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Mini Review

Heat shock transcription factors and sensory placode development

*Akira Nakai**

Department of Biochemistry and Molecular Biology, Yamaguchi University School of Medicine, Minami-Kogushi 1-1-1, Ube 755-8505, Japan

The heat shock transcription factor (HSF) family consists of at least three members in mammals and regulates expression of heat shock proteins in response to heat shock and proteotoxic stresses. Especially, HSF1 is indispensable for this response. Members of this family are also involved in development of some tissues such as the brain and reproductive organs. However, we did not know the molecular mechanisms that regulate developmental processes. Involvement of HSFs in the sensory development was implicated by the finding that human hereditary cataract is associated with mutations of the *HSF4* gene. Analysis of gene-disrupted mice showed that HSF4 and HSF1 are required for the lens and the olfactory epithelium, respectively. Furthermore, a common molecular mechanism that regulates developmental processes was revealed by analyzing roles of HSFs in the two developmentally-related organs. [BMB reports 2009; 42(10): 631-635]

INTRODUCTION

Heat shock response is characterized by induction of a set of heat shock proteins (Hsps), and is a fundamental adaptive response that maintains protein homeostasis (1, 2). This response is regulated mostly at the level of transcription by heat shock transcription factors (HSF1-4), which bind to a heat shock element (HSE) that is composed of at least three inverted repeats of the consensus sequence nGAAn (3, 4). Among HSF family members, HSF1 is required for induction of heat shock genes in mammals when cells or tissues are exposed to heat shock. Heat shock triggers conversion of an HSF1 monomer that is negatively regulated by heat shock proteins into a trimer that can bind to HSE with a high affinity, and bound HSF1 rapidly induces robust activation of the heat shock genes. This HSF1-mediated induction of Hsps is required for acquisition of thermotolerance (4, 5), and protection of cells from various pathophysiological conditions such as neurodegenerative diseases and other degenerative diseases (6). Furthermore, HSF1 is involved in lifespan expansion by up-regulating heat shock proteins. Members of HSF family also play critical roles in developmental processes such as gamatogenesis (7-12) and neurogenesis (13-16), in immune response (17-19), and in maintenance of the ciliated tissues (20) and the sensory organs (this review). These functions of HSFs are not always related with the regulation of heat shock proteins (21, 22). Therefore, we would like to understand molecular mechanisms that regulate developmental processes.

Cranial placodes are defined as transient embryonic thickenings of cranial ectoderm that contribute to the peripheral nervous system of the head (23, 24). The cranial placodes include the sensory placodes that will give rise to the nasal epithelium, the lens, and the inner ear, and the neurogenic placodes from which elements of cranial ganglia are derived. Among them, lens and olfactory precursors arise from a common territory surrounding the anterior neural plate at early developmental stages (25). Furthermore, a transcription factor Pax6 is expressed in both the olfactory and lens placodes, and is required for eye and nasal development (26-28). Unexpectedly, it was demonstrated that HSF1 and HSF4 is required for development of the olfactory epithelium and the lens, respectively. In this review, I summarize functions and mechanisms of HSFs in the sensory placode development.

Mutation of *HSF4* gene in human hereditary cataract

HSF4 is identified as a third member of the HSF family (29). HSF1 and HSF2 proteins are highly expressed in most cells and tissues, whereas the expression level of HSF4 protein is relatively low (30, 31). Different from HSF1 and HSF2, however, HSF4 is constitutively a trimer that can bind to HSE, suggesting that HSF4 may have physiological roles during development.

A first report implicating requirement of HSF4 in development came from the finding that mutations of *HSF4* gene are associated with dominant inherited cataracts in human (32). Thereafter, many patients of familial cataract are shown to be associated with the mutations of the *HSF4* gene (33-36). Interestingly, autosomal dominant cataract coincides with mutations in the DNA-binding domain, whereas recessive cataract does with mutations in the oligomerization domain or in the downstream of heptad repeat. Patients with mutated HSF4

*Corresponding author. Tel: 81-836-22-2214; Fax: 81-836-22-2315; E-mail: anakai@yamaguchi-u.ac.jp

Received 3 September 2009

Keywords: Cytokine, Heat shock protein, Placode, Sensory organ, Transcription factor

have no other symptom than cataract, suggesting that HSF4 should be dispensable in other tissues.

Inactivation of *HSF4* gene causes cataract in mice

The lens is characterized by its high degree of transparency. Development of the lens occurs by differentiation of epithelial cells into elongated fiber cells that accumulate in concentric layers and lose their nuclei and other organelles (37). The concentration of protein in the lens fiber cells is extremely high, as much as 450 mg/ml of protein in the center of the lens (38). As proteins in the center of the lens cannot turnover and must remain stable and soluble throughout the life of the organism (39, 40), it is remarkably important to stabilize proteins.

It was shown that level of HSF4 protein in the lens is extremely high compared with those in other tissues, and HSF4 consists of a major HSE-binding activity in the lens (41-43). Three laboratories demonstrated that inactivation of the *HSF4* gene in mice caused cataract at early postnatal days (42-44). Histological examination showed abnormal fiber cells, which contained nuclei and vacuole-like cavity. One report further noticed inclusion-like structures stained heavily with eosin in the fiber cells, implicating that expression of genes involving protein stability was affected by HSF4 deficiency (42). In fact, expression of heat shock proteins and crystallins was altered. Remarkably, Hsp27 was not induced during development of the HSF4-null lens, and expression of at least some γ-crystallin genes such as γF- and γS-crystallin genes decreased (42-44). One report demonstrated remarkable reduction of the expression of six γ-crystallins (γA- to γF-crystallin), which might consistent with severe phenotypes such as the appearance of inclusion-like structures (42). It would be noticeable that expression of many other genes and proteins also decreases in the HSF4-null lens (43, 44).

HSF4 regulates differentiation into fiber cells

Loss of HSF4 function also resulted in increased proliferation and premature differentiation of the lens epithelial cells (42). These processes are regulated by various cytokines such as fibroblast growth factors (FGFs) (45-47). It revealed that HSF4 inhibits expression of FGF-1, FGF-4, and FGF-7, and that at least the *FGF-7* gene is a direct target of HSF4. As FGFs regulate growth and differentiation of various cell types (48), regulation of FGF expression by HSF4 might be an important regulatory pathway for development in general. As the bow region of the lens, where the lens epithelial cells differentiated into fiber cells, was normal in two other HSF4-null mouse models (43, 44), expression of FGFs could be constant in these models.

Remarkably, HSF1 inversely activates the similar set of FGFs in the lens that is repressed by HSF4 (42). Therefore, abnormal levels of FGFs in HSF4-null or HSF1-null lens reversed to normal levels in double-null lens, and proliferation and differentiation of the epithelial cells were almost normal in double-null lens. These results demonstrate that HSF1 and HSF4 competitively regulate expression of FGFs that are essential for cell growth and differentiation (Fig. 1).

HSF4 binding regions on the genome

The lens is composed of only two cell types, and HSF4 starts to be expressed at early stage of lens development in both cell types (42). Therefore, it is suitable for comprehensive identification of HSF4-target genes and their analysis. Interestingly, levels of HSF1 and HSF2 in the lens were relatively high at embryonic days, but they decreased quickly after birth (49).

Analysis of the relative location of the HSF4 binding regions reveals that 53% maps to the intron and exons, and 40% to the

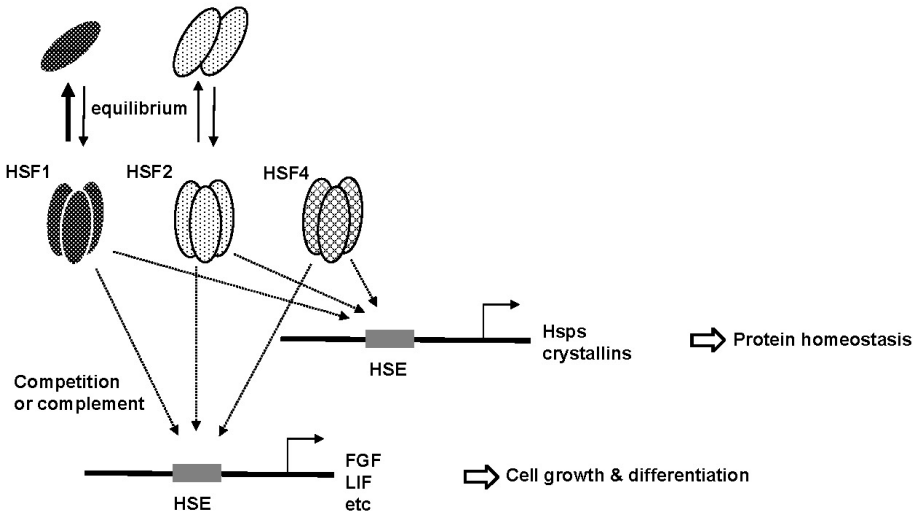

Fig. 1. Members of HSF family competitively or complementarily regulate expression of molecular chaperones and cytokines such as FGFs and LIF during the sensory placode development. Some developmental signal promotes a monomer-to-trimer transition of HSF1 in the olfactory epithelium. The trimeric HSF4 increases during lens development, which is associated with reduction of HSF1 and HSF2.

distal parts (>10 kb) of the genes (49). Remarkably, only 5% of HSF4 binding regions maps to 10 kb promoter-proximal regions. This result is in marked contrast to localization of the canonical HSEs on the 5′-proximal promoter of classical heat shock genes. Actually, genome-wide analysis of HSF1 binding regions was performed by a ChIP-on-chip experiment using a DNA microarray corresponding to human promoters (50, 51). Even though, 70% of the HSF4 binding regions is occupied by HSF1 and or HSF2. These observations suggest that HSF4 binding regions are distributed on whole genome and are co-occupied by members of HSF family.

It is a matter of great interest that HSF binding to genomic regions in stressed and unstressed conditions is linked to gene regulation. In Drosophila, HSF1 binds to multiple chromosome loci including non-heat shock loci on both conditions (52, 53). Genome-wide analysis showed that HSF1 binding to the promoter does not necessarily induce gene expression in yeast (54, 55) and in human (50, 51). Remarkably, HSF4 binding to genomic regions is closely associated with reduced histone H3K9 methylation, irrespective of the relative location of the HSF4 binding regions, and is not always correlated with developmental expression of genes on or near the HSF4 binding regions (49). HSF4-mediated chromatin modification would be necessary for gene activation, as HSF4 is required for induction of some non-classical heat shock genes during heat shock (49). This is a first semi-comprehensive analysis demonstrating that HSF-binding modulates chromatin modification.

HSF1 is required for olfactory neurogenesis

Analysis of sensory organs in HSF1- and HSF4-nul mice revealed that structure of the nasal cavity was abnormal and olfaction is disturbed in adult HSF1-null mice (56). HSF1 is not required for development of the olfactory epithelium until 3 weeks old, but is indispensable for maintenance of the olfactory epithelium in mice over 4 weeks old. Consistently, HSE-binding activity of HSF1 is induced in the olfactory epithelium in postnatal 4-week-old mice, which is associated with increased levels of major heat shock proteins (56). In the absence of HSF1, the heat shock proteins are not induced. Requirement of HSF1 in puberal mice is unique when it is compared with the requirement of transcription factors Mash1 and Ngn1 that control early olfactory neurogenesis (57, 58).

Proliferation and differentiation of the olfactory sensory neurons are regulated many cytokines (59-61), including *FGF* genes, whose expressions are regulated by HSFs in the lens (42). Expressions of most genes such as *FGF* genes were constant in the HSF1-null olfactory epithelium (56). In marked contrast, the level of leukemia inhibitory factor (LIF) expression was continued to be high during development of the olfactory epithelium. LIF is essential for normal development of olfactory sensory neurons (62). Continuous overexpression of LIF may greatly affect intracellular signalings in the olfactory sensory neurons, in which decreased expression of heat shock proteins causes altered protein homeostasis. LIF expression is inhibited by HSF1 during olfactory development whereas it is inhibited by overexpression of HSF4, suggesting that HSF1 and HSF4 have opposing effects on LIF expression (Fig. 1) (56).

Conclusion and future perspectives

It is interesting that HSF1 and HSF4 are required for post-natal development of the two sensory placodes, the olfactory placode and the lens placode, respectively. Each factor becomes dominant during post-natal development of each sensory organ. DNA-binding activity of HSF1 is induced in the olfactory epithelium, and HSF4 protein increased in the lens, which is associated with remarkably reduction of HSF1 and HSF2 proteins. As a result, each HSF induces some set of heat shock proteins and molecular chaperones. Simultaneously, HSFs regulates differently the expression of development-related cytokines such as FGFs and LIF. Although development of another sensory placode, the otic placode, is normal in young adult mice (63, 64), age-related hearing loss is suppressed by HSF1 activation (65), implicating that HSF1 may also play a role in the maintenance of the aged-inner ear function. The sensory organs are continuously exposed to external stimuli, but the observations suggest that the activation of HSFs during development might be programmed in the genome.

Acknowledgements
I thank members of our laboratory for their comments. This work was supported in part by Grants-in-Aid for Scientific Research and on Priority Area-a Nuclear System of DECODE, from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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