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Expression of Heat Shock Factor 2 in Mouse Testis: Potential Role as a Regulator of Heat-Shock Protein Gene Expression during Spermatogenesis[1]

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ABSTRACT

We have examined the expression and function of heat shock transcription factor 2 (HSF2) in spermatogenic cells of mouse testis. The results of in situ RNA hybridization analysis, RNA filter hybridization, and reverse transcription-polymerase chain reaction (RT-PCR) analysis indicate that HSF2 mRNA expression in testis is subject to developmental and cell type-dependent, as well as stage-dependent, regulation. Localized expression of HSF2 mRNA in testis first appears between Day 14 and Day 21 of postnatal development. In adult testis, HSF2 mRNA is found at highest levels in spermatocytes and round spermatids. Immunocytochemical staining and gel mobility shift analysis demonstrate that HSF2 protein is localized to the nuclei of spermatocytes and round spermatids and that this transcription factor exists in testis in a constitutively active DNA-binding state. We further demonstrate that the constitutive HSF2 DNA-binding activity present in testis is able to interact with promoter sequences of the hsp70.2 gene, a testis-specific member of the hsp70 gene family. Taken together, our results show that the expression and functional properties of HSF2 are regulated in spermatogenic cell types of the mouse testis, supporting a role for this transcription factor as a regulator of hsp gene expression during spermatogenesis.

INTRODUCTION

Spermatogenesis is the process by which immature male germ cells, through a complex series of progressive changes involving mitotic and meiotic cell division and cell differentiation, are transformed into mature spermatozoa [1, 2]. The dramatic changes in cellular morphology and function that are characteristic of cells undergoing spermatogenesis are brought about, at least to a large degree, by changes in gene expression that regulate the levels and types of proteins synthesized in each germ cell type [3–5]. One group of proteins that has received considerable attention is the heat shock protein (hsp) family. Several members of the hsp70 and hsp90 gene families (hsp70.2, hsc70t, and hsp86) display developmental or cell type-specific expression patterns during mammalian spermatogenesis, but the mechanisms controlling the expression of these genes have not been elucidated [6–12].

One of the best-studied regulators of hsp gene expression is heat shock transcription factor, or HSF [13], which is represented as a multigene family in a number of species including tomato, human, mouse, and chicken [14–18]. In the mouse there are two HSF genes, HSF1 and HSF2, which appear to have different functions with respect to the regulation of hsp gene expression. HSF1 has been shown to mediate the induction of hsp gene expression in response to elevated temperature and other environmental stresses [15, 18–20]; it has been demonstrated that HSF2 DNA-binding ability is activated during hemin-induced differentiation of human K562 erythroleukemia cells and that the activated HSF2 binds to hsp70 gene promoter sequences to induce transcription of this gene [21]. These results suggest that HSF2 may function to regulate the expression of hsp genes under non-stress conditions, perhaps in cells undergoing the process of differentiation.

In light of these results and the observations of regulated expression of several hsp genes in mouse germ cells described above, the present study was undertaken to determine whether the expression and/or functional properties of HSF2 are regulated in spermatogenic cells of the mouse testis and to examine whether this transcription factor may be involved in regulating hsp gene expression in these cells. Our results demonstrate that HSF2 mRNA exhibits complex patterns of regulation in the testis and that HSF2 protein exists in spermatogenic cell types in an active DNA-binding state; these findings support a role for HSF2 as a regulator of gene expression in spermatogenic cells of the mammalian testis.

MATERIALS AND METHODS

Experimental Animals

CBA/J mice were obtained from Jackson Laboratory (Bar Harbor, ME) and maintained under a controlled light cycle

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(14L:10D). Testes were removed from mice at the ages of 7, 14, and 21 days and 6–8 wk, rapidly frozen on dry ice, and then kept at −80°C until use. These studies were conducted in accordance with the Guiding Principles for the Care and Use of Research Animals promulgated by the Society for the Study of Reproduction.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) Analysis and RNA Filter Hybridization

Total RNA was prepared from mouse tissues by homogenization in guanidine isothiocyanate and centrifugation through cesium chloride [22]. Total RNA of isolated pachytene spermatocytes, round spermatids, and elongated spermatids was kindly provided by David Bunick (University of Illinois, Urbana-Champaign). RT-PCR of RNA samples was performed as described previously [23, 24]. Total RNA (2–5 μg) was reverse transcribed at 42°C through use of random hexamer primers and AMV reverse transcriptase in a 20-μl reaction. Two oligonucleotide primers, 22 nucleotides in length (55% GC content), were used to amplify a 153-bp PCR product from the HSF2 mRNA; a 104-bp fragment of the mouse ribosomal protein S16 mRNA was amplified as an internal control [25]. A mix including the oligonucleotide primers (500 ng each), [α-^{32}P]-dCTP (2 μCi at 3000 Ci/mmol), and *Taq* DNA polymerase (2.5 U) was added to each reaction; the total volume was brought to 100 μl with single-strength PCR buffer (10 mM Tris, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin), and the sample was overlaid with light mineral oil. Amplification was carried out for 20 cycles using an annealing temperature of 65°C in an MJ Research thermal cycler (Norwalk, CT). The samples were analyzed by electrophoresis on 5% polyacrylamide gels and film autoradiography.

For RNA filter hybridization, 30 μg of each total RNA was separated by electrophoresis on denaturing 1% agarose/formaldehyde gels. RNA was transferred to a nylon membrane (ICN, Irvine, CA), covalently attached by UV crosslinking, and detected by hybridization to the HSF2 cDNA (clone C9) [17] labeled with [^{32}P]-dCTP using random hexamer primers and the Klenow fragment of *E. coli* DNA polymerase [22]. Hybridization was performed in 50% formamide, 5-strength saline-sodium phosphate-EDTA (SSPE), double-strength Denhardt's reagent, 10% dextran sulfate, 0.1% SDS, and 100 μg/ml salmon sperm DNA. The membranes were subsequently washed in 0.1-strength SSC at 65°C and exposed to Kodak XAR-5 film (Rochester, NY).

In Situ RNA Hybridization and Immunocytochemistry

Testis sections, 20 μm, were cut using a Reichert 820 cryostat (Buffalo, NY) and mounted onto gelatin- and poly-L-lysine-coated glass slides for in situ hybridization as described previously [24]. Testis sections were fixed in 5% paraformaldehyde (pH 7.5) for 5 min, washed in double-strength SSC for 5 min, rinsed in distilled deionized water, washed in 0.1 M triethanolamine (pH 8.0), and incubated in 0.25% acetic anhydride in 0.1 M triethanolamine (pH 8.0) for 10 min. Sections were dehydrated through an ethanol series and vacuum dried until hybridization. The probes for HSF2 and hsp70.2 contained sequences between nucleotides 1317 and 1972 of the HSF2 cDNA [17] and nucleotides 2650 and 2960 of the hsp70.2 gene [12]. Antisense [^{35}S]UTP-labeled RNA probes were synthesized using T7 or SP6 polymerase [26]. The RNA probes (2×10^7 cpm/ml in hybridization buffer: 50% formamide, 5-strength SSPE, double-strength Denhardt's reagent, 10% dextran sulfate, 0.1% SDS, and 100 μg/ml yeast tRNA) were applied to the tissue sections, and the sections were overlaid with a coverslip. Slides were hybridized in light mineral oil in a shaking water bath at 47°C for 16–18 h. After hybridization, the coverslips were removed in 4-strength SSC and sections were treated with RNase A (20 μg/ml) at 37°C for 30 min, washed in increasingly lower concentrations of SSC down to 0.1-strength SSC at 55°C, and dehydrated through an ethanol series. The slides were exposed to Kodak XAR-5 film for 2–3 days and were then processed for liquid emulsion autoradiography using NTB-2 emulsion (Kodak). Slides were developed after 2 wk and stained with hematoxylin/eosin. All photography was performed with a Wild Makroscope (Wild Leitz Ltd., Heerbrugg, Switzerland) and a Nikon optiphot microscope (Tokyo, Japan).

Sections of adult mouse testis were subjected to immunocytochemistry with the HSF2 antiserum using the Vectastain ABC Elite Kit (Vector Labs., Burlingame, CA) according to the manufacturer's protocol for immunostaining of frozen sections. The specificity of the HSF2 polyclonal antibodies has been characterized as described previously [20]. Preimmune serum and HSF2 antiserum were both used at a dilution of 1:2000. The substrate for the immunoperoxidase reaction was 1 mg/ml diaminobenzidine tetrahydrochloride in 0.1 M Tris buffer (pH 7.2). After the immunostaining protocol, testis sections were stained with hematoxylin.

Native Gel Shift Analysis and Western Blot Analysis

Whole cell extracts of adult mouse testis were prepared by homogenization, in a Dounce homogenizer (Kontes, Vineland, NJ), of previously frozen testes in five volumes of buffer C (20 mM Hepes [pH 7.9], 0.42 M NaCl, 25% glycerol, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride). After centrifugation of the extract at 12 000 × *g* to pellet insoluble material, the native gel mobility shift assay was performed as described previously [17] with a self-complementary consensus HSE oligonucleotide (5′-CTAGAAG-CTTCTAGAAGCTTCTAG-3′) that contains four perfect inverted 5′ NGAAN 3′ repeats after annealing. For the experiments involving addition of polyclonal antibodies to whole cell extracts prior to gel mo-

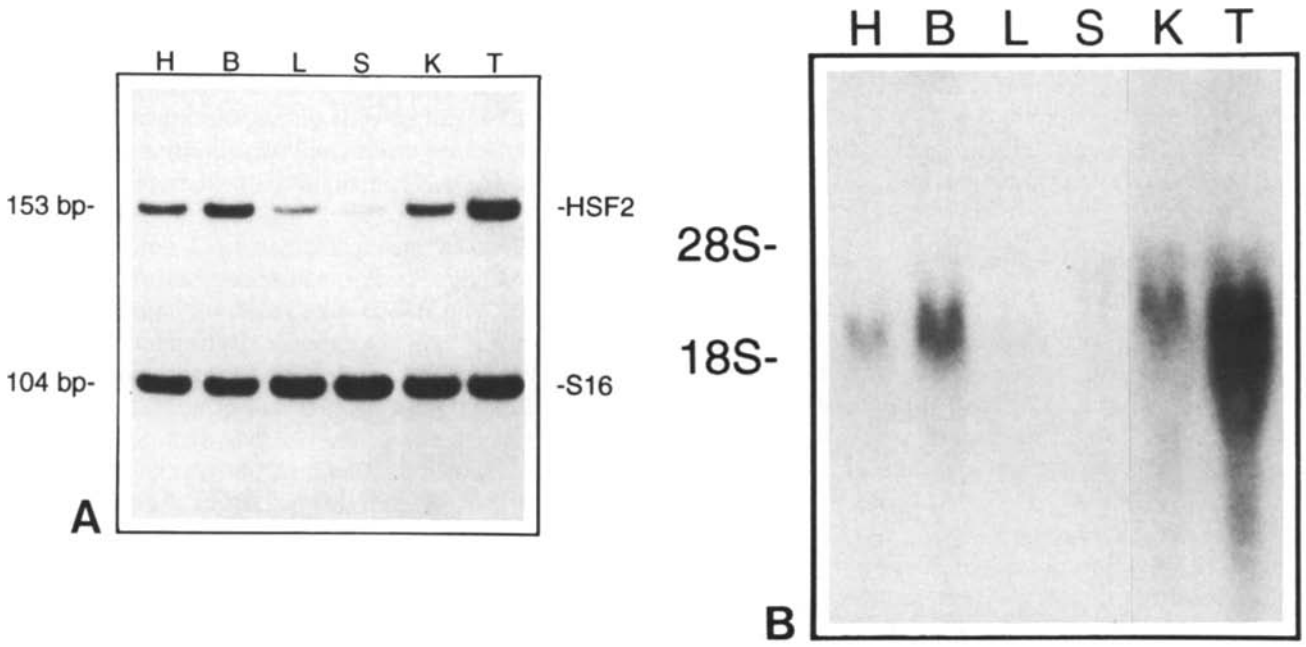

FIG. 1. Analysis of HSF2 mRNA in mouse tissues. A) Total RNA extracted from heart (H), brain (B), liver (L), spleen (S), kidney (K), and testis (T) was subjected to RT-PCR using oligonucleotide primer pairs specific for HSF2 mRNA (153-bp product) and S16 ribosomal protein mRNA (104-bp product) (internal standard). The amplification was linear with respect to input RNA at the assay point taken. B) Total RNA prepared from the mouse tissues listed in (A) was subjected to RNA filter hybridization using [^{32}P]-labeled HSF2 cDNA. The positions of migration of 18S and 28S rRNA bands are indicated to the left of the panel.

bility shift analysis, 1 μl of either preimmune serum (1:10 dilution) or immune serum (1:10, 1:50, or 1:250 dilution) was added to 2 μl of whole cell extract (10 μg protein) and incubated at 22°C for 20 min before DNA binding was assayed.

For analysis of HSF2 binding to hsp70.2 gene promoter sequences, two different restriction fragments containing promoter sequences of the hsp70.2 gene were isolated from plasmid pM 3.8, which contains the entire coding region of the hsp70.2 gene as well as approximately 600 bp of 5′ untranslated region and promoter sequences [12]. HSP70.2 promoter fragment A is a 355-bp *Eco*RI-*Eco*RV restriction fragment containing the 5′-most promoter sequences (from 1 to 355 numbered as described previously [12]) contained in this genomic clone. HSP70.2 promoter fragment B is a 206-bp *Eco*RV-*Eco*0109 fragment (nucleotides 356 to 561 numbered as described previously [12]). For analysis of binding specificity by competition, binding reactions were performed in the presence of a 50-fold molar excess of unlabeled HSE-containing oligonucleotide (24 mer) described above.

For Western blot analysis, whole cell extracts of adult mouse testes, prepared as described above, were run on an 8% SDS-PAGE gel and blotted to nitrocellulose through use of a Bio-Rad Semidry (Richmond, CA) transfer apparatus. The blot was then probed with the HSF2 polyclonal antibodies according to previously described methods [20].

RESULTS

Expression and Cellular Localization of HSF2 mRNA and Protein in Mouse Testis

In order to determine whether HSF2 mRNA is expressed in the mouse testis and if so, in what amounts and in which cell types, we analyzed the level and cellular localization of HSF2 mRNA in the testis. To examine the levels of HSF2 mRNA transcripts in mouse testis, we performed reverse transcription of mRNA coupled to the polymerase chain reaction (Fig. 1A) and RNA filter hybridization (Fig. 1B) using total RNA isolated from testis and other mouse tissues. For the RT-PCR assay, conditions were carefully determined to ensure that amplification was linear for each of the samples at the assay point taken (data not shown). The results of these analyses, shown in Figure 1, A and B, demonstrate that testis contains high levels of HSF2 mRNA transcripts.

To determine the cellular localization of HSF2 mRNA within the testis, we next performed in situ RNA hybridization analysis on sections of adult mouse testis using a ^{35}S-labeled antisense RNA probe specific for HSF2 mRNA (Fig. 2, A and B). This analysis revealed a striking pattern of HSF2 mRNA localization in some seminiferous tubules, but not others, in each testis section (Fig. 2, A and B [×100 BF and DF]). These results suggest that the expression of HSF2 mRNA is regulated with respect to the stages of the cycle of the seminiferous epithelium of the mouse testis. In situ RNA hybridization analysis using a sense probe for HSF2 mRNA

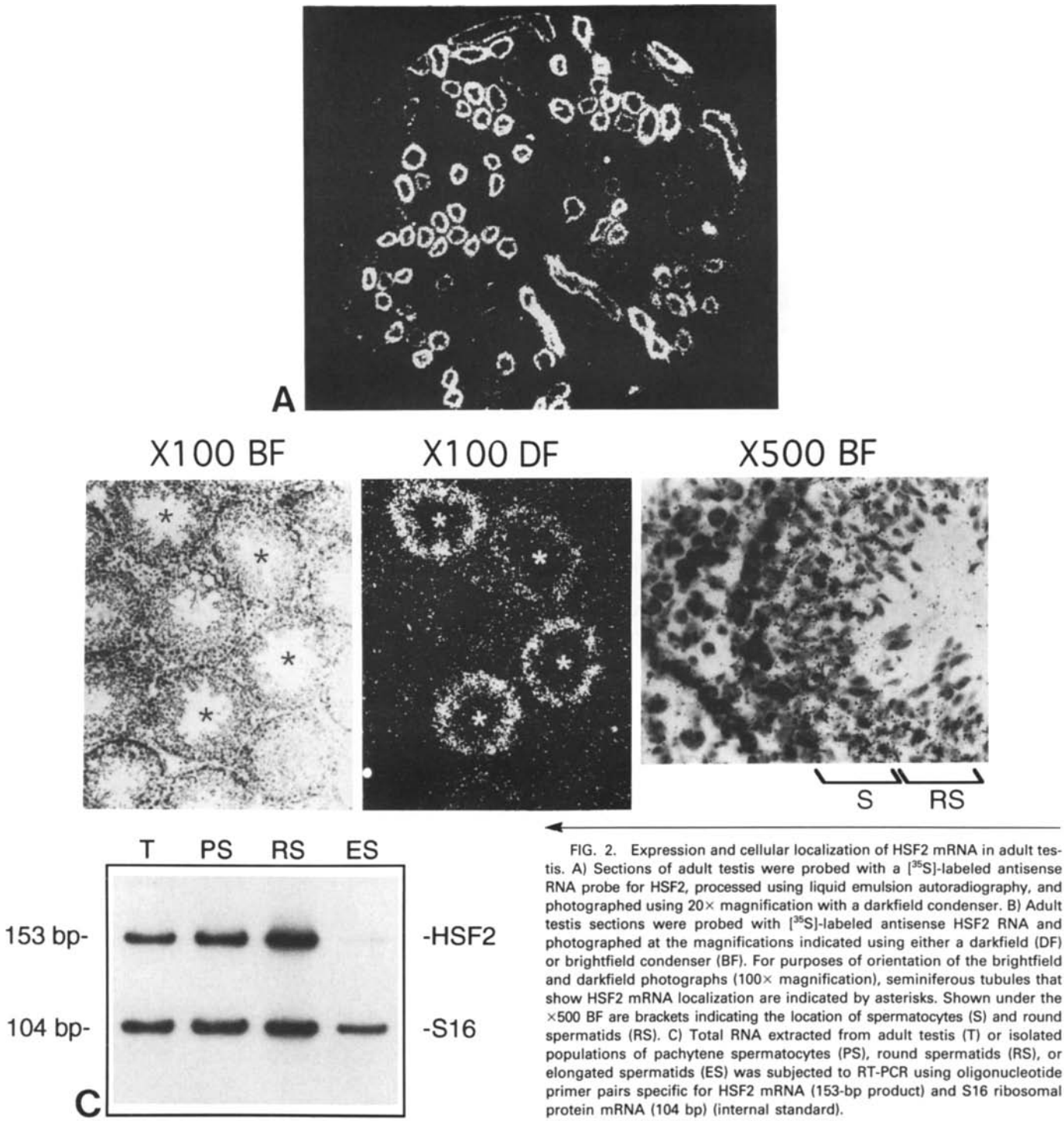

FIG. 2. Expression and cellular localization of HSF2 mRNA in adult testis. A) Sections of adult testis were probed with a [^{35}S]-labeled antisense RNA probe for HSF2, processed using liquid emulsion autoradiography, and photographed using 20× magnification with a darkfield condenser. B) Adult testis sections were probed with [^{35}S]-labeled antisense HSF2 RNA and photographed at the magnifications indicated using either a darkfield (DF) or brightfield condenser (BF). For purposes of orientation of the brightfield and darkfield photographs (100× magnification), seminiferous tubules that show HSF2 mRNA localization are indicated by asterisks. Shown under the ×500 BF are brackets indicating the location of spermatocytes (S) and round spermatids (RS). C) Total RNA extracted from adult testis (T) or isolated populations of pachytene spermatocytes (PS), round spermatids (RS), or elongated spermatids (ES) was subjected to RT-PCR using oligonucleotide primer pairs specific for HSF2 mRNA (153-bp product) and S16 ribosomal protein mRNA (104 bp) (internal standard).

did not yield any detectable hybridization signal (data not shown). This pattern of regulated expression of HSF2 mRNA in the testis is not a common feature of the HSF gene family, as in situ hybridization analysis of HSF1 mRNA did not reveal any pattern of localization that would indicate either cell type- or stage-dependent regulation of expression (data not shown).

Examination of the results of the in situ hybridization analysis at higher magnification indicates that in seminiferous tubules that display high HSF2 mRNA levels, this mRNA is localized predominantly to spermatocytes and round spermatids (Fig. 2B [×500 BF]). As an independent method for examining the cell type localization of HSF2 mRNA, we performed RT-PCR analysis using total RNA prepared from

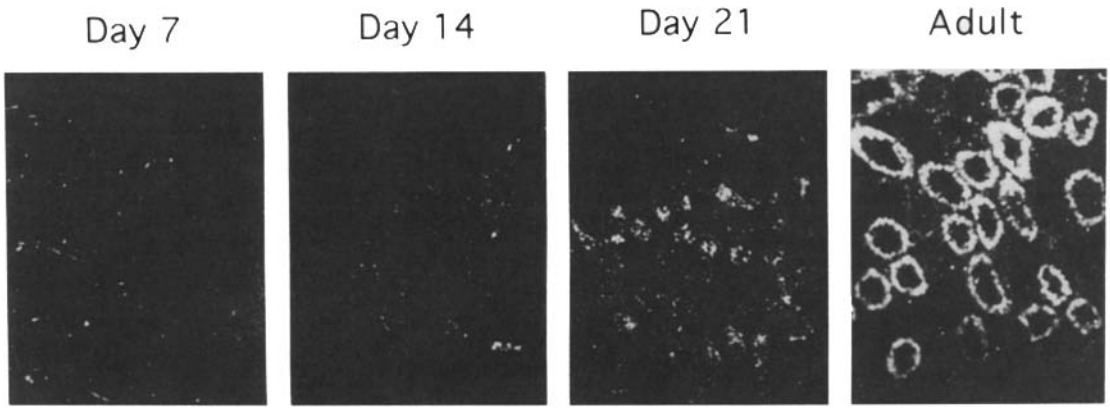

FIG. 3. Expression of HSF2 mRNA in testis during postnatal development. Sections of testes obtained from mice at different times after birth (Days 7, 14, 21, and adult) were probed with [^{35}S]-labeled antisense HSF2 RNA and photographed at 40× magnification using a darkfield condenser.

whole adult testis, isolated pachytene spermatocytes, round spermatids, and elongated spermatids. The results of this analysis, shown in Figure 2C, support the in situ hybridization results and indicate that HSF2 mRNA is abundant in pachytene spermatocytes and round spermatids but is present at significantly lower levels in elongated spermatids. The decrease in mRNA levels for the S16 ribosomal protein in elongated spermatids relative to the other spermatogenic cell types presumably reflects the general decrease in gene expression that occurs in cells in later spermatogenesis [27].

To determine whether HSF2 expression is also regulated during the postnatal development of testis, we performed in situ RNA hybridization analysis on sections of testis from mice at different times after birth (Days 7, 14, 21, and 8 wk [adult]). Low levels of HSF2 mRNA hybridization were observed in testes from Day 7 and Day 14 mice, with no apparent spatial localization of the mRNA (Fig. 3). However, beginning at Day 21, HSF2 mRNA was detected in some seminiferous tubules, and in adult testis the pattern of high HSF2 mRNA levels in some seminiferous tubules, but not others, is observed. These results are consistent with our observations that the pachytene spermatocyte is the stage of germ cell differentiation at which HSF2 mRNA first reaches high levels (Fig. 2B), as this germ cell type first becomes abundant in the developing seminiferous epithelium between Day 14 and Day 21 [28]. The results shown in Figures 1, 2, and 3, taken together, indicate that HSF2 mRNA expression in the testis is subject to developmental and cell type-dependent, as well as stage-dependent, regulation.

Next, in order to determine the cellular localization of the HSF2 protein in the mouse testis, we performed immunocytochemical staining of adult testis sections using polyclonal antibodies that specifically detect the HSF2 protein [20]. Intense immune staining (dark brown) of HSF2 protein was observed in the nuclei of spermatocytes and round spermatids (Fig. 4). No HSF2 staining was observed in elongated spermatids, indicating that the HSF2 protein is either degraded or removed into residual bodies prior to the final stages of germ cell maturation. Western blot analysis indicates that testis contains HSF2 polypeptide species with sizes similar to those found in mouse NIH 3T3 fibroblast cells (Fig. 5). The identity of the extra band of lower mobility that appears in the testis extract is unknown; but the possibilities that this band may represent a degradation product of one of the two major bands, a polypeptide product of an alternatively spliced HSF2 mRNA, or a distinct polypeptide recognized by the HSF2 antibodies, are currently being investigated.

Constitutive HSF2 DNA-Binding Activity Contained in Testis

To determine whether the HSF2 protein present in the testis exists in the inactive non-DNA-binding state or the active DNA-binding state, we performed native gel mobility shift analysis of extracts of adult testis using a labeled oligonucleotide probe that contains four inverted repeats of the consensus HSE binding site, 5′ NGAAN 3′. The results, shown in Figure 6, indicate that testis contains constitutive HSF DNA-binding activity. Co-incubation of the gel shift reactions with HSF1 or HSF2 polyclonal antibodies demonstrates that the constitutive HSF DNA-binding activity present in testis is composed of HSF2, because the mobility of this DNA-binding activity is altered by the HSF2 antibodies but not by the HSF1 antibodies (Fig. 6). Previous analyses have demonstrated the specificity of these antibodies in discriminating HSF1 and HSF2 DNA-binding activities in conjunction with the gel shift assay [20]. The specificity of the HSF2 activity for binding to the HSE was demonstrated by competition experiments using an unlabeled HSE oligonucleotide but not a CCAAT binding site oligonucleotide (data not shown).

Binding of HSF2 to HSP70.2 Promoter Sequences

Our results demonstrate that HSF2 expression is regulated in spermatogenic cell types and that this transcription factor exists in testis in the active DNA-binding state. These

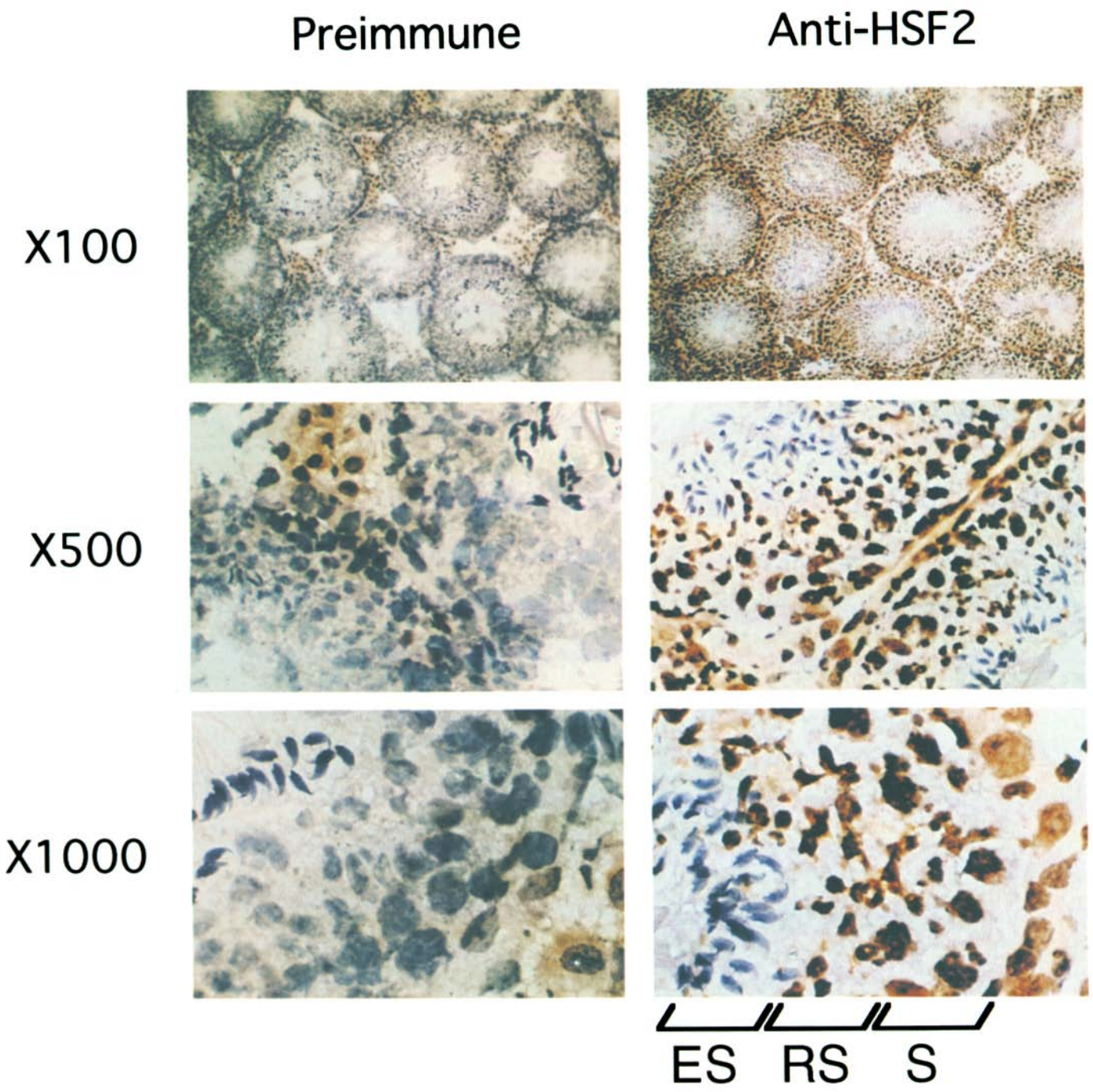

FIG. 4. Localization of HSF2 protein in adult testis. Frozen sections of adult testes were subjected to immunocytochemical staining with either preimmune serum or specific HSF2 antiserum [20] using an immunoperoxidase detection protocol (Vectastain ABC Elite kit, Vector Labs., Burlingame, CA) and then lightly stained with hematoxylin and photographed at different magnifications. Specific HSF2 immune staining appears as dark brown while nuclei with no immune staining appear light blue. Shown under the 1000× magnification of the anti-HSF2-stained section are brackets indicating the approximate localization of spermatocytes (S), round spermatids (RS), and elongated spermatids (ES).

observations suggest that HSF2 is involved in regulating gene expression in spermatogenic cells. One set of genes that represent obvious potential targets for regulation by HSF2 in male germ cells comprises members of the hsp70 gene family. In order to test the feasibility of this hypothesis, we examined whether the HSF2 DNA-binding activity present in the testis (as shown in Fig. 6) is able to bind to promoter sequences of a testis-specific member of the hsp70 gene family, the hsp70.2 gene. We chose to explore the possibility that HSF2 binds to the hsp70.2 gene promoter for several reasons. First, previous studies have indicated that the hsp70.2 gene product is expressed in pachytene sper-

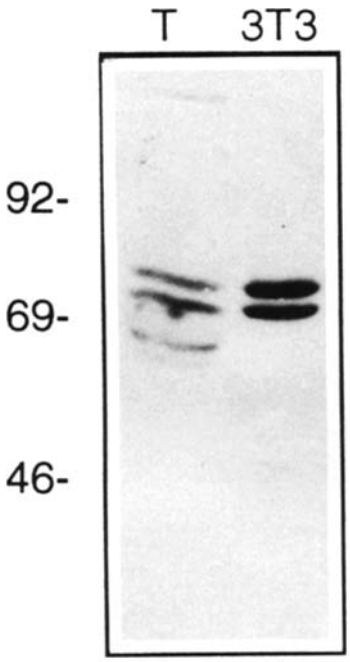

FIG. 5. Western blot analysis of HSF2 protein in testis. Whole cell extracts of adult mouse testis (T) and mouse NIH 3T3 fibroblast cells (3T3) were subjected to Western blot analysis using polyclonal antibodies specific for HSF2 [19]. Size markers (in kDa) are indicated to the left of the panel.

matocytes and round spermatids, with significantly lower levels in condensing spermatids [12, 29]; this correlates well with the pattern of HSF2 protein localization demonstrated by our immunocytochemical staining analysis (Fig. 4). Second, previous sequence analysis revealed the presence of an HSE motif in the hsp70.2 gene promoter [12].

For our analysis, [^{32}P]-labeled hsp70.2 promoter fragments were incubated either with purified recombinant HSF2 or with testis extracts and then subjected to gel mobility shift assay. Because the hsp70.2 gene promoter contains a number of potential HSE motifs and because using a single large fragment containing the entire promoter sequence would decrease the resolution of DNA-protein complexes in this assay, we used two restriction fragments containing different portions of the hsp70.2 promoter for the binding experiments. Fragments A and B comprise nucleotides 1–355 and 356–561 of the hsp70.2 gene promoter, respectively, numbered as previously described [12]. Both fragments contain at least two potential HSE motifs, centered at nucleotides 273 and 305 within fragment A and nucleotides 420 and 451 (the HSE noted previously [12]) within fragment B. As shown in Figure 7, recombinant HSF2 and HSF2 present in testis extracts bind to both hsp70.2 promoter fragments. The major DNA-protein complexes formed in each case are inhibited by an unlabeled synthetic oligonucleotide containing an HSE consensus sequence, indicating that these complexes contain HSF2 DNA-binding activity. The presence of additional, lower-mobility com-

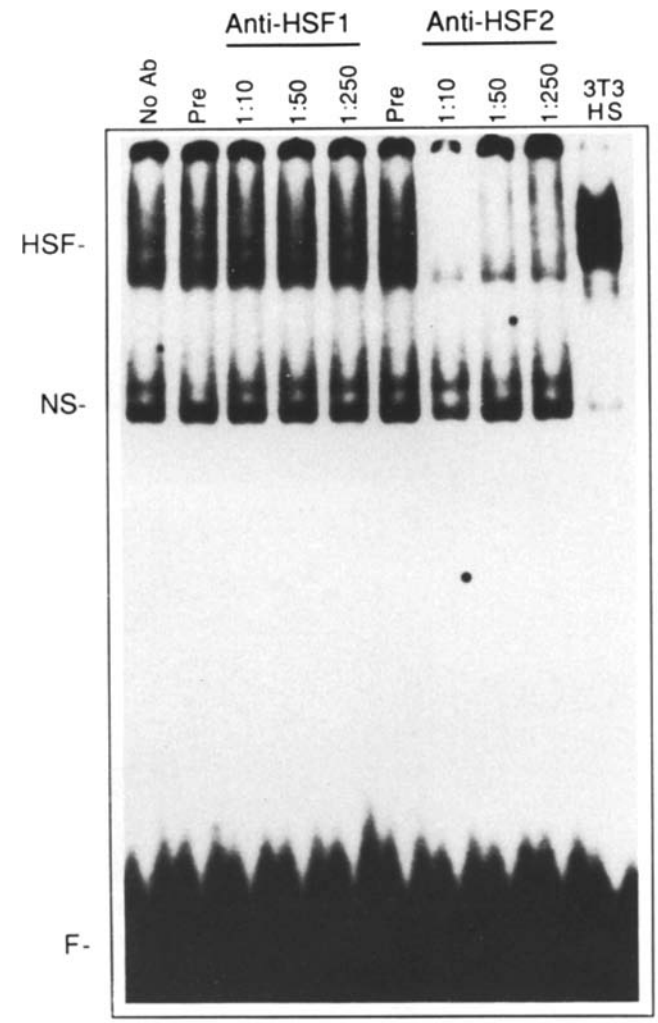

FIG. 6. Gel mobility shift analysis of HSF2 DNA-binding activity in testis extracts. An extract of adult (8 wk) testis was subjected to gel shift analysis using a labeled oligonucleotide probe containing four inverted repeats of the HSE consensus sequence 5′ NGAAN 3′ either in the absence of HSF1 or HSF2 antibody (No Ab), or after a preincubation with preimmune serum (1:10 dilution) or various dilutions (1:10, 1:50, 1:250) of the HSF1 or HSF2 antiserum. The migration of the heat-induced HSF DNA-binding activity in mouse fibroblast NIH 3T3 cells is shown in the last lane for comparison. Indicated at the side of the panel are the locations of the HSF:DNA complex (HSF), nonspecific complex (NS), and free probe (F).

plexes in the lanes containing recombinant HSF2 is consistent with previous observations in our laboratory showing that purified recombinant HSF2 is capable of forming multiple higher-order complexes on HSE-containing templates [30, 17].

DISCUSSION

The present study was undertaken to determine whether the expression of HSF2 is regulated in spermatogenic cells of the mouse testis and to examine whether this transcription factor may be involved in the regulation of hsp gene expression in these cells. We have found that 1) HSF2 expression is subject to developmental, cell type-depen-

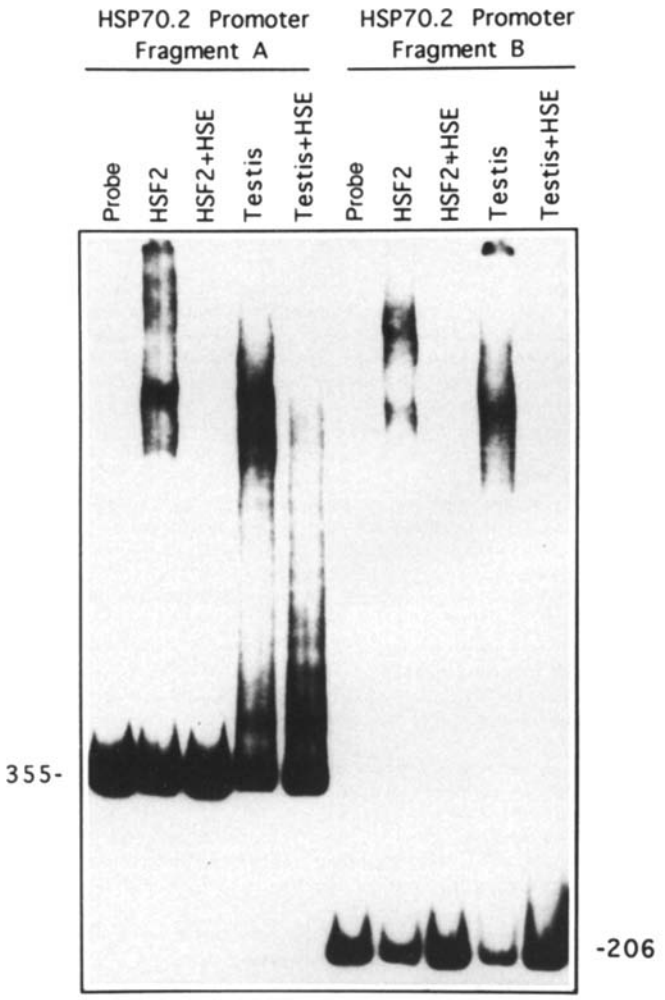

FIG. 7. Binding of HSF2 to hsp70.2 gene promoter sequences. Purified recombinant HSF2 or testis whole cell extracts were incubated with two [^{32}P]-end-labeled fragments corresponding to nonoverlapping portions of the promoter region of the hsp70.2 gene. Fragment A contains nucleotides 1–355 and fragment B contains nucleotides 356–561 of the 5′ region of the hsp70.2 gene, numbered as described previously [12]. Labeled probes were incubated either alone (Probe), with recombinant HSF2 (HSF2), with HSF2 and unlabeled competitor HSE-containing oligonucleotide (HSF2+HSE), with testis extract (Testis), or with testis extract and unlabeled competitor HSE-containing oligonucleotide (Testis+HSE). The sizes of the DNA fragment probes are indicated at the sides of the panel.

dent, and stage-dependent regulation in the mouse testis; 2) HSF2 protein is present in the testis in the active DNA-binding state; and 3) this constitutive HSF2 DNA-binding activity present in the testis is able to bind to promoter sequences of the hsp70.2 gene in vitro. These results support a role for HSF2 in regulating the expression of this testis-specific hsp70 gene, and perhaps other hsp gene family members, in spermatogenic cells of the mammalian testis.

Regulation of HSF2 in Spermatogenic Cells

The results of our in situ RNA hybridization analysis demonstrate that HSF2 mRNA is present at high levels in spermatocytes and round spermatids of some seminiferous tubules, but not others, indicating that expression of this mRNA is regulated in both a cell type-dependent and stage-dependent manner in the mouse testis. The mechanisms controlling these patterns of HSF2 mRNA expression in the testis are unknown, but one possibility we will explore in future studies is that the regulation is mediated by hormones—such as testosterone or FSH—that control the process of spermatogenesis by acting, both directly and indirectly, on Sertoli cells [31, 32]. A signal generated in Sertoli cells in response to FSH or androgen stimulation may be communicated to the adjacent germ cells and regulate HSF2 mRNA levels in these cells through either increased HSF2 gene transcription or increased mRNA stability. An example of this type of regulation was provided by studies on the expression of the transcriptional modulator CREM in spermatogenic cells; these studies demonstrated that FSH regulates levels of this factor by increasing CREM mRNA transcript stability [33]. Alternatively, HSF2 mRNA levels may be controlled by a regulatory molecule whose activity is modulated as part of the program of gene expression in spermatogenic cells. There are reports of regulated expression of other transcription factors during spermatogenesis, including members of the *fos* and *jun* family, octamer binding protein family, homeobox factor family, and CREB; but the mechanisms controlling the expression of these factors are poorly understood [3–5].

In addition to our observations demonstrating regulation of HSF2 at the level of mRNA expression in spermatogenic cells, we have obtained several pieces of data indicating that the functional properties of HSF2 protein are also regulated in these cells. First, gel shift analysis demonstrates that HSF2 protein exists in testis in the active DNA-binding state. The presence of constitutive HSF2 DNA-binding activity in testis is interesting because this transcription factor exists in most cell types in an inactive, non-DNA-binding state, which must be converted to the active, DNA-binding state in order to induce the transcription of hsp genes [13]. We do not know the mechanism by which HSF2 DNA-binding ability is activated in spermatogenic cells, but one possibility is that it occurs through oligomerization of HSF2 to the active trimeric form as a result of the elevated HSF2 protein levels in these cells. In support of this potential mechanism, we have previously demonstrated that overexpression of HSF2 in cultured mouse cells results in constitutive DNA-binding activity [20]. The second piece of data consistent with an activated state of HSF2 in spermatogenic cells is the localization of HSF2 protein in the nuclei of spermatocytes and round spermatids as demonstrated by the immunocytochemical staining analysis (Fig. 4). Nuclear localization appears to be a characteristic of the HSF2 activation process, as studies on human K562 erythroleukemia cells have shown that HSF2 activated by hemin treatment becomes localized to the nuclei of these cells [34].

Potential Functional Role for HSF2 in Spermatogenic Cells

Previous work by others has demonstrated that expression of several members of the hsp70 and hsp90 gene families, including the hsp70.2, hsc70t, and hsp86 genes, is regulated in spermatogenic cells of the mouse testis [6–12]. It has been hypothesized that these hsp genes are expressed in these cell types in order to provide for their specialized needs [12, 29]. During the process of spermatogenesis, germ cells undergo dramatic alterations in gene expression that result in changes in both the types and amounts of proteins present in each germ cell type [3–5]. These changes in protein constituency may require changes in the levels and/or species of hsp present in spermatogenic cells to meet the increased demand for molecular chaperones. Our results demonstrating that HSF2 expression is regulated in spermatogenic cell types and that the constitutive HSF2 DNA-binding activity present in testis binds to promoter sequences of the hsp70.2 gene suggest that this transcription factor may be involved in regulating the expression of this hsp gene, and perhaps other hsp genes, in spermatogenic cells.

Previous studies using cultured mouse and human cells showed that HSF1 and HSF2 both act as transcriptional regulators of hsp gene expression and bind to the same sequence motifs, the heat shock elements (HSE), in the promoters of hsp genes [13]. However, the functional properties of HSF1 and HSF2 are differentially regulated. The DNA-binding activity of HSF1, but not HSF2, is activated by exposure of cells to a variety of environmental stresses including elevated temperature and heavy metals [20], resulting in the induction of the cellular stress response. HSF2 DNA-binding ability is activated during hemin-induced differentiation of human K562 erythroleukemia cells, suggesting that this factor may function as a transcriptional regulator of hsp gene expression under non-stress conditions, particularly in cells undergoing the process of differentiation [21]. Our results are consistent with this proposed role for HSF2 in regulating gene expression in differentiating cells. More work will be required in order to determine the generality of the functional role of HSF2 in regulating gene expression during cell differentiation.

In future studies it will be important to determine the mechanisms responsible for controlling HSF2 expression in spermatogenic cell types and to identify the genes regulated by HSF2 in these cell types. The results of these studies will not only provide insight into the manner in which gene expression is regulated during mammalian spermatogenesis but will also increase our understanding of the roles played by heat shock proteins in the specialized functions of spermatogenic cells.

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