Chapter 8
HSF Is Required for Gametogenesis

Koji Shiraishi

Abstract Gametogenesis, spermatogenesis and oogenesis, is a unique process that includes meiosis, a step that produces haploid cells. Heat shock factors (HSFs), especially HSF1 and HSF2, play important roles during this process in cell type- and stage-specific manners; however, their functions in spermatogenesis and oogenesis are different because the cellular kinetics of spermatogenesis and oogenesis are completely different. Basically, HSF1 acts in cytoprotection and cell survival, playing an important role in quality control mechanisms by eliminating injured or abnormal cells during spermatogenesis, thereby protecting the organism from abnormal development in the next generation. The role of HSF2 in spermatogenesis is controversial, but it may be involved in the spermatogenic process. The disruption of HSF1 and HSF2 in oocytes led to multiple defects during meiotic maturation, causing female infertility and reduced oogenesis, respectively. The involvement of HSF1 and HSF2 has also been reported in pathological conditions, such as cryptorchidism, varicocele, and genotoxic stress response, where heat stress and oxidative stress are the major factors that inhibit spermatogenesis. The investigation of HSFs in gametogenesis per se may provide important information for understanding the physiology/pathophysiology of spermatogenesis and oogenesis and for establishing new therapeutic approaches for male and female infertility.

Keywords Heat shock factor • Heat shock proteins • Spermatogenesis • Oogenesis

8.1 Introduction

Responses against heat and other harmful cell stresses are regulated mainly by heat shock transcription factors (HSFs) (Wu 1995). By inducing the transcription of the molecular chaperones called heat shock proteins (HSPs), HSFs protect the cell from the deleterious consequences of protein-damaging stress. In vertebrates transcription of HSPs controlled mainly by four HSFs: HSF1, HSF2, HSF3, and HSF4 (Morimoto 1998; Nakai et al. 1995; Wu 1995). HSF3 is not expressed in human

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cells (Fujimoto et al. 2010). Basically HSF1 acts as a cell survival factor against various stresses and protects against cell death by upregulating a specific heat shock gene (Nakai and Ishikawa 2001; Inouye et al. 2003). HSFs play important roles during the process of gametogenesis, i.e., spermatogenesis and oogenesis, which includes meiosis, a step that produces haploid cells. Especially in the testis, which has the most rapid cell cycle in the whole body, the functions of HSFs in spermatogenesis are quite unique. The expression of HSF4 is restricted to the brain and lung and is required for ocular lens development and fiber cell differentiation (Nakai et al. 1997; Fujimoto et al. 2004). This chapter reviews the contribution of HSF1 and HSF2 in spermatogenesis and oogenesis based on findings mainly from mice and mentions the involvement of the HSFs in spermatogenesis during clinical situations.

8.2 Normal Spermatogenesis

Spermatogenesis is a developmental step that occurs in the seminiferous tubules in the testis, involving dynamic changes from spermatogonia to mature spermatozoa through mitosis, meiosis, and differentiation (Fig. 8.1). The final step of spermatogenesis, spermiogenesis, features unique chromatin remodeling as somatic histones

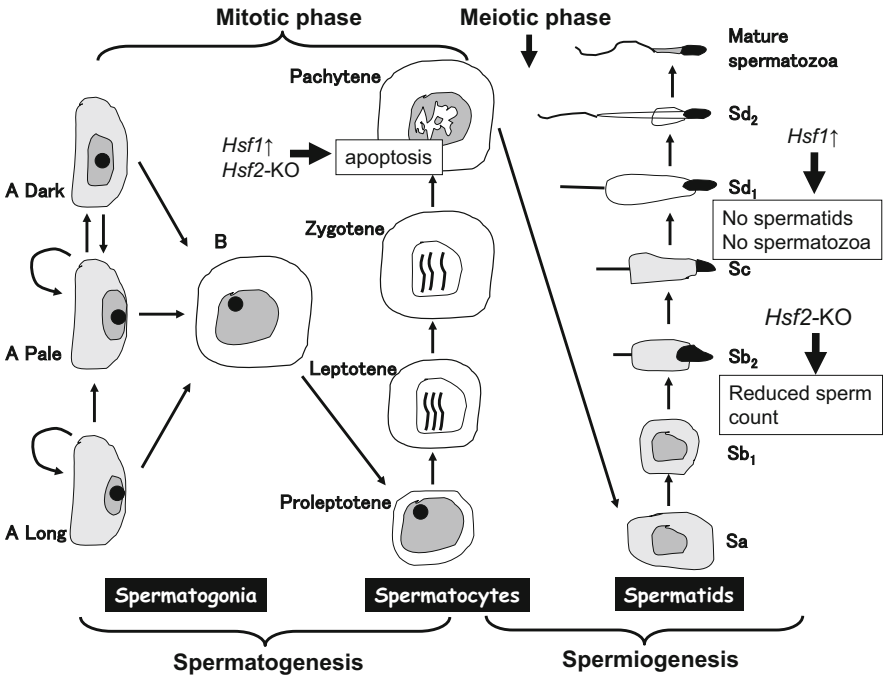

Fig. 8.1 A schema of mammalian spermatogenesis and the contribution of HSF1 and HSF2

and is replaced by DNA packing proteins, such as transition proteins and protamines. Germ cells continuously proliferate to supply a large amount of spermatozoa, whereas up to 75 % of potential spermatozoa are estimated to undergo apoptotic cell death in the seminiferous tubules of adult animals (Print and Loveland 2000). During the process of spermatogenesis, dramatic changes in the patterns of gene expression and chromatin structure are observed with each germ cell type and in a stage-specific manner.

8.2.1 Roles of HSF1 in Normal Spermatogenesis

HSF1 is a highly conserved transcription factor that coordinates stress-induced transcription through binding of heat shock response elements (HSEs) to the promoters of the HSP molecular chaperone genes (He et al. 2003). Basically, HSF1 promotes cell survival against various types of stress by inducing HSPs and other proteins. In the mouse testis, HSF1 is abundantly expressed from pachytene spermatocytes to round spermatids but cannot be detected in spermatogonial stem cells or elongating spermatids (Akerfelt et al. 2010). In the testis, where various types of cells are contained, there are also a variety of HSF1 expression levels in each cell type and the function in each cell type differs. In the normal human testis, distinct stage-dependent expression of HSF1 immunoreactivity was observed in primary spermatocytes (Fig. 8.2a) and postmeiotic spermatids (Fig. 8.2b) (our preliminary data).

Different from oogenesis and the other somatic cells, spermatogenesis is a unique process in which the cell kinetics are extremely high compared with the other organs. A large amount of low-quality spermatozoa are produced and released into the seminal tract, and simultaneously, immature spermatogenic cells undergo apoptosis and phagocytosis by Sertoli cells (Shiratsuchi et al. 1997). Primary spermatocytes are diploid cells, which are characterized by their nuclear appearance including the following: preleptotene, leptotene, zygotene, pachytene, or diplotene stages (Fig. 8.1). Recent studies have demonstrated the existence of a "pachytene checkpoint" in late meiotic prophase; when triggered, it leads to the elimination of defective germ cells by apoptosis (Roeder and Bailis 2000). Involvement of HSF1 is summarized in Table 8.1. Using transgenic mice, Nakai et al. showed that the active form of HSF1 increased the number of apoptotic pachytene spermatocytes and blocked spermatogenesis at this step, leading to spermatogenic dysfunction (Nakai et al. 2000). Further studies using HSF1 overexpression have shown massive degeneration of the seminiferous epithelium and the general absence of round and elongated spermatids (Izu et al. 2004; Hayashida et al. 2006). In other words, HSF1 paradoxically promotes apoptotic cell death of male germ cells rather than cytoprotection (Widlak et al. 2003; Nakai et al. 2000). On the other hand, *Hsf1*-KO male mice were fertile but produced approximately 21 % less sperm than wild-type mice (Salmand et al. 2008; Akerfelt et al. 2010). Taking these observations together, HSF1 could be considered to be

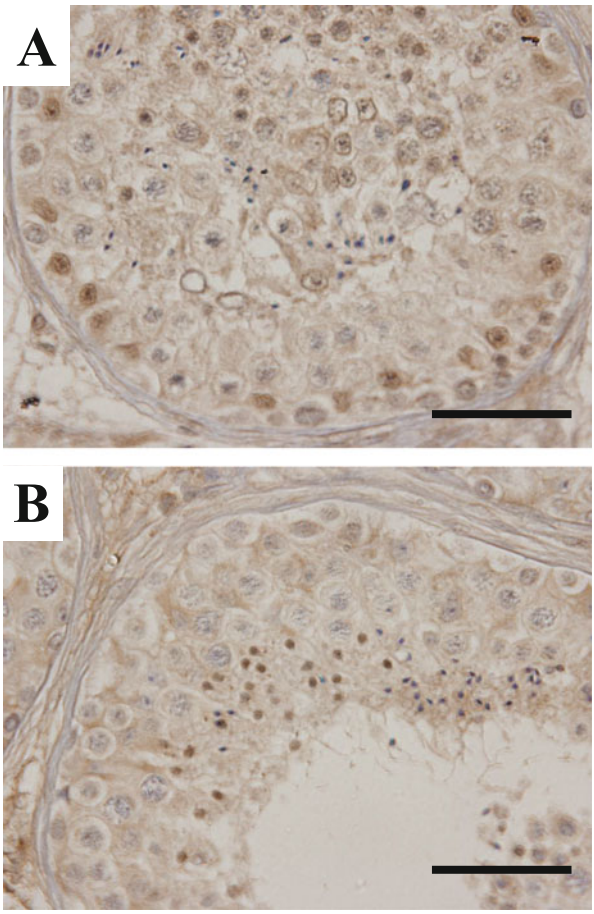

Fig. 8.2 Representative immunostaining for HSF1 in the human testis with normal spermatogenesis (×400, bar: 10 um). Stage-dependent expression of HSF1 was observed in primary spermatocytes (**a**) and postmeiotic spermatids (**b**). These microphotos were obtained from the same testis. Primary antibody was courtesy from Professor Nakai

dispensable for mouse spermatogenesis. Heat-induced apoptosis of primary spermatocytes is inhibited in mice lacking HSF1 (Izu et al. 2004). Furthermore, the inhibition of germ cell apoptosis by caspase inhibitors has also shown to have no merit for long-term spermatogenic function using a rat testicular ischemia-reperfusion model (Shiraishi et al. 2000). Although there is no information regarding the quality of spermatozoa produced from *Hsf1*-KO mice, HSF1 plays an important role in checking quality control mechanisms by eliminating injured or abnormal cells in spermatogenesis, thereby protecting the organism from abnormal development in the next generation.

Previous studies have shown that HSP27, HSP40, HSP60, HSP70, HSP90, and HSP105 are important for spermatogenesis; specifically, HSP70.1, HSP70.2, and HSP90 have been well studied (Sarge 1995; Dix et al. 1996, 1997). The expression levels of major heat shock genes, Hsp110, Hsp90α, Hsp90β, Hsp70.1, Hsp70, Hsp60, Hsp40, Hsp27, and testis-specific Hsp70.2, were not altered or even downregulated in the testes in *Hsf1*-KO mice compared with wild-type mice (Izu

Table 8.1 Reproductive phenotypes of *Hsf1* and *Hsf2* overexpression and knockout mouse

	Male phenotype	Female phenotype
Hsf1 transgenic	Apoptosis of pachytene spermatocyte no meiosis Protection against heat-induced spermatogonia death spermatocyte death	
Hsf1 knockout	No male infertility Lack of genotoxic proliferation block in spermatogonia Lack of genotoxic cell death in meiotic spermatocyte Lack of protection against heat-induced spermatogonia and spermatocyte death	Delay of germinal vesicle breakdown Meiosis arrest Abnormal cytokinesis Apoptosis of prophase oocyte Impaired granule exocytosis and pronuclei formation
Hsf2 transgenic	Not established	Not established
Hsf2 knockout	Reduced testicular size, sperm count, and male infertility Pachytene spermatocyte apoptosis Arrest of meiosis Complete spermatogenic defect in *Hsf1*/*Hsf2* double KO	No female infertility Reduced ovarian weight and female infertility Ovulation defect

et al. 2004). HSP70.2 is synthesized during the meiotic phase of spermatogenesis and is abundant in pachytene spermatocytes, and it is required for Cdc2 to form a heterodimer with cyclin B1, suggesting that it is a chaperone necessary for the progression of meiosis in the germ cells of male mice. Prior to the onset of massive apoptosis caused by the expression of active HSF1 in spermatocytes, a marked decrease in Hsp70.2 mRNA and protein levels in spermatocytes occurs (Widlak et al. 2007). The activation of HSF1 in spermatocytes does not activate these HSPs but leads to a massive activation of the caspase-3 pathway, a common pathway toward apoptosis (Widlak et al. 2003; Vydra et al. 2006), indicating that there are multiple pathways for HSF1-dependent apoptosis in primary spermatocytes. Possible HSF1-dependent factors involved in negative regulation of Hsp70.2 and other testis-specific genes remain to be identified. The level of HSP60 and HSP105 was rather increased in mice overexpressing *Hsf1*, which promotes the apoptosis of germ cells (Vydra et al. 2006).

On the other hand, the original function of HSF1, protection of cells from stress and escape from cell death, is observed in immature germ cells, mainly in spermatogonia (Izu et al. 2004; Akerfelt et al. 2010). Dark A spermatogonia divide to maintain their own numbers, whereas others differentiate into pale type A, then type B, which are the immediate precursors of the spermatocytes. Thus, the dark spermatogonia form a reservoir of resting cells, which divide into the primary spermatocytes (Fig. 8.1). Unlike the primary spermatocytes, diminishing these groups of cells causes permanent loss of germ cell supply, and these cells should be protected against a variety of stresses to maintain spermatogenic function. Therefore, how does HSF1 decide whether to prevent or promote apoptosis?

Hayashida et al. demonstrated a novel survival pathway, which requires a balance between HSP and TDAG51 (T-cell death-associated gene 51)-mediated pathways (Hayashida et al. 2006). TDAG51 is a member of the PHL (pleckstrin homology-like) domain family and acts upstream of the Fas-mediated cell death pathway. There are interplays between proapoptotic TDAG51 and antiapoptotic HSPs, both of which are direct target genes of HSF1. The unique balance between HSPs and TDAG51, in favor of HSPs in spermatogonia and in favor of TDAG51 in spermatocytes, would trigger HSF1-dependent cell survival and apoptosis, respectively.

Spermatids do not undergo further division but gradually mature into spermatozoa by changing their shape and are often described as "round" or "oval" spermatids and may also be called "early" or "late" spermatids. This step is called spermiogenesis (Fig. 8.1). Akerfelt et al. have shown that HSF1 is transiently expressed in meiotic spermatocytes and haploid round spermatids in the mouse testis and is localized to the sex chromatin both prior to and after the meiotic divisions (Akerfelt et al. 2010). HSF1 regulates the postmeiotic expression of X- and Y-chromosome genes that are required for correct packing of the DNA in the sperm (Akerfelt et al. 2010; Toure et al. 2004). These observations demonstrate the role of HSF1 in final spermatid differentiation as well as the mitotic and meiotic processes in spermatogonia and primary spermatocytes.

Testosterone, which binds and activates the androgen receptor (AR), suppressed HSF1 expression in Sertoli cells by promoting binding of the AR to the *Hsf1* promoter. As anticipated, HSF1 was upregulated in Sertoli cell androgen receptor-KO mouse testes compared with wild-type mice (Zhang et al. 2012), indicating that androgens may regulate HSF1 expression. Furthermore, HSF1 partially mediates the effects of androgens on the expression of HSPs in Sertoli cells through AR action (Yang et al. 2014). Germ cells localize to seminiferous tubules, which are isolated from the systemic circulation by the blood-testis barrier, indicating that somatic cells (i.e., Leydig, Sertoli, and endothelial cells) are easily affected by a variety of stresses. Understanding the role of HSF1 in these cells and their endocrinological regulation is therefore important for understanding spermatogenesis.

8.2.2 Roles of HSF2 in Normal Spermatogenesis

In addition to HSF1, HSF2 belongs to the family of HSFs and is most abundantly expressed in the testis, as well as other organs (Fiorenza et al. 1995). HSF2 plays important roles in regulating normal spermatogenesis process in mice (Goodson et al. 1995; Kallio et al. 2002; He et al. 2003; Wang et al. 2003; 2004; Akerfelt et al. 2008) with cell- and stage-specific expression profiles (Sarge et al. 1994; Alastalo et al. 1998; Björk et al. 2010). In rat, the expression of the alternatively spliced HSF2-alpha and HSF2-beta isoforms is developmentally regulated in a stage-specific manner (Alastalo et al. 1998). HSF2 mRNA first appears in the testis between day 14 and 21 of postnatal development and is found mainly in the nuclei

of early pachytene spermatocytes (stages I–IV) and round spermatids (stages V–VII) in the rat (Sarge et al. 1994; Alastalo et al. 1998).

The phenotype of *Hsf2*-KO in spermatogenesis is controversial. *Hsf2*-KO mice exhibit reduced testis and epididymis sizes, vacuolization of the seminiferous tubules, and lower sperm counts (Kallio et al. 2002; Wang et al. 2003). Kallio et al. reported that spermatocytes are eliminated via stage-specific apoptosis at the pachytene spermatocyte stage. They proposed that this apoptosis could be caused by an elevated frequency of synaptonemal complex abnormalities, indicated by the formation of loop-like structures or the appearance of separated centromeres, which can activate the pachytene checkpoint and trigger apoptosis (Roeder and Bailis 2000; de Rooij and de Boer 2003) in *Hsf2*-KO spermatocytes (Kallio et al. 2002). On the other hand, McMillan et al. found that *HSF2*-KO mice showed normal spermatogenesis (McMillan et al. 2002). This discrepancy may be caused by the technical methods for the generation of the knockout mice or the genetic background of the mice (Kallio et al. 2002; McMillan et al. 2002). The inactivation of both *Hsf1* and *Hsf2* results in a more severe phenotype, manifested by arrested spermatogenesis and complete sterility (Wang et al. 2004). It has also been proposed that HSF1 requires cooperation with HSF2 in development (Sandqvist et al. 2009) and that the synergistic action of both HSFs is crucial for spermatogenesis (Wang et al. 2003). On the other hand, the level of HSF2 protein in the testis of *Hsf1*-KO mice was the same as that in wild-type mice, indicating that there is no compensatory expression between HSF1 and HSF2 (Izu et al. 2004).

HSF2 may function to regulate the expression of *HSP* genes during germ cell differentiation; several studies have identified target genes for HSF2, whereas the target genes in spermatogenesis are largely undetermined and the vast majority of potential targets are not heat shock genes. Basically no correlation between the expression of HSF2 and classical HSF targets (e.g., HSPs) has been found (Sarge et al. 1994; Alastalo et al. 1998; Kallio et al. 2002; Wang et al. 2003). HSF2 has been shown to bind to the promoter of the *hsp70.2* gene, which is essential for spermatogenesis, in a cell type- and germinal stage-dependent manner (Sarge et al. 1994), whereas it is not a target of HSF2 (Kallio et al. 2002; Wang et al. 2003; McMillan et al. 2002; Akerfelt et al. 2008). Two splice variants, HSF2α and HSF2β, have been identified and HSF2α is predominantly expressed in the mouse testis (Goodson et al. 1995). Transfection experiments have revealed that the HSF2α isoform has 2.6-fold more transcriptional activity than the HSF2β isoform (Goodson et al. 1995). This alternative splicing of HSF2 is regulated during testis postnatal development, with a change in the expression level from predominantly HSF2β at day 7 to primarily HSF2-a in the adult testis. Different functions have been considered for these splice variants of HSF2 in spermatogenic cells.

It has been shown that HSF2 binds the promoters of a large set of targets and regulates the transcription of sex chromosome multicopy gene promoters in postmeiotic cells (Akerfelt et al. 2008). On the Y chromosome, the possible target genes for HSF2, as identified by ChIP-on-chip analysis, were the following: spermatogenesis-associated glutamate (E)-rich protein 4a (*Speer4a*), *Hspa8* (formerly *Hsc70*), ferritin mitochondrial (*Ftmt*), spermiogenesis-specific transcript on

the Y (Ssty2), Scyp3-like Y-linked (Sly), and Scyp3-like X-linked (*Slx*) (Akerfelt et al. 2008). In the postmeiotic spermatids (i.e., round spermatids), where *Ssty2* is thought to regulate chromatin remodeling, HSF2 is involved in chromatin remodeling during spermatogenesis through direct regulation of *Ssty2* and/or *Sly* (Toure et al. 2004; Akerfelt et al. 2008), which exist in multiple copies throughout the *MSYq* (male-specific Y-chromosome long arm) region. The presence of a Cor1 domain, which helps chromatin binding, in the Sly and Slx proteins and the fact that there is a high occurrence of sperm head abnormalities in some *MSYq* deletions (Ellis et al. 2005) indicate that *MSYq* may cause chromatin remodeling impairment during sperm head condensation. Taken together, the human data suggest that deletions in the MSYq region are the most common genetic cause for spermatogenic failures, resulting in oligo- and azoospermia (Krausz 2005); the role of HSF2 in regulating these genes is crucial for spermatogenesis. More directly, Mou et al. have identified nine synonymous mutations and five missense mutations in *Hsf2*, and the R502H mutation affected the transcriptional regulatory function of HSF2 as measured by the expression level of the target gene *HspA2* in patients with idiopathic azoospermia (Mou et al. 2013). Testis-specific HSPA2 is a member of the HSP70 family (Naaby-Hansen and Herr 2010). This molecular chaperone is present in spermatocytes during meiosis, participating as an element of the synaptonemal complex, and during the maturation stage of spermiogenesis (Georgopoulos and Welch 1993). These observations have obviously demonstrated that HSF2 plays a crucial role in spermatogenesis, in both model organisms and humans.

8.2.3 Another HSF in Spermatogenesis

A heat shock-like factor, HSFY, which shares partial homology with classical HSFs, was discovered on the human Y chromosome (Shinka et al. 2004; Tessari et al. 2004). HSFY contains a HSF-type A DNA-binding domain in the middle portion of the protein that is similar to other *Hsf* genes (Shinka et al. 2004; Tessari et al. 2004). In humans, the expression is observed to be mainly in the testis (Tessari et al. 2004), especially in Sertoli cells and spermatogenic cells (Shinka et al. 2004). The functions were presumed to be different in each cell type. However, the putative DNA-binding domain shows only 30 % homology to that of classical HSFs such as *Hsf1* and *Hsf2* (Shinka et al. 2004), and its exact function in spermatogenesis remains unknown (Vinci et al. 2005; Kinoshita et al. 2006). Therefore, HSFY is postulated to have functions different from those of the classical HSFs.

The HSFY gene is localized in the AZFb region of the Y chromosome, and its deletion causes azoospermia (Ferlin et al. 2003). On the other hand, high HSFY expression was observed in men with a normal spermiogram compared with infertile men (Ferlin et al. 2010), indicating that its expression is a good sign for

spermatogenesis. Furthermore, HSFY expression is altered in infertile patients with hypospermatogenesis (Sato et al. 2006).

8.3 Normal Oogenesis

Completely different from the testis, where huge amounts of sperms are produced every second, the cell kinetics of gametogenesis in the ovary are very low and only one oocyte matures in every month in humans. Mammalian oocytes are highly specialized cells engaged in complex meiotic cell division, characterized by several "stops and starts" prior to the formation of the zygote for fertilization (Mehlmann 2005) (Fig. 8.3). During development, oogonia enter meiosis at 13.5 embryonic days, and oocytes remain blocked at prophase I until they are fully grown. Then, in response to hormonal signals or after experimental isolation from their ovarian follicles, oocytes resume meiosis, enter meiotic maturation, and progress to metaphase II (MII), at which stage they can be fertilized. Basically, oocyte maturation ends with the first meiotic division. Meiosis is characterized by a tightly regulated chromosome chromatid separation, resulting in the extrusion of the first polar body, which contains less than 20 % of the oocyte volume. Although information regarding the role of HSFs in oogenesis is very limited compared with spermatogenesis, it was originally reported that HSF1 was highly expressed in mouse oocytes, whereas the expression of HSF2 was very faint (Christians et al. 1997). Accumulating

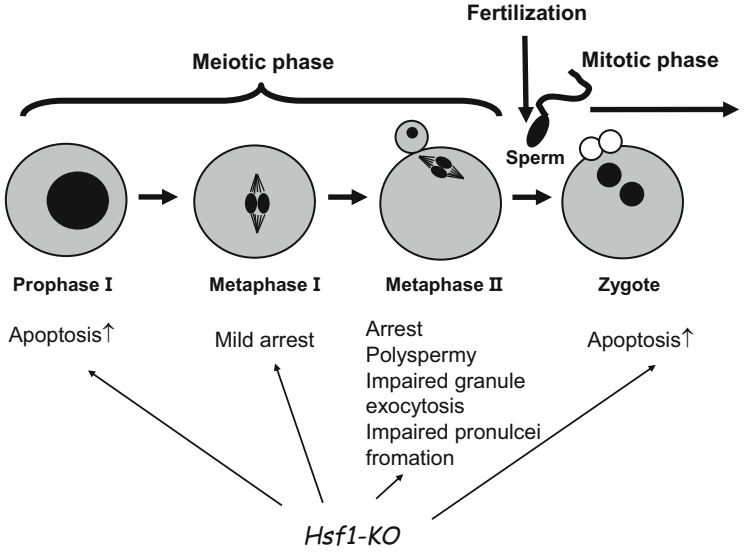

Fig. 8.3 A schema of mammalian oogenesis and the contribution of HSF1

evidence reviewed in this section has shown that HSF1 and 2 definitely play pivotal roles in oogenesis.

8.3.1 Roles of HSF1 in Normal Oogenesis

The involvement of HSF in oogenesis was first reported in a *Drosophila* study (Jedlicka et al. 1997) demonstrating that HSF is essential for oogenesis and HSF's contributions were complex because its role in oogenesis was only partially mediated by the regulation of HSP genes.

It has been reported that *Hsf1*-KO female infertility is linked to the inability of *Hsf1*-KO oocytes to produce viable embryos after natural mating (Christians et al. 2000). *Hsf1*-KO mice have normal ovaries and reproductive tracts, indicating that folliculogenesis and oogenesis were not influenced by the absence of HSF1. However, embryos without HSF1 were unable to develop properly beyond the zygotic stage. HSF1 acts as a transcription factor for meiotic genes during both embryonic and adult oocyte meioses. The resumption of meiosis, characterized by germinal vesicle breakdown (GVBD), was delayed in *Hsf1*-KO oocytes (Metchat et al. 2009). *Hsf1*-KO oocytes exhibited multiple defects during meiotic maturation, a marked block at the metaphase I (MI) stage, and alterations in meiotic asymmetrical cytokinesis, resulting to less than 16 % of mutant oocytes reaching the normal MII stage (Metchat et al. 2009). Subsequently, most of the MII oocytes were unable to cleave to the two-cell stage after fertilization, possibly due to mitochondrial damage and altered redox homeostasis (Bierkamp et al. 2010).

Involvement of HSP90, the major HSP expressed in fully grown oocytes, has been well documented. HSP90 is the major HSF1-dependent chaperone and is markedly downregulated by the absence of HSF1 (Metchat et al. 2009). Both *Hsp90*-KO and the HSP90 inhibitor, 17-(allylamino)-17-demethoxygelda-namycin (17AAG), caused a similar phenotype: impairment of meiosis progression. This finding suggests that Hsp90 deficiency contributes to the developmental defects seen in *Hsf1*-KO oocytes (Metchat et al. 2009). HSF1 directly regulates the transcription of Hsp90α, and the lack of Hsp90α leads to the degradation of kinases such as PLK1 and CDK1. In addition, the mitogen-activated protein kinase pathway was shown to regulate the asymmetry of the first meiotic division and MAPK activity was reduced in *Hsf1*-KO oocytes (Metchat et al. 2009). In addition to the downregulation of many HSPs in oocytes, other HSF1 target genes may contribute to the pleiotropic meiotic phenotype (Metchat et al. 2009): Pre-ovulated *Hsf1*-KO oocytes (1) have cytoskeletal deformities (i.e., Golgi apparatus, cortical actin cytoskeleton, cytoplasmic aggregates) (Christians et al. 2000); (2) have mitochondrial dysfunction, leading to the increased production of reactive oxygen species; and (3) are susceptible to oxidative stress (Bierkamp et al. 2010).

8.3.2 Roles of HSF2 in Normal Oogenesis

Female *Hsf2*-KO mice exhibited multiple fertility defects (Kallio et al. 2002): (1) reduced litter size, (2) the production of abnormal eggs (70 % of fertilized oocytes appear to be abnormal and unable to proceed to the two-cell stage), (3) reduction in ovarian follicle number and the presence of hemorrhagic cystic follicles, and (4) abnormal responses to gonadotropin due to abnormally elevated (50–60 times) levels of luteinizing hormone receptor mRNA (Kallio et al. 2002), which can be rescued by superovulation treatment. Taken together, these findings suggest that HSF2 plays a part in the oogenic process and defects in its function quantitatively alter oogenesis.

Because HSF1 has a role in testis Sertoli cells (Yang et al. 2014), there should also be a role in ovarian somatic cells (i.e., granulose cells and theca cells) to directly or indirectly regulate oogenesis. However, no information has been found to support roles for HSF 1 and 2 in the ovarian somatic cells. Furthermore, no placental defects were identified in the *Hsf2*- KO models, which could have explained embryonic lethality (Kallio et al. 2002).

8.4 HSF in Stressed Spermatogenesis and Its Clinical Significance

8.4.1 Role of HSFs on Heat-Induced Spermatogenic Cell Death

The testes in most mammals are maintained 2–7 °C below body temperature (in humans: 2–3 °C) by their location outside the body cavity in the scrotum and by a countercurrent heat-exchange process that cools the blood entering the testis (Kandell and Swerdloff 1988). Spermatogenesis is disrupted readily by slight increases in temperature or by other environmental stresses, implying that HSFs and HSPs play important roles in spermatogenic cells. In particular, primary spermatocytes are the most sensitive germ cells to heat stress (Yin et al. 1997). On the other hand, information regarding the effects of heat stress on oogenesis is very limited because the ovary is localized in the abdominal cavity and the cell kinetics are much lower than for spermatogenesis. The importance of precise testicular thermoregulation has been shown by the fact that even slight elevations in scrotal temperature, which are common in clinical practice, such as cryptorchidism, varicocele, sauna use, wearing of tight clothing, and even usage of laptop computers, are associated with male infertility. Studies on the effects of temperature on the testis have revealed that all testicular cell types, especially germ cells, are affected readily by heat stress (Shiraishi et al. 2012). Among a variety of cellular functions, HSF plays an important role in protecting the cells or causing apoptotic cell death.

It was found that HSF1 in pachytene spermatocytes was activated at 35 °C, whereas HSF1 from the somatic testis cells was activated at 42 °C (Sarge 1995), indicating that the mode of HSF1 activation is cell type specific. The stage-specific susceptibility to heat stress is supposed to be caused by the function of supporting Sertoli cells (Lue et al. 1999). Few pachytene spermatocytes at stage V died after heat stress in wild-type mice, whereas numerous pachytene spermatocytes at stages XI–XII died. In contrast, few pachytene spermatocytes at stages XI–XII died in HSF1-KO mice (Izu et al. 2004). These results demonstrate that HSF1 promotes heat shock-induced cell death of pachytene spermatocytes. In this section, literature reports describing the expression and role of HSF in pathophysiological conditions are reviewed.

8.4.2 Cryptorchidism

Although an experimental cryptorchidism model is often used to investigate the effects of in vivo heat shock stress on spermatogenesis, cryptorchidism per se is a congenital developmental disorder and the underlying pathophysiology causing deteriorated spermatogenesis still remains controversial. Heat stress in cryptorchidism is not a primary cause but an accompanying detrimental effect due to the retention of the testis in the abdominal cavity or inguinal tract. In other words, an experimental cryptorchidism model is a mild and long-term heat stress model for spermatogenesis. To examine the effects of short-term high-temperature exposure, anesthetized animals were simply submerged in a water bath at 43 °C for several minutes and then allowed to recover for up to several hours.

Shrinkage in the size of the testes, thinning of the germinal epithelium, and loss of germ cells (particularly spermatocytes and spermatids) has been reported in the initial stages of cryptorchidism. Apoptosis, rather than necrosis, has been considered the predominant mechanism of germ cell death in cryptorchidism (Kocak et al. 2002). *Hsf1*-KO and transgenic mice have shown than HSF1 is involved in cell death in primary spermatocytes without HSP induction in an experimental cryptorchid model (Izu et al. 2004; Hayashida et al. 2006). In fact, the constitutive expression of Hsp70.1 did not protect the seminiferous epithelium against cryptorchidism-induced cell death (Vydra et al. 2006). In contrast, it was shown by TUNEL staining that a substantial fraction of the apoptotic cells were observed in the outermost layer of the tubules in *Hsf1*-KO mice, indicating that cell death of undifferentiated germ cells exposed to heat shock is inhibited by HSF1 (Izu et al. 2004). Thus, HSF1 has two opposite roles in male germ cells. HSF1-mediated death signals may play major roles in pachytene spermatocytes, and the HSF1-mediated survival signals may dominate the death signals in undifferentiated germ cells (spermatogonia). Injured germ cells are selectively eliminated by an apoptotic mechanism that may be regulated by the p53 pathway (Yin et al. 1998, 2002). In response to cellular stress, p53 induces cell cycle arrest to allow the opportunity for DNA repair to occur before replication or mitosis. Alternatively, it induces

apoptotic cell death to eliminate irreparably damaged cells (Sionov and Haupt 1999). The choice between growth arrest and apoptosis is determined by many factors, including the cell type. Similar to p53, HSF1 is a major factor that senses proteotoxic stress and transduces it to the expression of proapoptotic and antiapoptotic genes, depending on the cell types. Hayashida et al. have determined that a target gene of HSF1 is Phlda1 (PHL domain, family A, member 1), also known as TDAG51, which subsequently activates apoptosis. They showed that apoptosis of the germ cells was inhibited by *Tdag51*-KO in heat stress (Hayashida et al. 2006). This suggests that the inhibition of spermatogenesis during cryptorchidism is partly attributable to the activation of the HSF1/Phlda1 pathway. Taken together, these observations suggest that HSF1 determines the choice between apoptosis and cell survival in response to heat stress by activating TDAG51 or several HSPs, respectively.

8.4.3 Varicocele

Varicocele is an abnormal enlargement of the pampiniform venous plexus, which is the most frequent cause of male infertility. Reflux of toxic metabolites of adrenal and/or renal origin, deficiency of the hypothalamic gonadal axis, and venous stasis leading to testicular hypoxia are among the main factors associated with varicocele. The most widely accepted mechanism underlying varicocele-dependent infertility is increased testicular temperature. This is because varicocele disrupts the countercurrent heat-exchange mechanism, leading to impaired testicular thermoregulation and increased testicular oxidative stress (Shiraishi et al. 2012). Taken together, the involvement of these factors and the alterations of several HSPs in men with varicocele suggest that HSFs play pivotal roles in the pathophysiology of varicocele.

mRNA expression levels of HSF1 and HSF2 were increased in oligospermic men with varicocele (Ferlin et al. 2010). Taking together the observation that HSF2 binds to the promoters for HsoA11 (Wilkerson et al. 2008), Hspb2, Hsp90aa1, and Fos in mitotic cells (Wilkerson et al. 2007), the expression of HSPA2 could be regulated by HSF2. HSPA2 was highly expressed in human testis and appeared to have an essential role during the meiotic phase of spermatogenesis (Son et al. 1999). The disruption of *Hspa2* resulted in meiosis failure, germ cell apoptosis, and male infertility (Dix et al. 1996, 1997; Govin et al. 2006). Therefore, the transcriptional regulatory function of HSF2 may be considered to be essential to assure the appropriate expression of HSPA2. Afiyani et al. observed significantly higher expression of HSPA2, which mainly localizes to postmeiotic cells such as round spermatid and sperm, in testicular tissue from men with varicocele (Afiyani et al. 2014). This HSP was downregulated in the ejaculated sperm of men with oligozoospermia and increased at 6 months after varicocelectomy (Yesilli et al. 2005). In varicocele patients, it has also been shown that the removal of varicocele by surgery improves HSPA2 expression (Yesilli et al. 2005; Afiyani

et al. 2014). Round spermatids and sperm in the varicocele group had higher HSPA2 levels compared with the control group. These results agreed with the report of increased HSP and HSF expression in sperm from oligozoospermic and varicocele individuals (Ferlin et al. 2010). HSP 32, also known as heme oxygenase-1 (HO-1), is one member of this family with enzymatic activity and is induced by heat stress, heavy metals, inflammatory mediators, and oxidized low-density lipoproteins; it has been shown to be increased in Leydig cells in patients with varicocele (Shiraishi and Naito 2005). In addition, the upregulation of HSP32 in Sertoli cells inhibits caspase-3 activity and alleviates heat-induced impairments in mouse testis (Li et al. 2013). HSF1 negatively regulates HO-1 gene expression in human Hep3B hepatoma cells in response to stimulation with 15-deoxy-delta12,14-prostaglandin J2 and arsenite (Chou et al. 2005); however, direct evidence regarding the role of HSF in Leydig and Sertoli cells is lacking.

Apparently, varicocelectomy improves the semen parameters of sperm concentration and motility. Even in patients whose semen parameters do not improve, the results of intracytoplasmic sperm injection therapy, a commonly used infertility treatment, can be improved after varicocelectomy (Shiraishi et al. 2012), indicating that some sperm factors may be improved. As HSF1 plays roles in the regulation of sperm DNA packing (Akerfelt et al. 2010), further investigation into HSF's role in sperm function is required.

8.4.4 Genotoxic Stress

Doxorubicin, an anticancer agent widely used against various types of neoplasms, belongs to the anthracycline antibiotic family, and it induces DNA damage by inhibiting topoisomerase II and also intercalating DNA. Doxorubicin is one of the famous agents that cause permanent azoospermia, if a majority of spermatogonia undergo cell death by its administration (Shiraishi and Matsuyama 2014).

At 2 days after a single intraperitoneal injection of doxorubicin (5 mg/kg), the proliferation of *Hsf1* wild type but not *Hsf1*-KO spermatogonia and preleptotene primary spermatocyte were inhibited, followed by a decreased number of meiotic cells in *Hsf1* wild type but not *Hsf1*-KO testes (Salmand et al. 2008). Proliferating cells were blocked or lost very rapidly as a result of an increase in cell death signals and phagocytic activity of Sertoli cells (Shiratsuchi et al. 1997). Nevertheless, spermatogenesis showed better signs of recovery in *Hsf1* wild type but not *Hsf1*-KO. These data indicate that acute response to genotoxic stress in the testis involves HSF1-dependent mechanisms that induce apoptotic cell death (Salmand et al. 2008), which is similar to the heat stress response orchestrated by HSF1 (Nakai et al. 2000; Izu et al. 2004). Most likely, these similar observations for HSF1 will be seen after the administration of other anticancer agents, and these responses should be further investigated.

8.5 Summary and Future Perspectives

Previous research, especially the generation of gene-manipulated animals, has greatly contributed to our understanding of the roles for HSF1 and HSF2 in gametogenesis. Obviously, HSF1 and HSF2 play important roles in both spermatogenesis and oogenesis. HSF1 checks the quality control of gametogenesis, whereas there is still controversy regarding the role of HSF2 in spermatogenesis. The generation of HSF2 transgenic mice will elucidate the function in spermatogenesis. Unfortunately, the simplest methodology, in vitro cell culture, is not useful for germ cells because male and female germ cell culture is very difficult in vitro.

Gametogenesis is strictly regulated by the gonadotropins: luteinizing and follicle-stimulating hormone. However, information regarding the effects of these hormones on the actions of HSFs is still lacking. The target cells in the testis and ovary, the Leydig, Sertoli, theca, and granulosa cells, are often cultured in vitro, and the role of HSFs in these endocrinological cells is readily investigated. This approach may contribute to the development of endocrinological stimulation in both spermatogenesis and oogenesis. Because HSFs are involved in a variety of signaling pathways in normal and stressed gametogenesis, the investigation of HSFs in gametogenesis per se may provide information for understanding the physiology/pathophysiology of spermatogenesis and oogenesis and for establishing new therapeutic approaches to combat male and female infertility.

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