Stress under the dam: meeting report of the Fourth International Workshop on the Molecular Biology of Stress Responses

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INTRODUCTION

The response of cells to a wide variety of stresses has been known to induce the synthesis of families of proteins known as the heat shock proteins (Hsps). Although first considered as special proteins that had cytoprotective properties under stress conditions, Hsps were subsequently found to play important roles in basic cellular processes such as protein folding and intracellular protein transport. The findings that Hsps were involved in disease processes provoked an increased interest in the study of the stress response in many cell models from bacteria to humans. Thus, if one looks for Hsps in the NCBI database, one is confronted with an astonishing 20 657 entries (15 July 2004), enough to discourage any newcomers to the field. As the knowledge gap between the “western societies” and the developing countries with respect to this fascinating field widened, Professors S.C. Lakhota (Varanasi, India) and W. Schumann (Bayreuth, Germany) organized a first workshop on the “Molecular Biology of Stress Responses” at Banaras University in Varanasi, India, in October 1997. The meeting aimed at permitting young scientists from developing countries to participate in the rapidly developing field of the basic aspects of the stress responses and its potential for applications to medical, agricultural, and environmental problems (Csermely

and Lakhota 1998). Given the enthusiasm of the young (and old) participants, a second workshop was organized in Wuhan, China, in 1999 (organizer Tangchun Wu) and in Mendoza, Argentina, in 2001 (organizer D. Ciocca) (reviewed by Grover 2002).

The Cell Stress Society International sponsored the Fourth International Workshop on the Molecular Biology of Stress Responses held in Wuhan and Yichang, China, on 13–16 May 2004. The workshop organized by Professors Tangchun Wu (Tongji Medical College), Lawrence E. Hightower (University of Connecticut), and Robert M. Tanguay (Université Laval) attracted around 100 scientists mainly from different regions of China and also from Canada, Denmark, France, Germany, The Netherlands, Russia, Sweden, the United Kingdom, and the United States. Many of the international scientists participating in the workshop arrived a day or two before the meeting and were treated to tours of the Yellow Crane Tower and the Hubei Provincial Museum in Wuhan.

THE CHAPERONE MACHINE AND ITS REGULATION

The workshop opened on 13 May at the conference center of Tongji Medical College by President Mingwu Fan and Vice-President Jizhou Xiang of the Huazhong University of Science & Technology, Professor Tangchun Wu, workshop chairman, and Professor Robert M. Tanguay, workshop cochairman. After the participants were welcomed,

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a photograph of the participants (front cover) was taken in front of the conference center. The first scientific session opened on the chaperone machine.

Following up on earlier work on the interaction between lipids and Hsps (Guidon and Hightower 1986; Arispe et al 2002), Antonio De Maio (John Hopkins University, Baltimore, MD, USA) discussed the possible significance of the interaction of Hsc70 and Hsp70 with phosphatidylserine moieties in membranes. He presented an interesting model suggesting that Hsp70 in excess interacts with membranes and opens a potassium channel leading to cell death. De Maio also showed that endocytosis and phagocytosis are enhanced by treatment with geldanamycin, a specific inhibitor of Hsp90. This effect also may be mediated by the presence of Hsp70, which may be interacting with the endocytic or phagocytic vesicles. Next, by creating hybrid molecules between monomeric domains of thioredoxin, DsbA, a member of the prokaryotic protein disulfide isomerase (PDI) family or the nonactive site of human PDI and a C-terminus of the N-terminal domain of DsbC, Chih-Chen Wang (Chinese Academy of Sciences, Beijing, People's Republic of China) showed evidence that dimerization creates chaperone and isomerase activity for these monomeric thiol-protein oxidases and reductases and that dimerization bestows new activities to the molecule revealing a strategy that living organisms may have used to achieve new or more efficient functions (Zhao et al 2003). She also presented data showing that the post-CTD domain of DnaJ is essential for its dimerization and chaperone activity.

Shifting to small Hsps, Zengyi Chang (Peking University, Beijing, People's Republic of China) and his students Xinmiao Fu and Wangwang Jiao (Tsinghua University, Beijing, People's Republic of China) presented work on the assembly and chaperone-like activity of the *Mycobacterium tuberculosis* Hsp16.3 nonamers (Fu et al 2003) and of the small IbpB protein of *Escherichia coli*. In a coupled transcription-translation assay, Hsp16.3 exists primarily as a trimer that can form nonamers through subunit exchange in the presence of purified Hsp16.3. Using mutants lacking the N-terminal 35-residue flexible domain, Xinmiao Fu presented new evidence that the N35 domain of Hsp16.3 was involved in formation of nonamers from trimers and also contained the substrate-binding site. However, nonamer formation is not required for chaperone activity. Interestingly, a mutant with truncated 9 C-terminal residues formed dimers, not trimers, but nonetheless retained chaperone activity. Wangwang Jiao presented work on the chaperone-like activity and oligomeric status of the small Hsp of *E. coli* IbpB that are temperature regulated. The flexible C-terminus of IbpB that is essential for chaperone activity and temperature-dependent regu-

lation of its oligomeric status was found to be highly susceptible to limited proteolysis. This is particularly interesting and may relate to the recently reported sensitivity and cleavage of the flexible N-terminal end of mammalian Hsp22 (Chowdary et al 2004). Why small Hsps from diverse organisms can act as monomers while others are found as dimers, trimers, or very large oligomers and how this relates to chaperone activity remains an intriguing problem of structural biology and evolution. Zhengyi Chang concluded with recent work aimed at identifying molecular chaperones in the periplasmic space. Surprisingly no typical chaperones were found in the periplasm, but instead many proteins that are very resistant to various denaturing conditions (heat, acid, or ethanol) were found in this fraction.

The opening session was followed by a remarkable official banquet in the Faculty dining room of Tongji Medical College. During the afternoon, the well-fed workshop participants were taken on a 4-hour bus trip from Wuhan to Yichang, where the meeting resumed. Yichang is situated at the beginning of the famous and beautiful Three Gorges and about 30 km downstream from the Three Gorges Dam on the Yangtze River. The buses arrived at the Longquan Hotel, Yichang, overlooking the first of the Gorges just in time for the evening meal (Fig 1).

On May 14, the first session was on Stress Response and Its Regulation. Wolfgang Schumann (University of Bayreuth, Germany) presented microarray data on identification of alkali-inducible genes regulated by the Sigma-W regulon (σ^W) (Schumann 2003). Normally σ^W is sequestered by its anti-sigma factor RsiW, and after alkali-shock, the anti-sigma factor RsiW is sequentially degraded by 3 different proteases. Next, Yu-Fei Shen (Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, People's Republic of China) presented her work on heat shock induced expression of Hsp90 α being regulated by the differential recruitment of individual factors to a regulatory element with GAS consensus sequence in the 5' upstream region of the gene. Her studies suggested that beyond the classical HSF-HSE, alternative pathways existed for regulating *hsp* genes in a heat shock response. Yong Zhang of Shen's Lab (Beijing, People's Republic of China) introduced data on the SWI-SNF chromatin-remodeling complex and the reversible acetylation on histones in the regulation of human hsp90 gene expression in Jurkat cells.

HSPS AND PROTEIN-FOLDING DISEASES

The booming interest in Hsps in recent years has been triggered by many reports suggesting that these chaperones may be key elements in numerous neurodegenerative diseases, which are now often referred to as

Fig 1. Some participants of the Fourth International Workshop. (A) Antonio De Maio (Baltimore). (B) John H. Williams (Chester). (C) Students of Professor Wu (Wuhan) waiting for dinner. (D) Xianzhong Xiao (Changsha) & Harm Kampinga (Groningen). (E) A Graham Pockley (Sheffield). (F) Dayue Duan (Reno). (G) Boris Margulis (Saint Petersburg). (H) The mighty Yangtze River in front of the rooms at Longquan Hotel. (I) Marja Jäätella (Copenhagen). (J) Earl Noble (London, Canada). (K) Miao Yang (Wuhan). (L) Participants at work.

protein-folding diseases (see reviews by Muchowski 2002; Barral et al 2004), as well as in the aging process (Morrow and Tanguay 2003). The third session dealt with various facets of Hsps in relation to protein-folding diseases. Harm Kampinga (Groningen, The Netherlands) started with a review of the Hsp70 chaperone machine. Although Hsp70 is effective at refolding heat-denatured proteins, coexpression of Hsp40 or Hip with Hsp70 further enhances refolding of heat-denatured proteins. Proteins containing long polyglutamine (poly-Q) stretches tend to form aggregates in cells, and their toxicity may be linked to the heat shock response. In poly-Q neurodegenerative diseases, Hsp70 has marginal or no effect on poly-Q inclusion formation; indeed, an impaired response can exacerbate the toxicity as shown for Hsp70 that becomes sequestered in the aggregates of poly-Q-expanded, truncated forms of the androgen receptor (Cowan et al 2003). However, Hsp70 overexpression does delay cell death caused by poly-Q proteins. Interestingly, coexpression of Hsp70 and Hsp40 protects against all cytotoxic features mediated by poly-Q expression. Patrick Arrigo (Université Claude Bernard Lyon 1, Lyon, France) discussed the oxidative burden in another cellular model of poly-Q proteins, Huntington's disease, and showed that while Hsp70 and Hdj-1 (Hsp40) were effective at reducing the oxidative burden of poly-Q expression, coexpression of Hsp27 with Hsp70 and Hsp40 were most effective at reducing oxidative stress in the cells. Interestingly Hsp27, a small Hsp that does not suppress the formation of aggregates and is not associated with the cellular aggregates can suppress poly-Q-induced cell death (Wytenbach et al 2002). Coexpression of Hdj-2 or of phosphorylation mutants of Hsp27 with Hsp70 was ineffective at reducing the oxidative stress caused by the expression of the poly-Q proteins. Finally, proteasome inhibitors enhanced protein granule oxidation, an observation of particular interest after the recent demonstration that overexpression of Hsp27 and its interaction with the proteasome can activate its activity and influence the degradation of ubiquitinated proteins (Parcellier et al 2003). Xiaojun Tan (Central South University, Changsha, People's Republic of China) showed that myocardial ischemic injury caused protein denaturation and protein aggregation and that heat shock treatment and expression of Hsp70 and α B-crystallin reduced the ischemia-induced protein aggregation. Protein aggregates were associated with mitochondrial membranes. Although Hsp70 accumulated in mitochondrial aggregates, α B-crystallin was associated with myocardial filament aggregates in the Z-line. Robert M. Tanguay (Université Laval, Québec, Canada) presented data on the effects of overexpression of 2 small Hsps in *Drosophila*, Hsp22 and Hsp23. Overexpression of

endogenous Hsp22 using the UAS-GAL4 system increased the lifespan of flies by more than 30% (Morrow et al 2004). This effect was particularly remarkable because the expression of Hsp22 was confined to brain motor neurons. Transgenic expression of Hsp23 induced a slightly lower extension of lifespan (~18%) but likely through a different mechanism. Flies overexpressing the mitochondrial Hsp22 were also more resistant to oxidative (Paraquat) and thermal stress. Geneviève Morrow (Université Laval, Québec, Canada) followed with a presentation on the life-threatening effects of suppressing expression of Hsp22 by transposon excision. Interestingly behavior and motor tests on flies showed that mitochondrial Hsp22 not only increased the longevity but also extended the flies' functional well-being because they retained motor activity longer. Overall, the data presented in this session support the increasingly convincing links between the oxidative status of a cell, its capacity to unfold or remove protein aggregates, and the appearance of degenerative traits including the phenotypes associated with aging.

In the afternoon of May 14, the workshop participants visited the Three Gorges Dam. The Dam Project facilitates control of the Yangtze flood waters downstream in highly populated cities like Wuhan, generation of electricity to meet China's growing energy needs, and irrigation of farm land. The Dam is 2.3 km long and when complete will raise water levels upstream by 175 m. A 5-step dual lock system allows boats and ships to pass the Dam.

STRESS RESPONSE IN CANCER

The past decade has seen a large increase in the number of observations relating the expression of Hsps and cancer. Although Hsps have been shown to negatively or positively regulate the apoptotic process at multiple levels, the exact mechanisms linking the stress response and the cancer process are still open. Stuart Calderwood (Harvard Medical School, Boston, MA, USA) opened the stress response cancer session by summarizing the current status on Hsp expression in cancer. In many cancers, the ErbB signaling pathway is activated. This pathway is regulated by heregulin binding to ErbB3 or ErbB4 receptors. Heregulin is a strong inducer of malignancy and protects cells from apoptosis. Heregulin also mediates HSF1 activation and hyperphosphorylation and, therefore, expression of Hsps, implicating the Hsps in the malignant process. George Chen (Chinese University of Hong Kong) showed that hydrogen peroxide and staurosporine have differential effects on the cell death of glioblastoma cells lacking deoxyribonucleic acid-dependent protein kinase and may be important for selecting or optimizing the treat-

ment for malignant brain tumors. All cancers have ways to evade and block apoptosis. Marja Jäättelä (Institute of Cancer Biology, Copenhagen, Denmark) reminded the participants of the workshop that in addition to necrotic and apoptotic cell death, there is also lysosomal-mediated cell death (Jäättelä 2004). She provided evidence that Hsp70 promotes cell survival by stabilizing lysosomal membranes and inhibiting cathepsin release. Interestingly, endosomal and lysosomal membranes of cancer cells contained Hsp70. Jäättelä concluded that Hsp70 functions by inhibiting the death-associated permeabilization of lysosomes. In summary, given the primary role of apoptosis, therapeutic attempts to regulate apoptosis through manipulation or regulation of the stress response appeal to the cancer field (see review by Sreedhar and Csermely 2004).

PHYSICAL ACTIVITY AND STRESS RESPONSE

The next session dealt with stress response and physical activity. Exercise is basically a physiological stress characterized by metabolic disturbances with increased intracellular Ca^{2+} , reactive oxygen species (ROS) production, altered hormonal milieu, and elevated temperatures in extreme cases. Earle Noble (University of Western Ontario, London, Canada) reminded us that men and women differentially regulate cardiac Hsp70 after exercise (Paroo et al 2002) and that the β -adrenergic system that is influenced by both estrogen and testosterone may be involved in this sex-specific difference. Understanding the molecular basis of this differential response has clear implications for the prescription of exercise for prevention of cardiac pathologies in men and women. Jun Xu (Institute of Hygiene and Environmental Medicine, Tianjin, People's Republic of China) showed that L-type calcium channels were markedly increased by stress. In addition, the steady activation characteristic of L-type calcium channels was changed, and the channels were activated more easily and earlier. Under stress, protein kinase A activity was positively regulated, causing the calcium current alteration. Next, Guo-Qiang Xiao (South China Normal University, Guangzhou, People's Republic of China) presented data on the effect of incremental exercise with and without taking a sauna on Hsp70 expression and aerobic capacity. Interestingly, incremental exercise induced higher levels of Hsp70 in white blood cells than sauna and exercise.

STRESS RESPONSE, INFLAMMATION, AND CARDIOVASCULAR DISEASES

Another field of growing interest is the significance of the stress response in inflammation and in cardiovas-

cular diseases. Graham Pockley (Sheffield, UK) reviewed the role of Hsps as regulators of inflammation and cardiovascular disease (Pockley 2002). Hsps are found in the peripheral circulation, and high levels of Hsp70 appear to be associated with a reduced risk of developing cardiovascular diseases. The mechanisms proposed include attenuation of inflammation, modified leukocyte adhesion, and effects on the endothelium. Dayue Duan (University of Nevada, Reno, NV) discussed cell volume perturbations and ion channel remodeling in cardiac diseases. Cell volume regulation has a critical impact on cell proliferation and apoptosis, and volume-regulated ion channels may play critical roles in heart remodeling. Volume-regulated ion channels may be attractive novel targets for therapeutics for the treatment of cardiovascular diseases. Xianzhong Xiao (Central South University, Changsha, People's Republic of China) provided data that apoptosis in cardiomyocytes induced by ischemia-reperfusion injury or oxidative stress involves the activation of both mitochondria and death receptor pathways. HSP70 suppressed apoptosis of myogenic cells induced by oxidative stress by inhibiting the activation of both receptor pathways, the release of the apoptogenic protein Smac-Diablo and cytochrome *c* (Cyt *c*) from mitochondria, and the cleavage of Bid into tBid. α B-crystallin suppressed oxidative stress-induced apoptosis of myogenic cells, the mechanism of which might be in part due to the inhibition of Cyt *c* release from mitochondria and the direct interaction of α B-crystallin with proapoptotic molecules p53 and Cyt *c*. Mingjun Hu (Yunnan Medical College, Shiyuan, People's Republic of China) presented recent work on the expression of Hsp70 and vascular endothelial growth factor (VEGF) in cerebral infarction with gene therapy for VEGF. Injection of plasmid encoding VEGF directly into the ischemic area of a stroke-like injury promoted expression of Hsp70 and angiogenesis while reducing the cerebral infarct volume. Lei Jiang (Central South University, Changsha, People's Republic of China) reported that intracellular delivery of recombinant α B-crystallin into neonatal rat cardiomyocytes had a protective effect against oxidative stress on the cells. Changzheng Jiang (Tongji Medical College, Wuhan, People's Republic of China) examined Hsp70 gene polymorphisms in patients with acute myocardial infarction. There was a significant association of homozygous B/B genotype of *HSP70-hom* with acute myocardial infarction, suggesting that the B/B genotype of *HSP70-hom* may play a role in coronary heart disease susceptibility in the Chinese population.

Homocysteine is a risk factor for coronary heart disease, is elevated in type 2 diabetes and Alzheimer's disease, and predicts cognitive decline in healthy elderly

(Austin et al 2004; Hayden and Tyagi 2004). John H. Williams (University College, Chester, UK) discussed how folate supplementation can reduce homocysteine-induced damages. Treatment with folic acid is accompanied by an increase in glutathione, an induction of heme oxygenase 1 (HO-1, Hsp32), but not Hsp70, and a decrease in serum-free Hsp70. Thus folate protection may act at least partially through Hsp32. This is consistent with the ROS-promoting activity of homocysteine and in line with the protective role of HO-1 in oxidative stress-related injuries and inflammation (Takahashi et al 2004). William Currie and Yu Chen (Dalhousie University, Halifax, Canada) concluded the session by presenting work on the angiotensin II model of induced hypertension and inflammation. Minipump infusion of angiotensin causes a progressive rise in blood pressure and histopathology. Remarkably, a single heat shock treatment, 24 hours before infusion of angiotensin, suppressed the hypertension and inflammation for up to about 7 days. This effect was mediated by depletion of I kappa kinase activity and suppression of nuclear factor- κ B activation (Chen et al 2004). The session concluded with a lively discussion among the participants on cell death pathways, the role of phosphorylation of Hsps, whether Hsps directly regulate inflammation signaling pathways, and on the origin of free Hsp70 in plasma.

ENVIRONMENTAL STRESS

The final session of the workshop (May 16) dealt with field pretranslational studies on the use of stress response in the treatment of work-associated diseases and on its use as biomarkers of environmental health hazards. Boris Margulis (Institute of Cytology, Saint Petersburg, Russia) discussed various applications of the stress response. After describing a new approach to measure Hsp70 by the enzyme-linked immunosorbent assay, he reported that addition of Hsp70 in culture medium protects mouse myeloma cells against apoptosis triggered by aggresomes resulting from mutant, misfolded Opsin (P23H) or poly-Q proteins (Q103-Huntingtin). This lead to basic questions on the mechanism(s) Hsp70 uses to enter cells (endocytosis) and exert its antiapoptotic action. Benzo(a)pyrene (BaP) is a ubiquitous polycyclic aromatic hydrocarbon environmental pollutant causing lung, gastric, and skin cancers. Tangchun Wu (Tongji Medical College, Wuhan, People's Republic of China) showed that exposure to BaP inhibits inducible Hsp70 expression without affecting the expression of the constitutively expressed Hsc73. Total HSF1 and HSE binding were downregulated by BaP as shown by Western blot and electrophoretic mobility shift assays, respectively. Interestingly vitamin C eliminated this BaP-induced reduction in

Hsp70. Lei Ke (Chongqing University, Chongqing) further elaborated on the combined effects of BaP and heat, a frequent combination in the workplace because BaP is a product of incomplete combustion. Using 2-dimensional gels to analyze A549 cells, these workers concluded that heat treatment at 42°C neither prevented the BaP-induced reduction in inducible Hsp70 at high concentrations of BaP nor did it affect the expression of Hsp73, confirming the observations of Professor Wu. The presence of antibodies against Hsps has been reported in sera of normal individuals (Pockley et al 1999) and has also been associated with numerous diseases ranging from exposure to pollutants to cardiovascular diseases (Wu et al 1996, 1998, see review by Pockley 2002). Miao Yang (Tongji Medical College, Wuhan, People's Republic of China) reported the intriguing observation that antibodies to Hsp60 and Hsp70 were associated with noise-induced hearing loss in workers exposed to high noise levels (Yang et al 2004). Although the mechanisms for such an association are unknown, she suggested that such antibodies could be involved in hearing loss. Cadmium is also an important environmental pollutant. Taiyi Jin (Fudan University, Shanghai, People's Republic of China) reported a dose-effect relationship between metallothionein (MT) messenger ribonucleic acid expression in lymphocytes and cadmium exposure. Interestingly, MT gene expression can be used as a biomarker inversely related to individual susceptibility to the renal toxicity of this compound. Finally, Yongyi Bi (Wuhan University, People's Republic of China) discussed the transcriptional activation of the MT I gene by the phenolic antioxidant tBHQ and showed that the redox cycling initiated by tBHQ released Zn from intracellular stores and activated the MTF1 signaling pathway.

CONCLUSION

Stress response has become a major player in multiple complex processes including cancer, cardiovascular diseases, degenerative neurological diseases, and aging. Understanding the mechanisms by which the Hsps can influence these processes is of primary importance for the design of therapeutic approaches to these problems. One question that was raised numerous times during the workshop is the origin of free-circulating Hsp70 and surface-associated Hsps70 because it clearly has relevance for regulation of the immune response and of inflammatory processes. A related point is the biological significance of antibodies to Hsps, which raises the question of whether the immune response to self-Hsps is beneficial or harmful. Ongoing work in many labs should bring answers to these questions in the near future.

Professor Wu closed the meeting on May 16, and the

participants expressed their gratitude to Professor Wu and his staff who have made this Fourth Workshop a very successful one (Fig 2). It was announced that the Cell Stress Society International will continue to support these workshops and that the Fifth International Workshop on the Molecular Biology of the Stress Response will return to the South American continent in 2006. It has been tentatively scheduled for the end of January 2006 in Concepcion, Chile, and is being organized by Virginia L Vega (Johns Hopkins University, Baltimore, MD, USA, and Universidad de Concepcion, Concepcion, Chile), Sandra Nicovani (Universidad de Concepcion, Concepcion, Chile), Antonio De Maio (Johns Hopkins University, Baltimore, MD, USA) and Robert M. Tanguay (Université Laval, Québec, Canada).

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Fig 2. (A) Robert M. Tanguay. (B) R. William Currie. (C) Tangchun Wu, authors of the report.