Heat shock in the springtime

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Abstract A collaborative workshop dedicated to the discussion of heat shock factors in stress response, development, and disease was held on April 22–24, 2014 at the Université Paris Diderot in Paris, France. Recent years have witnessed an explosion of interest in these highly conserved transcription factors, with biological roles ranging from environmental sensing to human development and cancer.

Keywords Heat shock factors · Stress response · Transcription · Epigenetics · Development · Disease

Introduction

Changes in gene expression in response to proteotoxic insults are primarily governed in eukaryotic cells by heat shock transcription factor 1 (HSF1), the founding member of a closely related family of DNA-binding proteins. As evidenced by their name, these regulatory factors are traditionally considered to be stress-responsive players that mediate cytoprotection based on elaboration of a broad transcriptional program that includes heat shock proteins, molecular

chaperones, antioxidant proteins, and other cellular components to promote protein homeostasis (proteostasis). While this is a mostly accurate description of HSF1, the other members of the vertebrate HSF family (HSF2–4) clearly play distinct roles in control of developmental gene expression and other cellular activities, not all of them as transcriptional activators. Moreover, recent work strongly implicates HSF1 as a driver of tumor growth and metastasis by controlling the expression of novel, non-heat shock genes (Fig. 1). In 2008, the first HSF-centered meeting was held in Roscoff, France (Jacques Monod Conference). In the years since then, the popularity and scope of HSF work have only increased, with established researchers breaking new ground and new investigators bringing a fresh, invigorating perspective. Thus, the time was right to ask two interconnected and critical questions in the field: where are we and where do we want to go?

The workshop (Fig. 2) was kicked off by Rick Morimoto (Northwestern University, USA), who delivered the keynote address focusing on a paradigm shift from cell-autonomous to organismal regulation of stress responses. Studies using *Caenorhabditis elegans* as a model demonstrate that stress responses are under neuronal control, communicated between tissues, and integrated with the metabolic processes of the organism (for further details, see recent reviews by (Taylor et al. 2014; van Oosten-Hawle and Morimoto 2014). Maintenance of proteostasis can be regulated by direct communication between different cell types, such as from neurons to cells in various somatic tissues and vice versa, or between and across different tissues. Coordinating how and when distinct stress signals are sensed and transferred to specific cell and tissue types in metazoans is therefore crucial. In this context, HSF (*hsf-1* in the worm) is thought to function as a survival factor or rheostat for acute stress and as a monitoring factor for chronic stress. With the exception of *hsf-1* being the first transcription factor identified linking thermosensory neuronal control and the heat shock response (Prahlad et al. 2008), the

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Fig. 1 A scenic view of HSF. Experimental approaches and technologies being brought to bear in the study of HSF are indicated *above* a model of the HSF1 trimer bound to DNA and superimposed over another famous Paris landmark. Biological roles and processes involving HSF are indicated *below*

signaling pathways and the transducing molecules therein remain to be identified. As part of efforts to understand the mechanism for aging-related proteostasis collapse (Ben-Zvi et al. 2009; Labbadia and Morimoto 2014), Morimoto presented data on the temporal relationship between aging and proteostasis as investigated in *C. elegans* at the early onset of adulthood. Proteostasis capacity is dramatically reduced in a very narrow timeframe as worms reach reproductive maturity. This is manifested as impaired stress response pathways, including the HSF-dependent heat shock response, mitochondrial and endoplasmic reticulum (ER) unfolded protein responses, as well as the oxidative stress response coordinated by *skn-1*. There is accumulating evidence that the proteostasis network sets a threshold for aging, or “healthspan,” very early in the life of an organism. Moreover, data were presented documenting HSF-dependent gene expression during normal development in the unstressed worm, suggesting control over physiology in the absence of unfavorable environmental conditions.

Environmental sensing and the role of misfolded proteins

HSF1 is clearly responsive to a broad array of disparate environmental insults, including heat shock, oxidative stress, and heavy metals. A key question arising from this remarkable

ability remains largely unanswered: how are these conditions sensed by HSF1, and is sensing direct or mediated by additional factors? Not surprisingly, multiple modes of regulation appear to be in play. Matthias Mayer (University of Heidelberg, Germany) has made considerable progress in this area working with recombinant human HSF1, a notoriously challenging protein to purify. Using hydrogen-deuterium exchange methodology, the DNA-binding domain (DBD) was found to be quite stable with low rates of exchange, consistent with the previous acquisition of DBD-DNA co-crystal structures. At low temperatures, the carboxyl-terminal heptad repeat (HR-C), thought to interact with the internal trimerization motifs (HR-A/B) to maintain the protein as a monomer, was also stable. Fascinatingly, as incubation temperature increased (above 37 °C), HR-C was observed to lose structure. This leads to the tantalizing speculation that this domain may be intrinsically responsive to thermal stress, resulting in HSF1 activation as the HR-A/B repeats are released and trimerized. A second level of regulation that has long been accepted as dogma is the role of protein chaperones, namely Hsp90 and its co-factors, in repressing HSF1 activities (Craig and Gross 1991). To date, the strongest evidence supporting this relationship has come from in vitro studies with purified human proteins and cell extracts and genetic approaches in budding yeast. Two groups presented results using parallel approaches demonstrating in vivo interactions between budding yeast

Fig. 2 Workshop participants. *Front row (l to r):* Rick Morimoto, Ji-Sook Hahn, Brian Freeman, Gabriella Santoro, Carmen Garrido, Lea Sistonen, Valérie Mezger, Sandy Westerheide, and Akira Nakai. *Middle row (l to r):* Cécile Caron, Michel Toledano, and Elizabeth Christians. *Back row (l to r):*

Kevin Morano, Allan Drummond, Matthias Mayer, Hans Reinke, Pia Roos-Mattjus, Claire Vourc'h, Gaëtan Jégo, Emmanuel Courtade, Délara Sabéran-Djoneidi, Marc Mendillo, and Quentin Thomen

Hsf1 and the constitutive Ssa class of Hsp70. Kevin Morano (University of Texas Medical School at Houston, USA) showed that affinity purification of Hsf1 followed by immunoblot revealed a robust interaction with Ssa1 in unstressed cells but intriguingly little to no interaction with Hsc82, the constitutively expressed Hsp90 homolog. This observation was impressively validated at the proteomic level by Allan Drummond (University of Chicago, USA) who detected interaction with both Ssa1 and Ssa2 by SILAC technology, with no enrichment of Hsp90. Importantly, versions of Hsf1 with different affinity epitopes installed were used in these experiments, and preliminary work from Morano suggested that the Ssa1-Hsf1 interaction weakens with increasing temperature. This is both consistent with regulatory models and indirectly confirms that the epitope-tagged Hsf1 is not interacting with Ssa1 as a misfolded substrate, as such an interaction would be expected to increase at higher temperature. Together, these results strongly implicate Hsp70 in Hsf1 regulation but raise new questions about a direct role of Hsp90.

One of the hoped for outcomes of studies of the heat shock and other stress responses is the ability to make therapeutic predictions based on empirically derived mathematical models. Attempts have been made to reach these goals (Petre et al. 2011; Rieger et al. 2005), but these models are extremely complex in nature and results are not completely consistent

with the published literature. With the goal of generating a more operational model of the heat shock response (HSR), Emmanuel Courtade and Quentin Thommen (PhLAM, Lille University, France) proposed a simpler model based on the experimental description of both HSF1 kinetics and cell viability under heat shock. Key features of this model are (i) elimination of the rapid variables of the HSR (e.g., trimerization, post-translational modifications, binding to HSE, transcription) and (ii) the introduction of Michaelis-Menten kinetics for the dissociation of heat shock protein: misfolded protein (HSP:MFP) complexes to take into account the saturation of the HSP pool by heat-induced misfolded proteins in the cell. This allows a simple description of HSR dynamics based on titration of chaperones from HSF1, which brings into focus dissociation of the (HSP:MFP) complex as the prevalent parameter of the HSR, compared with the sequestration of HSF1 by HSPs.

Courtade and co-workers have created novel methods to generate heat shock (1,250 nm) or oxidant stress (1,270 nm) by laser irradiation with tight kinetic and spatial control (Anquez et al. 2010; Anquez et al. 2012). On the one hand, this approach allows analysis of extremely rapid (~seconds) kinetics of activation that opens new avenues for temporal studies of stress response mechanisms. On the other hand, spatial control of laser-generated stress allows the

investigation of bystander effects of stress propagation within cell populations, for example through diffusion of long-lived reactive oxygen species (ROS) such as H_2O_2 or through cell-cell communication. How do cells experiencing oxidative stress recognize damaged, misfolded proteins and target chaperones to these substrates, which frequently aggregate in the cellular milieu? Real-time reporting of misfolding and aggregation in live cells can be achieved by analysis of fusions to fluorescent protein moieties. In the case of yeast cells, fusion of the disaggregase Hsp104 to the green fluorescent protein (GFP) reveals accumulation of the chaperone (and presumably substrates) as discrete puncta immediately after proteotoxic stress. Michel Toledano (CEA Saclay, France) presented work from his group and colleagues demonstrating the remarkable finding that Hsp104-GFP fails to form foci in cells challenged with proteotoxic concentrations of H_2O_2 in the absence of the peroxiredoxin/chaperone Tsa1, whereas heat shock-induced aggregates recruit the chaperone with normal efficacy in *tsa1*Δ cells. This result suggests that, while Hsp104 is capable of recognizing and/or being recruited to aggregates caused by heat shock, oxidant-induced misfolding generates distinct populations recalcitrant to Hsp104, and likely Hsp70, binding until an unknown step requiring Tsa1.

New insights into HSF regulation

Investigations into the roles of Hsf1 post-translational modifications have revealed a complex web of events that regulate activation and attenuation of the HSR indicative of both positive and negative impacts. The majority of past work has centered on phosphorylation, and Ji-Sook Hahn (Seoul National University, Korea) presented data demonstrating that budding yeast Hsf1 is phosphorylated by the nutrient-regulated protein kinases Rim15 and Yak1 in response to low glucose levels. These kinases do not appear to be responsive to heat shock, clearly delineating signaling from these two stressors. In keeping with this theme, the general protein phosphatase Ppt1 was found to be necessary for Hsf1 induction by ethanol, an important metabolic product of anaerobic yeast growth (Cho et al. 2014). While localizing these phosphorylation events to specific amino acid residues remains challenging, the genetic and physiological data demonstrating roles for these factors in Hsf1 function are clear. A role for protein acetylation in HSF1 regulation was reported by the Morimoto and Sistonen laboratories (Westerheide et al. 2009), and Sandy Westerheide (University of South Florida, USA) described her laboratory's efforts to understand cellular control of HSF1 acetylation via the deacetylase SIRT1 in both human cells and *C. elegans*. Deletion of worm Sir2.1 revealed that this factor is a convergence point for physiological effects of both caloric restriction and heat shock on both extrinsic stress tolerance and cellular polyglutamine toxicity. Two

SIRT1 regulatory factors, AROS and DBC1, also affect HSF1 acetylation status and activation, broadening the acetylation regulatory network (Raynes et al. 2013). Brian Freeman (University of Illinois, Urbana-Champaign, USA) presented both sides of the coin, documenting roles for both deacetylation and histone (more appropriately termed lysine) acetyltransferases (HATs/KATs) (Zelin et al. 2012). In vitro acetylation was shown to block DNA binding by HSF1, and overexpression of the KAT GCN5 reduced *hsp70* expression in human cells, consistent with prior data. Interestingly, using a panel of overexpressed lysine deacetylases (KDACs), deacetylation was seen to profoundly alter the magnitude, activation, and attenuation of the HSR in a KDAC isoform-specific fashion. Together, these results hint at a much more complex regulatory code of acetylation/deacetylation that could allow for tissue-specific control of the HSR based on selective KAT/KDAC expression.

What about additional regulatory partners of HSF1? One of the highlights of the workshop was the presentation by Akira Nakai (Yamaguchi University, Japan), whose group has performed extensive proteomic studies and ChIP-Seq analyses to identify HSF1 partner proteins under non-stress and stress conditions. In the absence of stress, a minute portion of HSF1 exists as a trimer and forms a complex with the replication protein RPA, which has access to nucleosomal DNA (Fujimoto et al. 2012). New data demonstrated a stress-inducible transcription complex where HSF1 interacts with the activating factor ATF1, a member of the ATF1/CREB family, and is required for *hsp70* induction. Interestingly, ATF1 needs to be phosphorylated in order to be recruited by HSF1 to the Hsp70 promoter, which in turn is required for recruitment of co-activators such as BRG1 and p300/CBP. This population of factors together facilitates HSF1 activity: the interaction between ATF1 and BRG1 leads to chromatin opening, whereas the interaction between ATF1 with p300/CBP accelerates the formation of closed chromatin. These findings pave the way for future studies aimed at unraveling HSF1 post-translational modifications and protein interactions specific for the distinct phases of the activation-attenuation cycle.

HSF as an oncoprotein

HSF1 is a versatile transcription factor with functions beyond regulating the inducible expression of HSPs in the heat shock response. Genome-wide screens for HSF1 occupancy in different organisms and cell types have revealed its plasticity as evidenced through driving distinct transcription programs depending on the cell type, developmental state, metabolic condition, and phase of the cell cycle (Vihervaara and Sistonen 2014). Association of HSF1 with a variety of human cancers has been widely recognized (reviewed by (Calderwood 2012;

Whitesell and Lindquist 2009)). In malignancy, HSF1 drives a transcriptional program that is distinct from acute heat stress and associated with poor clinical outcome (Mendillo et al. 2012). Following up on previous studies (Santagata et al. 2013), Marc Mendillo (Whitehead Institute, USA) presented data demonstrating a tight link between protein synthesis and HSF1 and how this link may be exploited as a therapeutic strategy in cancer. Although HSF1 has been shown to target open chromatin regions (Guertin and Lis 2010), it remains unknown by which mechanism HSF1 chooses its target genes and physically locates to target loci in distinct cell types and upon different stimuli. A major challenge is to unravel the relevant properties of HSF1 such as post-translational modifications or expression patterns that differ in malignant cells versus their non-malignant counterparts. Complementary clinical and molecular studies of HSF1 in specific cancer types, displaying distinct metastatic behavior, should inform how HSF1 is “hijacked” by malignant cells to facilitate oncogenesis.

HSF1 is a critical factor for both normal and pathological hematopoiesis, as demonstrated by Carmen Garrido (INSERM Research Centre, France). She discussed her group’s recent demonstration that the M-CSF-driven differentiation of monocytes into macrophages is regulated by a dual pathway involving HSF1 and Hsp70. During this differentiation process, HSF1 transiently increases in the nucleus of human monocytes and drives the expression of SPI1/PU.1, a master transcription factor in macrophage differentiation, which, when deregulated, causes leukemias and lymphomas. During macrophage differentiation, HSF1 also promotes *hsp70* expression, which in turn protects SPI1/PU.1 from proteasomal degradation (Jego et al. 2014). Garrido is now tackling the question of HSF1’s contributions to erythropoiesis in both *Hsf1* knock-out mouse and zebrafish/morpholino models. Because Hsp70 has proven to be critical in the development of many hematopoietic malignancies, she developed therapeutic tools by generating Hsp70 peptide aptamers (Goloudina et al. 2012; Mjahed et al. 2012). These aptamers can also be used to measure, by biolayer interferometry (OC-TET), the levels of Hsp70 released by tumors and circulating in the blood as a biomarker for cancers.

Interplay between HSF family members

Upon acute stress, HSF2 heterotrimerizes with HSF1 and was shown to co-localize with HSF1 to over half of its known target sites and modulate gene expression (reviewed by Akerfelt et al. 2010; Vihervaara and Sistonen 2014). A fundamental difference between HSF1 and HSF2 is that HSF1 possesses a potent transactivation capacity, whereas HSF2 is a poor transcription factor. Recently, it was shown that HSF2 is capable of binding to a number of loci in non-stressed mitotic

cells, whereas HSF1 is excluded from condensed pre-division chromatin as assessed in ChIP-Seq analyses (Vihervaara et al. 2013). Given the global inhibition of transcription in mitosis, including HSF1-mediated expression of HSPs, mitotic cells are considered more stress-sensitive than cells in interphase. Lea Sistonen (Åbo Akademi University, Finland) discussed the finding that HSF2 expression declines in many cell types and that the decline is more pronounced upon exposure to stress, raising the question of how compromised levels of HSF2 influence HSF1. In cells where HSF2 is downregulated, both HSF1 and RNA polymerase II, which are normally displaced from mitotic chromatin, are capable of accessing and activating the *hsp70* promoter, thereby contributing to enhanced survival of cells exposed to stress at this vulnerable phase of the cell cycle (Elsing et al. 2014). This property of certain cancer cells may explain their high tolerance toward various stress conditions.

The interplay of HSF1 and HSF2 is not restricted to acute heat stress. Gabriella Santoro (University of Rome Tor Vergata, Italy) presented data on the role of HSF1-HSF2 heterocomplexes in the regulation of a recently discovered stress-responsive gene, AIRAP (arsenite-inducible RNA-associated protein). Expression of AIRAP is strongly induced in human primary endothelial as well as peripheral blood monocytes and lymphocytes when treated with the anticancer drug bortezomib in clinically relevant concentrations used for multiple myeloma patients. Bortezomib is a potent proteasome inhibitor, and treatment with this drug differentially affects HSF1 and HSF2; HSF1 undergoes only a modest change in its phosphorylation status, which is clearly distinguishable from hyperphosphorylation caused by acute heat stress, whereas HSF2 levels are increased, consistent with the higher turnover of HSF2 as compared to HSF1 (Rossi et al. 2014). Of particular interest is the finding that repeated bortezomib treatments result in a profound accumulation of HSF2, which stands in stark contrast to the enhanced degradation of HSF2 upon acute heat stress (Ahlskog et al. 2010). In the case of bortezomib-mediated regulation of AIRAP, HSF1 and HSF2 are both recruited to HSEs where they can form heterocomplexes. However, these two HSFs have different effects on AIRAP expression; HSF1 acts as a positive regulator, whereas the elevated levels of HSF2 negatively regulate AIRAP expression after bortezomib treatments. This complicated interplay might have clinical relevance, given that HSF2 can reach high levels with the drug concentrations used for cancer patients.

HSF outputs as biomarkers and pathophysiological modifiers

Active HSF1-dependent transcription of human *Satellite III* (*Sat III*) regions in response to heat shock and diverse stress

results in accumulation of discrete foci termed nuclear stress bodies (nSB; (Vourc'h and Biamonti 2011)). These heterochromatin domains are formed by repeated sequences at pericentric (PC) regions, primarily in the 9q12 locus. In most adult tissues, PC regions are not transcribed, or are transcribed at low levels. Alteration of *Sat III* transcriptional status is also commonly observed in cancer, emphasizing their functional importance. Accumulation of satellite transcripts in mouse and human cells often correlates with DNA hypomethylation and chromatin remodeling, in pathological situations, or upon exposure to stress conditions (Eymery et al. 2009). *Sat III* transcripts therefore represent novel physiopathological biomarkers. As described by Claire Vourc'h (Albert Bonniot Institute, France), what cellular contexts promote the transcription of PC transcripts, the epigenetic status of these regions, and the different actors involved in their transcription represent major issues that remain to be addressed. *Sat III* transcription in response to stress is HSF1-dependent and occurs in both directions, although sense (G-rich) transcripts are much more abundant than antisense transcripts. As shown by Vourc'h, spontaneous nSB can be observed in tumors through HSF1 staining or detection of acetylated histones. Activation of *Sat III* transcription by heat shock is accompanied by the loss of the heterochromatin protein HP1 and recruitment of HATs, including CBP (Jolly et al. 2004), GCN5, and p300, with concomitant enrichment in acetylated histones (Fritah et al. 2009). It is also characterized by sequestration of splicing factors (e.g., SF2) and of TAF15, a non-canonical TAF protein with RNA-binding properties. Transient sequestration of these actors is likely to have a profound impact on global gene expression, alternative splicing, and other cellular functions. One of the most stimulating aspects in the field is that the foci induced by heat shock notably differ from those induced by the overexpression of HSF1 in terms of kinetics of recruitment. It is tempting to infer that the overexpression and aberrant activation of HSF1 in cancer might also rely on the specific recruitment of factors and contribute to the rewiring of the transcriptome characteristic of transformation (Mendillo et al. 2012). The identification of new factors in *Sat III* transcription by unbiased screening using human siRNA libraries and locked nucleic acid (LNA)-gapmers to knock-down *Sat III* expression will be determinant for unraveling the importance of *Sat III* expression during stress, cell cycle, senescence, and cancer. Understanding the nature and role of PC transcripts induced by HSF1 overexpression is a key question given the important role of HSF1 in embryonic development involving variation in HSF1 expression (reviewed in Abane and Mezger 2010).

A key component of physiological adaptation to environmental perturbations is anticipation of the daily changes generated by light and dark periods orchestrated by clock systems by a brain master pacemaker that controls peripheral clocks in other tissues. Hans Reinke (Düsseldorf University, Germany)

presented a fascinating account that HSF1 DNA-binding activity is regulated in a circadian manner and drives the expression of HSPs at the onset of the dark phase in the liver. Comparison between wild-type and *Hsf1*^{-/-} mice shows that HSF1 influences the period length of the mammalian circadian clock (Reinke et al. 2008). This means that HSF1 not only mediates cytoprotection through the induction of HSPs in a circadian manner, but, moreover, co-regulates the master clock. The expression of HSPs is circadian and systemically driven, but interestingly is independent of the circadian transcription factor BMAL1/CLOCK. Both body temperature and feeding were shown to control HSF1 activity (Note: feeding might act at least partially by modulating body temperature). A TAP-tag approach is currently underway in the Reinke laboratory to identify HSF1 partners that could mediate its effect on circadian cytoprotective adaptation. Additionally, loss of HSF1 leads to a dramatic reduction in *hsp90* mRNA levels in the liver. The control of Hsp90 production by HSF1 might be of profound importance, as Reinke presented evidence demonstrating that inhibiting Hsp90 chaperone activity markedly affected both the amplitude and phase oscillations of circadian rhythms in cultured cells, possibly due to a requirement for the chaperone to promote BMAL1 stability (Schneider et al. 2014).

The roles of HSFs in normal embryonic development raise the question of their putative protective versus detrimental role when environmental stress conditions interfere with developmental programs (Abane and Mezger 2010). Valérie Mezger (CNRS, Université Paris Diderot, France) presented data showing that the strong activity of HSF2 during normal brain corticogenesis provides a permissive state for the chronic activation of HSF1 in response to fetal alcohol exposure. During this process, HSF2 assumes a prominent role in driving the activation of HSF1, whose striking persistence is supported by an alcohol-specific set of post-translational modifications. The formation of alcohol-specific HSF1-HSF2 heterotrimers in vivo is responsible for perturbations in the expression of genes that control neuronal migration in the developing cortex. HSF2 is therefore a mediator of neuronal positioning defects characteristic of fetal alcohol syndrome, the most frequent cause of mental retardation (El Fatimy et al. 2014). Whether HSF2 might be protective in the context of other fetal stressors has been discussed, as well as the role of this atypical HSF activation in long-term alterations of brain performance via epigenetic mechanisms.

Models and methods for the field

Elisabeth Christians (CNRS, Université Pierre et Marie Curie, France) provided a thorough overview of the cellular and organismal models currently in use to study HSF, focusing on strengths and limitations. For example, HeLa cells have been

widely used in the study of HSF1 and HSF2 mechanisms of action. However, our understanding of the roles of HSFs in HeLa cells may be biased by the fact that they are hypertriploid and present a haplotype-specific activation of Myc by the insertion of HPV18, underscoring the need to investigate other cell choices. Mouse models have been extremely useful in deciphering the role of HSFs in development, particularly gametogenesis, brain, and lens development (reviewed in (Abane and Mezger 2010); (Le Masson and Christians 2011; Le Masson et al. 2011)). The maternal role of HSF1 is one of the most striking effects of HSFs on normal development and reproductive performance and was shown to mediate multi-step effects on a hub of meiotic genes involved in cohesin and synaptonemal complex, or controlling DNA recombination and spindle assembly checkpoint (Le Masson and Christians 2011). Inactivation of HSFs in mouse models results in diverse pathologies, including neurodegenerative-like and psychiatric-like diseases, cataracts, and lung disease (Shinkawa et al. 2011; Uchida et al. 2011; Wirth et al. 2004; Wirth et al. 2003). The mouse has also been invaluable in revealing the intimate crosstalk between HSFs, cooperative or antagonistic. One major limitation has been the generation of transgenic mice overexpressing HSFs, and numerous attempts have globally failed, until recently (Pierce et al. 2013; Pierce et al. 2010). This suggests that increasing HSF levels (or perturbing the ratio between HSFs) might be deleterious for organisms.

As described by Morimoto in the keynote address, the nematode *C. elegans* has proven invaluable in unraveling the genetic pathways involved in proteostasis diseases and longevity where HSF1 is a central player, as well as the non-cell autonomous aspects of the HSR and its neuronal control. In contrast, biochemical approaches are limited in the worm. Zebra fish (*Danio rerio*), which has been successfully and widely used in toxicology, cancer, and neurodevelopment or neuroscience approaches, has been somewhat underexploited in the field and should bring new possibilities in the future as underlined by very recent work studying the heat shock factor binding protein 1 (HSBP1; (Eroglu et al. 2014)). As stressed by Christians, the need for models other than rodents was made particularly clear in the case of the role of HSFs in oogenesis and pre-implantation development, where the scarcity of egg material has rendered mechanistic investigations difficult. The use of non-vertebrate models (e.g., sea urchins which produce $\sim 10^7$ of synchronized eggs) was promoted as an alternative model. In a similar vein, *Délara Sabéran-Djoneidi* (Université Paris Diderot, France) reviewed the new possibilities offered by the cutting-edge TALENS and CRISPR/Cas technologies (Pennisi 2013) in terms of knock-out and knock-in strategies to create novel versions (i.e., mutant or wild-type epitope-tagged) of HSF in diverse cells or organisms. She also pointed out the new opportunities offered by the SNAP-TAG system (Pennisi 2013) that could

be applied for in vivo labeling experiments, in order to study protein stability in stress contexts.

Perspectives and concluding remarks

The concept of combinatorial complexity in the HSF family arose as a major theme during the workshop. First, cells contain heterogeneous HSF populations that, in response to stress, physiological, or pathological situations, will distribute across the genome in very different ways. Fine-tuning of transcription in normal or stress conditions is likely achieved by combinatorial assembly of HSF trimers, with monomer-specific characteristics including alternative splicing (some of which are still to be characterized), and post-translational modifications. The precise “HSF signature” of each combinatorial possibility may depend on the type and severity of stress, as well as the DNA context of the HSE within promoters, introns, and gene bodies, all in a defined chromatin environment. How then to tackle this combinatorial issue? ChIP and ChIP-Seq data can give us access to the genome-wide binding profile of HSFs and possibly identify the nature of HSF molecules at a given location. However, it cannot provide conclusive evidence for the relative stoichiometry within a trimer (note that the use of isotopically labeled HSFs might bring new insights in ex vivo approaches). Neither can we, today, obtain accurate data on the profile of individual trimers, in terms of post-translational modifications. To date, the field has only experienced modest success in generating affinity tools, for example, antibodies against known post-translational modifications. Antibody-based approaches often encounter difficulties due to large protein complexes within the chromatin masking the study object, such as HSF1. To circumvent this issue, TALENS or CRISPR/Cas-guided knock-in of tagged HSF variants might be of use, as would the generation of high affinity binders for HSFs, using ribosomal display. Great expectations also come from the quickly evolving mass spectrometry technologies, in spite of current limitations of coverage as samples contain different proteins and the amount of protein necessary for total coverage is still very high. Finally, it was pointed out that the combinatorial nature of HSF post-translational modifications allows highly dynamic binding of very different proteins, including HATs/KATs or conversely, HDACs/KDACs, which, in turn, might profoundly remodel the local chromatin landscape.

A second aspect raised by the participants and reviewed by *Pia Roos-Mattjus* (*Åbo Akademi University, Finland*) was the necessity to develop a global strategy to collect, analyze, and compare genome-wide data generated by the field or, more generally, available on databases like *ENCODE* (e.g., ChIP-Seq, MNase footprints, RNA-Seq data). It was also discussed that a wealth of data has been generated using overexpressed or epitope-tagged proteins that might bias the obtained results.

In addition, while perhaps unavoidable to some degree, using certain cell lines of human cancer origin (e.g., HeLa, K562) might hamper investigations of HSF regulation and mechanisms of action. The decision was made to create a website to catalog tools, reagents, and data available in the field with pertinent information such as cell type and HSF status (tagged/untagged) to allow investigators to take all relevant experimental features into consideration before interpreting the data.

A third part of the discussion addressed the development of new therapeutic approaches targeting HSFs against cancer and neurodegenerative disorders. One difficulty has been to find modulators of HSFs that could either activate HSF, in order to target neurodegenerative, lysosomal, cardiovascular, or metabolic disorders or, conversely, inactivate HSF in cancer, or upon viral and bacterial infections. Notably, the search for compounds capable of modulating the actions of HSF1 has identified many HSF activators, but few inhibitors, and drug design has been severely restricted by the lack of structural information for full-length HSF1. In addition, putative pharmacological approaches should consider the necessity to target all HSFs or a specific family member and pay attention to the ratio between HSFs (e.g., HSF1/HSF2). Another layer of complexity emerges from the subtlety introduced by HSF isoforms in governing transcriptional control in normal or pathological situations. Moreover, it will be necessary to identify biomarkers of neurodegenerative disease, well before onset of pathology, to allow early intervention and start of any proteostasis-based treatment. Finally, the question remains of how well these compounds might work in aging cells.

More conceptually, the interesting notion of a “partial stress state” arose from discussions, which is characteristic of cancer and neurodegenerative disorders. This raised the question of how much we want or need to perturb the system, and pointed out the necessity to abandon the inherent bias of the “heat shock paradigm,” because of its extreme, high magnitude of HSF1 activation. In other words, future drug candidates may need to only modulate HSF1 activity in a subtle manner to be therapeutically effective, especially in a chronic treatment scenario. It was also pointed out that the discovery of drugs targeting neurodegenerative diseases has been hampered by difficulties in identifying the right cohorts, as well as the extended timeframe needed to evaluate efficacy in age-onset pathologies. One possibility for the near future would be to turn attention to other degenerative disorders like α 1-antitrypsin disease, which causes liver fibrosis and lung damage, sarcopenia, or retina-degenerative diseases, all of which hold promise for remediation by pharmacological manipulation of the proteostasis landscape. Lastly, synergy between HSF-targeted compounds and traditional drugs (i.e., anticancer molecules) should be strongly pursued and exploited when possible.

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