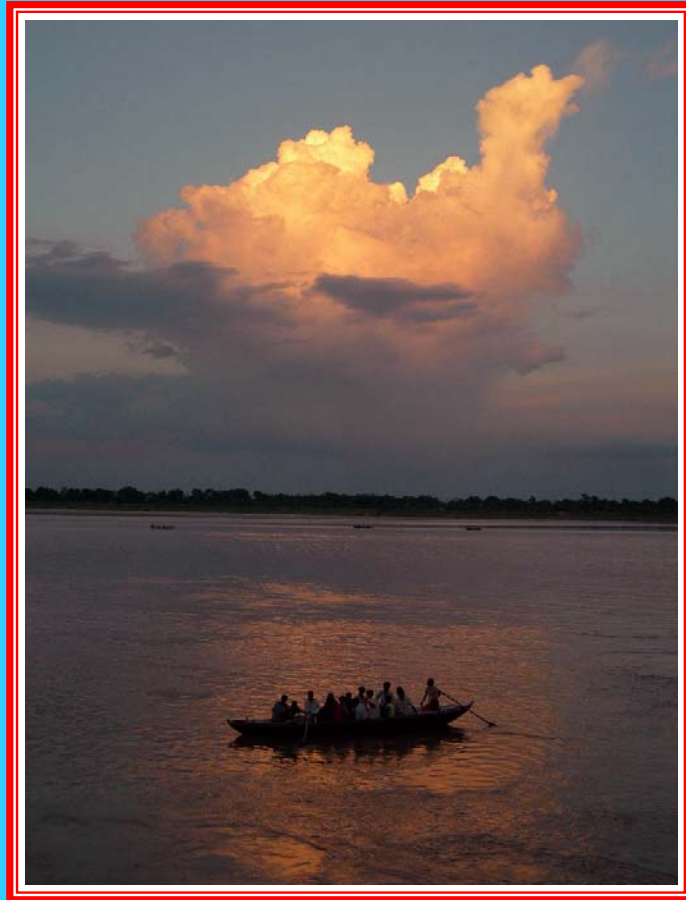


International Symposium
on
ENVIRONMENTAL FACTORS, CELLULAR STRESS AND EVOLUTION



Department of Molecular & Human Genetics
Banaras Hindu University
Varanasi 221 005, India

October 13-15, 2006

International Symposium
on
ENVIRONMENTAL FACTORS, CELLULAR STRESS AND
EVOLUTION

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ENVIRONMENTAL FACTORS, CELLULAR STRESS AND EVOLUTION

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Integrative Biology: a research and education paradigm for 21st century science

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Issues of great complexity face scientists, especially biologists, today. Many scientists, university departments, and institutes have taken the position that an 'integrative' approach is required to deal with complexity, no matter what 'part' of biology is emphasized. At the same time, there is no agreement on what 'integrative' biology is. Some believe that bringing together researchers of diverse expertise appropriate to the question being investigated, that is, multi-disciplinary teams, is integrative biology; others find that work on multiple taxa is integrative; still others accept that doing research that spans part or all of the hierarchy of biological organization is integrative biology.

A group of biologists, starting at Berkeley but now international in scope and affiliation, particularly facilitated by the International Union of Biological Sciences, is attempting to provide a general framework for the research and educational practice of integrative biology. We consider integrative biology to be both an attitude toward the practice of science and the practice itself. It encompasses the ideas mentioned above, but none alone is sufficient to be 'integrative biology'---all of them must be part of the conceptual framework, and then 'best practices' adopted for the study of complexity at hand. We find that many researchers are seeing the relevance of integrative approaches to their own research questions, and in fact a number have been intuitively practicing integrative biology throughout their careers. However, they often have not trained their students, either undergraduate or graduate, to be integrative biologists; rather, they have assigned them parts of complex questions so that they are well centered in a few concepts and techniques, but they lack the breadth of an integrative biologist.

We have articulated a set of general principles for integrative biology research and training. They are not complicated or restrictive; they are highly adaptable. We expect that much of research in biology, and science, including the social and humanities aspects of the applications of science, will reflect such principles during the current century, and we believe that the trend is well under way. We wish to give some coherence to the concept of integrative biology by offering ideas about principles and practices.

At the same time, because of constraints associated with techniques, rather than with ideas, research and especially teaching may be limited only to parts of the hierarchy of biological organization. Scientists who focus on the more organismal part of the hierarchy may look both 'downward' and 'upward'. We are approaching an integration of molecular and cellular, organismal, behavioral, ecological, and evolutionary biology in order to understand biology and its interactions. Integrative biology has a leadership role in the scientific exploration of complexity and social applications of science.

Molecular chaperones: stability and evolvability of cellular networks

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Molecular chaperones are inefficient enzymes, which have a large number of low-affinity interactions with hundreds of other proteins. Chaperones preferentially reside in the overlaps of various modules of protein-protein networks connecting hub proteins of these modules. In addition, chaperone-mediated weak links stabilize the protein, signaling and membrane-organelle networks in the cells. This central position gives an integrative role for the chaperones, efficiently de-coupling various network modules when stress occurs and damaged proteins become abundant. A similar reorganization of network modules during various diseases and aging may significantly modify the emergent properties of cellular networks contributing to the increased noise and disorganization of network responses in these states. Additionally, the increased noise leads to an increased sensitivity due to the phenomenon termed stochastic resonance. All these effects result in an increased diversity of the organisms. Based on the above properties, chaperones emerge as very sensitive regulators of the evolvability at the network level. In other words, chaperones smooth the rugged fitness landscapes and make hidden evolutionary possibilities accessible (1-6). Using the above properties, chaperone-related drugs affect a large variety of proteins and exemplify a novel class of efficient drugs acting at multiple targets. Network studies may help us to identify and design multi-target drugs, which may efficiently target polygenic diseases, and stabilize the cells in a simultaneous fashion (7).

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URLs: www.chaperone.sote.hu; www.weaklink.sote.hu

Evolution of heat shock regulation mechanisms in bacteria

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Bacterial species have developed genetic programs allowing them to rapidly adapt to drastic changes in their natural habitats including temperature, osmolarity, pH and oxidative stress. These genetic systems consist of a sensor able to register the appropriate environmental stressor, and a signal transduction system resulting either in the permanent or transient induction of a subset of genes the products of which allow adaptation to the adverse condition. Most research carried out over the last 20 years has been devoted to the understanding of the genetic programs allowing bacterial cells to respond to a sudden increase in temperature which occurs, e.g., when bacteria invade our body orally or by any other route. It turned out that two fundamentally different sensors have evolved termed direct and indirect sensor. The direct sensor consists of either an RNA or protein molecule that changes its conformation in a temperature-dependent way. It allows a high temperature response and ensures continued expression of the genes as long as the bacteria are exposed to the high temperature. In contrast, the indirect sensor consists of either a molecular chaperone or a protease able to sense non-native proteins, and the signal transduction pathway influences the activity of a transcriptional regulator. The heat shock response allows the transient expression of heat shock genes since the amount of non-native proteins is able to feedback to the sensor. Well-studied examples of direct RNA sensors are the *rpoH* and the *prfA* mRNA and of protein sensors the TlpA and the RheA repressors. Examples for indirect sensors are the DnaK chaperone system and the DegS protease of *Escherichia coli* and the HrcA/CIRCE and CtsR systems of *Bacillus subtilis*.

The role of stress proteins in responses of animals to environmental temperature variation

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Many organisms live in variable thermal environments, which pose substantial challenges to survival and reproduction. Understanding mechanisms by which animals respond to environmental variation has taken on new urgency, due to increasing effects of climate change on natural systems. I will discuss physiological responses to daily, seasonal, and regional variation in temperature in the willow leaf beetle *Chrysomela aeneicollis*, from populations living at the southern edge of their range at high elevations in the Sierra Nevada mountains, California, USA. Beetles experience extreme high and low temperatures in rapid succession during the short summer, while adults are mating and larvae developing. Daytime high temperatures routinely experienced by adults and larvae upregulate expression of a stress-inducible isoform of Hsp70. Hsp70 expression levels are greatest in the warmest drainages, and at lower elevations. Nighttime cold frequently causes mortality, though upregulation of Hsps during the day may afford protection from cold in some individuals. Regional warming observed since 1998 has coincided with local extinction of populations at low elevations, especially in the warmest drainage. In years prior to disappearance, Hsp70 expression levels of beetles living at these sites were the highest measured in this species. In these populations, variation at the glycolytic enzyme locus phosphoglucose isomerase (but not other polymorphic enzyme loci) correlates with differences in temperature between drainages. PGI allozymes differ in functional properties and Hsp70 expression and induction temperature differ among PGI genotypes. Differences in Hsp70 expression among PGI genotypes shows a high degree of phenotypic plasticity and corresponds in an adaptive way to differences in thermal tolerance and traits important for reproductive success (running speed and fecundity), both in the laboratory and in nature. This example demonstrates that variation in stress protein expression in natural populations may have profound impacts on fitness, and highlights the importance of Hsps for understanding how natural populations respond to climate change.

Studying the Ecological and Evolutionary Role of the Stress Responses in the Post Genomic Era

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The study of stress responses and evolution of stress tolerance is of interest to many scientific fields including biology, medicine and animal breeding. Like many other topics in biology, most investigations of the effects of and responses to stress exposures have been done on a limited number of model organisms and *in vitro* and/or the laboratory. Here much progress has been made in terms of identifying and describing beneficial and detrimental effects of stress, responses to stress and the mechanisms behind stress tolerance on various levels of biological organization from DNA to phenotypes. However, to gain further understanding of the importance, the interactions and the dynamics of responses to stress from an ecological and evolutionary perspective there is a need to combine studies on multiple levels of biological organization and to include ecological relevant traits and natural or semi-natural conditions. There is a wealth of information gathered in the laboratories that should be tested to verify the ecological and evolutionary significance of e.g. the thermal stress response and adaptation to environmental stress. Often this is not easy, however, there are several good examples where this has been done successfully using different approaches.

Specific and unspecific responses of plants to cold and drought stress

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Different environmental stresses of a plant may result in similar responses at the cellular and molecular level. This is due to the fact that the impacts of the stressors trigger similar strains and downstream signal transduction chains. A good example for an unspecific response is the reaction to stressors which impair the water relations of the plant tissue by cellular dehydration, e.g. drought, salinity and cold, especially frost. Frost resistant plant tissues tolerate freezing of the bulk of the cellular water, if it is exported from the protoplast into the apoplast before crystallization. Intracellular ice formation is lethal as it results in disintegration of the biomembranes. The stabilizing effect of liquid water on the membrane bilayer can be supported by compatible solutes and special proteins. Formation and accumulation of these compounds is induced by the strain itself (cold, drought) or by a signal preceding the incidence of the stress (e.g. decreasing day length induces cold hardening). At the metabolic level, osmotic adjustment by synthesis of low-molecular osmolytes (carbohydrates, betains, proline) can counteract cellular dehydration and turgor loss. Extremely hydrophilic proteins such as dehydrins are common products protecting not only the biomembranes in ripening seeds (late embryogenesis abundant proteins) but accumulate also in the shoots and roots of drought tolerant plants and during cold hardening. They are characterized by conserved amino acids motifs, like the K-, Ψ - or Y-segments. A high portion of random coil structures effects their exceptional water binding capacity and the conserved segments give rise to amphipathic α -helices which form lipid binding domains and thus can associate with, and protect lipid aggregates and hydrophobic domains of proteins. Accumulation of dehydrins can be induced not only by drought, but also by chilling, salinity, treatment with ABA and methyl jasmonate. Increasing the dehydration tolerance of transgenic plants expressing a heterologous dehydrin will be shown.

In addition to the more or less unspecific stress reactions, specific responses confer tolerance to strains which are associated with the primary stress, e.g. the decrease of the ambient temperature (cold stress) which causes an increase of the biomembrane viscosity and concomitant loss of function. Cold hardening therefore implies also reactions which result in a decrease of the membrane viscosity by increasing the ratio of membrane lipids to proteins, as well as the proportion of lipids with a low melting point.

Structure, Function and Regulation of Rice Hsp100 Proteins

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Hsp100 family proteins are ubiquitously distributed in plants. Western blotting experiments carried out using anti rice Hsp100 antibodies show that while heat stress to rice cause rapid accumulation of Hsp100 protein in varied cell types, high levels of this protein are present constitutively in rice embryos. *In silico* analysis of rice genome sequence from NCBI genome annotation initiative database, TIGR Rice genome database and KOME database reveals that there are five independent loci (namely Os05g44340, Os03g31300, Os04g32560, Os02g32520 and Os04g33210) that encode for OsHsp100 with various extent of homology to Hsp100 from other plant species. Hsp100 encoded by genomic locus Os05g44340 is the only species in rice that has been analyzed experimentally as of now (hereafter referred to as OsHsp100-1). *Oshsp100-1* cDNA encodes for 912 amino acid long protein (accession number - AF332981). Northern analysis carried out using *Oshsp100-1* 5'-UTR-specific probe as well as RT-PCR results show that expression of *Oshsp100-1* transcript is strictly heat-inducible in rice seedlings. Full-length *Oshsp100-1* cDNA complements yeast mutant disrupted for its own *hsp104* gene by insertional mutagenesis. Transgenic rice seedlings over-expressing *Athsp100* cDNA (corresponding to At1g74310 gene locus) possess increased high temperature tolerance. *Oshsp100-1* promoter (~2 kb) cloned upstream to *gus* gene, shows a clear induction of GUS expression in response to heat shock and heavy metal stress in transgenic rice cells. 5'-UTR of *Oshsp100-1* gene appears to have a role in translational regulation of the OsHsp100-1 expression. There are 24 entries in rice genome database for the heat shock transcription factors; however, detailed characterization of these proteins is yet to be undertaken.

Transgenics for abiotic stresses: targeting ion and glutathione homeostasis

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Salinity stress is a major threat to agriculture. We are adopting several established as well as novel strategies for raising plants with improved stress tolerance. Glyoxalase pathway enzymes viz. glyoxalaseI (glyI) and glyoxalaseII (glyII) are required for glutathione based detoxification of methylglyoxal (MG) which is a potent cytotoxic compound. We have earlier shown the potential of glyI as a probable candidate gene in conferring salinity tolerance. We further developed a technology involving both of these enzymes. Transgenic plants overexpressing glyII gene either alone or with glyI (double transformants) stably expressed the foreign protein and the enzyme activity was also higher. Compared to non-transformants, several independent glyII transgenic lines showed improved tolerance to high NaCl and were able to grow, flower and set seeds under salinity. The double transgenic lines always showed a response better than either of the single gene transformed lines. Ionic measurements in transgenic lines revealed higher accumulation of Na⁺ and K⁺ in old leaves and negligible accumulation of Na⁺ in seeds of the transgenic plants, thereby indicating that the seed quality is not compromised with the transgene overexpression under high salinity. Comparison of various growth and yield parameters demonstrated that there is hardly any yield penalty in the double transgenics under non-stress conditions and that these plants suffered only 5% loss in total productivity when grown in 200 mM NaCl. Maintenance of glutathione homeostasis is the possible basis for glyoxalase overexpression mediated enhanced salinity tolerance. The extended suitability of this engineering strategy for improved heavy metal tolerance in transgenic plants has also been recently demonstrated. These findings established the potential of manipulation of glyoxalase pathway for multiple abiotic stress tolerance in crop plants, which is being currently validated in rice in our laboratory. Another possible strategy which is being employed in our laboratory is the genetic engineering of NHX which is a sodium/proton antiporter and is known to be involved in ion homeostasis. A novel isoform of NHX has been cloned from pearl millet and overexpressed in rice and the transgenic plants have been analyzed for their enhanced stress tolerance. These strategies either alone or in combination may prove to be helpful in raising transgenic plants for saline areas.

Polyglutamine expansion in *Drosophila*: thermal stress and Hsp70 as selective agent

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Repetitive DNA sequences that encode polyglutamine tracts are prone to expansion and cause highly deleterious phenotypes of neurodegeneration. Despite this tendency, polyglutamine tracts ("poly-Qs") are conserved features of eukaryotic genomes. Poly-Qs are the most frequent protein-coding homotypic repeat in insect genomes, and are found predominantly in genes encoding transcription factors conserved from *Drosophila* through human. Although highly conserved across species, poly-Q lengths vary widely within species. In *D. melanogaster*, poly-Qs in 25 genes have more alleles and higher heterozygosity than all other poly-amino acid tracts. The heat shock protein Hsp70 is a principal suppressor of poly-Q expansions and may play a key role in modulating the phenotypes of the alleles that encode them. Hsp70 also promotes tolerance of natural thermal stress in *Drosophila* and diverse organisms, a role which may deplete the chaperone from buffering against poly-Q toxicity. Thus in stressful environments, natural selection against long poly-Q alleles more prone to expansion and deleterious phenotypes may be more effective. This hypothesis can be tested by measuring the phenotypic interactions between *Hsp70* and poly-Q transgenes in *D. melanogaster* undergoing natural thermal stress, an approach which integrates comparative genomics with experimental and ecological genetics.

Heat shock protein 90 and cancer: new roles and methods of regulation

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Heat shock protein 90 (Hsp90) is a molecular chaperone whose association is required for stability and function of multiple mutated, chimeric, and over-expressed signaling proteins that promote cancer cell growth and/or survival. Hsp90 client proteins include telomerase, mutated p53, Bcr-Abl, Raf-1, Akt, HER2/Neu (ErbB2), mutated B-Raf, mutated EGF receptor, and HIF-1alpha. Hsp90 inhibitors, by interacting specifically with a single molecular target, cause inactivation, destabilization and eventual degradation of Hsp90 client proteins, and they have shown promising anti-tumor activity in multiple preclinical tumor models. One Hsp90 inhibitor, 17-AAG, is currently in Phase II clinical trial and other inhibitors will shortly be entering the clinic. Like 17-AAG, most small molecule Hsp90 inhibitors bind to an identical N-terminal domain, but additional target-based screening has uncovered novobiocin as a structurally distinct small molecule inhibitor that recognizes a unique C-terminal motif in Hsp90. Small molecule inhibitors of Hsp90 have been very useful in understanding Hsp90 biology and in validating this protein as a molecular target for anti-cancer drug development. Recently, cell-impermeable Hsp90 inhibitors have revealed an important role for cell surface Hsp90 in tumor cell migration and metastasis. Thus, small molecules or antibodies targeting Hsp90 may have distinct anti-metastatic activity *in vivo*.

Hsp90 function depends on its ability to cycle between alternative conformations and to associate with distinct co-chaperone proteins that modulate this process. Hsp90 cycling is regulated in part by ATP binding and hydrolysis. Recently, however, post-translational modification of Hsp90 has been shown to play an important role in regulating the chaperone activity of this protein. These post-translational modifications include phosphorylation and acetylation, and we will discuss in detail how each process individually is a key determinant of Hsp90 activity both in mammalian cells and in yeast. From this review it will be clear that Hsp90 regulation in a cellular context is a very complex process. It is also likely that the cellular milieu in which Hsp90 exists helps to determine the chaperone's sensitivity to pharmacologic inhibition. Data will be presented to support the hypothesis that, to be effective, small molecule Hsp90 inhibitors need only interact with a small but phenotypically distinct pool of the chaperone. These observations may help explain the differential sensitivity of tumor and non-transformed cells to Hsp90 inhibition.

Protein misfolding and stress response: lessons from lymphocytes

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Ageing is characterized by a continuous accumulation of macromolecular, including protein damage, thought to be mainly induced by reactive oxygen species. Oxidized protein level increases exponentially with aging of all animal species. While modifications involving the highly susceptible sulfur-containing amino-acids are readily reversible by different enzyme systems, the majority of glycoxidative modifications to proteins are irreparable at the level of the primary sequence. However, they can either be repaired at the level of conformation or channeled to proteolytic routes.

Both function are provided by molecular chaperones. Chaperones are ubiquitous, highly conserved proteins playing a major role in the conformational homeostasis of cellular proteins. Diverse functions of chaperones in cells include (1) proper folding of nascent polypeptide chains, (2) facilitating protein translocation across various cellular compartments, (3) modulating protein activity via stabilization and/or maturation to functionally-competent conformation, (4) promoting multiprotein complex assembly/disassembly, (5) refolding of misfolded proteins, (6) protecting against protein aggregation, (7) targeting ultimately damaged proteins to degradation, (8) sequestering damaged proteins to aggregates, (9) solubilizing protein aggregates for refolding/degradation. Chaperones work in concert with co-chaperones and regulate local protein and signalling networks of the cell.

We summarize the current view of protein damage and turnover on ageing, with a special emphasis on the role of the stress response in longevity. Recent data on chaperone levels, function in ageing will be presented. Our ongoing experiments on lymphocytes from donors of different ages will be shown.

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Heat shock protein-mediated release: mechanisms and biological significance

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Heat shock proteins (HSP) are intracellular soluble proteins and despite the fact that they do not have a transmembrane domain, proteins like the sixty kilo-Dalton heat shock protein, HSP60, the seventy kilo-Dalton heat shock protein, HSP70 or the glucose regulated protein, gp96 are transported to the cell membrane following various kinds of stress including heat shock and infections. Currently there are two schools of thought as to how intracellular HSPs are released from cells into the extracellular milieu; a passive mechanism based on cell/tissue necrosis or trauma and active mechanisms based on controlled physiological release. Independent studies from several labs have shown that extracellular HSPs exert powerful immuno-stimulatory effects on the host's immune system. Recently this knowledge has been harnessed to develop various HSP-based therapies against cancer, viral infection, neuro-degeneration, tissue transplantation, cerebral and cardiac ischemia and ulcers. In order to develop even more powerful HSP-based therapies a clear understanding of the exact mechanism is required. This presentation will give a broad perspective of the current mechanisms of HSP release and discuss its biological significance.

Systems analysis of heat shock protein 90 from *Plasmodium falciparum*.

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Heat shock protein 90 (Hsp90) is a highly conserved molecular chaperone that is important for signal transduction, cell cycle regulation and control of gene expression in eukaryotes. Many Hsp90 client proteins are protein kinases and transcription factors resulting in a key role for Hsp90 in cell growth, differentiation, and apoptosis. We have been studying Hsp90 function in the malarial parasite, *Plasmodium falciparum*, using a combination of cell biological, pharmacological as well as computational approaches. Earlier work from our laboratory has demonstrated an essential role for PfHsp90 in the development of the parasite within human erythrocytes using geldanamycin (GA), a specific inhibitor of Hsp90 function. However, the precise molecular mechanisms of PfHsp90 function have not been deciphered. Towards better understanding the functions of PfHsp90 within the parasite, we have carried out an *in silico* analysis of the PfHsp90 interactome and examined its interconnectivity with other parasite chaperones by constructing a parasite chaperone interaction network. Analysis of the chaperone network reveals the presence of stress related as well as constitutive chaperone systems in the parasite. Examination of the PfHsp90 interactome predicts an important role for PfHsp90 in cell cycle progression, protein translation and stress tolerance within the parasite. Earlier work from our laboratory has suggested a role for PfHsp90 in modulation of parasite growth in response to environmental temperature. We have mathematically modeled the effect of repeated heat shock on Hsp90 dependent protein folding pathways within the cell. Using the model, we have also simulated the effect of GA on Hsp90 function in an attempt to understand the mode of action of GA on the parasite. In addition, we have carried out homology modeling of the structure of PfHsp90 in order to identify features that may contribute to our understanding of PfHsp90 function. Our work simulates the effect of repeated febrile episodes on PfHsp90 regulated pathways in the cells and predicts cellular processes likely to be regulated by PfHsp90.

Potassium signaling in bacteria: the cyano-deino story

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Potassium (K^+), the dominant intracellular cation, is required for several physiological processes like turgor homeostasis, pH regulation etc. In *E. coli*, a potassium dependent ATPase (KdpATPase), encoded by the *kdpFABC* operon, is an inducible high-affinity K^+ transporter synthesised under severe K^+ limitation or osmotic upshift. The *E. coli kdp* expression is transcriptionally regulated by the KdpD and KdpE proteins, which together constitute a two-component signal transduction system. The KdpD is a membrane bound sensor kinase with a cytosolic N-terminal domain (NTD) and a cytosolic C-terminal domain (CTD) interconnected by four transmembrane segments, while the KdpE is a cytosolic response regulator.

The Kdp system is widely dispersed among the different classes of bacteria including the cyanobacteria. The cyanobacterium *Anabaena* L-31 possesses, not one but, two *kdp* operons i.e. *kdp1* (consisting of *kdpA1B1G1C1D*) and *kdp2* consisting of (*kdpA2B2G2C2*) (1). The genome of radioresistant bacterium *Deinococcus radiodurans* shows the presence of a *kdp* operon with an unusual gene arrangement, *kdpBACD*. Of the two *kdp* operons, the *kdp2* is the major operon expressed in *Anabaena* L-31, in response to K^+ starvation and desiccation. In contrast to enteric bacteria, the *Anabaena kdp2* expression is not enhanced in response to osmotic stress (1).

The *kdpD* ORFs of *Anabaena* L-31 and *D. radiodurans* are naturally truncated when compared to the *kdpD* of other bacteria, while a *kdpE*-like gene is absent in these microbes. The short KdpD proteins show homology to the KdpD-NTD of *E. coli* KdpD, while CTD, the actual histidine kinase domain, is absent. The naturally short KdpD protein can be detected in *Anabaena* L-31 membranes (2). In *in vitro* experiments, the *Anabaena* L-31 KdpD could modulate the *E. coli* KdpD-CTD phosphatase activity when both the proteins were co-expressed in *trans*. The *E. coli* KdpD-CTD was also co-eluted with the *Anabaena* L-31 KdpD protein, indicating the ability of *Anabaena* L-31 KdpD to physically interact with *E. coli* KdpD-CTD (2).

A chimeric *kdpD* gene coding for AnacoliKdpD protein, in which *Anabaena* L-31 KdpD replaced the first 365 amino acids of *E. coli* KdpD, was expressed in *E. coli*. *In vitro*, the AnacoliKdpD protein was autophosphorylated and transferred the phosphoryl group to *E. coli* KdpE. The chimeric protein could also induce *kdpFABC* expression in response to K^+ limitation and osmotic upshock in *E. coli* (3). The presentation will discuss the expression of bacterial *kdp* operons in response to various environmental stress conditions, with special emphasis on potassium signaling in *Anabaena*.

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Multiplicity of heat stress transcription factors controlling the complex heat stress response of plants

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Heat stress proteins (Hsp) and heat stress transcription factors (Hsf) are structurally and functionally conserved elements of the eukaryotic heat stress response. Compared to other organisms, plants have much more elaborate systems of chaperones and at least 21 transcription factors controlling their expression. I will concentrate on the intriguing coexistence of functionally different types of plant Hsfs and show results on their interaction and cooperation in three phases of stress gene regulation: triggering, maintenance and attenuation with restoration of house-keeping gene expression.

In particular, tomato HsfB1 represents a novel type of general coactivator cooperating with class A Hsfs, e.g. with the tomato master regulator HsfA1. Both assemble into an enhanceosome-like complex resulting in strong synergistic activation of reporter gene transcription. HsfB1 also cooperates with other acidic activators, e.g. with the TGA bZip activator proteins binding to the cauliflower mosaic virus 35S promoter or with yet unidentified activators controlling house-keeping gene transcription. By these effects, HsfB1 can ensure the maintenance and/or restoration of transcription of viral or house-keeping genes during ongoing hs.

A recent new addition to this network of Hsfs is the interaction of HsfA4 as antiapoptotic Hsf with its repressor HsfA5. Both are highly selective in their interaction and do not mix with any other of the class A Hsfs.

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Basal transcription machinery: role in regulation of stress response in eukaryotes

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The basic transcription machinery has been conserved in evolution and its complexity is seen to increase tremendously from the simplest biological entities like viruses to the complex eukaryotic organisms. In prokaryotes, the sigma subunits of RNA polymerase dictate specificity towards the promoter while in eukaryotes this function of the sigma subunit appears to be distributed among several protein components that work as the basal transcription factors mediating interaction of the polymerase with the basal promoter. Most of the studies point to gene specific transcription factors and other ancillary factors rather than the subunits of the basal transcription machinery to be involved in transcriptional regulation.

While the transcription machinery and the response to a stress like heat shock are conserved, stresses like starvation and other environmental stressors elicit complex and varied responses dependent on the organism in question. Interestingly, it has been noted in bacteria that when an organism responds to one stress, it often shows ability to cope with other stresses as well and indeed, a particular stress is able to cause induction of genes required to function in response to an unrelated stress. In evolutionary terms, this may be justified in that a cell which encounters one stress is likely to face another stress and the chance of survival would be better if the given cell is prepared to cope with more than one stress at the same time. Mechanistically, it might mean that the regulation of stress responses is linked so that once the mechanism is activated by one stress, the cell might be predisposed for efficient activation in response to another stress. In prokaryotes, a stress sigma factor, σ^S , seems to regulate several stress response genes in conjunction with stress specific regulators. What would such a factor, a counterpart of σ^S , be in eukaryotic transcription machinery that is closest to the core polymerase and would dictate transcription of stress regulated genes in general? In this review we discuss the logic behind the suggestion that as in prokaryotes, eukaryotes also have a common functional unit in the transcription machinery through which the stress specific transcription factors regulate rapid and highly controlled induction of gene expression associated with generalized stress response and point to some candidates that would fit the bill for the eukaryotic equivalent of σ^S .

Heat shock transcription factors (HSFs) and stress induced cell death pathways in a rat histiocytoma

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HSFs are highly conserved transcriptional factors, which regulate the transcription of heat shock genes. Between the two major HSF isoforms, HSF1 regulates the induced transcription of heat shock genes whereas HSF2 has been reported to function under non-stress conditions especially during development and differentiation. Since posttranslational modification of HSFs is mandatory for the induced transcription of HSPs, induced HSF expression alone was thought to be dispensable. A rat histiocytic tumor cell line, BC-8 failed to synthesize HSPs upon sub-lethal heat shock and in the absence of inducible HSP synthesis these cells undergo apoptosis which is both extrinsic and intrinsic activation of apoptotic pathways. Mild heat shock induced only HSF2 expression and its DNA-binding activity that is associated with the activation of survival death pathway, chaperone-mediated macroautophagy. Similarly anticancer drugs such as geldanamycin/17AAG induced both HSF1 and HSF2 expression but the DNA-binding activity was observed only with HSF2 but not HSF1, however cells subsequently have gone through intrinsic apoptosis. Activation of apoptosis was correlated with the induction of reactive oxygen species, increased intracellular calcium and a change in mitochondrial membrane permeability transition. Activation of autophagy was correlated with the expression of lysosomal marker *lamp2a* and formation of autophagosomes. Under sub-lethal stress conditions compromising HSF1 by antisense oligonucleotides increased oxyradicals and damage to mitochondria, whereas compromising HSF2 has no effect. In contrast under mild stress conditions compromising HSF2 inhibited macroautophagy however cells subsequently experienced necrosis. It seems that HSFs do play a role in the regulation of cell death pathways acting as sensors of the changes in the intracellular environment. For the first time we demonstrate that HSF2 is inducible by anticancer drugs or heat shock and is required for the activation of survival pathways under stress conditions.

Role of heat shock genes in apoptosis

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Heat shock induced gene expression helps limit the consequences of stress-induced damage and facilitates cellular recovery. Hsps normally act as 'chaperones' ensuring proper folding of nascent polypeptide chains and modulate protein activity by changing protein conformation. In addition, they also shuttle proteins between cellular compartments and regulate protein degradation. Evidences accumulated over the past decade have revealed roles for Hsps in regulation of cell survival by modulating apoptosis induced by both the intrinsic and extrinsic pathways. Amongst the various Hsp gene families, Hsp70 is the most widely known anti-apoptotic player. The chaperoning functions of Hsp70 family proteins are required to inhibit translocation of Bax into mitochondria, the release of cytochrome-C, formation of apoptosome and activation of initiator caspase. Independent of the chaperoning activity, Hsp70 family members are also known to modulate JNK, NF- κ B and Akt signaling pathways involved in regulating the key players of the apoptotic cascade. Hsp90, though not required for either maturation or maintenance of most proteins, acts *in vivo* as a chaperone for unstable signal transducers and keeps them poised for activation. It interacts with RIP-1 kinase and Akt and promotes NF- κ B mediated inhibition of apoptosis. Hsp27 exerts its anti-apoptotic influence by inhibiting cyt-C and TNF-mediated cell death. Hsp α B-crystallin suppresses caspase-8 and cyt-C mediated auto-activation of caspase-3. In contrast to the anti-apoptotic roles of the major heat shock proteins, Hsp60 plays both anti- and pro-apoptotic roles in the cell. Cytosolic Hsp60 forms a complex with the pro-apoptotic protein Bax, thereby preventing it from being translocated into the mitochondria. On the other hand, it promotes maturation of procaspase-3 resulting in cell death. Apart from Hsps, some noncoding RNAs, like miRNA, are also known to modulate the apoptotic response in *C. elegans*, *Drosophila* and HeLa cell lines. Our recent *in vivo* studies have demonstrated pro-apoptotic roles for the *Hsp60D* and the noncoding *hsw* genes of *Drosophila melanogaster*.

Chaperone Hsp90 as a molecular mechanism of genetic and environmental canalization

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We have demonstrated that manipulation of Hsp90 results in the expression of altered phenotypes, which are partially due to uncovering of normally hidden genetic variation. Neither the frequency nor the identity of such “buffered” genetic polymorphisms in natural populations have yet been assessed.

We have performed a pilot study using geldanamycin to inhibit Hsp90 to assess the potential of quantitative genetics to identify Hsp90 buffered polymorphisms. Specifically, we undertook QTL analysis of an *A. thaliana* developmental response, hypocotyl elongation in the dark. Our study identified two novel genomic regions (QTLs) contributing to hypocotyl length. Fine mapping of one QTL revealed that multiple loci responded to a decrease in Hsp90 function, suggesting that such loci may be frequent. Analysis of hypocotyl elongation across 60 divergent accessions discovered previously unknown correlations to geographical factors such as latitude and environmental factors such as temperature variance and yielded association data supporting our linkage analysis.

As the light sensitivity of geldanamycin precludes its use for the analysis of most other traits, we have created a set of 120 recombinant inbred Col/Ler lines that are genetically reduced in Hsp90 through an RNAi approach and a corresponding control set. These lines have been analyzed for a variety of life-history traits. Our preliminary data suggest the existence of novel Hsp90 buffered loci in several previously studied traits such as flowering time. To generate high-resolution physical maps for these 240 RILs, we have developed a high-throughput affordable microarray-based mapping technique using Insertion and Deletion markers, producing maps with markers spaced on average at 2 cM intervals. Data on mapping of Mendelian mutants in F2 populations and suitability of the array for other RIL populations will be presented.

Developmental plasticity in *Bicyclus* butterflies as a response to alternating seasons with different levels of environmental stress

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Abstract: Invertebrates faced with the challenge of persisting through alternating wet and dry seasons in the tropics have frequently evolved developmental plasticity as an adaptive response to the temporal variation in the environment. *Bicyclus* butterflies in Africa exhibit seasonal polyphenism with alternating adult generations of a wet season form and dry season form. These differ in wing pattern but also show numerous other adaptations, either to a favourable (wet) season in terms of resources or to one (dry) that is more stressful. This divergence has led us to examine not only the bases of the developmental plasticity in wing pattern in a model species, *B. anynana*, but also the evolution of key life history traits including adult starvation resistance and longevity. This has been done both in terms of the processes that generate variation and plasticity in the phenotype and in the ecological context of adaptive responses to variation in the occurrence of environmental stress.

Integrative biology of stress: molecular actors, the ecological theater, and the evolutionary play

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Stress, whether in natural environments or in disease, is of special interest to biological investigators because it can reveal both challenges and mechanisms obscured in more moderate regimes. One view of stress is that it limits biodiversity, survival, and evolution by overwhelming defense mechanisms and genetic variability. A contrasting view is that stress enhances biodiversity, survival, and evolution by engendering novel mechanisms and combinations of genes not evident under "normal" conditions. I will review the evidence for each view, and advocate a synthetic approach that seeks to reconcile them.

URL: http://pondside.uchicago.edu/~feder/Martin_Feder.html