Stress goes Far East: meeting report of the Sixth International Workshop on the Molecular Biology of Stress Responses

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Introduction

The heat shock response was discovered in 1962 by mistake. F. Ritossa was studying the puffing pattern of *Drosophila* larvae when he discovered a new pattern 1 day (Ritossa 1962, 1996). It turned out that one of his colleagues had raised the temperature of the incubator from 25°C to 32°C leading to the new puffing pattern—and the heat shock response was born. Twelve years later, A. Tissières studied the heat shock response at the level of proteins using a method of separating radioactively labeled proteins by the newly developed method of polyacrylamide gel electrophoresis. By comparing the protein pattern of different larval tissues grown either at 25°C or heat-shocked to 32°C, Tissières detected several proteins present at 32°C but absent or nearly absent at 25°C. He coined the term heat shock proteins (Hsps; Larminat et al. 1992). At the end of the 1970s, the heat shock response was discovered in *Escherichia coli* (Neidhardt and VanBogelen 1981; Yamamori and Yura 1982), and we know now that it occurs in all organisms, from bacteria to man (Shamovsky and Nudler 2008). We also know that the heat shock response is genetically regulated. Important

heat shock proteins are molecular chaperones (Saibil 2008) and adenosine triphosphate (ATP)-dependent proteases (Baker and Sauer 2006). But molecular chaperones also play an important role in causing diseases in humans, animals, and plants (Calderwood 2007). Recent books deal with their role in cancer (Calderwood et al. 2007), in inflammation and immunity (Asea and De Maio 2007), and in neurodegenerative diseases (Asea and Brown 2008).

Stresses in health and diseases

Alexzander Asea (Temple, TX, USA) led off the workshop by talking about the possibility of Hsp-based immunotherapies. He focused on Hsp72 which is released from tumors after thermal stress. Hsp72 with bound peptides are taken up by antigen-presenting cells leading to a release of the peptides which are further processed.

Neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis have been termed "protein misfolding disorders" in which protein aggregates form in neurons causing specific populations of neuronal cells to die prematurely (Ian Brown, Toronto, Canada). While differentiated neurons poorly induce Hsps, a compound (celastrol) has been identified that induces a wide set of Hsps in differentiated human neurons grown in tissue culture, thus offering the possibility of suppressing the formation of protein aggregates in these cells.

Tangchun Wu (Wuhan, China) presented studies that there are individual differences in the levels of Hsps in plasma, lymphocytes, and tissues from normal, stressed, and ill people. His new results suggest that genetic variation of Hsp70 and Hsc70 in the general population regulates the expression levels of each protein under normal and stressed

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conditions. The various levels of expression of Hsp70 and Hsc70 contribute to different risks for coronary heart disease and lung cancer.

Bacterial infection causes inflammation and fever, but mechanisms connecting heat shock and inflammatory responses are as yet unclear (Akira Nakai, Ube, Japan). In this talk, Nakai showed a new feedback loop consisting of HSF1-ATF3-IL-6. The expression of most of the inflammatory genes is negatively regulated under the control of this loop.

William Currie (Halifax, Canada) discussed his work on insulin-induced expression of Hsps and myocardial protection. Since the early 1960s, surgeons have used insulin to improve cardiac function after ischemia. A single injection of insulin into rats yields myocardial protection and increased phosphorylation of Hsp27 after 1 h and increased expression of Hsp70 after 6 h. Inducible myocardial protection as seen at 1 and 6 h after insulin treatment is temporally due to multiple signaling pathways and mechanisms.

Alberto Macario (Baltimore, USA) talked about diseases called chaperonopathies in which the main cause is a deficient molecular chaperone due to a genetic mutation or to an acquired modification, such as aberrant posttranslation modifications and/or abnormal quantitative levels. He presented data showing that the Hsp70 and CCT family display a great diversity at the gene, transcript, and protein levels predicting a wide array of cellular locations, functions, pathologies, and potential applications as therapeutic targets or agents.

Contrary to the common belief that Hsp60 family proteins in animal cells are mitochondrial chaperones, the bulk of Hsp60 proteins in *Drosophila* cells appear to be outside mitochondria (Subhash Lakhota, Benares, India). It was further shown that, of the four Hsp60 genes (Hsp60A-D) known in flies, three seem to have distinct developmental functions. Recent work revealed the specific requirements of Hsp60C protein in tracheal development, early stages of spermatogenesis, and oogenesis, essential both in somatic follicle and germ line cells for proper organization of cytoskeletal elements. In the absence of the Hsp60D protein, induced apoptosis is blocked. Evidence was presented to suggest that Hsp60D is required for removal of DIAP1 from the inactive and/or activated caspases for completing the apoptotic cascade.

Yoshiro Saito (Osaka, Japan) reported that toxic lipid oxidation products can induce a beneficial stress response termed adaptive response. Lipid oxidation products induce the adaptive response through upregulation of the cellular antioxidant system via different mechanisms in primary cortical neuronal cells. Electrophilic lipids containing the reactive alpha, beta-unsaturated carbonyl group depend on transcriptional factor Nrf2, while lipophilic arylating quinone, gamma-tocopheryl quinone, induces a slight ER stress response via the activation of ATF4.

Michael Lynes (Storrs, USA) spoke on metallothionein, a stress response protein that can be induced in cells by reactive oxygen species (ROS), divalent heavy metal cations, endotoxin, acute phase cytokines, or interferon. In light of these inducing agents, it is not surprising that metallothionein has many influences on the development of adaptive and innate immune functions. Metallothionein can be found in a variety of extracellular spaces, and one consequence of this extracellular metallothionein is the induction of leukocyte chemotaxis. A number of parallels were drawn between metallothionein effects as a potential danger signal and the Hsps.

Harm Kampinga (Groningen, The Netherlands) showed data on a chaperome-wide screen for suppressors of aggregation of proteins with expanded polyglutamine repeats. In this screen, two members of the DnaJ (Hsp40) family, DnaJB8 and DnaJB6, were identified as the best suppressors of aggregation. These chaperones also protected against polyglutamine-related cytotoxicity and they effectively suppressed aggregation in vivo using the *Xenopus* model. Finally, a novel nomenclature for the human chaperone families was proposed, largely based on the more consistent nomenclature used by the National Center of Biotechnology Information for heat shock genes. The new nomenclature will soon appear in *Cell Stress & Chaperones*.

Renu Wadhwa (Ibaraki, Japan) presented how stress chaperone mortalin/mthsp70, which was first cloned as a mortality factor in mouse cells is present at multiple subcellular sites, and has multiple functions that range from mitochondrial transport, chaperoning, and protection against oxidative stress. Recently, this chaperone has been implicated in Alzheimer's and Parkinson's diseases, with proteomic studies consistently identifying oxidatively damaged mortalin as a potential marker for such neurodegenerative diseases. Hence, it is possible that mitochondrial dysfunction coincides with the collapse in the mitochondrial chaperone network that aim not only to import, sort, and maintain integrity of protein components within the mitochondria but also to act as a buffer to the molecular heterogeneity of damaged and aging mitochondrial proteins in an ROS-rich microenvironment. Or, inversely, it may also seem that vulnerability to mitochondrial dysfunction could be precipitated from the detrimental (antichaperone) gain of function of a sick mortalin.

Bacterial stress response

Dipankar Chatterji (Bangalore, India) discussed the effect of nutritional stress on bacteria like *Mycobacterium smegmatis* taken as a model system. This has major relevance with respect to the infectivity of the organism,

as we know the latent or dormant state of *Mycobacteria* is very difficult to eliminate completely from the host. Under starvation conditions, the dodecameric DNA-binding and iron-binding protein Dps is one of the most abundant proteins in the cell. Its oligomerization states have been studied by different methods.

François Baneyx (Seattle, USA) reported on the complex network of stress-inducible molecular chaperones and proteases of *E. coli*. He focused on homodimeric Hsp31, an ATP-regulated chaperone collaborating with DnaK team, and HchA which is induced by oxidative and acid stresses.

Valakunja Nagaraja (Bangalore, India) dealt with proteins able to inhibit the enzyme DNA gyrase. In particular, GyrI and YacG have been shown to interact with the gyrase prior to or after binding to DNA. While GyrI is member of the SOS regulon, YacG is induced by oxidative stress.

Wolfgang Schumann (Bayreuth, Germany) reported on a five-component signal transduction system of *Bacillus subtilis* which is induced by alkali stress. This system consists of a membrane-anchored anti-sigma-factor sequestering the alternative sigma-W factor. Upon an alkaline shock, a site-1 protease cleaves the anti-sigma-factor within its extracytoplasmic domain followed by a site 2. Finally, the truncated anti-sigma-factor is released into the cytoplasm where its degradation occurs by the third protease, finally leading to the release of sigma-W and transcription of the approximately 60 genes of the sigma-W regulon.

Alastair McEwan (Brisbane, Australia) described a new family of bacterial MerR-like transcriptional regulators: the *Neisseria gonorrhoeae* MerR-like regulators (NmlR) responding to disulfide stress. It was shown that NmlR regulated expression of a thioredoxin reductase, a copper efflux ATPase, and an esterase that were essential for defense against nitrosative stress in this bacterium. The NmlR system is an essential part of neisserial stress defense during interactions with human epithelial cells. The diversity of NmlR regulators in bacteria was also described and their link to glutathione-based stress responses was noted.

Bernd Bukau (Heidelberg, Germany) reported on protein aggregation in heat-treated *E. coli* cells. His group noticed that protein aggregates are normally formed at the cell poles and, if they are formed somewhere in the cell, they move towards the cell poles. He further talked about ClpC which can completely unfold substrate proteins.

Jung-Hye Roe (Seoul, South Korea) reported on a regulatory system of *Streptomyces coelicolor*, which senses and responds to thiol stress. This species produces mycothiol as antioxidation buffer, and the genes involved in the production of the compound are controlled by a sigma factor, which is sequestered by the anti-sigma-factor RsrA. RsrA itself contains a thiol–disulfide redox switch. While oxidative stress causes oxidation of the redox switch

and release of the sigma factor, mycothiol is able to reduce RsrA thus establishing an autoregulatory loop.

Skorn Mongkolsuk (Bangkok, Thailand) focused on host–bacterial interactions in their environment, where bacteria are exposed to organic hydroperoxides generated from host cells, chemicals in the environment, and aerobic respiration. Their research activities have focused on the *ohrR–ohr* system, where *ohr* encodes a thiol peroxidase metabolizing organic hydroperoxides to less harmful alcohol derivatives, and *ohrR* encodes a transcription regulator belonging to the MarR superfamily and acting as a sensor of organic hydroperoxides. Molecular characterization of OhrR interaction with target promoters indicates that in the reduced form OhrR binds to operator sites and blocks RNA polymerase from binding to the promoter. Exposure of the cells to organic hydroperoxides leads to oxidation of the sensing cysteine residue and disulfide bond formation follows. The oxidation of OhrR and subsequent reactions resulting in major structural changes, which prevent the regulator from binding to the operator site and derepressing the expression of the target genes.

A complication in understanding stress responses is the heterogeneity in stress resistance in bacteria like *E. coli* (Tom Ferenci, Sydney, Australia). Global regulators like RpoS and ppGpp, both influential in directing transcription, also vary among strains due to *rpoS* and *spoT* mutations as well as other factors. The reason for this variation is the need for bacteria to adapt rapidly to fluctuating environments and to satisfy mutually exclusive nutritional or stress-resistance capabilities.

Rojana Sukchawalit (Bangkok, Thailand) presented data on the repressor RirA of *Agrobacterium tumefaciens*, which regulates iron uptake systems. In the absence of this repressor, cells exhibited an iron overload, increased sensitivity to oxidants, and a defect in tumorigenesis on tobacco leaves. These data demonstrate an important function of RirA during plant–pathogen interaction.

Heat shock response in bacteria

Hitoshi Nakamoto (Saitama, Japan) uses Cyanobacteria as a model system. These are phototrophic prokaryotes that probably exhibit the widest range of diversity in growth habitats of all photosynthetic organisms. His group focuses on the understanding of expression and function of the molecular chaperones GroEL and small Hsps. Expression of these chaperones is modulated by light involving novel DNA elements. Of the two *groEL* genes present, one is dispensable under normal growth conditions but plays an important role under stress conditions such as cold stress for a thermophilic cyanobacterium *Thermosynechococcus elongatus*. Small

Hsps play an important role in quality control of thylakoid membranes and the membrane-associating proteins that are components of photosynthetic apparatus. Association of molecular chaperones with thylakoid membranes and the membrane-associating proteins may have been essential in order to sustain photoautotrophic life under stress.

The presentation of Pongpan Laksanalamai (Baltimore, USA) dealt with a small Hsp and a Class II chaperonin Cpn encoded by the psychrophilic Archaeon *Methanococcoides burtonii*. Purified α -subunit of Cpn had an optimal ATPase activity at 16°C. The small Hsp forms homogeneous 24-mer multimeric complexes able to bind denatured lysozyme.

Heat shock proteins and drug development

Linda Hendershot (Memphis, USA) dealt with solid tumor cells encountering an environment that has decreased nutrient and oxygen supply, which results in activation of the unfolded protein response (UPR). They found that there was an induction of proangiogenic factors like vascular endothelial growth factor (VEGF), fibroblast growth factor, angiogenin, and interleukin 8, where VEGF is regulated at several different levels. Firstly, VEGF is transcriptionally upregulated by direct binding of XBP1 to its promoter, which plays a significant role in the upregulation of VEGF expression. Secondly, VEGF mRNA stability is increased via activation of the adenosine monophosphate and p38 mitogen-activated protein kinases. Lastly, previous data demonstrated that processing and secretion of VEGF protein is dependent on the upregulation of ER chaperones that occur during UPR activation. These data suggest that the UPR plays a number of important roles in regulating VEGF expression and is likely to play a critical role in angiogenesis and tumor development.

Hideo Iwasaka (Oita, Japan) reported on the acute respiratory distress syndrome (ARDS), a common, devastating clinical syndrome of acute lung injury that affects both medical and surgical patients. An improved understanding of the pathogenesis of ARDS has led to the assessment of several novel treatment strategies. Hsp47 is a procollagen–collagen-specific molecular chaperone essential for the early stages of collagen biosynthesis, whose expression increases in parallel with the expression of collagens during the progression of various fibrosis models. The administration of Hsp47 antisense oligonucleotides markedly suppressed the increased production of collagens and was also effective against other kinds of tissue fibrosis such as myocardial infarction and liver cirrhosis.

Len Neckers (Bethesda, USA) asked the question whether Hsp90 is a cancer chaperone. Hsp90 is required

for the stability and function of a number of signaling proteins that promote cancer cell growth and/or survival. Since only part of the Hsp90 proteins is subject to pharmacological inhibitors, the insensitive Hsp90 proteins are protected by acetylation and phosphorylation.

John Williams (Chester, UK) raised the question of how Hsp70 is getting in the right place at the right time, in particular how it moves to the extracellular environment and how it then interacts with other cells. He further discussed how Hsp70 impacts on diseases of old age and how they might be manipulated therapeutically.

Shi Chung Ng (San Diego, USA) reported on experiments involving arimoclomol (AC), which stabilizes the active form of HSF1. Rats with an induced stroke exhibited a highly significant improvement in functional recovery when treated with AC 1 h after stroke. Even animals whose AC treatment started 48 h after stroke were halfway to complete recovery by day 7 after stroke.

Stress in plants

Hervé Chrestin (Montpellier, France) reported on a main physiological disease of *Hevea brasiliensis*, the rubber tree, called “Tapping Panel Dryness” that greatly impairs productivity in plantations. Many of the highly differentially expressed genes in the bark between healthy and TPN trees are water-stress-related genes indicating that water stress may be one of the major causes of TPN.

Sunil Kaul (Ibaraki, Japan) presented the molecular evidence for the anticancer and antistress activity of the leaf extract of Ashwagandha, a plant widely used in home medicine in India. Although the root extract of this plant has been in use as a herb remedy to treat a variety of ailments and to promote general wellness, its leaf extract was shown to have withanone, a component that exhibited selective killing of cancer cells. Kaul also showed that the extract gives protection against UV and hydrogen-peroxide-induced damage.

Posters

The poster sessions took place in the Congress Center of the Chulabhorn Research Institute and were well attended. A total of 28 posters dealt with the different aspects of the workshop. At the end, the three best posters were awarded to Suresh Thakur in first place, Aekkapol Mahavithakanont in second place, and Tatsanee Chuchue and Nisa Patikarnmonthon in joint third place.

Concluding remarks

We wish the best of luck to Professor Ingyol Jin from the Kyungpook National University of Deagu, South Korea, who will organize the seventh workshop.

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