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Molecular biology of stress responses in India

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Vaasansi jeernani yathaa vihaaya, navaani grihnaati naroparaani
Tathaa shareeraya vihaaya jeerna, nanyaati sanyaati navaani dehee.

Just as a person sheds old clothes for new ones, similarly,
the eternal being sheds old bodies and acquires new ones.
(from the Indian philosophical book, *Gita*)

INTRODUCTION

A meeting on the molecular biology of stress responses organized by Subhash C. Lakhotia and Wolfgang Schumann was held 14–17 October 1997 in the 3000-year-old holy city of Varanasi, India. The meeting drew approximately 100 participants from 11 countries and provided an excellent opportunity to discuss some of the latest developments in molecular stress research in a relaxed atmosphere.

HEAT SHOCK RESPONSE IN EUBACTERIA

The heat shock response in *Escherichia coli* depends primarily on σ^{32}, a specific heat shock transcription factor encoded by the *rpoH* gene. A detailed comparative analysis of *rpoH* homologs from seven Gram-negative bacteria by T. Yura (*tyura@hsp.co.jp*) suggested that the chaperone-mediated feedback control involving region C and the Q(R/K)(K/R)LFFNLR 'RpoH box' evolved as a primary regulatory strategy early during σ^{32} evolution, whereas the translational control mediated by mRNA secondary structure was adopted as an additional strategy during evolution of γ proteobacteria (McCarty et al 1996; Yura 1996). W. Schumann (*wolfgang.schumann@uni-bayreuth.de*) reported suggestive evidence that the GroE chaperonin machine of *Bacillus subtilis* enhances the activity of the HrcA repressor protein. This helps in the termination of the heat shock response by enhancing the binding of the HrcA repressor to the *CIRCE* element that shuts down the synthesis of the class I heat shock genes, *dnaK* and *groE* (Mogk et al 1997). Class II heat shock genes of *B. subtilis* are regulated by the alternative σ factor, σ^B. A detailed description of the stress response of the eight-gene σ^B regulon was presented by U. Voelker (*voelker@su1701.biologie.uni-marburg.de*). For the eight genes, six regulators of σ^B activity are arranged in two partner-switching modules each consisting of a phosphatase, an antagonist and a switch protein/kinase (Hecker et al 1996; Voelker et al 1996, 1997).

SALT STRESS AND OTHER STRESSORS IN BACTERIA

D. Rudulier (*leruduli@unice.fr*) gave an excellent introduction to the osmoprotection mechanisms used by various bacteria. He also presented some recent findings on the pathways of *Sinorhizobium meliloti* for the synthesis of the osmoprotective glycine betaine and choline-O-sulfate (Pocard et al 1997). E. Bremer (*bremer@mailer.uni-marburg.de*) summarized his group's data on the synthesis and uptake of glycine betaine by *B. subtilis* (Boch et al 1996; Kappes et al 1996). He also emphasized

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Figure A devotee offers holy Ganga water to the Sun God (photograph of Ashok Khanna) Reproduced from the book *Banaras* with kind permission of Rupa & Co. 7/16 Ansari Road, Daryaganj, New Delhi 110 002, India.

the importance of the heretofore uncharacterized stretch-activated channels ensuring the efficient and fast efflux of osmoprotective molecules when water suddenly arrives after the salt stress periods. A. Tripathi (*anil@banaras.ernet.in*), who did an excellent job in the organization of the meeting as its Convener, talked about the salinity stress responses of *Azospirillum* giving details on the function of the periplasmic glycine betaine-binding protein, GBBP (Tripathi and Mishra 1998). A. R. Strom (*arnestr@kjemi.unit.no*) compared the uptake and synthesis of glycine betaine by *E. coli* with the synthesis of the 'backup' osmoprotectant of this organism, trehalose (Rokenes et al 1996; Lamark et al 1996). S. K. Apte (*cbd@magnum.barc.ernet.in*) gave a detailed account on the stress responses of the nitrogen-fixing cyanobacteria, *Anabaena* strains, including its more than 15 osmotic stress proteins, 12 heat shock proteins and 20 potassium deficiency-induced proteins (Apte et al 1997; Ramani and Apte 1997). Interestingly, an *E. coli* GroEL-specific antibody detects four different polypeptides in *Anabaena* ranging from 57 to 61 kDa in molecular mass. Lon (also called La) plays a primary role in the degradation of abnormal proteins in a great variety of stressed bacteria. M. Bazzicalupo (*marcobazzi@dbag.unifi.it*) reported that Lon-deficient *Azospirillum brasilense* cells survive heat shock better, which may indicate its involvement in the control of the heat shock response in this organism (Mori et al 1996). V. Braun (*volkmar.braun@mikrobio.uni-tuebingen.de*) and H. Ohtake (*hohtake@ipc.hiroshima-u.ac.jp*) gave excellent summaries of the bacterial iron and phosphate uptake mechanisms, respectively. Bacterial adaptation to cold temperatures was discussed by M. K. Ray (*malay@ccmb.globemail.com*) and S. K. Mahayan (*skmaha@magnum.barc.ernet.in*). S. Srinivasan (*s.srinivasan@unsw.edu.au*) talked about the starvation survival of non-differentiating bacteria and R. Chowdhury (*iichbio@giascIO1.vsnl.net.in*) reported interesting data on the virulence of *Vibrio cholerae* that emphasized the importance of using more in vivo experimental systems like isolated segments of rabbit ileum for studies of the etiology of *V. cholerae* infections.

HEAT SHOCK FACTORS – EUKARYOTIC STRESS RESPONSE

S. H. Sanjeev (*ssatyal@casbah.acns.nwu.edu*), from Rick Morimoto's lab, reported the cloning of heat shock factor binding protein 1 (HSBP1), a novel conserved 76-amino acid protein containing two arrays of hydrophobic repeats that interact specifically with the heptad repeats of heat shock factor 1. HSBP1 attenuates the activity of heat shock factor 1 in both in vitro and in vivo experiments. P. Sadhale's (*pps@mcbl.iisc.ernet.in*) studies raised the possibility that the RBP4 and RBP7 subunits of RNA-polymerase II of *Saccharomyces cerevisiae* may link the stress-specific transcription factors to the basal transcription machinery. K.-D. Scharf and L. Nover (*nover@cellbiology.uni-frankfurt.d400.de*) gave a detailed account of the one constitutive and two inducible plant heat shock factors HsfA1 and HsfA2/B1, respectively. Participants of the workshop were happy to receive the AHA-experience by learning the activator function of the *A*romatic-*H*ydrophobic-*A*cidic element in the C-terminal region of these transcription factors. It was also interesting to learn that HsfA2 relocates to cytoplasmic heat stress granules, 40 nm aggregates thought to be involved in the recovery of housekeeping mRNA after heat shock (Nover and Scharf 1997). U. K. Srinivas (*ushaks@ccmb.globemail.com*) described the surprising finding that albumin is a heat shock protein in early embryonic rat liver.

Though the Indian philosophical book *Gita* describes our eternal being as one which 'sheds old bodies and acquires new ones', our normal cells do not seem to fit perfectly to this definition during their whole life span. Mammalian cells cultured in vitro initially proliferate but stop dividing after a finite number of divisions, a phenomenon termed replicative senescence. In addition, it has been observed that cells from progressively older animals undergo progressively fewer divisions in culture before undergoing senescence, suggesting that there is a correlation between the age of the animal and the in vitro life span of the derived cells. Since cells from different animal species appear to undergo a relatively constant number of divisions and the number of population doubling of a given cell type is highly reproducible, it has been suggested that cellular senescence may be a programmed event controlled by the expression of a set of genes. E. S. Gonos (*sgonos@eie.gr*) suggested that constant, greater stress may accelerate the clock of cellular

senescence, while lesser, repeated stress may slow it down or reset it (Gonos et al 1996; Derventzi et al 1996; Toussaint et al 1998).

Studies of D. N. Deobagkar (*dndeo@unipune.ernet.in*) showed that induction of heat shock proteins by the stress response of mosquitoes results in an increased competence of the mosquito to carry a higher load of parasites of malaria, dengue, filariasis and other diseases. These findings must serve as a serious warning against the indiscriminate use of insecticides which might, in fact, enhance vectorial capacity of adapted mosquitoes. D. Pomerai (*plzddp@pln1.nott.ac.uk*) presented some very interesting data showing that microwave radiation at frequencies and power levels comparable to those used in mobile phones induce a profound stress response by a non-thermal proteotoxic mechanism (Daniells et al 1998). D. Karchowdhuri (*i.t.r.c.@vikram.doe.ernet.in*) summarized studies on the effects and possible mechanisms of lead poisoning of mice.

MOLECULAR CHAPERONES

R. Tanguay (*rmtang@rsvs.ulaval.ca*) reported a novel link between molecular chaperones and protein degradation by demonstrating the interaction of small heat shock proteins of *Drosophila melanogaster* with UBC9, a ubiquitin-conjugating enzyme. A. Richardson (*alexandra.richardson@medecine.unige.ch*), from Costa Georgopoulos's lab, in collaboration with Samuel J. Landry analyzed the structure and function of Gp31, a bacteriophage T4-encoded co-chaperonin of the host GroEL complex. While Gp31 is able to substitute for GroES, GroES can not function as Gp31, most probably because of its roof loop resulting in a smaller opening on the top of the GroES-heptamer. Another loop structure on the 'bottom' of Gp31 connects the co-chaperonin to GroEL and contributes to the specific interaction of Gp31 with its target, Gp23, the major capsid protein of the phage. C. K. K. Nair (*rbbd@magnum.barct1.ernet.in*) reported the characterization of the *dnaK* locus in *Halorefax mediterranei*.

The 90 kDa chaperone family gained quite a lot of attention at the meeting. P. Csermely (*csermely@puskin.sote.hu*) summarized the status of our present knowledge on Hsp90 emphasizing that the vast abundance of this protein in most of the resting cells might imply its involvement in building and reorganizing the cellular architecture of the cytoplasm and possibly the cell nucleus. Recent data from a large number of laboratories confirmed that Hsp90 is indeed a low-affinity ATP/ADP-binding protein and that nucleotides may play a role in the regulation of Hsp90 protein complexes. J. K. Pal (*jkpal@unipune.ernet.in*) presented nice data demonstrating that Hsp90 interacts with the heme-regulated eIF-2α kinase during stress and that this association leads to the activation of the kinase and thereby to the inhibition of translation initiation (Pal et al 1996; Xu et al 1997). The *hsp83* locus is known to be involved in the signalling of several kinases such as those encoded by *sevenless* and *torso* in *Drosophila*. *hsp83* also interacts with *hsr-omega*, a stress-inducible non-protein coding gene of *Drosophila*. Data from S. C. Lakhotia's (*lakhotia@banaras.ernet.in*) lab show that the *hsp83*, *hsr-omega* and the *ras Roughened* loci genetically interact with each other, which suggests that the *hsr-omega* RNA transcripts may be involved in the ras-related signalling pathway (Lakhotia and Ray 1996). P. K. Srivastava (*srivastava@nso2.uchc.edu*) summarized his pioneering work on heat-shock protein-peptide complexes as participants in antigen presentation by MHC molecules. Evidence was presented showing that Grp94 is a calcium-dependent aminopeptidase – a property that is consistent with its proposed role in ER peptide trimming. Grp94 (and to a lesser extent Hsp70 and Hsp90) most probably carries the 'peptide fingerprint' of the parent cell and therefore can be used for the immunization of animals against their own tumors. Corresponding studies of cancer patients are in progress: results from a pilot study show that such vaccination is feasible, non-toxic and elicits tumor-specific immunological responses (Blachere et al 1997; Tamura and Srivastava, 1997).

PLANT STRESS

The expression pattern of the 90-kDa heat shock proteins in rice has been examined by A. Grover (*pmb@dusc.ernet.in*) and co-workers (Pareek et al 1997). Induction of a 110 kDa homologue of the yeast Hsp104 (termed OsHSP110) can be also observed in these plants after heat shock and a great variety of other stresses such as salinity, desiccation, low temperature stress, etc. OsHSP110 is also regulated during development. Rice also contains another related stress protein with a molecular weight of 104 kDa (termed OsHSP104 or SAP104), which has a homologous ATP-binding region to OsHSP110 and to the yeast Hsp104 (Singla et al 1997). Engineering studies of polyamine and carbohydrate metabolism to achieve higher stress tolerance against osmotic stress were reported by M. V. Rajam (*gen@duscernet.in*). K. C. Upadhyaya (*kailash@jnuniv.ernet.in*) presented some interesting data showing that the *Arabidopsis ACaM5* calmodulin is a heat shock protein. The *ACaM5* gene was also induced by touch, irrigation, water spray, light and UV both in transgenic tobacco plants and in DNA-protein interaction assays using protein extracts from *Brassica* tissues. One of the take-home messages from the sessions dealing with plant stress was the warning made by L. Nover, A. Strom and others that stress-resistant transgenic plants may not be

as potentially lucrative as they seem at first glance if stress tolerance is achieved by the overproduction of compounds that significantly affect the taste or odour of the tissues involved. Special care must be taken to ensure that the accumulation of the protectant does not affect the part of the plant used for nutrition. Ideally, its level would return close to normal by the time of the harvest.

PROCEEDINGS VOLUME AND STRESS ORGANIZATION

The proceedings volume of the workshop will be published as a special issue of the *Journal of Biosciences*, published by the Indian Academy of Science, Bangalore, India (expected date of publication May 1998). Those who are interested may order the issue from Prof. S. C. Lakhotia. The stress of workshop organization was transformed to the organization of stress by the end of the meeting. A proposal is being made to the International Union of Biological Sciences to consider the field of 'molecular stress research' as a priority area in 1998–2000. The proposal suggests the sponsorship of stress-related conferences *not* in Europe or in USA/Canada as a vehicle to propagate the newest results of stress research to the interested scientific community of the developing countries. More fellowships should be established to enhance scientific collaboration between countries of Europe/USA/Canada and Asia/South-America/Africa. The possibility of setting up a 'stress-site' on the Internet will be also explored.

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