A murine world without HSFs: meeting report

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INTRODUCTION

Since its discovery in 1962, the Heat Shock Response (HSR) has been studied extensively in efforts to define the molecular mechanisms by which cells and organisms adapt to environmental challenges. HSR is a paradigm of inducible gene expression including the 3 inter-dependent steps classically described: (1) an inciting stress signal (eg, heat, oxidative stress, chemicals), (2) activation of transcriptional regulators, and (3) downstream effector targets (ie, hsp genes encoding heat shock proteins [Hsps]). The reader is referred to several recent reviews that describe such cellular and molecular dissection (Christians et al 2003; Pirkkala et al 2001; Voellmy 2004).

Beginning in the early nineties, molecular cloning of the heat shock transcriptional regulator (HSF) in mammals led to the exciting discovery of a multigene family (HSF1, 2, 4) (Nakai et al 1997; Sarge et al 1991). Unlike the single HSF regulator found in yeast and flies, the presence of multiple HSFs immediately raised several intriguing questions about their biological functions, physiological specificity, and evolutionary pressure for maintaining the heat shock response in higher vertebrates. While answers using gene targeting sought to resolve their complex functions in vivo, this recent year marks an important milestone as knockout mice are now available for the three known mammalian HSFs.

A cross section of investigators, with diverse research interests in HSF activation and regulation, recently met for a special one-day mini-symposium entitled: "Making sense of HSF mutations in mice: Does the field need another knockout model?" (Salve Regina University, Newport, RI, USA, 16 July 2005). Participants were challenged

to provide a critical analysis of results from studies performed on HSFs knockout mice. With this short note, we wish to acknowledge the emerging efforts of these investigators tackling the unexpected and sometimes conflicting results of mutational analysis of HSFs in mice.

HSF KNOCKOUT ERA IN MICE

This Hsf knockout series started in 1998 with the group of I. Benjamin who pioneered this genetic approach in the Cell, Stress, and Chaperones field. In the first study, McMillan and coworkers (1998) identified HSF1 as the major regulator of HSR, without which Hsp transcription as well as resistance to apoptosis were abolished following a thermal stress. Next, Xiao et al (1999) provided a detailed picture of the complex phenotype for HSF1 deficiency in extra-embryonic development, growth, and endotoxemic-induced survival and female infertility further characterized by Christians (Christians Xiao et al 1999). The mouse embryonic fibroblasts and animals derived from this Hsf1 mutant line were widely distributed to a large number of investigators worldwide who have been addressing independent questions using this robust system during physiologic or pathologic challenges. Two other groups working independently have generated and characterized Hsf1 mouse models. All Hsf1 mutants exhibited female infertility, indicating this factor's essential role in oogenesis and, perhaps, postfertilization development (Izu et al 2004; Zhang et al 2002). Akira Nakai's group also implicated HSF1 in male reproduction where it acts as a protective agent for immature germ cells and a proapoptotic regulator for meiotic germ cells (Izu et al 2004).

How does HSF1 mediate these different important physiological roles? A first explanation would be that HSF1 acts as a transcriptional factor since it has been shown that basal expression of several Hsps and non-hsp

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genes are regulated by HSF1 under nonstress conditions (Trinklein et al 2004). In addition, previous studies supported a repressive property of HSF1 in the absence of DNA-binding activity. This intriguing possibility should be considered when further HSF1 functions are investigated (Chen et al 1997).

While Hsf1 knockouts confirmed expected functions and revealed new roles for HSF1, the status of HSF2 remains far more obscure. HSF2 was thought to be the developmental factor of the HSF family because of its regulation during in vitro erythroleukemic differentiation and different levels of expression throughout the first two-thirds of mouse gestation (Rallu et al 1997; Sistonen et al 1992). To demonstrate these developmental functions in vivo, Hsf2 knockout experiments were undertaken in the same time as Hsf1 knockout. This approach proved to be more laborious to handle through the procedure of gene targeting and further, Hsf2 knockout observed by McMillan and coworkers (2002) in Benjamin's group in Dallas was unexpectedly viable and without gross abnormalities. Such a situation always prompts scientists to extend their observations and experiments to minimize the possibility of having missed subtle abnormalities. Finally, conflicting data were published by Mezger-Morange's group working independently in France on another version of Hsf2 knockout (Kallio et al 2002). Hsf2 knockout performed by V. Mezger and coworkers (Kallio et al 2002) implicated this factor's role in development of both central nervous and reproductive systems. The third Hsf2 knockout line generated by Mivechi's group reached similar conclusions (Wang et al 2003). Without doubt, significant differences in experimental strategies used for gene targeting and variability in mouse genetic backgrounds might account for the observed disparate phenotypes. More importantly, this variable phenotype also goes with and reinforces the idea that HSF2 function is highly influenced by other factors (potentially encoded by modifier genes depending on genetic background) and environmental conditions.

Finally, HSF4, the most recently discovered HSF, established itself as an essential regulator in a very specialized organ, the ocular lens (Fujimoto et al 2004; Min et al 2004). The mysterious factor was well served by the gene-targeting approach, confirming linkage data identifying the human Hsf4 locus as a good candidate for an inherited form of cataracts (Bu et al 2002).

With all 3 HSFs knockouts in hand, redundant functions in members of the HSF family can be evaluated by using double and, in theory, even triple knockouts. Double Hsf1-Hsf2 and Hsf1-Hsf4 knockouts were reported, and confirmed that members of the HSF family interact at least functionally to regulate complex cellular differentiation such as spermatogenesis or lens formation through regulation of expression of gene encoding Hsps

but also non-Hsps such as fibroblast growth factors (FGFs) (Fujimoto et al 2004; Min et al 2004).

Is this the end of the story or merely the beginning? We believe the answer to this rhetorical question will be especially aided, in part, by wider distribution from the originators of these mouse models to the broader community based on the collegiality that fosters scientific inquiry. Reinforcing these processes was the goal of this summer's mini-symposium, which brought together biologists, embryologists, cardiologists, neuroscientists, and specialists in cancer, inflammation, or chromatin conformation. In the end, such steps are likely pivotal in bridging important gaps in knowledge and existing limitations in studying stress response pathways in mammals, especially ones pertaining to an organism's physiological and metabolic requirements under nonstress conditions and pathological responses during disease.

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References

- Bu L, Jin Y, Shi Y, et al. 2002. Mutant DNA-binding domain of HSF4 is associated with autosomal dominant lamellar and Marner cataract. *Nat Genet* 31: 276–278.
- Chen C, Xie Y, Stevenson MA, Auron PE, Calderwood SK. 1997. Heat shock factor 1 represses Ras-induced transcriptional activation of the c-fos gene. *J Biol Chem* 272: 26803–26806.
- Christians E, Davis AA, Thomas SD, Benjamin IJ. 2000. Maternal effect of Hsf1 on reproductive success. *Nature* 407: 693–694.
- Christians E, Qi Z, Renard J, Benjamin IJ. 2003. Heat shock proteins in mammalian development. *Semin Dev Biol* 14: 283–290.
- Fujimoto M, Izu H, Seki K, et al. 2004. HSF4 is required for normal cell growth and differentiation during mouse lens development. *Embo J* 23: 4297–4306.
- Izu H, Inouye S, Fujimoto M, Shiraishi K, Naito K, Nakai A. 2004. Heat shock transcription factor 1 is involved in quality-control mechanisms in male germ cells. *Biol Reprod* 70: 18–24.
- Kallio M, Chang Y, Manuel M, et al. 2002. Brain abnormalities, defective meiotic chromosome synapsis and female subfertility in HSF2 null mice. *Embo J* 21: 2591–2601.
- McMillan DR, Christians E, Forster M. 2002. Heat shock transcription factor 2 is not essential for embryonic development, fertility, or adult cognitive and psychomotor function in mice. *Mol Cell Biol* 22: 8005–8014.
- McMillan DR, Xiao X, Shao L, Graves K, Benjamin IJ. 1998. Targeted disruption of heat shock transcription factor 1 abolishes thermotolerance and protection against heat-inducible apoptosis. *J Biol Chem* 273: 7523–7528.
- Min JN, Zhang Y, Moskopidhis D, Mivechi NF. 2004. Unique contri-

- bution of heat shock transcription factor 4 in ocular lens development and fiber cell differentiation. *Genesis* 40: 205–217.
- Nakai A, Tanabe M, Kawazoe Y, Inazawa J, Morimoto RI, Nagata K. 1997. HSF4, a new member of the human heat shock factor family which lacks properties of a transcriptional activator. *Mol Cell Biol* 17: 469–481.
- Pirkkala L, Nykanen P, Sistonen L. 2001. Roles of the heat shock transcription factors in regulation of the heat shock response and beyond. *Faseb J* 15: 1118–1131.
- Rallu M, Loones M, Lallemand Y, Morimoto R, Morange M, Mezger V. 1997. Function and regulation of heat shock factor 2 during mouse embryogenesis. *Proc Natl Acad Sci U S A* 94: 2392–2397.
- Sarge KD, Zimarino V, Holm K, Wu C, Morimoto RI. 1991. Cloning and characterization of two mouse heat shock factors with distinct inducible and constitutive DNA-binding ability. *Genes Dev* 5: 1902–1911.
- Sistonen L, Sarge KD, Phillips B, Abravaya K, Morimoto RI. 1992. Activation of heat shock factor 2 during hemin-induced differentiation of human erythroleukemia cells. *Mol Cell Biol* 12: 4104–4111.
- Trinklein ND, Murray JI, Hartman SJ, Botstein D, Myers RM. 2004. The role of heat shock transcription factor 1 in the genome-wide regulation of the mammalian heat shock response. *Mol Biol Cell* 15: 1254–1261.
- Voellmy R. 2004. Transcriptional regulation of the metazoan stress protein response. *Prog Nucleic Acid Res Mol Biol* 78: 143–185.
- Wang G, Zhang J, Moskophidis D, Mivechi NF. 2003. Targeted disruption of the heat shock transcription factor (hsf)-2 gene results in increased embryonic lethality, neuronal defects, and reduced spermatogenesis. *Genesis* 36: 48–61.
- Xiao X, Zuo X, Davis AA, McMillan DR, Curry BB, Richardson JA, Benjamin IJ. 1999. HSF1 is required for extra-embryonic development, postnatal growth and protection during inflammatory responses in mice. *Embo J* 18: 5943–5952.
- Zhang Y, Huang L, Zhang J, Moskophidis D, Mivechi N. 2002. Targeted disruption of hsf1 leads to lack of thermotolerance and defines tissue-specific regulation for stress-inducible Hsp molecular chaperones. *J Cell Biochem* 86: 376–393.