural areas in the developing world.

Asia and the Pacific have been the most active regions in inland fisheries development mainly due to the long tradition and huge market demand. China is the largest freshwater fish producer in the world. Its freshwater fish production from aquaculture accounted for 72.21% of the world total in 1997. Its catch from inland water reached 5.07 million tons in 1998, while the world inland fish production from capture was 7.7 million tons in 1997.

The traditional inland fisheries, largely depending on extensive use of natural resources, sometimes at the cost of environment deterioration, is now facing a strong challenge. Sustainability has become vitally important to the future growth of this food production sub-sector. Environmentally friendly aquaculture systems and various types of technologies suitable for sustainable use of inland waters have been developed in the region. The training workshop is to provide opportunities for the inland fisheries and aquaculture personnel from China and other countries in the region to share existing experiences and management skills and exchange views over the future trend of development. It is envisaged to promote the long-term sustainability of inland fisheries and aquaculture development in the region.

The training workshop will be a participatory process, and the topics will be opened with introductory lectures by resources persons. The participants are required to contribute their own experiences and opinions in the open discussion. Study tours and field trips will be organized to expose participants to the present inland fisheries and aquaculture practices in China.

Organizers

The training workshop is jointly organized by the *Freshwater Fisheries Research Centre* (FFRC) of the *Chinese Academy of Fishery Sciences* and the *Network of Aquaculture Centres in Asia-Pacific* (NACA), which is an inter-governmental organization with the mandate of promoting regional cooperation for aquaculture development.

Date, Duration and Venue

The training workshop will be 4 weeks, (tentative dates: 15 October - 10 November 2001, at the Freshwater Fisheries Research Centre, Qitang, Wuxi City, China, which is equipped with appropriate facilities for training and conferences.

Syllabus

- Inland fisheries and aquaculture development in participating countries
- Inland fisheries and aquafarming systems
  - Integrated fish farming
  - Rice-fish integration
  - Enclosed culture system with least pollutant discharge
  - Aquaculture and culture-based fisheries in open waters (with case study of reservoir fisheries management)
  - Enhancement of fishery resources (with two case studies of open water fisheries management)
  - Small-scale / rural aquaculture development
- Transplantation / introduction of new species
- Health management and environment control in aquaculture
  - Health management in aquaculture
  - Biological regulation of culture environment
- Genetic improvement and genetic resource conservation for sustainable development
- Legislature and policy development concerning inland fisheries and aquaculture
  - Legislature and policy development
  - Law and regulation enforcement
- Special lectures: China fisheries service and extension

Participants

The participants should be technical or managerial personnel with relevant experiences and currently involved in inland fisheries and aquaculture from the Asia-Pacific region.

Financial Issue

The training workshop will be organized thanks to funds provided by the *APEC Technological and Scientific Collaborative Fund* of the Chinese government. The organizer will bear all the local costs and expenses of overseas participants in China pertaining to the training workshop activity, including the registration fee, board and lodging (arranged meals and double-occupancy accommodation at FFRC hostel), local transportation, etc. In addition, the participants will be provided with a daily pocket allowance of thirty Chinese Yuan (*CNY 30*) to meet daily needs. Candidates are required to cover their international travel to and from Shanghai, China, from their own sources.

Health and insurance

The candidates/applicants must be physically and mentally fit for international travel and stay in a foreign country for four weeks to attend the training workshop. An appropriate international travel accident and sickness insurance policy must be arranged to protect each participant for the entire period of the training workshop prior to his/her departure from home country by the participant, or his/her sponsor.

Guideline / format of country paper preparation and presentation on inland fisheries and aquaculture development

1. The time allocated to each participant for presentation in 15 - 20 minutes;
2. Multi-media instruments such as LCD projector, video player, slide and overhead projector are available. Participants are encouraged to use these tools for the presentation.
3. Format of the report:

The paper should basically composed of the following components:

(1) Overall development and present status of inland fisheries and aquaculture in sense of importance of national economy and contribution to people's food supply
(2) Inland fisheries resource and present utilization
(3) Fish farming systems and technology presently adopted for inland fisheries and aquaculture and their implication in the sustainable development
(4) Constraints and technological development need for sustainable inland fisheries and aquaculture

Application

For further information, regarding the training course, please contact:

NACA Secretariat P.O. Box 1040 Kasetsart Post Office Bangkok 10903 Thailand Tel: +66-2-5611728; 5611729 ext. 111 Fax: +66-2-5611727 E-mail: naca@mozart.inet.co.th zhoux@fisheries.go.th Contact person: Mr. Zhou Xiaowei	**Freshwater Fisheries Research Centre** Qitang, Wuxi City Jiangsu 214081 China Tel: +86-510-5558719, 5555112 Fax: +86-510-5555112, 5553304 E-mail: wmmiao@public1.wx.js.cn rlcc@public1.wx.js.cn Contact persons: Mr. Miao Weimin Mr. Yuan Xinhua

Announcement
Fundacao Luso-Americana para o Desenvolvimento/
Luso-American Development Foundation (FLAD/NSF)
INTERNATIONAL BIOETHICS INSTITUTE

(Second edition)

30 June – 6 July, 2001, Lisbon, Portugal

The FLAD/NSF International Bioethics Institute (FNIBI) is an innovative co-operation between the EU and the USA, aiming at helping life science faculty to guide their students in bioethics. Consisting of a series of one week long faculty development summer workshops, FNIBI will be held at FLAD's headquarters in Lisbon, Portugal. Participants listen to lectures from European and American experts, participate in sessions devoted to ethical theory, pedagogy, and policy, and write case studies for use in their biology, marine, agricultural, biotechnology, animal, or environmental science classes. The Institute emphasizes active learning skills, with the goal of promoting the integration of ethics discussions into the life sciences curriculum. Particular attention is paid to ethical issues in agricultural biotechnology, as well as issues such as environment, marine sciences, biodiversity, and animal welfare and rights. The programme includes a guided visit to the Oceanarium of Lisbon, and a field trip to Arrabida's Natural Park, with sessions in the 15th century monastery Convento da Arrabida.

All participants receive lunches, books, case studies, exercises, and bibliographies. Participants from European countries other than Portugal receive 1000 Euro as travel and living expense allowance. Participants from Portuguese Universities outside the area of Lisbon receive a 500 Euro travel and living expense allowance. Participants from Portuguese Universities in the area of Lisbon receive a 250 Euro travel and living expense allowance. Participants from the US receive a travel stipend of $ 1950.

The workshop is conducted in English. Applicants must be tenured or tenure-track life science faculty members. Applicants are favoured who are interested in integrating discussions of ethics into existing agronomy/biotechnology courses and who apply with colleagues as a team from their institution.

The series is funded by FLAD, the National Science Foundation (USA), DG Research/European Commission (EU), the Foundation for Science and Technology/Ministry of Science and Technology (P); supported by the Iowa State University's Bioethics Program, Office of Biotechnology, and Plant Sciences Institute (USA), and the Fundacao Oriente (P). Hosts include the Centre of Environmental Biology and the Centre of Philosophy of the University of Lisbon.

Deadline for applications: 15 April, 2001. Late applications will be considered, if space is available. For more information and application form, go to:

http://www.biotech.iastate.edu/Bioethics/Institute/flad.html
or
http://www.flad.pt/pt/bioethic.html

PUBLICATIONS REVIEW

ANCIENT LAKES
Biodiversity, Ecology and Evolution

Edited by A. Rossiter and H. Kawanabe.
Published in *Advances in Ecological Research* (Vol. 31) Academic Press, 2000 (624 pages).

This volume presents a diverse range of exciting new perspectives and hypotheses on ancient lakes biota. Ancient lakes, with a unique and uninterrupted history of over 100 000 years, are increasingly recognized as important models for evolution and speciation.

Presenting the latest research results and theories from a wide range of studies in these lakes, together with many suggested areas for future research, this volume will be an essential reading for all biologists and developers interested in ecology, evolution, conservation and biodiversity.

PROGRESS AND PROSPECT OF MARINE BIOTECHNOLOGY

Edited by Huai-Su Xu and Rita R. Colwell.
Published by China Ocean Research (Vol. 31) Academic Press, 1999 (433 pages).

This volume represents the Proceedings of the "International Symposium on Progress and Prospects of Marine Biotechnology" (ISPPMB '98) held on 6-9 October, 1998 in Qingdao, China, as an activity of the International Year of the Ocean.

This volume presents the major developments in marine biotechnology that have occurred during the past decade, and the areas of research and development that will have the greatest potential for improvement of human health and the environment in the 21st century and, most importantly, proposes those fields of marine biotechnology having the greatest value for developing countries.

The proceedings' volume contains the collection of 134 papers presented at ISPPMB '98, consisting of 17 plenary lectures, 28 keynote lectures, and 89 oral and poster presentations.

THE BIOLOGY AND FERTILITY OF TROPICAL SOILS:
TSBF Report 1997-1998

By Mike Swift. Published by TSBF IUBS/UNESCO, 2000 (96 pages).

The Report of Activities of The Tropical Soils Biology and Fertility (TSBF) Program, co-sponsored by IUBS and UNESCO for the Years 1997 and 1998, is organised geographically. It presents reports of soil biology research from Africa, South Asia and the rest of the world.

As the balance of pages indicates, the major concentration of TSBF activities remains in East and Southern Africa. The Program's capacity to facilitate and support TSBF research outside this regions is still limited, but there is little doubt that this period has seen a worldwide increase of interest and demand for research and capacity building in soil biology.

This growing global interest in the conservation of biodiversity is now reaching beyond issues such issues as saving the 'charismatic megafauna' and preserving the rainforests to the realisation that there is a rich world below ground, which is critical to both agricultural productivity and the maintenance of many of the environmental services, which constitute our life support system.

CALENDAR OF MEETINGS

IUBS – sponsored meetings are indicated in bold-type face
Additional information may be obtained from addresses in () parentheses

2001

ANTARCTIC BIOLOGY
VIII SCAR International Biology Symposium "A Global Context"
27 Aug.-1 Sept., Amsterdam, Netherlands
(Contact: VU Conference Service, De Boelelaan 1105, 1081 HV Amsterdam, The Netherlands
Tel : +31 (0) 20 444 5790
Fax : +31 (0) 20 444 5825
E-mail : vu_conference@dienst.vu.nl)

BIODIVERSITY
Biodiversity as a Source of New Medicines
16-19 August, Cali, Colombia
(Contact: Dr. Ligia Pabon de Majid
Tel/Fax: 3302461
e-mail : ligpabon@mafalda.univalle.edu.co)

3rd IUPAC International Conference on Biodiversity (ICOB-3)
3-8 November, Antalya, Turkey
(Contact: Dr. Bilge Sener, Gazi University Faculty of Pharmacy, Ankara, Turkey
Tel: +90 312 2122267
Fax: +90 312 2133921
E-mail: blgsener@tr-net.net.tr)

CHEMISTRY
The World Chemistry (IUPAC 38th) Congress
1-6 July, Brisbane, Australia
(Contact: Congress Secretariat, PO Box 177, Red Hill Qld. 4059, Australia
http://www.ccm.com.au/wcc)

CROP PROTECTION
The British Crop Protection Council (BCPC) Conference Weeds 2001
12-15 November, Brighton, UK
(Contact: The BCPC Conference Secretariat, 5 Maidstone Buildings Mews, Bankside, London SE1 1GN, UK
Tel: +44 (0)20 7940 5555
Fax: +44 (0)20 7940 5577
E-mail : conference@bcpc.org
http://www.bcpc.org)

DEVELOPMENTAL BIOLOGY
14th International Congress of Developmental Biology
8-12 July, Kyoto, Japan
(Contact: Prof. Masatoshi Takeichi, Kyoto University, Kyoto, Japan)

ECOLOGY
ABUDIV 2001 Conference
"Theory and Application of Statistical Ecology"
28 August, Balatonfured, Hungary
(Contact:
E-mail : padisak@tres.blki.hu
http://www.terra.hu/abudiv/index.html)

2nd International Conference on Plants & Environmental Pollution
15-19 November, 2001 Lucknow, India
(Contact: Dr. K. J. Ahmad, Secretary, International Society of Environmental Botanists, National Botanical Research Institute, Rana Pratap Marg, Lucknow-226001, India
E_mail :NBRI@lw1.dot.net.in)

ENDOCRINOLOGY
14th Int'l Congress of Comparative Endocrinology
26-30 May, Sorrento, Italy
(Contact: Studio Congressi Cicala de Pertis, Via S. Anna dei Lombrdi, 38
80134 Napoli, Italy
E-mail : studiocongressi@napoli.com
http://www.napoli.com/studiocongressi)

FOOD SCIENCE
XI World Congress of Food Science & Technology
22-27 April, Seoul, Korea
(Contact: Prof. W.E.L. Spiess, President, IUFoST, Institute of Process Engineering, Federal Research Centre for Nutrition, Haid-und-Neu-Str. 9, D-76131 Karlsruhe
Tel: + 49 (0) 721 6625-300
Fax: + 49 (0) 721 6625-303
E-mail: walter.spiess@bfe.uni-karlsruhe
http://www.congress2001.or.kr/pre_internet.html)

GENERAL BIOLOGY

1st Int'l Conference on Biosystem Science & Engineering ICBSE &
Int'l Conference on Endocrinology & Molecular Morphogenesis &
Int'l Conference on Transgenic Animals and Bio-Logic Engineering
21-27 Oct., Beijing, China
(Contact: Bangzhe J. Zeng, An der Hohnhorst 11, 31535 Neustadt a. Rbge., Germany
E-mail: Bangzhe@hotmail.com
http://www.genbrain.net)

GLOBAL CHANGE

The Global Change Open Science Conference
10-13 July, Amsterdam, The Netherlands
(Contact: Open Science Conference, Congrex Holland BV, PO Box 302, 1000 AH Amsterdam, The Netherlands
Tel: +31 20 5040208
Fax: +31 20 5040225
http://www.sciconf.igpb.kva.se)

Detecting Environmental Change: Science and Society
16-20 July, London, UK
(Contact: Dr. Catherine E Stickley, Environmental Research Centre, Dept. of Geography, University College London, 26 Bedford Way, London WC1H 0AP, UK
Tel: +44 (0) 20 7679 5562
Fax: +44 (0) 20 7387 7565
E-mail: c.stickley@ucl.acl.uk)

HISTORY & PHILOSOPHY OF SCIENCE

21st International Congress of History of Science on "Science and Cultural Diversity"
8-14 July, Mexico City, Mexico
(Contact: Prof. Juan José Saldana, Chairman of the Organizing Committee, Apartado Postal 21-873, 04000 Mexico City, Mexico
Fax: (525) 544 63 16
E-mail: xxiichs@servidor.unam.mx
http://www.smhct.org)

INVERTEBRATE SURVEY

Int'l Colloquium of E.I.S. "Recent Changes in Ranges of Invertebrates: Invertebrates on the Move"
1-5 September, Leiden, The Netherlands
(Contact: European Invertebrate Survey, Darwinweg 2, 2333 CR Leiden, Netherlands
Fax: + 31(0) 71 56 87 666
E-mail : eis@nnm.nl)

LABORATORY ANIMALS

ICLAS-CCAC International Symposium on Regulatory Testing and Animal Welfare
21-23 June, Québec City, Canada
(Contact: The Secretary, ICLAS-CCAC International Symposium on Regulatory Testing and Animal Welfare, Centre de Recherche du CHUL (CHUQ), 2705, Laurier Boulevard, suite T-4-41, Sainte-Foy, Québec, Canada, G1V 4G2. Tel: + 1 (418) 656 4141 ext. 8669
Fax: + 1 (418) 656 2761
E-mail: ICLAS.CCAC@crhul.ulaval.ca
http://www.crchul.ulaval.ca/ICLAS2001)

LAKE BIODIVERSITY

ILEC Conference – 9th International Conference on the Conservation and Management of Lakes (Biwako 2001)
11-16 Nov., Oroshimo, Kusatsu, Shiga, Japan
(Contact: Secretariat of Biwako 2001, Shiga Prefectural Government, 4-1-1 Kyomachi, Otsu, Shiga, 520-8577, Japan
Ph: +81 77 528 3465, Fax: +81 77 528 4849
E-mail: lake2001@pref.shiga.jp
http://www.pref.shiga.jp/lake2001)

MEDICINAL & AROMATIC PLANTS

(WOCMAP 2001)
8-10 July, Budapest, Hungary
(Contact: Dr. Oszkár Köck, Nat'l. Inst. for Agricultural Quality Control, P.O. Box 30, 93. H-1525 Budapest, Hungary. Ph: +36 (0)1 2123 127
Fax: +36 (0)1 2122 673
E-mail: map.congr@ommi.hu)

PLANT PROTECTION

Seed Treatment – Challenges and Opportunities
26-27 Feb., Wishaw, North Warwickshire, England
(Contact: British Crop Protection Enterprises
49 Downing Street, Farnham, Surrey GU9 7PH, UK
Ph: +44(0)1252 733 072, Fax: +44(0)1252 727 194
E-mail: md@bcpc.org

Resistance 2001 – Meeting the Challenge
23-26 Sept., Harpenden, Herts., England
(Contact: IACR-Rothamsted, Harpenden, Herts. AL5 2JQ, UK. Ph: +44 (0)1582 763113
Fax: +44 (0)1582 760981
E-mail: res.2001@bbsrc.ac.uk
http://www.iacr.bbsrc.ac.uk/tmeeting.html)

SUSTAINABLE DEVELOPMENT
12th Annual Meeting and Conference of the Caribbean Academy of Science on Science and Technology for Sustainable Development in the 21st Century
9-13 June, Georgetown, Guyana
(Contact: John Caesar, Chairman Organizing Committee, Le Bureau Business Centre, Meridien Pegassus Hotel, Seawall Road, Kingston, Georgetown, Guyana
Fax: +592 226 4276
E-mail: lebureau@webworksgy.com
http://www.was2001.org.gy)

15th Int'l Environmental Informatics Symposium: Sustainability in the Information Society
10-12 October, Zurich, Switzerland
(Contact: Dr. Lorenz M Hilty, Swiss Federal Laboratories for Material Testing and Research (MPA) Lerchenfeldstr. 5, CH-9014 St Gallen, Switzerland
Tel: +41 71 2747 345
Fax: +41 71 2747 862
E-mail: lorenz.hilty@empa.ch
http://www.empa.ch/iep01)

VERTEBRATE MORPHOLOGY
International Congress of Vertebrate Morphology
21-26 July, Jena, Germany
(Contact: ICVN-6, Institute of Systematic Zoology and Evolutionary Biology, University of Jena, Erbertstrasse 1, D-07743 Jena, Germany
Tel: + 49 5641 949155
Fax: +49 5641 949152
E-mail : icvm-6@pan.zoo.uni-jena.de
http//icvm-6.zoo.uni-jena.de)

2002

HORTICULTURAL SCIENCE
XXVI Int'l Horticultural Congress: Horticultural Art & Science for Life
11-17 August, Toronto, Canada
(Contact: IHC c/o Congress Canada, 49 Bathurst St., Suite 100, Toronto, Ontario, Canada M5V 2P2
Ph: +1 (416) 504 4500
E-mail: IHCreg@congresscan.com
http://www.ihc2002.org)

ICSU
27th General Assembly of the International Council for Science & Associated Meetings
20-28 September, Rio-de-Janeiro, Brasil
(Contact: Larry Kohler, Executive Director, ICSU, 51 Bld de Montmorency, 75016 Paris, France
Ph: +33 (0)1 45 25 03 29
Fax: +33 (0)1 42 88 94 31
E-mail: secretariat@icsu.org
http://www.icsu.org)

ORNITHOLOGY
23rd Int'l Ornithological Congress
11-17 Aug., Beijing, China
(Contact: Prof. Xu Weishu, 1-1-302 Beijing Sci. and Tech. Commission, Apt. 30, Lingnan Rd. Beijing 100037, P.R. China. Ph/Fax: +86 (0)10 6846 5605
E-mail: abstract@ioc.org.cn
http://www.ioc.org.cn

PALEOBOTANY
6th European Paleobotany-Palynology Conference
29 August- 2 September, Athens, Greece,
(Contact: Prof. Evangelos Velitzelos, Dept. of Historical Geology and Paleontology, Faculty of Geology, University of Athens, 15784 Athens, Greece
Tel/ Fax: +30 1 727 4162
E-mail: velitzel@geol.uoa.gr)

PARASITOLOGY
Xth Int'l Congress of Parasitology
August, Vancouver, Canada
(Contact: Prof. M. Zia Alkan, Dept. of Parasitology, Medical Faculty of Ege Univ., Bornova-Izmir 35100, Turkey. Fax: +90 (0)232 388 134
E-mail: alkan@med.ege.edu.tr)

PATHOPHYSIOLOGY
4th Int'l Congress of Pathophysiology
29 June-5 July, Budapest, Hungary
(Contact: Prof. Lajos G. Szollar, Institute of Pathophysiology, Semmelweiss University Medical School, Budapest, P.O.B. 370, H-1445 Hungary)

SOIL SCIENCE
17th World Congress of Soil Science (WCSS)
14-21 August, Thailand
(The Secretariat, 17th Kasetsart University, PO Box 1048, Bangkok 10903, Thailand
Tel: (662) 9405787
Fax: (662) 9405788
http://www.17wcss.ku.ac.th)

2003

MEDICINAL AND AROMATIC PLANTS
3rd World Congress on Medicinal and Aromatic Plants for Human Welfare (WOCMAP-III)
3-7 February, Chiang-Mai, Thailand
(Contact: K. H. Baser, Secretary General, ICMAP, Anadolu University Medicinal and Aromatic Plant and Drug Research Center (TBAM), 26470 Eskiesehir, Turkey
Tel: +90 222 3352952
Fax: +90 222 3350127
E-mail: info@icmap.org
http://www.icmap.org)

IUBS
IUBS 28th General Assembly and Associated Scientific Symposia
September-October, Cairo, Egypt
(Contact: IUBS Secretariat, 51 Bld Montmorency, 75016 Paris, France
Tel: +33 (0) 1 45 25 00 09
Fax: +33 (0) 1 45 25 20 29
http:// www.iubs.org)

TRAINING COURSES 2001

China APEC Scientific and Technological Foundation International Training Course on Integrated Fish Farming
May-July, Qingdao, China
(Contact: Ms. Zhang Jinmei or Mr. Zhou Enhua, Asia-Pacific Regional Research and Training Centre for Integrated Fish Farming, Wuxi-City, Jiangsu Province 214081, P.R. China
Tel: +86 510 5555112/5569005
Fax: +86 510 5555112/5553304
Email: RLCC@public1.wx.js.cn)

ICRO/UNESCO TRAINING COURSES

The Use of Expression Systems for Studying Structure, Function and Regulation of Membrane Proteins
11-24 April, Shanghai, China
(Contact: Dr. Jian Fei, Shanghai Institute of Cell Biology, 320 Yue-Yang Lu, 200031 Shanghai, China
Fax: +86 21 6271 3169
E-mail: fei@guomai.sh.cn)

Gene Transfer in Plants - Cellular and Molecular Aspects
7-19 May, Kostinbrod, Bulgaria
(Contact: Prof. Atanas Atanassov, Institute of Genetic Engineering, 2232-Kostinbrod-2, Bulgaria
Tel: + 359 721 2552
Fax: + 359 721 4985
E-mail: geneng@mtel.net)

Frontiers in Reproduction: Molecular and Cellular Concepts and Applications
20 May-1 July, Woods Hole, Massachusetts, USA
(Contact: Ms. Carol Hamel, Admission Coordinator, 7 MBL Street, Woods Hole, Massachusetts 02543-1015, USA
Tel: (508) 289 7401
E-mail: admissions@mbl.edu)

Signalling to Growth and Cell Division in Arabidopsis
22-28 July, London, UK
(Contact: Dr. Bogre Laszlo, School of Biological Sciences, Royal Holloway, University of London, Egham, TW20 OEX, UK
Tel: +44 (0) 1784 434326
e-mail: l.bogre@rhbnc.ac.uk)

Ion Pumps and ABC Transporters Overexpressed in Yeast as Drug Targets
10-21 September, Cape Town, South Africa
(Contact: André Goffeau, Laboratoire de Génétique Moléculaire, Ecole Normale Superieure, 46 rue d'Ulm, 75230 Paris, France
Tel: + 33 1 44 32 30 41
E-mail: Goffeau@biologie.ens.fr)

Biotechnological Tools for Plant Improvement
10-21 September, Bahia Blanca, Argentina
(Contact: Dr. Viviana Echenique, Departemento de Agronomia (UNS), San Andrés 800, 8000- Bahia Blanca, Argentina
Tel: + 54 291 4595127
E-mail: echeniq@criba.edu.ar)

BIOLOGY INTERNATIONAL

The News Magazine of the International Union of Biological Sciences

Three regular issues of *Biology International* will be published in 2001. Free copies are offered to Ordinary and Scientific Members of the Union. The annual subscription rate for individuals is 45 Euros.

Signed articles express the opinion of the authors and do not necessarily reflect the opinion of the Editor of *Biology International*. Prospective authors should send an outline of the proposed article to the Editor, with a letter explaining why the subject might be of interest to readers.

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http://www.iubs.org

The International Union of Biological Sciences is a non-governmental, non-profit organisation, established in 1919. Its objectives are to promote the study of biological sciences, to initiate, facilitate, and co-ordinate research and other scientific activities that require international cooperation, to ensure the discussion and dissemination of the results of cooperative research, to promote the organisation of international conferences and to assist in the publication of their reports.

The membership of the IUBS presently consists of 43 Ordinary Members, adhering through Academies of Science, National Research Councils, national science associations or similar organisations, and 80 Scientific Members, all of which are international scientific associations, societies or commissions in the various biological disciplines.

National Adhering Organisations

ARGENTINA - Consejo Nacional de Investigaciones Cientificas y Técnicas
AUSTRALIA - Australian Academy of Science
AUSTRIA - Österreichische Akademie der Wissenschaften

BELGIUM - Royal Academy of Science, Letters & Arts
BRAZIL - Conselho Nacional de Desenvolvimento Cientifico e Tecnologico
BULGARIA - Bulgarian Academy of Sciences
CHILE - Sociedad de Biologia de Chile
CHINA - Association for Science and Technology, Beijing
CHINA - Academia Sinica, Taipei
CUBA - Academia de Ciencias
CZECH REPUBLIC - Czech Academy of Sciences
DENMARK - Det Kongelige Danske Videnskabernes Selskab
EGYPT - Academy of Scientific Research and Technology
FINLAND - Delegation of Finnish Academies of Sciences & Letters
FRANCE - Académie des Sciences
GERMANY - Deutsche Forschungsgemeinschaft
HUNGARY - Hungarian Academy of Sciences
INDIA - Indian National Science Academy
IRELAND - Royal Irish Academy
ISRAEL -Academy of Sciences and Humanities
ITALY - Consiglio Nazionale delle Ricerche
JAPAN - Science Council of Japan

LEBANON - National Scientific Research Council

MEXICO - Consejo Nacional de Ciencia y Tecnologia
MONACO - Centre Scientifique de Monaco
NETHERLANDS - Koninklijke Nederlandse Akademie van Wetenschappen
NEW ZEALAND - The Royal Society of New Zealand
NORWAY - Det Norske Videnskaps Academi
PHILIPPINES - National Research Council of the Philippines
POLAND - Polish Academy of Sciences
PORTUGAL - Ordem dos Biólogos
ROMANIA - Romanian Academy of Sciences
RUSSIA - Russian Academy of Sciences
SAUDI ARABIA - King Abdul Aziz City for Science & Technology
SLOVAK Republic - Slovak Academy of Sciences
SOUTH AFRICA - Foundation for Research Development
SPAIN - Comision Interministerial de Ciencia y Tecnologia Tecnologia
SWEDEN - Kungliga Vetenskapsakademien
SWITZERLAND - Swiss Academy of Sciences
TUNISIA - Association Tunisienne des Sciences Biologiques
UNITED KINGDOM - The Institute of Biology
U.S.A. - National Academy of Sciences- National Research Council

VENEZUELA - Consejo Nacional de Investigaciones Cientificas y Tecnicas