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MEETING REVIEW

Small heat shock proteins: big folding machines

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Abstract The workshop was entitled “The Small HSP World” and had the mission to bring together investigators studying small heat shock proteins (sHSPs). It was held at Le Bonne Entente in Quebec City (Quebec, Canada) from October 2 to October 5 2014. Forty-four scientists from 14 different countries attended this workshop of the Cell Stress Society International (CSSI). The small number of participants stimulated interesting discussions, and the resulting informal atmosphere was appreciated by everybody. This article provides highlights from talks and discussions of the workshop, giving an overview of the latest work on sHSPs.

Keywords Small heat shock protein (sHSP) · HspB · Structure · Function · Oligomerisation · Chaperone · Alpha-crystallin domain (ACD) · Arabidopsis · Drosophila · Artemia · Synechocystis · Mammalian

Introduction

The small heat shock proteins (sHSPs) were initially reported in the seminal paper of Alfred Tissières on the labeling of proteins after a heat shock in *Drosophila melanogaster* (Tissières et al. 1974). sHSP gene cloning started at the end of the 1970s (Storti et al. 1980; Wadsworth et al. 1980; Craig and McCarthy 1980). In 1982, Elizabeth Craig (Ingolia and Craig 1982) reported that their sequences were related to alpha-crystallin identifying the hallmark of sHSP as the alpha-crystallin domain (ACD). Interest in the functions of the sHSP came in the late 1980s when it was reported that the induction of Hsp27 in mammalian cells could be correlated to the phenomenon of thermotolerance (Landry et al. 1989). Members of this family of heat shock proteins have been found in most domains of life including viruses (Maaroufi and Tanguay 2013). In addition to being inducible by most of the stressors that induce the so-called heat shock response, sHSPs are also expressed in the absence of stress. Thus, in many different organisms, the expression patterns of sHSPs differ depending on the organ or in a developmental-stage-specific manner.

The Cell Stress Society International (CSSI) first International Workshop on the “Small HSP World” was held in Québec, Canada, on October 2–5, 2014. The workshop organized by Robert M. Tanguay (Université Laval, Québec, Canada) brought together 44 scientists from 14 different countries to discuss the current state of the art in the structure and functions of these fascinating albeit still poorly understood chaperones. Figure 1 shows all the speakers as well as a photo of the Bonne Entente, the hotel where the workshop took place.

Opening lecture

The opening lecture was given by Harm H. Kampinga (Groningen University, Netherlands). Following a summary of key findings on sHSPs, he presented an extensive comparison of each of the ten sHSPs in humans (Kampinga et al. 2009). Although sharing the alpha-crystalin domain (ACD), human sHSPs have very distinctive features regarding their heat-induced expression, tissue and intracellular localizations, assembly, substrate preference, and function. Due to these differences, human sHSPs have different abilities to protect

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◀ **Fig. 1** Speakers of the CSSI first International Workshop on the "Small HSP World." Speakers in order of talks and photo of the Bonne Entente, the hotel where the workshop has taken place. *First row* (*left to right*): Harm H. Kampinga, Elizabeth Vierling, André-Patrick Arrigo, Justin Benesch with Heath Ecroyd, and Cecilia Emanuelsson. *Second row* (*left to right*): Justin Benesch, Krzysztof Liberek, Nikolai B. Gusev, Stéphanie Finet, and Stephen D. Weeks. *Third row*: Sevil Weinkauf. *Fourth row*: Johannes Buchner. *Fifth row*: Wilbert C. Boelens. *Sixth row* (*left to right*): Ivor J. Benjamin, Tangchun Wu, Roy A. Quinlan, Serena Carra, Josée N. Lavoie, and Lawrence E. Higtower. *Seventh row* (*left to right*): Melinda E. Tóth, Nikola Golenhofen, R. William Currie, Martin Haslbeck, Thomas H. MacRae, and Robert M. Tanguay.

against acute and different types of chronic (disease-related) stress.

Session 1 and 2—structure-function of sHSPs

Elizabeth Vierling (University of Massachusetts, Amherst, USA) continued with the idea that each sHSP may have evolved in its own way, using the plant *Arabidopsis thaliana* as a supportive model. Small HSP mutants that retain a small subunit conformation are more effective chaperones than those that form large oligomers. Interestingly, such mutants have also led Vierling's team to reexamine the quaternary structure of wheat Hsp16.9 and to suggest that the crystal structure of Hsp16.9 as a double doughnut dodecamer may represent only one of several possible structural arrangements. Ongoing work in *Synechocystis* was also presented in which the analysis of Hsp16.6 defect suppressors suggests that there are different ways to acquire thermotolerance.

Based on the multiple unrelated functions of constitutively expressed human sHSPs, André-Patrick Arrigo (Université de Lyon, France) suggested that human sHSPs may interact with many essential polypeptides in a manner similar to HSP90. Thus, different substrates or clients may interact with different oligomeric forms of HSPB1. Using three different substrates of HSPB1 (pro-caspase 3, histone deacetylase 6 (HDAC6), and signal transducer and activator of transcription 2 (Stat2)), it was shown that the dynamic structural organization of HSPB1 governed by phosphorylation generates different platforms that recognize specific substrates. Adding the ability of HSPB1 to form hetero-complexes with other sHSPs further increases the possibility of specific substrate (client) recognition sites. One such substrate is glucose-6-phosphate dehydrogenase (G6PDH), which interacts with highly phosphorylated HSPB1 following HSPB1-HSPB5 interaction. Considering the multiple reports of HSPB1 expression in different diseases, drugs could be designed to target sHSP interactions with a specific client protein.

Heath Ecroyd (University of Wollongong, Australia) then reported that HSPB5 (alphaB-crystallin) can inhibit aggregation of different substrates via weak and transient interactions rather than by forming a stable complex with them. HSPB5 is also capable of binding to amyloid fibrils, and by doing so, it stabilizes them and prevents the deleterious effect of their fragmentation and/or secondary nucleation. Binding to the fibrils appears to require the N- and/or C-terminal extension of the sHSP rather than the ACD. sHSPs therefore interact with substrates at multiple points along their aggregation pathway. It was therefore suggested that sHSPs would be best described as stabilizers rather than holdases due to their ability to interact transiently with substrate, an activity that most likely predominates in the cell under basal conditions. A new way to assess chaperone activity in vivo using IRES vectors was also presented.

Cecilia Emanuelsson (Lund University, Sweden) presented the work of her team on the chloroplast Hsp21 from *Arabidopsis*. Compared to cytosolic plant sHSPs such as wheat Hsp16.9, Hsp21 has a longer N-terminal domain and extended IXI/V motif in the C-terminal region and adopts a dodecameric conformation. It was also shown that approximately half of the amount of Hsp21 translocated to thylakoid membranes upon plant heat stress and that this property is shared by a few proteins but never to the same extent as this sHSP. By using mutants, the dimeric form of Hsp21 was tentatively identified as the form going to membranes.

Justin Benesch (University of Oxford, UK) documented the dynamism of HSPB5 oligomers by taking advantage of the sensitivity and separation afforded by native mass spectrometry. It was shown that for polydisperse sHSPs, oligomers with an even number of subunits were typically more abundant than oligomers with odd subunits, indicative of a weak dimer interface. Using different assays, a disulfide-locked dimer of the HSPB5 ACD was shown to prevent the aggregation of different substrates and reduce the toxicity of the aggregates, while the equivalent domain of HSPB1 was not efficient. It was also shown that phosphorylation weakened the dimer interface of HSPB5 and that this is linked to an increased rate of subunit-exchange and ultimately enhanced anti-aggregation activity in vitro.

In bacteria, heat shock results in protein aggregation in cell poles. Krzysztof Liberek (University of Gdańsk, Poland) reported that the simultaneous presence of IbpA and IbpB (the orthologs of human HSPB) during substrate aggregation was required for subsequent Hsp100- and Hsp70-dependent substrate disaggregation and renaturation and that IbpB needed IbpA to associate to the aggregate in order to accomplish its function. However, IbpA by itself was able to change the morphology of protein aggregates. Both N- and C-terminal ends of IbpA were required for interaction with substrate and chaperone functions. Lastly, using a peptide approach, a new structural element involved in oligomerisation and chaperone activity of IbpA was identified in position -1 of the IXI motif.

Nikolai B. Gusev (Moscow State University, Russia) compared the bio-physical properties of different HSPB1 disease-causing mutants. The effects of the mutations were different

depending if they were located in the ACD or in N- and C-terminal ends. Mutations located in the beginning of ACD affected phosphorylation-dependent dissociation of large HSPB1 oligomers, thus probably modulating its physiologically important properties. As a rule, mutations led to decreased thermal stability and chaperone-like activity of HSPB1. The quaternary structure and interaction with HSPB6 were also affected but sometimes in different ways. Two mutations found in neurological diseases displayed physico-chemical properties similar to those of the wild-type protein (K141Q and T180I).

Stéphanie Finet (UPMC Paris 6, France) continued on the analysis of correlation between structure and in vitro function of HSPB1, HSPB4, HSPB5 wild type, and HSPB5 R120 mutants. The physico-chemical properties were determined using dynamic light scattering, multi-angle light scattering, and small-angle X-ray scattering. The number of subunits of HSPB1 and HSPB5 increased with temperature while the smallest HSPB4 oligomer was obtained at 37 °C. The subunit exchange rate was faster between HSPB1 and HSPB5 than between HSPB4 and HSPB5. It was further shown that sHSPs had different affinities to substrates and that hetero-complexes had a different efficiency to prevent protein aggregation. With HSPB5 R120 mutants, she demonstrated a direct correlation between the modification of structure and the loss of chaperone function. No cavity was found in the oligomers.

The subject of Stephen D. Weeks' (KU Leuven, Belgium) talk was the HSPB1-HSPB6 hetero-oligomer. This hetero-complex has been previously shown to exist in vivo and, at a lower level of assembly, composed preferentially of hetero-dimers. Using a combination of native mass spectrometry, analytical gel filtration, and disulfide cross-linking, numerous deletion constructs and mutants were characterized in an attempt to identify the sequence determinants that drive this exclusive association. Surprisingly, the data point to an essential role of the unstructured N-terminal region (NTR) rather than of the ACD, the latter forming the principle dimer interface in the oligomers. Iterative mapping highlighted a conserved sequence in the NTR of HSPB6 that Stephen and his colleagues have recently reported to be also involved in defining chaperoning activity of this particular sHSP (Heirbaut et al. 2014). In addition, data were shown related to the effect of HSPB1 congenital mutations on the hetero-oligomerisation process. Curiously some mutants showed opposing behavior, either inhibiting or promoting association with HSPB6. Coming full circle, this raises the question as to the role of these species in vivo.

Session 3—crystallins

Sevil Weinkauf (TUM, Garching, Germany) explained how her team has managed to analyze the quaternary structure of HSPB5 using single-particle cryo-electron microscopy. They obtained a 9.4 Å resolution quaternary structure of the 24-mer of HSPB5 (αB-crystallin) and were almost able to discriminate helices located in the N-terminal extension. This latter extension seemed to play a decisive role in higher-order oligomer assembly. Many oligomeric assemblies of HSPB5 ranging from 6 to 48 subunits were obtained. The work on HSPB4 (αA-crystallin) structure is ongoing but difficult due to its polydispersity. At this time, there is only little evidence for the existence of a symmetric 24-mer of HSPB4.

Johannes Buchner (TUM, Garching, Germany) demonstrated that the phosphorylated residues in the NTR of HSPB5 were in close proximity and that they enhanced flexibility. Using phosphorylation mimicry, it was shown that phosphorylation shifted the oligomer equilibrium to smaller assembly forms and increased in vitro chaperone activity as well as the suppression of aggregation of cytosolic proteins. One of the client proteins, p53 was found to bind preferentially to smaller oligomers. Hetero-complexes between different human sHSPs were shown to have a different ability to prevent aggregation suggesting means to regulate activity and potentially substrate specificity. It was also commented that the chaperone activity of different sHSPs varied depending on the substrate used as illustrated for malate dehydrogenase, GAPDH, and citrate synthase.

Wilbert C. Boelens (Radboud University, Nijmegen, Netherlands) presented the work his laboratory did in collaboration with Pierre Goloubinoff (Université de Lausanne, Switzerland). Using different techniques, they were able to show protein refolding activity of IbpB and HSPB5. Furthermore, it was found that phosphorylation inhibited the anti-aggregation activity of HSPB5, which is contradictory to results reported by other groups (Ecroyd and Buchner). The observed differences with chaperone-like activity assays will need further examination in order to try to standardize these types of assays.

Session 4—sHSPs in the clinic

Ivor J. Benjamin (Medical College of Wisconsin, Milwaukee, USA), a practicing cardiologist as well as a researcher, brought the audience into the clinic and introduced them to roles for sHSPs in myofibrillar diseases. He proposed that heart failure can be viewed as a protein folding problem. He demonstrated that mutations in HSPB5 caused myopathies through reductive stress in *Drosophila* and that it was responsible for cellular hypertrophy in cardiomyocytes derived from induced pluripotent stem cells. The results presented suggested that metabolic remodeling of redox pathways with high NADPH generation are genetic modifiers of reductive stress-induced pathology.

Tangchun Wu (Huazhong University of Science and Technology, Wuhan, China) discussed the relationships between sHSP variants and several human diseases. Because of their varied functions, sHSPs can participate in a large number of fundamental cellular processes such as controlling protein folding, F-actin-dependent processes, cytoprotection/anti-apoptosis, differentiation, cell proliferation, and gene expression. As a consequence, they are involved in pathologies such as neurodegenerative diseases, cancer, and cardiovascular diseases. Genetic mutations in sHSP genes may change their expression levels and affect protein functions, thus contributing to cellular malfunctions especially during stress. Here, he examined current reports regarding some of these mutations or variations of sHSP genes and analyzed their associations with the development, progression, and prognosis of several human diseases (Guo et al. 2010).

Roy A. Quinlan (Durham University, UK) described how together the dynamic duo of sHSPs and intermediate filaments (IF) maintains cell homeostasis, resists cellular stress, and enables evolution in cells and tissues. He proposed that IFs are used by sHSPs as reaction centers to bring proteins together, and he introduced the concept of synergy between sHSPs and IFs when combined in the sHSP-IF complex and the role played by both of them in the stress response. This interaction may regulate the diverse functions of sHSPs found in multiple processes. For example the R120G mutation in HSPB5 may reduce mitochondrial movement within cells.

Serena Carra (Universita di Modena e Reggio Emilia, Modena, Italy) reported on the alterations of proteostasis and RNA metabolism in Amyotrophic Lateral Sclerosis (ALS) and on the beneficial effect of HSPB8 towards ALS-associated proteins (TDP43) in motor neuronal cells and in the *Drosophila* fly model. The results further showed that upon proteotoxic stress HSPB8 dissociated from the HSPB8-Hsp70-Bag3 complex. Whereas HSPB8 was recruited into stress granules, Bag3-Hsp70 colocalized with ubiquitinated defective ribosomal products adjacent to the stress granules.

Continuing on HSPB8 and Bag3, Josée N. Lavoie (CHU, Québec, Canada) demonstrated how the silencing of Bag3, HSPB8, or the autophagic adaptor protein p62/SQSTM1 impaired mitosis, suggesting a role for the HSPB8-Bag3 complex in actin dynamics, spindle positioning, and proper chromosome segregation. Significantly, the Bag3-dependent mitotic phenotype could be corrected by increasing cortical rigidity from the outside upon addition of the lectin concanavalin A. These novel findings suggested the involvement of a quality control mechanism regulated by Bag3-HSPB8 in the proper remodeling of actin-based mitotic structures.

Lawrence E. Hightower (University of Connecticut, Storrs, USA) explored the effects of heat shock and the mechanistic role of Hsp27 in cell movement using fish keratocytes, a well-established model of rapidly moving cells. Previously, he and his co-workers used a human colon cancer cell line and nontumorogenic colonocytes to show that HSPB1 is critical for wound healing (Doshi et al. 2009). In fish scale keratocytes, heat shock caused a decrease in cell speed and changes in cell morphology indicating both cytoskeletal rearrangements and increased adhesion to substrata. A model for how Hsp27 may regulate actin filament dynamics, cell speed, and morphology was discussed.

Working with the hypothesis that cellular membranes are thermal sensors, Melinda E. Tóth (Biological Research Centre, Szeged, Hungary) presented her work and Prof. László Vígh's work on how *Synechocystis* Hsp17 and bacterial IbpA and IbpB interacted with lipid membranes and how their deletion affected membrane fluidity and fatty acid composition of *Escherichia coli*. She then followed up by describing diverse neuroprotective effects of HSPB1 in transgenic mice treated or not with ethanol and amelioration of certain symptoms of Alzheimer's disease (AD) using AD model mice. Cholesterol was also suggested to control the interaction of HSPB1 with lipid rafts.

Nikola Golenhofen (University of Ulm, Germany) followed with the work of her team on HSPB5 function in neurons. They observed a phosphorylation-dependent recruitment of HSPB5 to dendrites and axons. Furthermore, overexpression of HSPB5 (but not of the other sHSPs) in cultured hippocampal neurons increased the complexity of the dendritic tree. This stimulating effect on dendrites was not observed using a non-phosphorylable HSPB5-mutant. Thus, a function of HSPB5 in protecting the dendritic arbor during pathological situations (when HSPB5 is upregulated and phosphorylated) was suggested.

Finally, R. William Currie (Dalhousie University, Halifax, Canada) commented on the preferential use of males in many animal studies and asked whether there were sex differences in the expression of Hsp70 and HSPB1. Using the rat hippocampus as a model, he reported that after heat shock, males had a greater heat shock induction of Hsp70 and HSPB1 than females. However, the localizations of Hsp70 in microglia and blood vessels and HSPB1 in astrocytes were similar in male and female animals.

Session 5—sHSPs, development and pathology

The last session was dedicated to sHsps in different developmental models. Martin Haslbeck (TUM, Garching, Germany) presented recent work on Sip1, a sHsp only expressed in embryos of *Caenorhabditis elegans*. This sHSP was not induced by heat shock but was necessary for survival of the embryo. The expression of Sip1 also enhanced lifespan and survival upon heat shock. Sip1 acted as a pH-activated chaperone in vitro with its optimum of activity at the physiological pH of the *C. elegans* embryo; while the pI of most sHSPs was

acidic, that of Sip1 was 7.9. Using immunoprecipitation followed by mass spectrometry, some clients were identified among which vitellogenins were rather prominent.

Thomas H. MacRae (Dalhousie University, Halifax, Canada) described the effects of independent RNAi knock down of the three *Artemia franciscana* sHSPs on diapause. The results obtained suggest that p26 enhances stress tolerance of the cyst, affects the rate of embryo development, and prevents spontaneous diapause termination. ArHsp21 RNAi had only small effects on cysts while ArHsp22 RNAi killed the adults. Altogether, these results show that *Artemia* sHSPs are functionally distinct.

Using *D. melanogaster* as a model, Robert M. Tanguay (Université Laval, Québec, Canada) described to what extent the overexpression of Hsp22, which was shown to extend lifespan and protect against oxidative stress, altered the mitochondrial proteome. These results complemented some biochemical assays suggesting that Hsp22 altered mitochondrial metabolism and mitochondrial protein quality control to increase longevity.

Round table—the follow-up

A final round table discussion was chaired by Johannes Buchner and Elizabeth Vierling. One of the points that what raised often during the workshop was the need to define a set of assays and conditions that investigators should use prior to claiming that their sHSP possesses chaperone activity and to allow comparison between different members of the family or modified forms. There was general consensus that while the in vitro assays report on the principle biochemical and biophysical properties of sHSPs, they do not necessarily reflect what happens in the cell. Designing new functional assays will be essential to understand the complex functions of these proteins in living cells and animals. Using cell lysates as "substrate" could be a first step in this direction. It was also pointed out that the community should define on what basis a protein can be identified as a substrate of sHSPs and should decide which of the words "substrate", "client," or "target" is the more appropriate. Concerning nomenclature, the terms substrate and client are at the moment used as synonyms.

During the course of the workshop, it was noticed that people working with crystallins only infrequently use the HUGO-approved nomenclature proposed by Kampinga et al. (2009). Because this nomenclature has not been broadly adopted by the sHSP community and because this nomenclature only applies to human sHSPs, its general utility was questioned. Overall, what seemed important was that it should be clear which sHSP was actually being discussed. For other HSP families, there is little doubt that the new nomenclature has improved communication of data and electronic database organization.

Essentially, all of the participants have acknowledged that the formula of the workshop strongly encouraged discussion and it was proposed to hold a workshop on sHSPs every 2 years. It was also proposed to add a session dedicated to address new technologies and experimental protocols. Finally, it was emphasized that the participation of young people was good and should be encouraged in the future.

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