The Role of Heat Shock Factors in Mammalian Spermatogenesis

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Abstract Heat shock transcription factors (HSFs), as regulators of heat shock proteins (HSPs) expression, are well known for their cytoprotective functions during cellular stress. They also play important yet less recognized roles in gametogenesis. All HSF family members are expressed during mammalian spermatogenesis, mainly in spermatocytes and round spermatids which are characterized by extensive chromatin remodeling. Different HSFs could cooperate to maintain proper spermatogenesis. Cooperation of HSF1 and HSF2 is especially well established since their double knockout results in meiosis arrest, spermatocyte apoptosis, and male infertility. Both factors are also involved in the repackaging of the DNA during spermatid differentiation. They can form heterotrimers regulating the basal level of transcription of target genes. Moreover, HSF1/HSF2 interactions are lost in elevated temperatures which can impair the transcription of genes essential for spermatogenesis. In most mammals, spermatogenesis occurs a few degrees below the body temperature and spermatogenic cells are extremely heat-sensitive. Pro-survival pathways are not induced by heat stress (e.g., cryptorchidism) in meiotic and postmeiotic cells. Instead, male germ cells are actively eliminated by apoptosis, which prevents transition of the potentially damaged genetic material to the next generation. Such a response depends on the transcriptional activity of HSF1 which in contrary to most somatic cells, acts as a proapoptotic factor in spermatogenic cells. HSF1 activation could be the main trigger of impaired spermatogenesis related not only to elevated temperature but also to other stress conditions; therefore, HSF1 has been proposed to be the quality control factor in male germ cells.

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1 Spermatogenesis Overview

Spermatogenesis is a tightly coordinated developmental process under hormonal control which enables the continuous production of sperm: from initial spermatogonial diploid cells, millions of haploid spermatozoa are produced daily that are able to fertilize a mature oocyte. In mammals, the process occurs in the seminiferous tubules of the adult male testes. Seminiferous tubules, the functional units of the mammalian testis, are composed of epithelium containing germinal cells and supportive Sertoli cells localized close to the tubule membrane. Sertoli cells, besides providing a nourishing function, maintain the integrity of spermatogenesis by forming the blood–testis barrier (rev. in: Brehm and Steger 2005). The interstitial, testosterone producing Leydig cells fill the extratubular space (rev. in: Davidoff et al. 2009). In the course of spermatogenesis, the germ cells continuously undergo mitotic, meiotic, and postmeiotic phases of maturation, moving towards the center of the seminiferous tubules. The following developmental stages are thereby passed through: spermatogonia (A, intermediate, and B), which divide

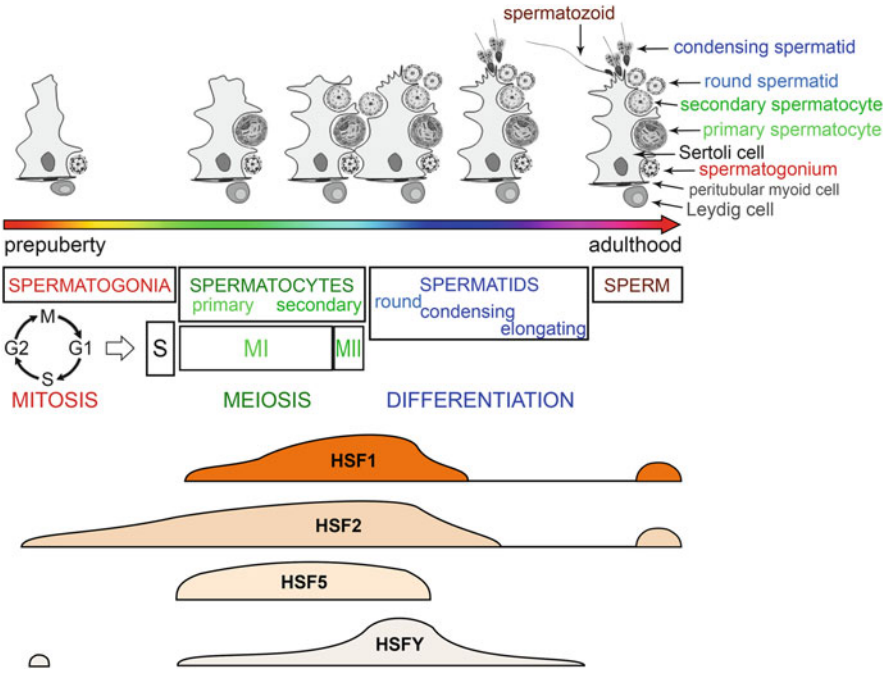

Fig. 1 A scheme of mammalian spermatogenesis and pattern of HSFs expression. The *upper schematic drawing* shows the cellular composition of developing testes (from prepuberty to adulthood). Developing germ cells are imbedded in a somatic Sertoli cell. HSFs are expressed predominantly in spermatocytes and spermatids undergoing meiosis and differentiation, respectively. Distribution of HSF3, HSF4, and HSFX in testes has not been determined yet. M, mitosis; G1, gap 1; S, synthesis of DNA; G2, gap 2; MI, meiosis I; MII, meiosis II

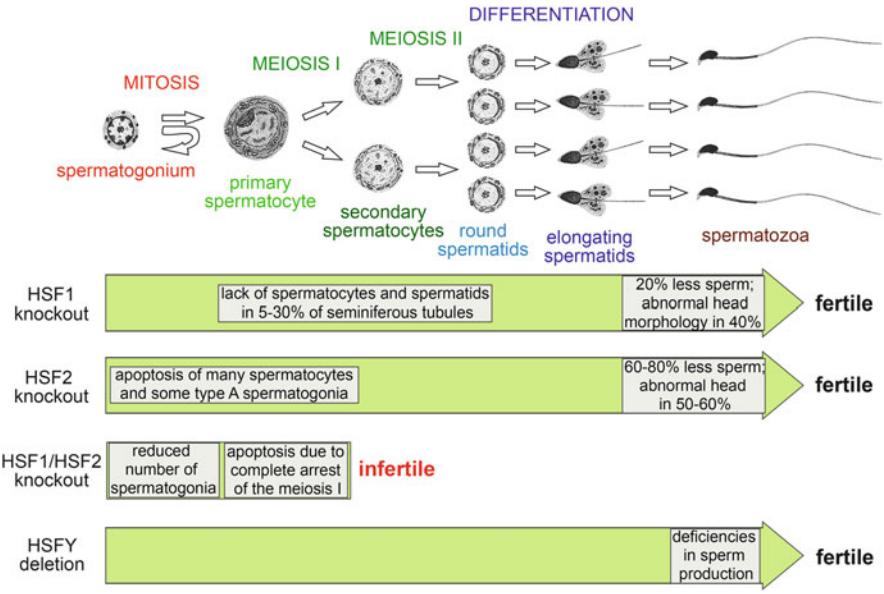

Fig. 2 Contribution of HSFs to male germ cell development. Consequences of HSFs loss are summarized based on mouse knockout models (HSF1 and HSF2) and naturally occurring microdeletions in the human genome (HSFY)

mitotically, spermatocytes (primary and secondary), which divide meiotically, and spermatids, which undergo morphological changes and differentiate into spermatozoa (Figs. 1 and 2). The duration of spermatogenesis varies considerably between species ranging from 35 to 70 days. During the prophase of the first meiotic division (which is the longest phase of meiosis), chromosomal synapsis and genetic recombination take place. After the completion of meiosis, during differentiation of spermatids, histones are replaced by transition proteins, then by protamines, which enables the tight packing of the paternal DNA, so it can be delivered unaffected to the oocyte (rev. in: Toshimori 2009). Spermatogenesis proceeds in synchronized waves along the seminiferous tubules, and every given cross section of the tubule contains only certain cell types in a specific combination (Kotaja et al. 2004). Maintenance of the proper ratio of each type of cell in the tubule is necessary for coordinated germ cell differentiation. Excessive (as well as aberrant) cells are removed by apoptosis, which is an important mechanism providing the homeostasis of male germ cells (rev. in: Shukla et al. 2012).

Spermatogenesis is temperature-dependent in most mammals. It occurs optimally at a temperature substantially lower (by around 4–5 °C) than the normal body temperature. Elevated testicular temperature has a detrimental effect on spermatogenesis and can compromise sperm quality and increase the risk of male infertility. A number of external and internal factors could disturb thermoregulation of the testes (which normally is controlled by the scrotum) and increase the risk of

abnormal spermatogenesis. These thermogenic factors include lifestyle and behavioral factors, occupational and environmental factors (external factors), and clinical factors resulting from pathophysiological conditions (internal factors). Impaired spermatogenesis related to elevated temperature is observed in certain disorders such as cryptorchidism, retractile testes, acute febrile diseases, obesity, varicocele, or as a result of occupational heat exposure (bakers, welders, drivers, or computer operators) and in men preferring a sitting lifestyle (rev. in: Agarwal et al. 2008; Skakkebaek et al. 2016).

The morphological changes that take place in seminiferous epithelium under an elevated scrotal temperature are well described using animal models. Reduced weight and smaller size of testes, disorganization of the germinal epithelium with concurrent reduced cell population, appearance of multi-giant cells, and reduced fertility were commonly observed in mouse and rat testes subjected to experimental cryptorchidism or hot baths (Reid et al. 1981; Loughlin et al. 1991; Yin et al. 1997; Lue et al. 1999; Rockett et al. 2001; Chaki et al. 2005). A significant decrease in sperm concentration and total sperm count were also observed in men exposed to transient scrotal hyperthermia (Rao et al. 2015). The most temperature-sensitive germ cells are primary spermatocytes (in the first meiotic division) (Yin et al. 1997; Rockett et al. 2001; Khan and Brown 2002). The loss of testicular germ cells triggered by heat shock occurs primarily by apoptosis. Damage of spermatocytes is manifested by giant cell formation, karyolysis, and appearance of abnormal chromosomal patterns as well. Moreover, decreased repopulation of seminiferous epithelium was observed in mice exposed to heat that could result from impairment of stem cells and delay in the reappearance of spermatocytes and spermatids (Gasinska and Hill 1990).

Exposition of most somatic cells to sublethal heat conditions induces a cytoprotective reaction called the heat shock response (rev. in: Richter et al. 2010). The fundamental component of this reaction is de novo synthesis of heat shock proteins (HSPs), which is regulated by heat shock transcription factors (HSFs). Accumulation of heat shock proteins renders somatic cells thermotolerant and allows them to survive subsequent otherwise lethal heat stress. However, diverse mechanisms are induced by elevated temperatures in spermatogenic cells, which are described below.

2 General Characteristics of Heat Shock Transcription Factors

Heat shock factors are well known as transcriptional regulators of genes encoding heat shock proteins, functioning as molecular chaperones. Eukaryotic cells express a number of different classes of HSPs, which can differ by their molecular weight, pattern of expression (constitutive vs. inducible), or cell localization (Kampinga et al. 2009). Expression of some HSPs is developmentally regulated or restricted to

specific cells (rev. in: Rupik et al. 2011; Ji et al. 2012; Dun et al. 2012). The general function of HSPs is to assist protein folding either during de novo synthesis or under stress conditions associated with protein misfolding. HSPs accomplish this by binding to stretches of hydrophobic amino acids in misfolded proteins and thus prevent formation of protein aggregates (rev. in: Vabulas et al. 2010). Besides the regulation of cell adaptation to stress mediated by induction of HSPs expression, members of the HSF family contribute to processes associated with development and growth. This function is probably associated with regulation of genes involved in diverse cellular processes that extend far beyond protein folding (which is the general role of HSPs).

The mammalian HSF family includes several members: HSF1 through HSF5, HSFY, and HSFX, which are grouped in one family due to high similarity of their DNA-binding domain. This domain enables binding to specific regions in the genome called Heat Shock Elements (HSEs). HSE consensus sequence is a tandem array of at least three oppositely oriented "nGAAn" motifs or a degenerate version thereof. In promoters of *HSP* genes, a number of such pentanucleotides in HSE can range from three to eight. Also the number of functional HSEs varies. The HSE architecture is an important determinant of specific HSF binding during gene regulation in diverse cellular processes (Yamamoto et al. 2009).

Although members of the HSF family have some common structural features, they are functionally distinct (for more information about the structure and properties of HSFs see, e.g., in: Fujimoto and Nakai 2010; Xu et al. 2012; Vydra et al. 2014). They generally exist in several isoforms and can undergo multiple post-translational modifications (phosphorylation, acetylation, sumoylation, ubiquitination). Moreover, they can interact with each other as well as with hundreds of different proteins (depending on the context) that modulate their activity. HSF1 (as well as avian HSF3, which among mammals was identified only in mice; Fujimoto et al. 2010) is activated by heat shock and numerous forms of physiological stress. HSF2 and HSF4 are considered as developmental factors, while the function of HSF5, HSFY, and HSFX, although not exactly known, is putatively associated with gametogenesis.

HSF1 is the main regulator of the heat shock response; thus, it has been studied the most extensively yet. Under physiological conditions, it exists as an inactive monomer in complexes with HSPs as well as other proteins. During stress conditions, when the level of unfolded proteins increases, HSPs are released from complexes with HSF1 and serve as molecular chaperones for unfolded molecules. An elevated level of unbound HSF1 promotes its trimerization, hyperphosphorylation, and high affinity binding to HSEs (each DNA-binding domain in the trimer recognizes a single nGAAn motif), which initiates the transcription of HSF1-dependent genes (rev. in: Pirkkala et al. 2001; Voellmy 2004; Fujimoto and Nakai 2010). HSF2 is supposed to form inactive dimers at physiological temperature and under several stress conditions trimerizes to a transcriptionally active form (Sistonen et al. 1994). No phosphorylation of HSF2 has been reported (although ubiquitination and sumoylation are possible) (Xu et al. 2012). HSF2 is a short-lived protein regulated mainly at the level of its expression. It can

autoregulate its own transcription (Park et al. 2015) and can be posttranscriptionally regulated by miR-18, which belongs to the Oncomir-1 cluster of microRNA (Björk et al. 2010). HSF3 also exists as a dimer at physiological conditions (Nakai and Ishikawa 2000), while HSF4 forms a transcriptionally competent trimer even at physiological temperature (Nakai et al. 1997).

3 The Role of HSFs in Spermatogenesis

3.1 Patterns of HSF Expression in Mammalian Testes

According to publicly available databases (e.g., Human Protein Atlas available from www.proteinatlas.org and BioGPS: http://biogps.org; Uhlén et al. 2015; Wu et al. 2016), all HSF family members are expressed in mammalian testes; however, HSF5 and HSFY are expressed exclusively in testes, while expression of the other family members is more ubiquitous. Among them, HSF2 is expressed primarily in testes, then in brain, whereas HSF4 is primarily in lenses.

In mouse and rat testes, HSF1 protein was detected in spermatocytes (mainly in nuclei, although it is excluded from metaphase plate during meiotic divisions) and round spermatids, then its level diminishes in elongating spermatids (Alastalo et al. 1998; Akerfelt et al. 2010). A very similar pattern of expression was observed in the case of HSF2 (Sarge et al. 1994; Goodson et al. 1995; Alastalo et al. 1998), but it is possible that *Hsf2* gene expression starts in spermatogonia and is retained up to elongating spermatids (Kallio et al. 2002; Björk et al. 2010; Chalmel et al. 2012). In rat testes, HSF2 was additionally found in cytoplasmic regions identified as cytoplasmic bridges between germ cells (Alastalo et al. 1998). Furthermore, both HSF1 and HSF2 were found in the heads of epididymal spermatozoa (Wilkerson et al. 2008). The testes express predominantly the larger α isoform of HSF1 and HSF2 (Goodson et al. 1995; Neueder et al. 2014). It is noteworthy that transcription of HSF1 is downregulated by the androgen receptor in Sertoli cells; thus, it is under the influence of testosterone (Yang et al. 2014).

Expression of HSF5 protein is restricted to spermatocytes and round spermatids (documented in human and rat testes; Chalmel et al. 2012); thus, it is essentially the same as HSF1 and HSF2. Also human HSFY was found in these stages of spermatogenesis and additionally in elongated spermatids or occasionally in type A spermatogonia and Sertoli cells (Shinka et al. 2004; Sato et al. 2006). In situ hybridization revealed that mouse *Hsfy* was predominantly expressed in round spermatids (Kinoshita et al. 2006). Up to date, there is no information on localization of HSF3, HSF4, and HSFX proteins in spermatogenic cells. Summarizing, almost all HSFs are expressed at the highest level in spermatocytes and round spermatids (Fig. 1), which coincides with the phases of active transcription, synaptonemal complex formation, crossing-over, and intensive chromatin remodeling that take place in these cells.

3.2 Relevance of HSFs for Normal Spermatogenesis

To understand the physiological roles of HSFs in vivo, knockout mice for these factors were generated (rev. in: Christians and Benjamin 2006). Targeting of the *Hsf1* gene resulted in increased prenatal lethality (as a result of defects in the placenta) and postnatal growth retardation. Although many physiological functions were affected (e.g., thermotolerance, systemic body temperature regulation, redox homeostasis and antioxidative defenses, immune response, motor activity, smell, hearing, memory, and others), under normal conditions such mice could survive to late adulthood. However, *Hsf1* knockout females were infertile due to defects in oogenesis and preimplantation development, while males exhibited normal reproductive ability yet produced about 20% less sperm than wild-type mice (Xiao et al. 1999; Salmand et al. 2008; rev. in: Abane and Mezger 2010). Moreover, approximately 40% of epididymal spermatozoa displayed abnormal head morphology. Reduced number of sperm was a consequence of a slightly disrupted spermatogenesis: disorganized or missing layers of germ cells were found in 5–30% of the *Hsf1*−/− seminiferous tubules. Most of the affected tubules contained only spermatogonia, some of them additionally contained spermatocytes, but all disrupted tubules lacked spermatids. Abnormal sperm head morphology of *Hsf1*−/− sperm could arise from defects in the chromatin packing due to disturbed replacement of transition proteins with protamines (Fig. 2) (Salmand et al. 2008; Akerfelt et al. 2010).

Defective spermatogenesis was also observed in *Hsf2* null mice, whereas other defects included increased embryonic lethality, nervous system developmental disturbances, female hypofertility, and ovary abnormalities (rev. in: Abane and Mezger 2010). The synaptonemal complex, which is formed between homologous chromosomes during the pachytene stage of meiotic division, was frequently disorganized in such mice (Kallio et al. 2002). Many primary spermatocytes and some type A spermatogonia died by apoptosis. As a result, testes of *Hsf2*−/− males were significantly smaller and the number of epididymal sperms was reduced by 60–80% in comparison to wild-type animals (Kallio et al. 2002; Wang et al. 2003). Moreover, similarly to *Hsf1* knockout mice, sperm head abnormalities were detected in more than half of spermatozoa that suggested defects in the chromatin condensation (Akerfelt et al. 2008). Despite markedly reduced sperm count and quality, only a small reduction in fertility was observed in *Hsf2*−/− male mice (Fig. 2). More severe defects of spermatogenesis (block at the spermatocyte stage and infertility) were detected in men with a dominant-negative mutant form of HSF2 (Mou et al. 2013). However, such an inhibitory mutation was detected only in one of 766 patients infertile due to unknown causes, and coexisting alterations in other important genes cannot be excluded.

Importantly, mice with the double knockout of both *Hsf1* and *Hsf2* exhibited severe defects of spermatogenesis associated with a reduced number of dividing spermatogonia and arrest of spermatogenesis at pachytene stage of the first meiotic division leading to apoptosis. As a consequence, such males completely lacked

postmeiotic cells and were infertile, despite normal mating performance (Fig. 2). This observation indicates the complementary roles of HSF1 and HSF2 in spermatogenesis (Wang et al. 2004).

Knockout models reveal the lens-specific role of HSF4 because *Hsf4*-null mice developed cataract. However, males and females were fertile, and the histology of the testis and ovary was normal (Fujimoto et al. 2004). Other HSF (i.e., HSF3, HSF5, HSFY, and HSFX) mouse knockout models have not been established yet. However, a few cases of *HSFY* deletions were found in human. Functional *HSFY* and *HSFX* genes both exist in two identical copies on the human Y or X chromosome, respectively (Bhowmick et al. 2006). *HSFY1* and *HSFY2* are located within rapidly evolving male-specific region of Y (MSY) that contains several testis-specific genes organized in palindromes. Deletions in MSY are associated with deteriorated spermatogenesis and azoospermia (complete absence of spermatozoa in the ejaculate). *HSFY* genes are usually deleted together with other protein coding genes from that region. Microdeletions restricted to *HSFY* locus could also occur. They result in deficiencies of the sperm production; however, spermatogenesis is not completely arrested (Fig. 2). Thus, it was concluded that *HSFYs* (and overlapping noncoding genes) are not absolutely required for human male fertility. On the other hand, functional *HSFY* genes have been conserved on the Y chromosome for 100,000 years during the evolution of modern humans, indicating that HSFY does make a real contribution to the reproductive fitness of men (Kichine et al. 2012). Nevertheless, detection of *HSFY* deletion and assessment of *HSFY* mRNA level were proposed as useful diagnostic markers for the presence of retrievable testicular sperm in assisted reproductive technologies (Stahl et al. 2011).

In conclusion, a largely overlapping distribution of HSFs in spermatogenic cells and insights from knockout models implicate that different HSFs could cooperate to maintain proper spermatogenesis. Moreover, it cannot be excluded that HSFs may be able to some extent compensate each other.

3.3 Interplay between HSF1 and HSF2 in Spermatogenesis

Although HSF1 and HSF2 are functionally distinct, they can cooperate either under physiological conditions or during stress (Mathew et al. 2001; He et al. 2003; Ostling et al. 2007; Shinkawa et al. 2011). Co-localization of HSF1 and HSF2, most probably in the form of heterotrimers, was shown in the nuclear stress granules/bodies, which were formed in response to heat shock in human cells (Alastalo et al. 2003). Moreover, HSF1-dependent transcription could be modulated by the HSF1/HSF2 ratio (He et al. 2003; Loison et al. 2006; Ostling et al. 2007; Sandqvist et al. 2009; Elsing et al. 2014). HSF1/HSF2 complexes/heterotrimers also exist in mouse spermatogenic cells (Sandqvist et al. 2009; Akerfelt et al. 2010; Korfanty et al. 2014). Complete male infertility observed specifically in double knockout mice due to arrest of meiosis (Wang et al. 2004) suggests that HSF1/HSF2 cooperation could be critical for proper spermatogenesis.

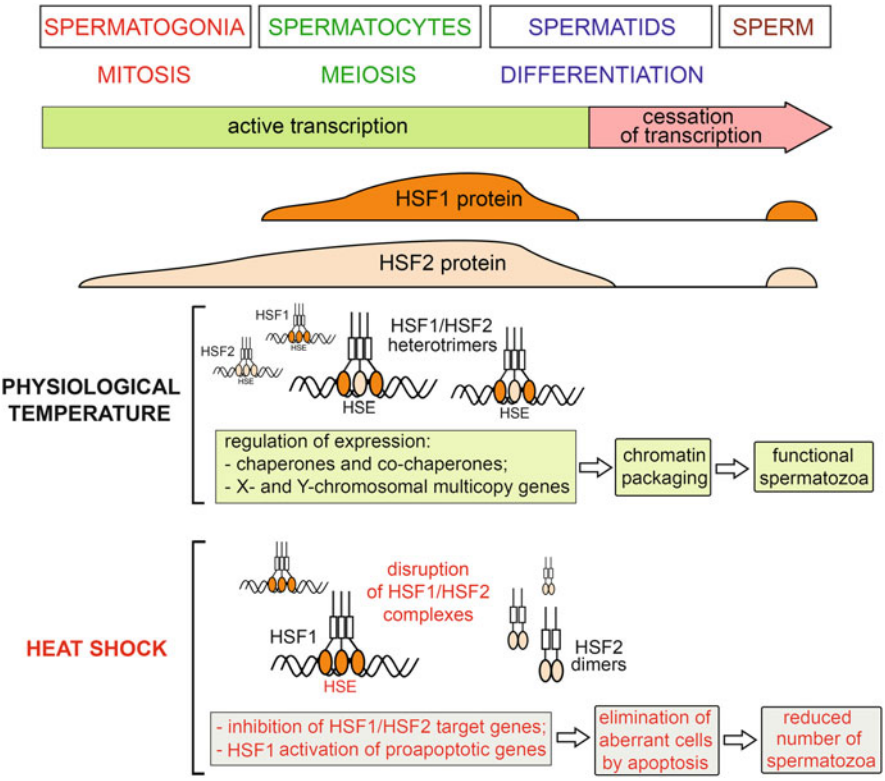

Fig. 3 Interplay between HSF1 and HSF2 in spermatogenesis at physiological and elevated temperatures. Observed cooperation of HSF1 and HSF2 in spermatocytes and round spermatids is critical for normal spermatogenesis. Heat shock-induced disruption of HSF1/HSF2 interactions is connected with release of HSF2 from chromatin and increased HSF1 binding. Proapoptotic genes could be activated by HSF1 leading to the elimination of damaged germ cells. HSE, heat shock element

HSE-binding activity of both HSF1 and HSF2 was detected in mouse testes (Fiorenza et al. 1995). However, genome-wide analyses of HSF1 and HSF2 DNA target sites revealed that both transcription factors generally occupy different genomic targets, and only ~15% binding sites are shared (Akerfelt et al. 2008, 2010). Genes whose promoters are co-occupied in mouse spermatogenic cells by both HSF1 and HSF2 encode primarily for chaperones and co-chaperones that facilitate protein folding (Korfanty et al. 2014). Thus, both factors could participate in the regulation of the basal level of transcription of these genes at the physiological temperature (Fig. 3). Interestingly, expression of the testis-specific *Hspa2* gene, which is restricted to spermatocytes and spermatids and is indispensable for spermatogenesis (rev. in: Scieglinska and Krawczyk 2015), seems to be independent on HSF1 or HSF2 binding (Wang et al. 2004; Korfanty et al. 2014).

It has to be noted that there is a huge inconsistency among HSFs binding sites mapped at the physiological temperature by different technologies (Akerfelt et al. 2008, 2010; Kus-Liśkiewicz et al. 2013; Korfanty et al. 2014), which suggests that the binding is relatively weak in such conditions. Observed variability could result from a transient sequence-independent chromatin binding, corresponding to the HSFs search for more specific targets that was suggested for HSF1 by Herbomel et al. (2013).

The exact HSF1 and HSF2 role in specific germ cell types is unknown; however, their involvement in the regulation of postmeiotic expression of certain X- and Y-chromosomal multicopy genes (e.g., *Sly*, *Slx*, *Ssty1/2*) required for the correct packing of the chromatin in sperm was documented (Fig. 3) (Akerfelt et al. 2008; Akerfelt et al. 2010). The X and Y chromosomes are largely non-homologous; hence, they synapse during meiosis in their short homologous regions only, while unsynapsed regions are silenced and incorporated into a distinct chromatin domain at the cellular periphery called the sex body (rev. in: Turner 2007). HSF1 and HSF2 binding allows the multicopy genes to escape postmeiotic sex chromosome repression; hence these genes can be active in round spermatids. Consistently, lack of HSF1 or HSF2 results in deregulated expression of the multicopy genes and altered sperm head morphology equivalent to that observed in mutant males with partial deletion of the MSY long arm (Ellis et al. 2005).

HSF2 occupies hundreds of loci in the chromatin during mitosis; among others it binds to the promoter of inducible *HSP70* (*HSPA1*) gene preventing its full compaction (Xing et al. 2005). Such gene bookmarking maintains the promoter in a transcription competent state, and its induction could occur even in the early G1 phase if stress were to arise. Similar mechanism of *Hsp70* bookmarking in condensed chromatin was demonstrated in mouse spermatogenic cells. HSF2 linked to the promoter of *Hspa1b* gene already in late spermatids most likely enables subsequent binding of HSF1 as well as SP1 transcription factor in mature sperm. Keeping the promoter chromatin in open conformation would make the *Hspa1b* gene ready for early activation that occurs shortly after fertilization in the one-cell stage embryo (Christians et al. 1995; Wilkerson et al. 2008). In contrary to HSF2, HSF1 is rapidly excluded from chromatin when the metaphase plate is formed, both in mitosis and meiosis (Vihervaara et al. 2013).

3.4 Contribution of HSF1 and HSF2 to the Heat Shock Response in Testes

Extensive studies revealed that HSF1 activation protects cells by inducing a set of HSPs, which enable cells to survive otherwise lethal stress. This function of HSF1 has been described in various model systems ranging from cell culture to stress-response analyses at the organismal level, such as yeast, nematodes, and rodents. Accordingly, accelerated heat-induced apoptosis was commonly observed in cells

with reduced HSPs expression due to the HSF1 downregulation (McMillan et al. 1998; Xiao et al. 1999). Searching for a temperature set point for induction of HSF1 binding to DNA revealed that HSF1 activation takes place at temperatures of 41 °C or higher in most mammalian cell types and tissues (liver, kidney, lung), whereas in testes HSF1 is activated at significantly lower temperatures (35–38 °C) (Sarge 1995). This phenomenon is a unique property of male germ cells, with a minor exception of T-lymphocytes where HSF1 is activated at febrile temperatures (i.e., 39 °C and above; Gothard et al. 2003). Somatic types of testicular cells (peritubular myoid and Leydig cells) exhibit HSF1 activation profile identical to that observed in other somatic mammalian cells (Sarge 1995). It has to be emphasized that despite HSF1 activation, pro-survival pathways are not induced in meiotic and postmeiotic cells in response to stress. Transcription of major heat shock genes is induced only in somatic compartment of testes and possibly in premeiotic germ cells, although activated HSF1 binds to *Hsp* gene promoters also in spermatocytes (Huang et al. 2001; Izu et al. 2004; Kus-Liśkiewicz et al. 2013). Moreover, the constitutively expressed testis-specific variants of *Hsp70* (*Hspa2* and *Hspa1l*) genes are downregulated following heat shock (Widlak et al. 2007a). Furthermore, ectopic expression of HSPA1 (the major heat inducible HSP70 family member) do not protect spermatogenic cells from apoptosis induced by heat shock (Widlak et al. 2007b).

Severity and consequences of heat shock depend on temperature and duration of exposure (rev. in: Balogh et al. 2013). Hence, responses of spermatogenic cells to hyperthermia at 37–38 °C (e.g., in cryptorchidism) and at 42–43 °C are different. Even relatively short (15–20 min) heat shock at 43 °C resulted rather in changes of critical proteins' localization then in changes of their levels. Redistribution of BAX from cytoplasmic to perinuclear space, release of cytochrome c, and subsequent activation of the initiator caspase 9 and the executioner caspases 3, 6, and 7, as well as resulting chromatin fragmentation and PARP cleavage were observed within 0.5–2 h after heating (Yamamoto et al. 2000; Hikim et al. 2003). In experimental cryptorchidism (i.e., approximately at 37 °C), massive apoptosis is observed much later, in three to four day time frame (Barqawi et al. 2004; Chaki et al. 2005; Tao et al. 2006). Differences in the early response of spermatogenic cells to the temperature shift up to 38 °C (mild stress) or 43 °C (acute stress) were also visible at the transcriptional level. At the acute stress, only ~2.5% of all transcribed genes were affected (mostly repressed), whereas about 10% of genes were affected at the mild stress (Kus-Liśkiewicz et al. 2013). The rapid early response to the severe stress observed in spermatogenic cells seems to be independent of activation of HSF1 as the transcription factor. However, significantly lower number of spermatocytes undergoing apoptosis induced both by mild or severe heat shock in HSF1 deficient mice suggests some role of HSF1 also in this early response (Izu et al. 2004).

HSF2 protein is less stable than HSF1. It is ubiquitylated by the ubiquitin E3 ligase anaphase-promoting complex/cyclosome (APC/C) and directed to proteasomal degradation within one hour in response to stress (Ahlskog et al. 2010). Thus, the general ability of HSF2 to bind HSEs diminishes along with

heat shock duration and temperature increase (Sarge et al. 1991). However, following heat shock both HSF1 and HSF2 could bind concurrently in some HSPs promoters (Trinklein et al. 2004; Ostling et al. 2007; Ahlskog et al. 2010; Shinkawa et al. 2011). A ChIP-Seq study performed in human K562 erythroleukemia cells also showed the involvement of both HSFs in the regulation of genes coding for chaperones and co-chaperones, where the total number of HSF1 and HSF2 target loci was increased after temperature elevation (Vihervaara et al. 2013). A similar study performed in isolated mouse spermatogenic cells revealed that temperature elevation resulted in increased binding of HSF1 (mainly to promoters of chaperones and other stress-related genes), while HSF2 binding gradually decreased up to complete loss at 43 °C. In agreement with this observation, the number of HSF1/HSF2 complexes detected in spermatogenic cells in situ was markedly reduced at elevated temperatures (Korfanty et al. 2014). It is noteworthy that increased HSF1 binding was not associated with activation of the target genes transcription but rather with their repression upon hyperthermia (Kus-Liśkiewicz et al. 2013). This could be caused by disruption of HSF1–HSF2 interactions following the release of HSF2 from chromatin, which apparently resulted in the suppression of several genes essential for spermatogenesis during stress conditions (Fig. 3).

3.5 HSF1 as a Quality Control Factor in Male Germ Cells

Disturbed interactions of HSF1 and HSF2 and resulting misregulation of transcription of target genes could be an important consequence of temperature elevation in spermatogenic cells. However, it is also known that activation of HSF1 alone is sufficient to trigger apoptosis in meiotic and postmeiotic cells. Expression of mutated, constitutively active HSF1 in spermatogenic cells of transgenic mice leads to massive degeneration of seminiferous epithelium and male infertility (Nakai et al. 2000; Widłak et al. 2003). In contrary to the majority of somatic cells, where activation of HSF1 is an important element of the cytoprotective system, HSF1 acts as a proapoptotic factor in spermatocytes and spermatids (Fig. 4). Active HSF1 do not induce *Hsps* genes in spermatogenic cells, instead, it initiates caspase-3-dependent apoptosis to eliminate target cells (Vydra et al. 2006). Such HSF1-dependent mechanism apparently prevents transition of the potentially impaired genetic material to the next generation. Since huge amount of spermatozoa are produced daily, elimination of spermatogenic cells damaged during meiosis or differentiation seems to be a more reasonable strategy than their repair. Thus, HSF1 has been proposed to be a quality control factor in male germ cells (Izu et al. 2004).

Mechanisms of proapoptotic action of HSF1 are still not fully resolved. PHLDA1, whose expression is activated directly by HSF1 in some cells including mouse testes, could play an important role here. Overexpression of PHLDA1 may induce apoptosis depending on the cell context. As long as HSF1 (alone or in cooperation with HSF2) activates expression of *Hsps* genes, HSPs interact with

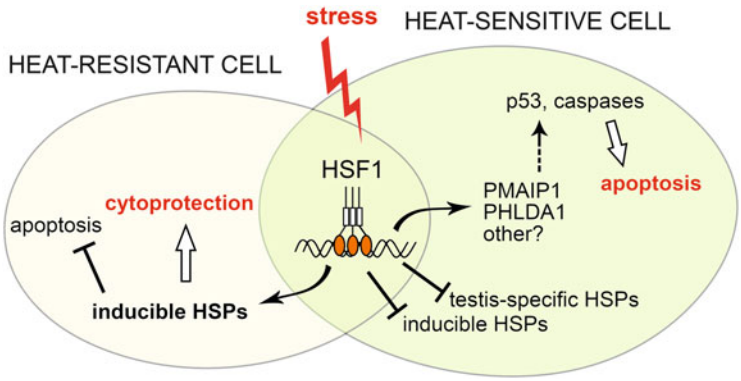

Fig. 4 Proposed model of the HSF1-dependent heat shock response in heat-resistant and heat-sensitive cells. HSF1 activated by different forms of cellular stress induces expression of heat shock proteins (HSPs) that prevent apoptosis and provide cytoprotection in heat-resistant cells (most somatic cells). Expression of HSPs (both inducible and testis-specific variants) is blocked in heat-sensitive meiotic and postmeiotic male germ cells. Instead, HSF1 activates expression of proapoptotic proteins (PMAIP1, PHLDA1) that in the absence of HSPs could induce apoptosis, which actively eliminates potentially impaired cells

PHLDA1 and inhibit cell death mediated by this protein. Since expression of *Hsps* is repressed in spermatogenic cells exposed to high temperatures, PHLDA1 could promote apoptosis when it does not form a complex with HSPs. In fact, heat-induced apoptosis is to some extent diminished in PHLDA1 null mice testes (Hayashida et al. 2006). HSF1 can also directly upregulate the expression of a strictly proapoptotic gene—*Pmaip1* (*Noxa*) after heat shock treatment (Korfanty et al. 2015). PMAIP1 is a proapoptotic member of the BCL2 family (rev. in: Ploner et al. 2008). Similarly to PHLDA1, overexpression of PMAIP1 can induce apoptosis (Oda et al. 2000). PMAIP1 promotes apoptosis by targeting the pro-survival protein MCL1 for proteasomal degradation. Involvement of PMAIP1 in heat-induced apoptosis was already shown in human acute lymphoblastic T-cells (Stankiewicz et al. 2009). Overexpression of HSP70 stabilized MCL1 protein levels in heat-shocked cells and prevented heat-induced BAX activation and apoptosis. It is noteworthy that *Pmaip1* is the most induced gene by active HSF1 in 15-day-old transgenic mice testes (when the transgene is already expressed in the first pachytene spermatocytes, while massive apoptosis is not visible yet; Vydra et al. 2006; http://dx.doi.org/10.6070/H4P26W5M). Interestingly, HSF1 binding was detected not in the promoter, but in the second intron of the *Pmaip1* gene (Korfanty et al. 2014).

Data presented above strongly suggests that cell fate on proteotoxic condition is determined by the balance between antiapoptotic HSPs and proapoptotic proteins including PHLDA1 and PMAIP1, which are differently regulated by HSF1 in heat-sensitive and heat-resistant cells. This paradoxical activation of both pro- and antiapoptotic mechanisms by HSF1 in response to stress ensures that neither aberrant cellular survival nor inappropriate cell death arises.

4 Summary and Future Directions

Decreased semen quality and sperm concentration are the major factors of male infertility observed over the past several decades in many areas of the world. The etiology of these declines is complex and not well understood, yet involvement of environmental stress is generally appreciated. Hence, explanation of the role of heat shock transcription factors and their cooperation during normal spermatogenesis as well as during cellular stress would apparently contribute to understanding the problem of male subfertility. Below, we have pointed out the relevant data which was addressed in the current review.

1. All HSF family members are expressed in mammalian testes, mostly in spermatocytes and round spermatids: HSF5 and HSFY are expressed exclusively in testes while expression of the other family members is more ubiquitous (although HSF2 is expressed primarily in testes).
2. HSF1, HSF2, or HSFY deficient males reveal some defects in spermatogenesis. Moreover, double HSF1 and HSF2 knockout is connected with complete arrest of meiosis and males infertility. Hence, cooperation of different HSFs in a proper maintenance of spermatogenesis is suggested.
3. HSF1 and HSF2 partially share genomic-binding sites, and in mouse spermatogenic cells both factors could cooperate in the regulation of the basal level of transcription of several genes coding for chaperones and co-chaperones that facilitate protein folding.
4. The postmeiotic expression of certain X- and Y-chromosomal multicopy genes (e.g., *Sly*, *Slx*, *Ssty1/2*) required for the correct packing of the chromatin in sperm depends on both HSF1 and HSF2; hence, a deficiency of HSF1 or HSF2 results in sperm head abnormalities.
5. HSF2 is not excluded from the condensing chromatin during meiosis maintaining some promoters in a transcription competent state; hence, binding of other transcription factors (including HSF1) and rapid induction of target genes remains possible.
6. Spermatogenesis requires a temperature substantially lower than normal body temperature; thus, an elevated testicular temperature has a detrimental effect on spermatogenesis and can compromise sperm quality and increase the risk of male infertility (primary spermatocytes during the first meiotic division are the most heat-sensitive).
7. Death of germ cells could be induced by either mild (37 °C) yet extended elevation of temperature exemplified by cryptorchidism (massive apoptosis observed after three-four days) or acute heat shock at 42–43 °C (massive apoptosis observed after a few hours).
8. Interactions between HSF1 and HSF2, which are crucial for correct spermatogenesis, are disrupted during heat shock, when activation of HSF1 and its increased binding to chromatin correlates with destabilization and decreased binding of HSF2; consequently, transcription of many genes essential for spermatogenesis is inhibited during stress.

9. HSF1, the main regulator of the heat shock response and important element of the cytoprotective system, is activated in spermatogenic cells at significantly lower temperatures than in somatic cells (starting from 35 to 41 °C, respectively).
10. Despite the HSF1 activation, pro-survival pathways are not induced in male meiotic and postmeiotic cells in response to stress: although activated HSF1 binds to promoters of *Hsp* genes in both spermatocytes and somatic cells, transcription of cytoprotective HSPs is induced only in the somatic compartment of testes. Moreover, the constitutively expressed testis-specific variants of *Hsp70* (*Hspa2* and *Hspa1l*) genes are downregulated following heat shock, and ectopic expression of HSPA1 (the major heat inducible HSP70 family member) do not protect spermatogenic cells from heat shock-induced apoptosis.
11. Transcriptionally active HSF1 induces caspase-3-dependent apoptosis in spermatocytes and spermatids, which allows the elimination of potentially damaged spermatogenic cells. Hence, HSF1 has been proposed to be a quality control factor that prevents transition of the potentially damaged genetic material to the next generation.
12. HSF1 may play a dual role in response to heat shock, either cytoprotective or cytotoxic. Besides the upregulation of cytoprotective HSPs, HSF1 can directly activate the expression of proapoptotic PHLDA1 and PMAIP1 (NOXA); thus, the final response to stress could be determined by the balance between antiapoptotic and proapoptotic factors regulated by HSF1 differentially in heat-sensitive and heat-resistant cells.
13. Molecular mechanisms of the proapoptotic action of HSF1 in heat-sensitive cells as well as the functions of other HSF family members (especially those testis-specific, i.e., HSF5 and HSFY) during spermatogenesis are not fully resolved and further studies are needed.

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