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Idées neuves pour une vieille famille : les facteurs de choc thermique HSF au carrefour entre stress, épigénétique et développement

New ideas for an old family: Heat Shock Factors at crossroads between stress, epigenetic and development

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Rapport sur la Conférence

Conference Report

RESUME DU RAPPORT

Conférence Jacques Monod intitulée : Idées neuves pour une vieille famille : les facteurs de choc thermique HSF au carrefour entre stress, épigénétique et développement

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L'activité des facteurs de transcription HSFs (*Heat Shock Factors)* a été découverte incidemment par Ritossa en 1962 qui étudiait des drosophiles soumises à un choc thermique. Les cellules réagissent en induisant la synthèse de polypeptides très conservés, les HSPs (*Heat Shock Proteins*), dont la plupart codent des chaperons moléculaires. La réponse des organismes au stress thermique (HSR, *Heat Shock Response*) s'est avérée être universelle et s'étend à toute une variété de stress environnementaux, protéotoxiques. De plus, l'activité des facteurs HSFs ne se limite pas à la régulation de la HSR, mais s'étend aussi aux processus de développement embryonnaire, aux mécanismes épigénétiques et à diverses pathologies.

Cette conférence a abordé la complexité des HSFs au travers de 9 sessions qui ont réuni 24 conférenciers dont les contributions récentes ont fait progresser le domaine. De plus, 16 communications orales courtes ont été sélectionnées sur la base des résumés soumis. En tout, 68 chercheurs de 16 pays ont participé à la Conférence qui s'est déroulée dans une grande convivialité et a fourni l'émulation et les conditions de l'établissement de nombreuses collaborations.

Les avancées cruciales exposées recouvrent la notion que HSF est impliqué dans la longévité et les maladies neurodégénératives à agrégats de protéines, dont les voies de régulation sont très intriquées. La présence d'une seule protéine encline à l'agrégation suffit à compromettre gravement la « protéostasie » et à réduire ainsi la longévité de l'organisme. L'activité des facteurs HSFs s'intègre aussi dans le contexte d'un organisme où différents types cellulaires interagissent. Bien que la réponse au choc thermique soit réputée universelle, toutes les cellules d'un organisme ne répondent pas de façon autonome, mais il existerait un contrôle intégratif de la réponse au niveau d'un organe ou d'un organisme.

Quoique classiquement identifié comme un activateur génique, HSF intervient dans l'établissement et la modification de marques épigénétiques. Il interagit avec des complexes de « silencing », de remodelage de la chromatine ou par la synthèse d'ARN non codants pour la structuration de l'hétérochromatine.

L'implication des HSFs dans diverses pathologies cardiaques, cancéreuses, inflammatoires, neurodégénératives et aussi des problèmes de développement (gamétogenèse, cataractes, syndrome d'alcoolisation foetale) a fait l'objet de nombreuses interventions. Les mécanismes de régulation de l'activité de HSF (ARNs non codants, nouvelles modifications post-traductionnelles) ont connu des avancées importantes alors qu'était soulignée la nécessité de comprendre la régulation de l'expression des facteurs HSFs eux-mêmes : le niveau de HSF dans une cellule est en effet crucial. Ces niveaux se révèlent bénéfiques ou délétères pour un même organe, comme le cœur ou selon certains types de tumeurs, et sont trouvés modifiés dans de nombreuses pathologies.

La modulation positive ou négative de HSF s'avère donc un enjeu thérapeutique très important. Ceci a ouvert la voie à de nombreuses recherches de traitements, basées sur le contrôle de l'activité des facteurs HSFs. Ces travaux, développés tant par des laboratoires académiques que ceux des firmes privées, exploitent des cribles à grande échelle, en s'appuyant sur divers organismes modèles (levure, cellules humaines, nématode, drosophile). Il en ressort une extraordinaire variété d'inhibiteurs ou d'activateurs de l'activité de HSF1: composés de synthèse ou issus de la médecine traditionnelle, ARN aptamères, RNAi… L'identification des cibles des HSFs à grande échelle a permis de mettre en évidence la présence de sites de liaison de HSF dans des introns ou des parties distales des gènes pour les gènes cibles non-*Hsp* qui semblent surtout impliqués dans le développement. Enfin, le croisement avec les approches bioinformatiques théoriques et de modélisation mathématique de la HSR a suscité des collaborations déjà fructueuses, dont la portée prédictive pourra être mise au service de la recherche d'approches thérapeutiques efficaces.

CONFERENCE REPORT

Final report from the Jacques-Monod Conference entitled

New ideas for an old family: Heat Shock Factors at crossroads between stress, epigenetic and developement

Roscoff, September 17-21, 2008

Scientific report

Abbreviations: HS, Heat shock ; HSR, heat shock response ; HSF, Heat Shock Factor, HSP, Heat shock protein.

Keynote lecture

The Conference was launched by a Key Note by **Rick Morimoto** (Evanston, IL, USA). Rick Morimoto enthusiastically demonstrated how C elegans was instrumental to emphasize the role of HSF1 in environmental stresses, aging and diseases. In particular, alteration of the proteome health, protein folding diseases represent life threatening pathologies as cells appear poorly adapted to chronic protetoxic stress.

His talk addressed one of the major questions related to aging and proteotoxic stress considering the fact that protein homeostasis becomes less efficient in older organisms. A major breakthrough was the discovery that the insulin-like signaling (ILS) could coordinately influence aging and protein homeostasis through the action of two factors that detect and respond to misfolded proteins, DAF-16 and HSF1. A second important step was the demonstration that one single aggregation-prone protein can deeply disturb the cell folding landscape and cause a global imbalance in folding homeostasis.

A third stricking new observation awaiting further investigation in higher organism is the fact that cellular HSR is organized in *C. elegans* as an integrated system-wide at the organismal level which requires thermosensory neurons. Mutant worms, developmentally deficient in those neurons, are unable to elicit HSR in their body cells but still respond to other stress. One can infer that the existence of two interacting levels of stress regulation either cell-autonomous cell or non-autonomous, implying an organismal control of HSF activity.

Rick Morimoto ended his Key Note by pointing out the future challenge: how to manipulate the folding landscape to rescue misfolding events. One way to reach this would be by fine tuning HSF1 activity which could capture misfolded proteins and let occuring mutations being simple polymorphism without pathological phenotype.

Session I *HSF dynamics and activation in response to stress*

John Lis (Ithaca,NY, USA) gave a plenary lecture on the probing of dynamics and function of *Drosophila* HSF *in vivo*. He added interesting and recent findings on the mechanisms of HSF action on the transcription and consequences for the therapy of cancer.

EGFP-tagged HSF was monitored in *Drosophila* live tissue, in particular in the salivary glands which contain polytene chromosomes by taking advantage of multiphoton

microscopy. John Lis nicely showed how dHSF binds and unbinds very rapidly at *Hsp70* loci in control cells, whereas activated dHSF after HS stably sits on the promoter and provides a plateform for many rounds on transcription. The activation of *Hsp70* gene is not affected by its nuclear localization and no repositioning is observed during activation. There is also no correlation between nucleosome loss and the RNA polymerase movements. A RNAi screen identified factors responsible for rapid nucleosome loss. These includes HSF itself, GAGA factor, poly(ADP)-ribose polymerase (PARP) which was found to interact with HSF. Experimentally designed RNA aptamer inhibits HSF DNA-binding to *Hsp83*, a target gene occupied by the factor even in uninduced cells. This potent inhibition decreased Hsp83 levels and result in fly phenocopies of *Hsp83* mutations. This *Drosophila* model using aptamers turned out to be effective in supressing abnormalities induced by constitutively active forms of EGFr and Raf oncogenes, in line with the role of HSF1 in carcinogenesis. The effects of HSF1 inhibition by aptamers are now explored in various cancer cell lines.

Evgeny Nudler (New York, NY, USA) brought new insights on HSF1 activation mediated by the ribonucleoprotein complex containing the translation elongation eEF1A and the non-coding RNA HSR1. HSR1 is not only present mammals, but also in *Xenopus*, *Drosophila* and plants. HSR1 serves as a cellular thermosensor that determines the temperature threshold at which the HS response is triggered, while eEF1A is sensitive to other proteotoxic stressors and is linked to translational shutdown and cytoskeleton collapse associated with HS stress. HSR1 and eEF1A are new therapeutic targets in cancer, inflammatory diseases associated with HSF1 dysregulation.

Lea Sistonen (Turku, Finland) presented an overview on the role and functional hierarchy of posttranslational modifications regulating HSF activity in mammals and on their. HSF1 and 4 are at the origin of the discovery of a bipartite motif PDSM (Phosphorylation – Dependent Sumoylation Motif) which mediate their repression, and more recently of NDSM (Negatively charged-Dependent Sumoylation Motif). HSF1 is also regulated by acetylation at several lysine sites, including the site which is a SUMO target within PDSM. An interesting concept is the possible switches that could happen between sumoylation and acetylation. HSF2 is not phosphorylated but sumoylated on a hydrophobic SUMO-binding motif (SBM). Interestingly, although HSFs are sumoylated and although sumoylation by SUMO-2 is very strongly induced by stress, the SUMO-2 targets identified by mass spectrometry were found to be involved in chromatin structure, DNA and RNA topology or translation rather than transcription factors. This emphasized the role played by the peculiar set of regulatory mechanisms controling HSF fonction.

Significant progress was reported by **Sandy Westerheide** (Evanston, IL, USA) on the role of HSF1 acetylation. SIRT1 is the HSF1 deacetylase and thus activates HSF1 upon HS. In contrast, HSF1 is acetylated in normal conditions and during the attenuation phase, which leads to the disruption of HSF1-DNA interactions. The discovery of this HSF1 regulation by SIRT1 will help to further build the interactive network between stress-regulated transcription factors, including not only HSF1 but also p53 and FOXO, all important in cell proliferation, differentiation and aging.

In human cells, heat shock induces the HSF1-dependent production of SatIII RNAs that remain associated to their site of transcription. **Giuseppe Biamonti** (Pavia, Italy) brought new insights on the identity and role of these enigmatic non-coding RNAs. Most of the SatIII RNAs contain the G-rich strand and are induced by several stresses at levels and kinetics that are highly stress-dependent. Notably, their induction by hyperosmotic stress does not depend

on HSF1. SatIII RNAs have been suggested to have a role in the control of a heterochromatic state. The presence of short RNAs derived from SatIII transcripts was detected but does not depend on the endogenous RNAi pathway, nor seem to be produced by splicing.

Michael Schroda (Freiburg, Germany) dissected the HSR in the unicellular algae *Chlamydomonas reinhardtii* which contains two HSFs, one of which, HSF1 is a crucial regulator of HSR. HS-induced HSF1 phosphorylation seems pivotal for the regulation of HSF1 activation and for the induction of heat shock genes. HS seems to upregulate the levels of HSF1 mRNAs, in contrast to animal cells, and in line with previous plant studies, but does not act on HSF1 oligomeric state. HSP70A interacts with HSF1 in all stress conditions and HSP90 appears important for the attenuation of the stress response.

In the search for new therapeutic approaches to treat diseases linked with aging and neurodegeneration, **Dan Garza** (Novartis, Cambridge, MA, USA) described their research program using transgenic flies with reporter systems specific for the three stress pathways (heat stress, ER stress and genotoxic stress). This elegant approach allowed efficient pharmacological/RNAi screen to identify drugs/genes involved in the different pathways.

Session II ***HSFs and maintenance of organ integrity and longevity***

Ivor Benjamin (Salt Lake City, UT, USA) gave a plenary lecture discussing previous work on REDOX homeosatis and fonctional consequences observed in case of oxidative or reductive stress triggered by HSF1 deficiency or mutated chaperone (R120G αBC), respectively. In the second part of his talk, I. Benjamin described recent studies on a new mouse model overexpressing a constitutively active HSF1 at low (CaLow expressor lines) or high (CaHigh expressor lines) levels in heart. By inducing cardiac specific HSPs, CaLow HSF1 reduces the transition of the mitochondrial pore, a key player in apoptosis. CaHigh HSF1 mice die from cardiac hypertrophy and massive fibrosis and display a high increase in hypertrophy gene markers. HDACs and PGC-1a, a co-activator of mitogenesis are upregulated. Exercice provokes pathological cardiac hypertrophy in CaHigh HSF1 mice, with sudden death, suggesting that, in human, the HSF1 levels could be linked to prediposition of heart failure that occur in professional sports.

Maria Saraiva (Porto, Portugal) demonstrated that ablation of HSF1 accelerates protein misfolding and tissue deposition of in an animal model of familial amyloidotic polyneuropathy (FAP), a neurodegenerative disease that selectively affects the peripheral nervous system (PNS). The *Hsf1* KO displays brain ventriculomegaly, astrogliosis and disregulation of HSPs. Mouse model of FAP expressing mutated human transthyretin (TTR) does not recapitulate the human pathology. Therefore it was remarkable to notice that, in the null-*Hsf1* background, the TTR mutant exhibited extensive TTR tissue deposition and the degeneration of unmyelinated nerve fibers with the upregulation of markers of inflammatory stress as in human patients.

Earl Noble (London, ON, Canada; short talk) brought a deeper insight in the understanding the HSF1-dependent induction of Hsp70 by skeletal muscle exercise. His data suggest a non-cell autonomous or epigenetic regulation of HSF1-mediated Hsp70 expression. Indeed, unlike HS and although the phosphorylated form of HSF1 accumulates in all nuclei, only a select group of muscle fibers increase *Hsp70* upon exercise. This suggests an integrative control of the response to exercise at the whole muscle level.

Session III ***HSFs in physiopathological situations I***

Gabriella Santoro (Roma, Italy) gave a plenary lecture on the role of HSF1 at crossroads between inflammatory and survival signaling pathways. In cancer cells, HSF1 has a Janus-like behavior, because it is found associated with anti- and pro-apoptotic responses. On the one hand, HSF1 disruption is considered therapeutic in several tumor types in which HSP expression confers thermoresistance and resistance to chemotherapy. In the case of cervical carcinoma, HSF1 silencing surprisingly does not affect cancer cell sensitivity to cisplatin or to hyperthermia when administered separately. However, it causes a dramatic increase in sensitivity to the combined treatment, hyperthermochemotherapy, leading to massive apoptosis. On the other hand, HSF1 activation may result in apoptosis in agressive cancer with aberrations in survival signaling dependent on NF-kB. In such tumors, synthetic cyclopentenones are promising tools in anticancer, anti-inflammatory therapy which, through their ability to drive HSF1 activation, lead to potent inhibition of NF-kappaB activation.

While HSF1 and HSF2 are ubiquitously found among vertebrates, two other HSFs HSF3 and HSF4 were identified only in chicken and mammals, respectively. **Mitsuaki Fujimoto** (Yamaguchi, Japan) reported the discovery of the missing HSF in mammals, mHSF3, as well as the missing HSF in avian (cHSF4). Mouse HSF3 undergoes trimerization and nuclear translocation when ectopically expressed in COS 7 cells, but is not able to drive the induction of *Hsp* genes in the absence of HSF1, although it possesses a functional transactivation domain. The fact that mHSF3 protects the cell against cell death upon HS and suppresses aggregation of polyglutamine protein suggests a role in cell protection.

Agnieszka Szczepek (Berlin,Germany ; short talk) reported data on the transcriptional regulation of HSF1 and Hsp70 in the auditory pathway in rats subjected to emotional stress. In the midbrain, the *inferior colliculus* integrates and routes the sensory perception. In rat cumulating emotional stress and ultrasonic, unpleasant but not ototoxic ultrasonic sound, the transcriptome analysis of the *inferior colliculi* reveals the upregulation of *Hsf1* and *Hsp70* gene expression. These findings might open a new link between psychological and cellular stress with important implication in posttraumatic disorder (PTSD) understanding and treatment.

Garry Shen (Winnipeg, Canada ; short talk) demonstrated that HSF1 is involved in the transcriptional increase of plasminogen activator inhibitor-1 (PAI-1) which plays an important role in thrombosis, metabolic syndrome, atherosclerosis and inflammation. Oxydized or glycated LDL (low-density lipoprotein)-induced PAI-1 is dependent on the binding of HSF1 to a HSE element by and does not occur in *Hsf1*-null mouse cells. Correlated high levels of HSF1 and PAI-1 are found in a tissue specific manner in STZ-diabetic mice or apoE-KO mice.

Session IV ***Poster session***

Session V ***HSF in physiopathological conditions***

This session was mostly dedicated to the complex roles of HSF1 in cancer.

Luke Whitesell (Cambridge, USA) gave a plenary lecture on the multifaceted role of HSF1 in cancer. He gave insights on the mechanism by which HSF1 acts on the initiation, maintenance and progression of the tumors, suggesting that HSF1 might induce a unique epigenetic state that confers high malignant potential. This would help to understand why some tumor cells express high level of HSF1. Therefore HSF1 is confirmed as a therapeutic target and Luke Whitesell described a screening strategy to identify inducers and inhibitors of HSF1. He provided the first data of 200 identified and verified HSF1 inducers, many of which were never previously reported to have bioactivity and whose direct targets have to be currently identified.

Stuart Calderwood (Boston, MA, USA) demonstrated how HSF1 is involved in the metastatic progression of estrogen-dependent breast cancers by associating with MTA1, a component of the NURD repressor complex. This interaction HSF1-MTA1 is responsible for the silencing of estrogen-dependent genes by transcriptional but also likely by non-transcriptional mechanisms.

Nahid Mivechi's work (Augusta, GE, USA) suggested that HSF1 could influence the nature of the cells undergoing cancer transformation as, in a *Hsf1*$^{-/-}$*p53*$^{-/-}$ background, the number of lymphoma is reduced through altered cytokine signaling and inflammatory factors, while the incidence of solid tumors is increased, likely through genome instability. She commented more recent studies performed on chemically-induced hepatocarcinoma and showed that the absence of HSF1 favors a reduction of cancer incidence, likely *via* its action on the JNK pathway.

John Price (Clayton, Australia) showed that the invasive and metastasis potential of the human breast cancer cell line MDA-MB-231 rely on HSF1 through the regulation of genes involved in cell migration and growth factor pathways.

This session also highlighted the role of HSF1 in a novel area: **Hans Reinke** (Geneva, Switzerland) showed that HSF1 was discovered in a screen for new regulators of circadian gene expression. Although *Hsf*1 is not a clock gene by itself, its activity is highly rythmic and regulates a number of genes of various networks involved in stress and immune responses.

Session VI *Interplay between HSFs*

It becomes more and more evident that multiple HSFs offer a higher level of regulation through cooperation or competition.

In his plenary lecture, **Klaus-Dieter SCHARF** (Frankfurt, Germany) presented a very comprehensive landscape of HSF diversity in various plant species where the numerous HSFs are grouped in three classes. He underlined the specific cooperation between Class A HsfA1 (the master-stress regulator) and HsfA2 which becomes dominant in thermotolerant cells, and the repressive interactions between HsfA4b and HsfA5. Class B HsfB1 has no transactivation potential but contributes synergistically to the activity of Class A and, interestingly of other transcription factors involved in other stresses or in development.

In mammals, the role of HSF2 and how HSF1 and HSF2 interact remain poorly understood. **Yves Le Dréan** (Rennes, France) brought important data showing that the complex formed by HSF1 and HSF2 is crucial for the response to proteotoxic stress due to

proteasome inhibition. He provided some evidence that HSF2 is needed for the regulation of the basal expression of proteasome subunits, modulating its catalytic activity.

Anton Sandqvist (Turku, Finland) presented new data showing that HSF1 and HSF2 form heterotrimers and that this heterotrimerization influences the transcription of SatIII sequences, a major site of stress-induced, HSF1-dependent transcription in human cells. He indicated that this might be through differential recruitement of partners for HSF1 and HSF2.

Session VII ***Posters***

Session VIII ***HSFs and Epigenetics***

Làzlò Tora (Illkirch, France) explored the mechanisms of chromatin remodeling and the importance of gene positioning in the nucleus in stressed *Drosophila* cell. The SAGA-type histone acetyl transferase E(y)2 interacts with the nuclear pore complex and with a new export complex (Xmas-2) to regulate mRNA transport. These types of interactions modify the attachment of *Hsp70* gene to the nuclear envelope and modulate transcription, in normal and heat shock conditions.

Kevin Sarge (Lexington, KY, USA) extended his previous observation on the role of human HSF2 in *Hsp70* gene bookmarking during mitosis to other HSE-containing genes like *Hsp90*, *Hsp27* and *c-fos*, which in contrast to Hsp70, also need HSF2 for their basal expression. Mouse HSF2 as well as HSF1 would influence chromatin structure in mature spermatozoa and would be bound to the *Hsp70* gene, possibly in correlation with the transcription of *Hsp70* at the zygotic genome activation.

Claire Vourc'h (La Tronche, France) explored by RepChIP global centromeric (CT) and pericentromeric (PCT) sequence expression in different cell context including human normal, stressed or cancerous cells. Epigenetic marks and HSF1 play an important role in regulating expression of the PCT repetitive sequences. The impressive quantity of data collected so far clearly indicated specific behavior of those CT and PCT sequences according to tissue, cancer cell type and stress-physiological conditions. The mechanisms controling this behavior and their impact on cell life are under current investigations.

In yeast, RNAPol II recruitment on *Hsp* genes correlates with the onset on chromatin remodeling. However, **Alexandre Erkine** (Vermillion, SD, USA) showed that histone displacement differs drastically between even closely related promoters, *HSP12*, *SSA4*, and *HSP82* and that this seemed to correlate with preferential requirements for Msn2/4, HSF1 binding and SWI/SNF, RSC complex. Genetic screen based on the search for functional substitution of the C-terminal activation domain of HSF led to a very interesting and provocative suggestion : the role of activation domains would be to target positively charged and hydrophobic partners and would thus help to modify the surface of nucleosomes in order to turn them into better targets for chromatin remodeling.

Session IX ***HSFs, gametogenesis and preimplantation development***

In her plenary lecture, **Elisabeth Christians** (Toulouse, France) gave an overview on the role of HSFs in gametogenesis and early embryonic development from *Drosophila* to mouse. She emphasized her recent discoveries on how HSF1 exerts its maternal effect, showing that HSP90α, as a HSF1 target, acts at different phases of oogenesis : the onset of germinal vesicle breakdown, meiosis I progression, polar body formation through control of asymetric division and formation and positioning of the spindle. HSF1/ HSP90α likely act in influencing the MAPK pathway with marked effect on CDK1. Finally, she provided new insights on the role of HSF2 during preimplantation development in the establishement of a functional heat shock response.

Eva Henriksson (Turku, Finland) reported the amazing occupancy of mouse chromosome Y by HSF2 on the long arm harboring multicopy of a few genes. Aberrant levels of chromatin packaging proteins and DNA fragmentation are detected in HSF2-deficient sperm, suggesting that HSF2 is necessary for the proper chromatin organization. HSF1 role was also investigated since it is expressed at different stages of spermatogenesis. Novel HSF1 target promoters were identified with again accumulation on the long arm of Chromosome Y. A number of common targets between the HSF2 and HSF1 screen is suggestive of simultaneous binding and raises the question of the role of HSF1/2 heterotrimers in spermatogenesis.

Remi Dumollard (Villefranche-sur-Mer, France) gave an overview of the drastic changes in energetic metabolism that occurs early development in order to adapt to the increasing demand of energy. By imaging intracellular levels of NAD(P)H, GSH and cytoplasmic and mitochondrial ATP concentrations, he could identify the outcome of the metabolism of energetic substrates on the setting of intracellular redox potential and feeding of mitochondrial ATP production. The role of HSF1 in regulating this process was then approached with a special interest in the potential involvement of SIRT1, an NAD-dependent histone-deacetylase which was shown to modify HSF1 in a previous talk of the conference.

In her short talk**, Wieslawa Widlak** (Gliwice, Poland) described the paradoxal response to heat shock of spermatocytes, the most damage-sensitive germ cells. Transgenic spermatocytes expressing a constituvely active HSF1 downregulate testis specific *Hsp* genes and, consequently, induce caspase-dependent apoptosis, involving mitochondria- and death receptor-dependent pathways. The role of HSF1 in this process seems to misdirect a network of transcription factors required for the expression of those testis specific *Hsp* gene. Differential regulatory properties of HSF1 was further commented, based on transcriptome data comparing somatic cells and spermatocytes under stress.

Juan Jordano (Sevilla, Spain) explained how the numerous HSFs in plants exert redundant functions in seed longevity and dessication tolerance. Gain of function approaches determined that HsfA9 contributes to these processes. In contrast, loss-of-function led to impaired seed longevity, in correlation with a reduction of seed Hsps, while resistance to developmental dessication was maintained. This talk highlighted the necessity to better understand HSFs roles in plant survival in a context of climate changes.

Session X ***HSFs and postimplantation development***

This session started with the new data of the role of HSF in developement and ended with a transition for the last session by illustrating the way yeast has been used for the modeling of HSR in human and how yeast can be used as a model to understand and manipulate the human HSF1 for therapeutic purposes.

Akira NAKAI (Yamaguchi, Japan) gave a major breakthrough in the field by unravelling the non-classical molecular mechanisms by which HSF4 is involved in sensory placode development in mice. HSF4 binds to various regions in the genome, including introns, exons and distal parts of protein-coding genes in mouse lenses, some of which are also occuped by HSF1 and 2. These findings brought a kind of revolution in the field of HSF where the historical *Hsp* gene are reputed to classically contain HSEs in rather proximal locations upstream the transcription start. Apart from its effects on the expression on many genes, HSF4 induces a specific epigenetic mark by demethylating histone H3K9 in the bound regions.

Valérie Mezger (Paris, France) explored the consequences of the involvement of HSF2 in the normal migration of young neurons in stress-exposed fetal brains. The stress chosen is exposure to ethanol since the fetal alcohol syndrom (FAS) is reputed to include neuronal migration defects. In contrast with the activation of HSF1 by HS, which does not affect HSF1 levels and is only transient, alcohol induces an unexpected transcriptional activation of the *Hsf1* gene and triggers specific post-translational modifications of HSF1, which are distinct from those observed after HS. Through these specific signatures, alcohol is able to sustain a prolonged HSF1 activation, which modifies the expression of genes involved in neuronal migration. HSF1 and HSF2 are therefore actors on the still elusive molecular mechanism of FAS.

Dennis Thiele (Durham, NC, USA) demonstrated the power of yeast model to identify new genes and molecules that could regulate HSF1. Human HSF1 is unable to substitute to yeast HSF and rescue cell viability. Yeast deleted in the essential yHSF and containing huHSF1 were therefore screened by a life/death screen for small molecules that would activate human HSF1. HSF1A, one identified activator allows yeast growth, activates protein chaperon expression in mouse cells in a HSF1-dependent manner, stimulates HSF1 accumulation, and synergizes with thermal stress. Moreover, HSF1A ameliorates polyQ-GFP protein aggregation. The mechanism by which HSF1A activates HSF1 is under current investigation.

David Neef (short talk, Durham, NC, USA) went on using this approach by identifying protein kinase inhibitors that would activate human HSF1 in yeast. He identified a very potent inhibitor which acts on casein kinase 2, tyrosine kinase and ERK, and a more modest inhibitor which blocks the activity of GSK3, AMPK. In line with their activating HSF1, these two inhibitors reduced HSF1 Ser303/307 phosphorylation. Those two sites are highly phosphorylated during heat shock and when they are mutated make the modified hu HSF1 able to complement yeast HSF .

Session XI ***Bioinformatics and mathematical modeling***

As bioinformatics and mathematical modeling are key components of postgenomic biology, this session aimed to present recent update on those tools applied to the identification of HSF DNA binding sites and to the theoritical and predictive understanding of HSR. One of the goals was to foster collaborations across HSF scientific community.

Tim Westwood (Toronto, ON, Canada) discussed how ChiP on CHIP analyses revealed the presence of 430 HSF binding sites in *Drosophila* genome, out of which promoters of *Hsp* genes represented only 3%. Half of the binding sites are located in within 1kb uptstream of transcription start of non-heat shock genes. Around 40% of other sites are found in non-heat shock genes including 6 sites in the introns of developmental genes, which are responsive to ecdysone-, the hormone involved in post-embryonic development. This finding is reminiscent of what is found for HSF4 in sensory placode development. In flies deficient for HSF (*Hsf*^{4} mutant line) a small set of genes, including a few chaperones, were found upregulated by HS, not in a HSF-dependent manner, but by increase of message stability. Interestingly, all the genes involved in the ecdysone response are repressed by HSF upon HS, the biological meaning of which could be to stop development in inappropriate life conditions.

Understanding the details of the heat shock response has broad ramifications for the the biology of the cell and response to cellular insults and for the onset and treatment of a number of diseases, including neurodegenerative disorders, cancer, aging, and cardiovascular diseases. **Ion Petre** (Turku, Finland) proposed a model based solely on well-documented molecular reactions. The model captures in mechanistic details all key aspects of the regulation, as it is able to account for the swift heat-induced transactivation of the genes encoding heat shock proteins, the backregulation of the transcription and its return to the original level once the stress is removed. The mathematical model was validated based both on existing data from the literature, as well as on novel experimental evidence. The current model can be extended to include several other aspects of the heat shock response, such as the misfolding/refolding of the chaperones and of the heat shock factors. Adding the phosphorylation of heat shock factors and its effect on their activity seems to be however a more difficult problem that may require novel mathematical techniques.

Jacques van HELDEN (Brussel, Belgium) approached the most recent breakthroughs in bioinformatics for the detection of transcription factor binding sites, and in a recent collaboration with Tim Westwood, which have been established thanks to this Conference, applied them to HSFs. To circumvent the impossibility of simple pattern matching approaches in the search for HSF binding sites, several strategies can be combined to estimate and improve the reliability of the predictions : the building of position-specific scoring matrices (PSSM), the use of which can be optimized by estimating the risk of false positive as a function of the score ; the occurrence of various motifs for the prediction of enhancer regions ; and the powerful application of comparative genomics to identify conserved sites and regions.

Administrative report

This was the first Jacques Monod Conference held on this subject and therefore represented a challenge. It succeeded in bringing together scientists from disciplines as diverse as molecular biophysical and biology, physiopathology, development, epigenetics and bioinformatics. This first Conference devoted to HSFs matched the same high standards as the meetings 'Molecular chaperones & the heat shock response' organized at the Cold Spring Harbor or the Gordon Conferences entitled 'Stress proteins in Growth, Development & Disease' in which HSF topic has been represented so far. The quality of the talks was excellent and poster sessions were very active and full of participants. The unique venue at Roscoff markedly contributed to the very positive atmosphere at the Conference, which was extremely lively, constructive and gave rise to many collaborations.

We were fortunate enough to attract to Roscoff 24 world-leading scientists to present their unpublished results from all over the world and from the various disciplines cited before. Ten came from overseas (USA, Japan) and 10 from European countries (Belgium, Finland, France, Germany, Italy, Portugal, Switzerland) and 4 from France. In addition, 16 speakers were selected for shorter oral presentations (Australia, Canada, Finland, Germany, Japan, Poland, Spain, USA). One of these speakers had to unfortunately withdraw at the last minute due to personal issues after the hurricane problems in the US. One registered chinese scientist could not attend the meeting due to visa problems. 68 scientists (35 seniors and 33 juniors) from 16 different countries attended the meeting (Hungary, Korea and Sweden in addition to the previously mentioned countries) among which 29 were women (around 42%).

Two poster sessions were scheduled, one in the evening and one in the morning. Attendees presented 30 posters, which were kept up at the two separate sessions. This provides ample opportunity to participants to present and/or see and was very helpfull in generating unformal discussions.

The Conference was highly appreciated by the participants. Many of them expressed their satisfaction to the organizers and pointed out how much they have felt the need for such a Conference in this field. They enjoyed the small size and the organization of the Conference which allowed friendly and constructive discussions and the initiation of new collaborations. Many students and postdocs also mentionned how much they appreciate this opportunity to get an overview of this very rich and diverse field and invaluable chance to discuss with world-leaders.

The Jacques Monod Committee, and especially of Mrs Dominique Lidoreau was invaluable in contributing to the success of the Conference by providing very efficient guidance, constant help and diligent advice to the meeting organizers.

During the Conference, the presence of Alain Paoli during the sessions allowed a very reactive help for all the technical computer problems. The teams at the Gulf Stream Hotel and Hotel de France provided the participants with extreme comfort and good atmosphere.

As a consequence, the organizers were gratified by an overwhelming support from the participants to prepare another meeting in 3 years. Elisabeth Christians (Toulouse, France) who already helped the president and vice-president in this conference accepted to be president and to submit the required an application for the next conference. She will be helped

in her task by Luke Whitesell from Susan Linquist's lab (Whitehead Institute, US) as vice-president.

Suggestions for improvements

Raising funds fo the Conference proved to be a challenge. Most companies declined to sponsor when they understood that they could not present their products during the poster sessions and the coffee breaks. From their point of view, the fees were too high for the modest advertissement opportunity of being listed on the Conference book. One company (Diagenode) had already registered the Conference on her web site as a Conference at which they had been willing to be present but finally decided to withdraw. Some of them (Roche Diagnostics, Dominique Dutscher, Leica Microsystems, Eppendorf France and Promega) accepted to sponsor the Conference but to a very limited extent as they provided a few hundreds Euros each.

A larger venue to the coffee breaks would be welcome which would also allow the exhibition of Companies as well.

Roscoff site is a wonderfull place and participants enjoyed it. Nevertheless, the walking distance between the meeting room and the restaurant could have been challenging for some participants that might encounter difficulties to walk such a distance several times each day.