Stress at the Korean Mountains: meeting report of the 8th International Workshop on the Molecular Biology of Stress Responses

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Abstract South Korea is a country exemplified by a combination of upscale new technology and ancient mysticism. The busy streets of Seoul hustle and bustle like any large, active metropolis, yet the city's inhabitants radiate an intrinsic sense of peace, creating a timeless atmosphere. The combination of emerging technology and

profound respect for the Korean culture and heritage makes this country a unique environment in which to organize a successful scientific meeting. Cell Stress Society International, in its quest to cross the cultural frontiers of science and propagate research on the stress response, partnered with the newly created Korean Cell Stress Society to hold the 8th International Workshop on the Molecular Biology of Stress Responses on June 1–4, 2010.

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South Korea is a country exemplified by a combination of upscale new technology and ancient mysticism. The busy streets of Seoul hustle and bustle like any large, active metropolis, yet the city's inhabitants radiate an intrinsic sense of peace, creating a timeless atmosphere. The combination of emerging technology and profound respect for the Korean culture and heritage makes this country a unique environment in which to organize a successful scientific meeting. Cell Stress Society International (CSSI), in its quest to cross the cultural frontiers of science and propagate research on the stress response, partnered with the newly created Korean Cell Stress Society (KCSS) to hold the 8th International Workshop on the Molecular Biology of Stress Responses on June 1–4, 2010. The workshop venue was the beautiful Seorak Mountains in the northwest part of the country, which provided a peaceful environment for a productive discussion and many entertainment possibilities, including climbing one of the highest peaks in the country and enjoying a spectacular view of the Korean landscape, visiting a historic monastery, and enjoying sushi at the seaport. Images of the meeting site and participants appear in Supplement 1 (online only).

The meeting was organized by KCSS president, Professor Chang Duck Kim and CSSI president, Professor Antonio De

Fig. 1 CSSI President De Maio prays at the Seorak Mountain for more research funding

Maio (Fig. 1). However, the majority of the workshop was put together through the incredible work of Professor Eunil Lee, assisted by a great group of Korean scientists. Unfortunately, Professor Lee became ill the day before the meeting and missed the great event that he organized. Hopefully, he has fully recuperated, and we will see him soon at other CSSI events.

After an enjoyable bus trip from Seoul to the Seorak Mountains, the workshop was initiated by the traditional pre-workshop symposium, which provided basic knowledge on the field of the stress response to newcomers, particularly students and postdocs, to this fascinating discipline. The pre-workshop session was composed of four interactive lectures, the first of which was presented by Antonio De Maio (University of California San Diego, USA), who provided a general introduction to the cell stress field and heat shock proteins (hsps). Dr. De Maio was followed by Harm Kampinga (University of Groningen, Netherlands) who spoke about chaperones and protein folding. After Dr. Kampinga, Linda Hendershot (St. Jude

Children's Research Hospital, USA) offered a provocative overview of the endoplasmic reticulum (ER) stress, and, finally, Robert M. Tanguay (University of Laval, Canada) discussed the biology of small heat shock proteins. The welcome ceremony for all participants was held during an exuberant Korean-style dinner, with words from both KCSS and CSSI presidents and an introduction of all the international speakers. After dinner, participants were summoned to a midnight poster session/discussion where they enjoyed a wonderful display of science by several of the participating students and postdocs. The main body of the workshop took place from Tuesday to Thursday, with excellent presentations and great discussions after each topic, followed by more opportunities to enjoy delicious Korean cuisine and lively interaction among participants during midnight discussions. The workshop closed out the week with an exciting tour of the region on the last day of the conference followed by an exquisite dinner and an enjoyable selection of traditional Korean music and dancing.

The initial session of the workshop was devoted to chaperones in cellular physiology. HtpG is a prokaryotic homologue of Hsp90, whose function has been enigmatic, as indicated by Hitoshi Nakamoto (Saitama University, Japan). HtpG was demonstrated to be vital for the survival of the cyanobacterium *Synechococcus elongates* PCC 7942 after heat shock. HtpG interacts with a polypeptide component of the phycobilisome, the light-harvesting apparatus of cyanobacteria. In addition, HtpG collaborates with DnaK, DnaJ, and GrpE in protein folding. CSSI former president Kazuhiro Nagata (Kyoto Sangyo University, Japan) presented very exciting results on the vectorial redox in the ER, which is critical for the proper formation of disulfide bonds within nascent polypeptides. He reported that ERp46, ERp57, P5, and protein disulfide isomerase (PDI) were oxidized by ERO1 α , which was subsequently oxidized by ERO1 α /PDI regulatory hub complex. These innovative observations demonstrated a regulatory mechanism for counterbalancing the redox homeostasis of the ER. This topic was continued by Linda Hendershot (St. Jude Children's Research Hospital, USA). She discussed the quality control mechanism in the ER, which is the central compartment for synthesis of the majority of secreted and membrane proteins, fondly referred to as the "incubator for baby proteins." Proteins in this compartment need to be folded for their subsequent transport to their final destinations. Those proteins that fail to fold properly ought to be discarded through translocation into the cytosol where they are degraded by the 26S proteasome system. Dr. Hendershot emphasized that the oxidizing environment of the ER is critical for folding, but unnecessary for the degradation of polypeptides. She discussed the question of how molecular chaperones and co-chaperones within the ER lumen discern between folding and transloca-

tion/degradation of newly synthesized proteins. She pointed out the important role for DnaJ proteins in such decisions. Sang Yeol Lee (Gyeongsang National University, South Korea) presented data regarding the redox protein 2-Cys Prx, which changes functions from thiol peroxidase to molecular chaperone, depending on conditions from heat to oxidative stress. Similarly, AtTDX, a plant-specific type-II thioredoxin, isolated from *Arabidopsis* formed homopolymers of low or high molecular weight and exhibited multiple roles as disulfide reductase and foldase chaperone (low MW) and holdase chaperone (high MW). The holdase chaperone function of AtTDX provided heat resistance to plants. Iron homeostasis was introduced by Claudina Rodrigues-Pousada and Catarina Pimentel (Instituto de Tecnologia Química e Biológica, Portugal). They discussed that iron is an essential element for many cellular processes and that iron deficiencies or excesses result in several human diseases, including hemochromatosis and Friedreich ataxia. In *Saccharomyces cerevisiae*, Yap5 is an iron-responsive factor that regulates the transcription of the vacuolar transporter CCC1. Genes encoding proteins involved in iron metabolism were normally repressed by Yap5, which was overcome by changes in iron homeostasis. However, CCC1 mutants exhibited higher sensitivity to iron excess than Yap5 mutants, suggesting an alternative mechanism. Dr. Pimentel proposed that Hap4 was responsible for basal expression of CCC1. An unexpected observation that was introduced by Dr. Rodrigues-Pousada was that treatment with arsenite resulted in the expression of genes encoding for iron-cluster protein biosynthesis, such as YFH1, the ortholog of human frataxin, the iron transporter AFT2, and the ferric reductase FRE2/FRE1. The expression of these genes was dependent on Yap1, another member of the Yap family.

The initial talk on cancer and the stress response was given by Renu Wadhwa (National Institute of Advanced Industrial Science and Technology, Japan), who showed that Mortalin, also known as mitochondrial Hsp70, was overexpressed in cancer cells. This protein, resident of many cell compartments (mitochondria, cytosol, ER), was involved in sequestration of p53, inhibiting its transcriptional activity and subsequently increasing carcinogenesis. In particular, mortalin interacted with p53 mutants. Disruption of mortalin-p53 interaction by shRNA or pharmacological inhibition resulted in p53-mediated, tumor-cell-specific apoptosis. This pathway may lead to an interesting therapeutic strategy against cancer. Toshihiko Torigoe (Sapporo Medical University, Japan) discussed cancer initiating cells/stem cells (CIC/CSC), which correspond to a small population of transformed cells with tumor-initiating capacity, being likely responsible for cancer recurrence and metastasis. Comparative gene expression analysis using DNA microarrays of purified CIC/CSC populations from various tumors revealed novel specific gene markers for these cells, such as SOX2, SMCP,

and DNAJB8. In particular, overexpression of all three of these proteins in cancer stem cells increased their tumor-initiating capacity, which was reversed by RNAi approaches. In addition, DNA immunization with DNAJB8 could suppress tumor growth. Overexpression in normal cells did not lead to cell transformation, indicating that DNAJB8 cannot be considered as an oncogene, but apparently participates in sustaining cell viability and growth after transformation. These findings are striking given the strong activity of DNAJB8 against aggregation and toxicity of polyglutamine proteins (see discussion below). Sangnam Oh, a graduate student in Eunil Lee's laboratory (Korea University, South Korea) presented a fascinating study on the sensitization of cancer cells to TNF-induced apoptosis by hydrostatic pressure. She showed that the mechanism involved included upregulation of the decoy receptor DR5, caspase activation, decrease in mitochondrial membrane potential, and reactive oxygen species (ROS) production. These studies were extended to other transformed cell lines, obtaining similar results. However, it was not observed in non-transformed human cells. Dae Ho Kwon (Korea University, South Korea) presented the problem encountered during many cancer therapies, which is the development of drug resistance. He showed that cells pretreated with 2,3,7,8-tetrachlorodibenzo-para-dioxin displayed reduced death after cisplatin treatment, which is widely used against gastric cancer. Finally, **Joohun Ha** (Kyung Hee University, South Korea) talked about kinase regulation by 5' AMP-activated protein kinase (AMPK) in cancer cells. He discussed that AMPK plays a central role in adenosine triphosphate (ATP) homeostasis in response to various metabolic stresses. AMPK antagonizes extracellular-signal-regulated kinase (ERK) activation induced by low glucose via induction of dual-specificity phosphatase 1 and 2 expressions, thereby revealing a novel signal pathway between AMPK and ERK in cancer cells.

The topic of chaperones in protein folding diseases was discussed by Boris Margulis (Russian Academy of Sciences, Russia) and Harm Kampinga (University of Groningen, Netherlands). Dr Margulis spoke about Huntington's disease, which is characterized by the formation of protein aggregates in the cells as the result of a large repeat of glutamine residues in the Huntington gene. These polyglutamine (polyQ) chains resulted in aggregates that could covalently bind glyceraldehyde-3-phosphate dehydrogenase by a reaction catalyzed by transglutaminases. The formation of these polyQ aggregates could be inhibited by the presence of molecular chaperones, particularly Hsp70 and Hdj1/2. Dr. Margulis presented data demonstrating that Hsp70 in a dose-dependent manner reduced the formation of these aggregates. Moreover, inhibitors of transglutaminase also reduced the aggregate size. Dr. Kampinga reported on a comparative screen in his laboratory, in which all members of the human

HSP110, HSP70, and DNAJA and DNAJB protein families were compared for their effectiveness in reducing aggregation and toxicity of polyQ proteins. Dr. Kampinga's group identified two members of the DNAJB family (DNAJB6 and DNAJB8) as the most potent suppressors and found that their activity was largely independent of collaboration with HSP70s and that it was regulated by histone deacetylases. In addition, chaperones can protect against folding diseases. Mutations in chaperones themselves may be causative to folding diseases, which have been coined "chaperonopathies" and were discussed by Alberto Macario (University of Maryland, USA). So far, the identified chaperonopathies are caused by mutations in members of the cytosolic chaperonin containing TCP-1 family of cytosolic chaperonins, in several members of the small heat shock protein (HSPB) family of small heat shock proteins, and a few in DNAJ members. Whether the mutations lead to loss of function of their role in protein quality control and/or have dominant gain-of-function characteristics (like the polyQ proteins, for example) are, in most cases, unknown. In this regard, Ivor Benjamin (University of Utah, USA) presented an interesting study on the genetically heritable cardiomyopathy caused by an autosomal dominant small Hsp CryAB mutation. This mutation results in multisystem disease of the lens, heart, skeletal muscles, and causes lifespan shortening. Studies using a genetically modified mouse model indicated that mutations on CryAB caused a reductive stress rather than oxidative stress. Thus, an excess of reducing equivalents in the form of nicotinamide adenine dinucleotide phosphate or reduced glutathione were observed. Dr. Benjamin indicated that G6PD, the rate-limiting enzyme in the pentose phosphate pathway, was necessary and sufficient for the development of CryAB mutation-induced cardiomyopathy.

Another important aspect of the workshop was infectious disease. Dong-Kwon Rhee (Sungkyunkwan University, South Korea) discussed the importance of *Streptococcus pneumoniae*, a gram-positive bacterium, as a human pathogen. *S. pneumoniae* is the most common cause of acute otitis media, sinusitis, pneumonia, and meningitis. This bacterium is exposed to several environmental stresses, such as heat stress during the transition from nasal mucosa (30–34°C) to the blood stream (37°C), triggering the expression of heat shock proteins. ClpL, a member of the Hsp100 family, from *S. pneumoniae* repressed adherence of the bacterium to epithelium and triggered the secretion of TNF- α via a mechanism dependent on actin polymerization during the initial stage of infection. Alejandro Barbieri (Florida International University, USA) also presented an exciting study illustrating how *Pseudomonas aeruginosa*, a gram-negative bacterium, uses the endocytic pathway to propagate. *P. aeruginosa* translocates toxins (e.g., ExoS and ExoT) into host cells via a Type III secretion apparatus, altering Rab5 and EEA1, which are part of the early endosome and play a critical role in early vesicle trafficking. He showed that ExoS specifically regulates the

activity of Rab5, blocking its interaction with guanine exchange factor Ras interference 1, facilitating infection. Investigations on the molecular mechanism of this pathway may result in novel therapeutic interventions to ameliorate *pseudomonas* pathogenesis, which is a serious health problem. More news about the endocytic process was presented by Laura Santambrogio (Albert Einstein College of Medicine, USA), who talked about the delivery of cytosolic components into the lysosomes during autophagy. She described a novel mechanism in which cytosolic proteins are delivered between late endosomes and lysosomes. This delivery of soluble cytosolic proteins was mediated by chaperones, Hsc70 (HSPA8) in particular, which intersected the endosomal sorting complex required for transport (ESCRT) I and III systems that result in the formation of vesicles within multiple vesicular bodies. As elegantly demonstrated, HSPA8 interacted with PS on the endosomal membrane by a highly positive-charged region in this molecule. This interaction was ATP-dependent.

Antonio De Maio (University of California San Diego, USA) talked about the interaction of Hsp70 with lipid membranes and the different mechanisms for the export to the extracellular space of this otherwise cytosolic protein. He presented evidence that membrane-free Hsp70, which is mainly a product of necrosis, could induce cell death, whereas membrane-bound Hsp70 was capable of activating cells of the immune system. He discussed the novel concept of the stress observation system, which is a new form of cellular communication during injury/infection conditions. In this system, hsps, particularly Hsp70, are incorporated in the lipid bilayer of vesicles, named stress cellular vesicles, which act as an "alert" signal for the occurrence of injury.

The role of hsps was also discussed in an array of other disease conditions. Virginia Vega (University of California San Diego, USA) talked about diabetes mellitus type 1 (DMT1), which is an autoimmune disease that affects one in every 400–500 children in the United States. This disease is characterized by the destruction of β cells in the pancreas. She explained that a major risk for DMT1 patients is the high incidence of infections. She hypothesized that macrophages from DMT1 patients displayed a lower phagocytic and killing capacity, resulting in overwhelming infections. Using non-obese diabetic (NOD) mice, she showed that macrophages derived from these animals during pre-diabetic (8 weeks old) and diabetic (12 weeks old) conditions showed reduced phagocytic/killing capacity than did cells from wild-type animals. Finally, she presented clear evidence that exposure of macrophages to heat shock and recovery increased the phagocytic capacity of NOD-derived macrophages. These observations may lead to therapeutic alternatives to reduce infections in DMT1 patients. Jinoh Kim (University of California Davis, USA) presented an interesting study on

the role of secretory pathways in the development of Cranio-lenticulo-sutural dysplasia (CLSD). This disease is characterized by late-closing fontanels, sutural cataracts, facial dysmorphisms, and skeletal defects. The condition is the result of a missense mutation in SEC23A, which is a component of the coat protein complex II (COPII). In fact, fibroblasts from CLSD patients were secretion deficient and displayed an enlargement of the ER. However, there was no apparent overexpression of PDI or BIP (Grp74), suggesting the absence of ER stress. Irina Guzhova (Russian Academy of Sciences, Russia) presented novel research demonstrating protective activity associated with Hsp70 against seizures. She showed that pretreatment with exogenous Hsp70 (injected intracerebroventricularly) into animals in which seizures were induced by treatment with N-methyl-D-aspartic acid (NMDA) or pentylenetetrazole resulted in reduced insult severity. The Hsp70-induced anticonvulsatory effect was associated with the presence of the hsp in neurons and terminals of the limbic seizure complex of the brain. In addition, Hsp70 co-localized with NMDA receptors, synaptophysin, gamma-aminobutyric acid (GABA)-synthesizing enzyme, and L-glutamic acid decarboxylase. Thus, the anticonvulsant property of exogenous Hsp70 is likely part of a mechanism to modulate GABA neurotransmission, contributing to the maintenance of the excitatory–inhibitory balance of the CNS. Kwon Moo Park (Kyunpook National University, South Korea) showed that a mild ischemia/reperfusion episode resulted in subsequent tolerance of a second ischemic insult, which is known as preconditioning. The protective effect was associated with the production of ROS. In fact, antioxidants abolished the preconditioning effect. Sung Soo Kim (Kyung Hee University, South Korea) talked about cyclophilins (Cyps), which correspond to a family of proteins containing a peptidyl cis–trans isomerase and chaperone activity. Cyps participate in an array of physiological and pathological events, including muscle differentiation, T cell activation, viral infection, and atherosclerosis. Moreover, CypA is upregulated in many human cancers, and its expression is directly under transcriptional regulation by p53 and HIF-1 α . Overexpression of CypA prevented hypoxia, which was associated with the suppression of ROS generation and depolarization of mitochondrial membrane potential. Another member of the family, CypB, was critical in the protection from ER stress via its peptidyl prolyl cis-trans-isomerase activity. Finally, Tohru Mizushima (Kumamoto University, Japan) discussed an effect of geranylgeranylacetone, which is an anti-ulcer drug, on the expression of hsps. He proposed a therapeutic effect of this drug on inflammatory bowel disease and idiopathic pulmonary fibrosis.

Patrick Arrigo (University of Lyon, France) reported on the response of small hsps, in particular HSPB1 (Hsp27) in response to a variety of apoptotic stimuli. Human HSPB1 is

an oligomeric protein that has tumorigenic and metastatic roles. He showed that the subcellular (re)localization, level of oligomerization, and phosphorylation of HSPB1 were very different depending on the type of (chemotherapeutic) stress that was used, which may reflect the different ways in which this protein can act to prevent stress-induced cell death. In addition, Dr. Arrigo reported the development of various aptamers that can either abrogate HSPB1 function or stimulate it. Such aptamers may have great therapeutic value as illustrated by one HSPB1-inhibiting aptamer that could abrogate growth of a squamous cell carcinoma. The biology of small hsps was further discussed by Robert Tanguay (Laval University, Canada), CSSI President-elect. Following up on the earlier demonstration that the overexpression of a small mitochondrial hsp of *Drosophila* (DmHsp22) increased lifespan and resistance to oxidative stress, he discussed the mechanisms by which this small hsp might influence longevity. Microarray, mitoproteomic, and immunoprecipitation approaches suggested that DmHsp22 may protect or stabilize components of the oxidative phosphorylation complex. When expressed in mammalian cells, this small hsp increased the lifespan of normal fibroblasts but caused malignant transformation of human breast cancer cells. Interestingly, DmHsp22 also interacted with p53, like mortalin (as discussed by Renu Wadhwa above). Continuing on the subject of aging, Joong-Jean Park (Korea University, South Korea) presented data on clusterin (apolipoprotein J), which is a disulfide-linked heterodimeric glycoprotein. Expression of the human secretory form of clusterin increases with aging, and expression of this human protein in *Drosophila* resulted in lifespan extension. Moreover, *Drosophila* expressing human clusterin was more resistant to heat shock, wet starvation, and oxidative stress. In particular, the levels of ROS were reduced in transgenic flies with respect to controls. In vitro experiments revealed an antioxidant capacity associated with clusterin as it inhibits glutamine synthetase activity. Thus, clusterin seems to function as an antioxidant, reducing ROS and delaying aging.

Nonsense-mediated mRNA decay (NMD) is a surveillance mechanism to avoid the production of aberrant mRNAs. Yoon Ki Kim (Korea University, South Korea) described how human proline-rich nuclear receptor coregulatory protein 2 (PNRC2) is involved in NMD. Down regulation of PNRC2 reduced NMD and artificially tethered PNRC2 downstream of the normal termination codon, reducing mRNA abundance. Thus, an interaction between PNRC2 and the decapping complex was presented. RNA-induced silencing complexes are involved in the stability and translation of cellular mRNAs. RISC and targeted mRNA accumulates in discrete cytoplasmic structures known as GW bodies. Young Sik Lee (Korea University, South Korea) demonstrated that GW bodies were associated

with late endosomes (multivesicular bodies [MVB]). Interfering RNA was enhanced by blocking the maturation of late endosomes into lysosomes. In contrast, RNA silencing was reduced by blocking MVB ESCRT depletion. Dr. Lee suggested that recycling of RISC was promoted by MVB, which resulted in a more efficient RNA silencing. Finally, Joon Kim (Korea University, South Korea) brought us to the world of ribosome biogenesis. He discussed how the ribosomal protein S3 plays multiple roles, including translation as part of the ribosome, DNA repair, apoptosis, and metastasis. The role of S3 in DNA repair has been associated with phosphorylation of this protein by PKC δ kinase after oxidative stress conditions. Phosphorylated S3 was found in the non-ribosomal fraction within the nucleus. The endonuclease activity was reduced after dephosphorylation by phosphatase PP2A.

The workshop was a great opportunity for direct interaction among participants, particularly for students who enjoyed

talking with professors about their research and future plans. The final day was an experience of picture taking to preserve the memories of the good time everyone had. Seven students received travel awards from the CSSI (Catarina Pimentel, Kim Yeonghwan, Md Abdur Rashid, Tak Eunyoung, Thao Dang-Hien Tran, Choi, Ran Gao, and Kim Hyun-Ju). Images from the awards presentations appear in Supplement 1 (online only). In addition, two poster awards were presented. Several of the international speakers remained in South Korea to enjoy the country's hospitality, excellent food, and sightseeing around the many beautiful locations of this great country. The 8th International Workshop on the Molecular Biology of Stress Responses was a great success due to the outstanding organization provided by the KCSS. We are sure that this workshop will result in sustainable growth for the KCSS and the possibility of developing an Asian Federation of Stress Societies, which will include other nations such as Japan and China.