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Constitutive and Heat-Shock Induced Expression of Hsp70 mRNA During Chicken Testicular Development and Regression

Belén Mezquita,[1] Jovita Mezquita,[1] Mercè Durfort,[2] and Cristóbal Mezquita[1]*

[1]Laboratori de Genètica Molecular, Institut d'Investigacions Biomèdiques August Pi i Sunyer, Facultat de Medicina, Universitat de Barcelona, 08036 Barcelona, Spain
[2]Departament de Biologia Cel·lular Animal, Facultat de Biologia, Universitat de Barcelona, 08028 Barcelona, Spain

Abstract The constitutive and heat shock induced expression of Hsp70 mRNA was investigated in normal adult chicken testis and in adult testis after testicular regression induced by diethylstilbestrol (DES) treatment. In addition to the canonical form of Hsp70 mRNA, we have detected transcripts with an extended 5'UTR and transcripts containing, in the 5'UTR, sequences of the 18S ribosomal RNA. Hsp70 was expressed in unstressed male gonads in adult and regressed testis, being the expression much lower in regressed testis. Upon heat shock at 44°C or 46°C, Hsp70 was highly induced in both tissues. However, when testicular seminiferous tubules were incubated at the chicken internal temperature of 39°C, no induction of Hsp70 was observed in mature testis, while the expression markedly increased in regressed testis. Induction at 39°C was completely inhibited in the presence of 6 mM aspirin. Aspirin in the range 3–10 mM decreases the expression of Hsp70 in unstressed and stressed testicular cells, in striking contrast with the effect observed in other tissues as liver. These data suggest that the expression of Hsp70 is regulated in a specific manner in chicken testis and particularly in the male gonad undergoing regression. J. Cell. Biochem. 82: 480–490, 2001.

Key words: Spermatogenesis; 18S ribosomal RNA; 5'UTR; aspirin; apoptosis; heat shock; snoRNA U20; snoRNA U14

When cells are exposed to stressful conditions, including heat shock, the survival response implies the rapid expression of a small number of highly conserved proteins known as heat shock proteins (hsp) [Morimoto et al., 1994]. Hsp, particularly Hsp70, can repair proteins that unfold, misfold or aggregate upon stress, assisting this way in the recovery of the cell [Bukau and Horwich, 1998]. In addition, Hsp70 may play an important role in cell survival by interfering with apoptotic programs [Beere et al., 2000; Buzzard et al., 1998; Creagh et al., 2000; Gabai et al., 1997; Jaattela et al., 1998; Li et al., 2000; Meriin et al., 1999; Mosser et al., 1997; Mosser et al., 2000; Nylandsted et al., 2000; Volloch et al., 1998; Yaglom et al., 1999]. Hsp70 is highly expressed in many tumor cells and the ability of Hsp70 to prevent apoptosis may enhance tumorigenesis and limit the efficacy of cancer therapy [Jaattela, 1999; Vargas-Roig et al., 1998]. While we have a great deal of information about the heat shock response in somatic cells, the mechanisms involved in the expression of heat shock genes during spermatogenesis and their potential role in the survival mechanisms of testicular cells are less understood.

Marked differences in the expression of Hsp70 between somatic and testicular cells have been reported. For instance, primary spermatocytes of Drosophila are unable to raise a heat shock response [Michaud et al., 1997]. Lack of the typical somatic induction of Hsp70 after heat shock also has been reported in trout [Le Goff and Michel, 1999] and mouse testicular cells [Mezquita et al., 1998a; Zakeri et al., 1990; Zakeri and Wolgemuth, 1987]. However,

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*Correspondence to: Cristóbal Mezquita, Departament de Ciències Fisiològiques I. Facultat de Medicina. Universitat de Barcelona, Casanova 143, 08036 Barcelona, Spain.
E-mail: mezquita@medicina.ub.es

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targeted gene disruption of Hsp70-2 produces a dramatic increase in spermatocyte apoptosis in mice and clearly shows that this testis specific protein is linked to the mechanisms that inhibit apoptosis during spermatogenesis [Dix et al., 1996]. Birds are unique among homeothermic animals in developing spermatogenesis at the elevated avian internal body temperature of 39–41°C [Beaupre et al., 1997]. We have shown that in avian spermatogenesis, in prepuberal and adult testis, heat shock increases the amount and polyadenylation of Hsp70 transcripts [Mezquita et al., 1998a]. Herein we have characterized different testicular transcripts of Hsp70 and examined the expression of Hsp70 mRNA in unstressed and heat-shock-stressed testicular cells obtained from mature testis and testis undergoing regression after DES treatment. In addition, we have compared the effect of aspirin on the expression of Hsp70 in the different tissues studied.

METHODS

Animals and Treatment

Experiments were performed on adult 6–12 month-old chickens. To induce testicular atrophy, adult chickens were given for two weeks daily subcutaneous injections of DES purchased from Sigma (5mg in corn oil). Animals were killed by exsanguination and the testes were quickly dissected and weighted.

Morphological Methods

Small fragments of the testis were fixed by immersion in 3.5% glutaraldehyde. Fixed tissue specimens were rinsed in PBS, progressively dehydrated in graded ethanol (50%–70%–90%–absolute) and embedded in paraffin according to standard procedures. Paraffin sections of 7 μm thickness were cut on a rotatory microtome and deposited on glass slides. Histological examination was performed after staining with hematoxylin-eosine.

Culture of Seminiferous Tubules and Cell Suspensions

Testes were decapsulated and the seminiferous tubules, gently dispersed with forceps, were cultured in 10 vol of minimum essential medium (Eagle) at the appropriated temperatures. In other experiments cell suspensions were prepared from the seminiferous tubules or from liver. The tissues were finely minced with scissors, suspended in 10 volumes of minimum essential medium (Eagle) containing 0.1% trypsin, incubated at 31°C for 30 min in an orbital air incubator, and filtered through four layers of surgical gauze. Then, fetal calf serum was added to 10%. Aliquots of $1–6 \times 10^9$ cells (10 ml) were used as control and heat-shock samples.

Heat-Shock Conditions

Heat shock was conducted at 44°C or 46°C for 2 h in a water bath orbital incubator. Controls were incubated at 39°C for 2 h. Cells or tissue were centrifuged at $1{,}000 \times g$ for 5 min. The pellet was extracted with TriPure (Boehringer).

Preparation of RNA, Electrophoresis, Northern Hybridization and Immunological Detection

Total RNA was prepared with the TriPure Isolation Reagent from Boehringer Mannheim, according to the specifications of the manufacturers. Samples of total RNA (20–40 μg), obtained from adult (25-week-old) chicken testis, were electrophoresed through 2M formaldehyde, 1.2–1.6% agarose gels, in 0.02M MOPS, 5 mM sodium acetate (pH 7) and 1 mM EDTA and transferred to nylon membranes positively charged (Nytran Plus, Schleicher and Schuell) in 10 × SSC, then fixed by UV crosslinking. Blots were hybridized using ExpressHyb hybridization solution (Clontech) and non-radioactive-digoxigenin specific probes labeled as described below. Hybridization conditions and the immunological chemiluminescence detection procedure were as described in [Engler-Blum et al., 1993].

Rapid Amplification of cDNA Ends (RACE) and RT-PCR for the Characterization of Hsp70 5′UTRs

First-strand cDNA from adult testis was obtained by using the Amplifinder RACE or the SMART RACE protocol from Clontech (Palo Alto, CA) as previously described [Mezquita et al., 2000; Mezquita et al., 1999]. cDNA was amplified after two consecutive PCR reactions. The first PCR was done with the anchor primer AGGTTCCAGAATCGATAGTG or the UPM primer (Clontech), and the specific primer TTGGGCTTGCCACCCTCG. The second PCR was done with the anchor primer or the NUP primer (Clontech) and a nested specific primer (GAATACGTGGTGCCCAGATC, or D4: GAT-

CTCCACTTTGCCATGCTGGAAGAC). RACE-PCR conditions were as follow: 5 min at 94°C and 30 cycles at 94°C, 30 sec; 60°C/52°C, 30 sec, and 68°C, 3 min. For Smart RACE PCR conditions were as recommended by Clontech.

First-strand cDNA from heat-shocked adult testis was obtained using Moloney Murine Leukemia virus (M-MuLV) Reverse Transcriptase and an oligo d(T) primer (Amersham Pharmacia Biotech) as previously described [Mezquita et al., 2000]. This cDNA was used for PCR-amplification with specific primers located in the 18S rRNA sequence (upstream primer, RU: TGATGGGGATCGGGGATTGC) and in the hsp70 sequence (downstream primer: GAATACGTGGTGCCCAGATC). Two consecutive amplification reactions were carried out. The second PCR reaction was done with the RU primer and the hsp70 nested primer CGGCCCTTTGCCAGA CATG. PCR conditions were as follow: 3 min at 94°C and 30 cycles at 94°C, 30 sec; 58°C, 30 sec, and 68°C, 3 min. Reamplification was done using the same conditions except for the annealing temperature that was 62°C.

Analysis of PCR Products

PCR products were polished, cloned in the pPCR-Script Amp SK(+) vector (Stratagene, La Jolla, CA), and sequenced as described [Mezquita et al., 2000]. PCR products as well as RACE products (1/10 aliquots) were also analyzed in 2% agarose gels stained with ethidium bromide and photographed under UV light (Gel Doc 1000, BioRad). Alternatively, RACE products were alkali-blotted in NaOH 4N for Southern transfer and hybridization in the conditions described previously for Northern blots.

DNA Probes

Probes were obtained from recombinant clones containing the whole cDNA or specific segments. Labeling was done by PCR amplification using Taq DNA polymerase and a dNTP mix containing DIG-11-dUTP (Boehringer Mannheim). Amplification conditions were as follow: 94°C, 3 min; 30 cycles at 94°C, 1 min; 52°C, 1 min; 72°C, 3 min; and final extension at 72°C, 7 min. To obtain the probe for Hsp70, the primers ATGTCTGGCAAAGGGCCGGC and TTGGGCTTGCCACCCTCG, that amplify the first 308 bp of the coding region of the gene, were used. For the 18S rRNA specific probe, the primers used were RU and GACGGGCGGTGTGTACAAAG.

RESULTS

Characterization of hsp 70 Transcripts Expressed in Adult Chicken Testis

In addition to the major canonical form of Hsp70, with a short 5′UTR [Morimoto et al., 1986], we have sequenced, in the testis, two minor variant forms with extended 5′UTRs. One of these forms, resulting from an alternative initiation of transcription, includes, in the 5′UTR, the TATA box and part of the proximal heat shock regulatory element (Fig. 1A). A second variant, obtained by 5′RACE from adult chicken testis, contained 112 nucleotides of the highly phylogenetically conserved 3′ end of 18S ribosomal RNA (Fig. 1B). Using specific primers, located within the 18S rRNA sequence and the 5′UTR of Hsp70, we have amplified by RT-PCR a hybrid sequence comprising the entire 3′ end of chicken 18S rRNA (220 nt) and the 9 nt immediately preceding the coding sequence of Hsp70 (Fig. 1C). The heptanucleotide AGAGGAA, present in both the 18S rRNA 3' end and the Hsp70 5′UTR, is in the boundary region of the hybrid sequence. The heptanucleotide is flanked by repeats: ACTATCTAGAGGAATCTATCA. Interestingly, in this boundary region a sequence of 22 nt shows a perfect complementarity with the small nucleolar RNA U20 (Fig. 2). No amplification of hybrid molecules was obtained using primers with genomic DNA, indicating that the hybrid forms are not present in the genome. The amount of Hsp70 mRNA containing sequences of 18S rRNA in its 5'UTR markedly increased when testicular cells were exposed to heat shock (Fig. 3).

Effect of DES Administration on Testis Weight and Morphology

Administration of the estrogen agonist DES to roosters for 2 weeks induced more than a 90% reduction in testis weight. This drastic atrophy was accompanied by a substantial reduction of seminiferous tubule diameter, collapse of tubular lumen and severe decrease in germ cell number (Fig. 4). Cells lining the basement membrane of the tubules, presumably spermatogonia and Sertoli cells, are the main cells remaining after DES treatment. Recently we have reported changes indicative of vascular

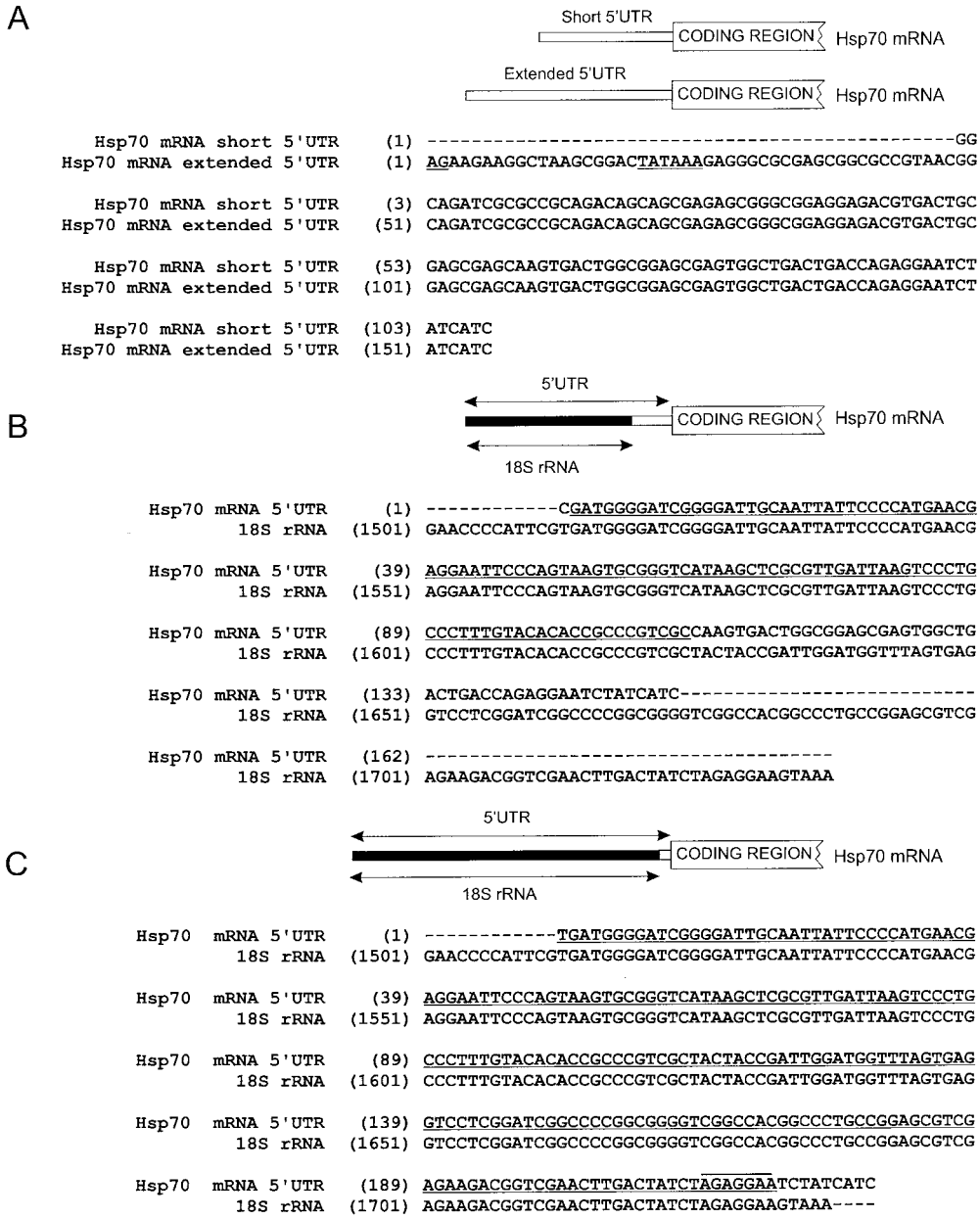
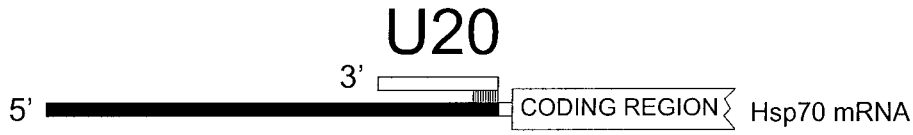


Fig. 1. 5′UTR sequences of the Hsp70 mRNA isolated from mature chicken testis. In addition to the canonical 5′UTR, forms were detected with an extended 5′UTR containing the ''TATA box'' and part of the heat shock regulatory elements (underlined in **A**). In addition, other forms of Hsp70 mRNA showed 5′UTRs containing sequences of 18S rRNA (underlined in **B** and **C**). The heptanucleotide AGAGGAA flanked by repeats in C is overlined. The sequences have been deposited in the EMBL Nucleotide Sequence Database under the accession numbers AJ301878, AJ301879, AJ301880 and AJ301881.

regression after testicular atrophy induced by DES treatment [Mezquita et al., 2000].

Effect of DES Administration on the Amount of Hsp70 Transcripts Expressed in Adult Chicken Testis Cells

Since DES is known to induce a dramatic increase in apoptosis of testicular cells [Nonclercq et al., 1996] and Hsp70 functions as an antiapoptotic protein in several systems [Beere et al., 2000; Buzzard et al., 1998; Creagh et al., 2000; Gabai et al., 1997; Jaattela et al., 1998; Li et al., 2000; Meriin et al., 1999; Mosser et al., 1997; Mosser et al., 2000; Nylandsted et al., 2000; Volloch et al., 1998; Yaglom et al., 1999], we wanted to know whether the response to the

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snoRNA U20     (1) TGGATATGATGACTGATTACCTGAGAAATAATTGATGAAATCTCAAGAAA
Hsp70 5'UTR  (229) ----------------------------------------GATGATA

snoRNA U20    (51) -ATTCCTCTAGATAGTCAAGTTCTGATCCAG------------------
Hsp70 5'UTR  (222) GATTCCTCTAGATAGTCAAGTTC-GACCGTCTTCTCGACGCTCCGGCAGG
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Fig. 2. Complementarity between the small nucleolar RNA U20 and the 5′UTR of Hsp70 containing 18S rRNA sequences. The complementary sequence is underlined.

estrogen agonist is accompanied by a decrease in the amount of Hsp70 transcripts in testicular cells. To examine this possibility total RNA was obtained from adult testis with and without DES treatment. Northern blot hybridization with a probe from chicken Hsp70 showed a marked decrease of Hsp70 mRNA after DES administration for two weeks (Fig. 5). In addition, another striking difference between normal testicular tissue and DES treated testicular cells was the response to incubation at 39°C of testicular tissue in vitro. When seminiferous tubules from adult testis were exposed to the internal body temperature of 39°C, no increase of Hsp70 mRNA was detected (Fig. 5). However, when tubules from DES treated animals were incubated at 39°C, a marked increase of Hsp70 mRNA occurred (Fig. 5). These results show a remarkable difference in the Hsp70 expression between normal testicular cells and testis undergoing regression upon DES treatment.

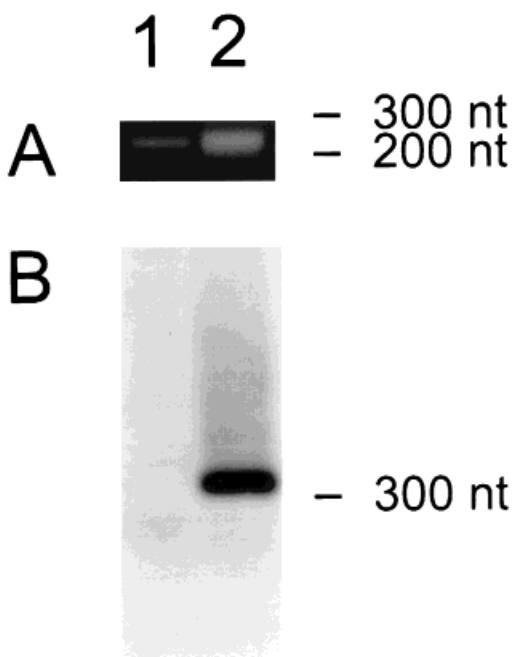

Fig. 3. Effect of heat shock on the amount of Hsp70 mRNA containing 18S rRNA sequences within the 5′UTR. cDNA was obtained from mature testis before (1) and after (2) heat-shock. **A:** Ethidium bromide analysis of PCR products obtained with primers located within 18S rRNA (RU) and Hsp70, as described in Methods. **B:** Southern blot analysis: the RACE products obtained with the anchor and D4 primers were hybridized with a 18S specific probe (see Methods).

Effect of Aspirin on the Expression of Hsp70 in Testicular and Somatic Cells

Aspirin and salicylates modify the expression of Hsp70 in different tissues and could change cell resistance to apoptosis [Fawcett et al., 1997; Giardina and Lis, 1995; Jurivich et al., 1995; Jurivich et al., 1992; Winegarden et al., 1996]. We examined whether the increase in expression observed at 39°C in testicular cells upon DES treatment was modified by the presence of 6 mM aspirin. In the presence of aspirin, no increment in the levels of Hsp70 mRNA was detected (Fig. 6). This observation indicates that aspirin can completely inhibit the expression of Hsp70 at 39°C. The inhibitory effect of aspirin was much less pronounced when cells were exposed to heat-shock at 44°C (Fig. 6). Aspirin (6 or10 mM) also decreases the amount of Hsp70 mRNAs in normal testicular cells incubated at 39°C or 46°C (Figs. 7 and 8) in striking contrast with the effect observed in

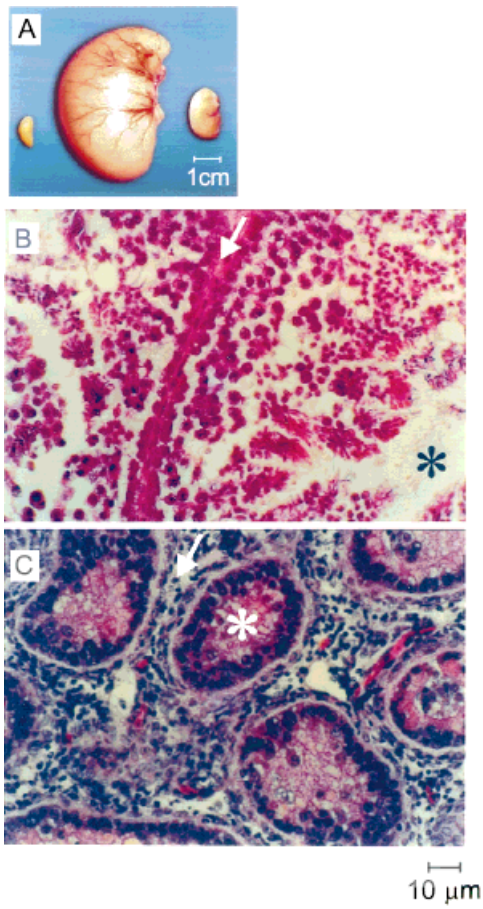

Fig. 4. Testicular regression induced by DES treatment. Immature testis from a 6-week-old chicken (**A**, left). Mature testis from a 6-months-old rooster (A, middle). Mature chicken testis after testicular regression induced by two-week daily injections of DES (A, right). Histological staining with hematoxylin-eosine of paraffin sections of normal adult testis (**B**) and adult testis after DES exposure for two weeks (**C**). The normal tissue, without DES treatment (B), shows two contiguous seminiferous tubules separated by a narrow intertubular space (arrow). Upon DES exposure (C) the cross-sectional area and the germ cell number of the tubules drastically decrease and the tubular lumen (black asterisk in B) collapses (white asterisk) while the intertubular space widens and is occupied by an abundant population of cells. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

somatic cells such as hepatocytes, where aspirin increases the expression of Hsp70 (Fig. 9). This result suggests that the expression of Hsp70 at the internal body temperature of 39°C or in heat-shocked cells at 44–46°C is different in somatic cells, in normal testicular cells and in testicular cells upon DES treatment. The effect of aspirin in testicular cells decreasing the expression of Hsp70 may increase the vulnerability of these cells particularly after DES treatment.

DISCUSSION

Variant Forms of Hsp70 mRNA

We have characterized two minor variant forms of Hsp70 mRNA in chicken testis differing in their 5′UTR from the canonical sequence. One of the forms possesses an extended 5′UTR resulting from an alternative initiation of transcription from upstream promoters with read-through of downstream promoters. A second form of Hsp70 mRNA incorporates sequences of 18S rRNA into the 5'UTR.

Previously we have studied the expression of several genes during spermatogenesis that utilize alternative promoters to 'TATA box' in meiotic and postmeiotic cells [Mezquita et al., 1993; Mezquita et al., 1994; Mezquita et al., 1997; Mezquita et al., 1998b; Mezquita et al., 1999]. The extended 5′UTRs of the mRNAs, resulting from the alternative initiation of transcription, possess increased secondary structure that can block premature translation. Since during spermatogenesis transcription ceases before translation, the mRNAs must be stabilized and their translation postponed until needed in non-transcribing cells. The presence of extended 5′UTRs is particularly paradoxical in transcripts of heat shock genes where it has been emphasized the importance of short 5′UTRs. The artificial introduction of a minimal secondary structure in the 5′UTR of Hsp70 mRNA blocks translation during heat shock [Hess and Duncan, 1996]. On the other hand, we do not know the function of the somatic promoter elements incorporated in the extended 5′UTR of testicular mRNAs. One possibility is that these sequences could act as cis-regulatory elements in the control of translation, in a similar way as previously reported for the Drosophila's TCE element [Kempe et al., 1993] and beta2 tubulin gene core promoter [Santel et al., 2000].

The presence of rRNA-sequences in the 5′UTR of several mRNAs, both in the sense and antisense orientation, has been previously reported [Mauro and Edelman, 1997]. Complementary sequences between the 5′UTR of Hsp70 mRNAs and 18S rRNA have been postulated as a mechanism for translation by ribosome shunting [Yueh and Schneider, 2000] or by internal ribosome entry sites [Chappell et al., 2000] and also as a mechanism that can inhibit translation [Verrier and Jean-Jean, 2000]. The presence of rRNA sequences in the

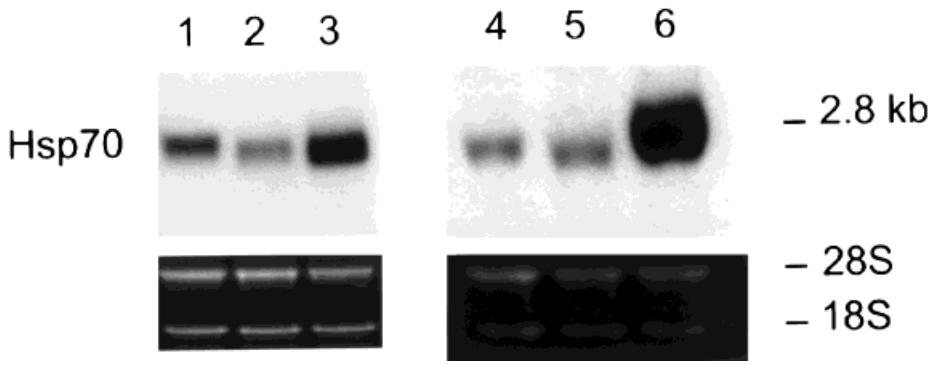

Fig. 5. Effect of testicular regression induced by DES treatment on the expression of Hsp70 mRNA. Northern blotting analysis of Hsp70 mRNA obtained from adult chicken testis (**lane 1**), regressed testis (**lane 2**), regressed testis incubated in vitro for 2 h at 39°C (**lane 3**), adult chicken testis (**lane 4**), adult chicken testis incubated in vitro for 2 h at 39°C (**lane 5**) and adult chicken testis incubated in vitro for 2 h at 44°C (**lane 6**). Panels with ribosomal RNAs show the equalization of RNA samples used for analyses.

sense orientation has been documented in the 5′UTR of several mRNAs, including the mouse heat shock protein 86, which contains a sequence of 129 nt of 28S rRNA [Mauro and Edelman, 1997]. Although neither the origin nor the function of these sequences is known at present, it has been speculated that the large amount of rRNA within the cell may drive forward processes that are otherwise rare events, such as trans-splicing [Mauro and Edelman, 1997]. Sequence complementarity between Hsp70 mRNA and 18S rRNA [Yueh and Schneider, 2000] can facilitate transplicing as has been demonstrated in vitro for other RNA duplexes [Solnick, 1985]. Ribosomal RNA sequences incorporated into mRNA may interact with ribosomal proteins located within or outside the ribosome, controlling translation or delivering the messages to particular domains of the cell [Mauro and Edelman, 1997].

Our observation on the presence of 18S rRNA sequences in the 5′UTR of Hsp70 mRNA with perfect complementarity with the small nucleolar RNA U20 (snoRNA U20) suggests, in addition, the possibility of interaction with these small RNAs. SnoRNA U20 is encoded by an intron of the nucleolin gene in mammals, and its expression appears to be the result of intronic RNA processing [Nicoloso et al., 1994]. Hsp70 and nucleolin are co-expressed in different cell systems [Konishi et al., 1995]. SnoU14, another related small nucleolar RNA, is located in three

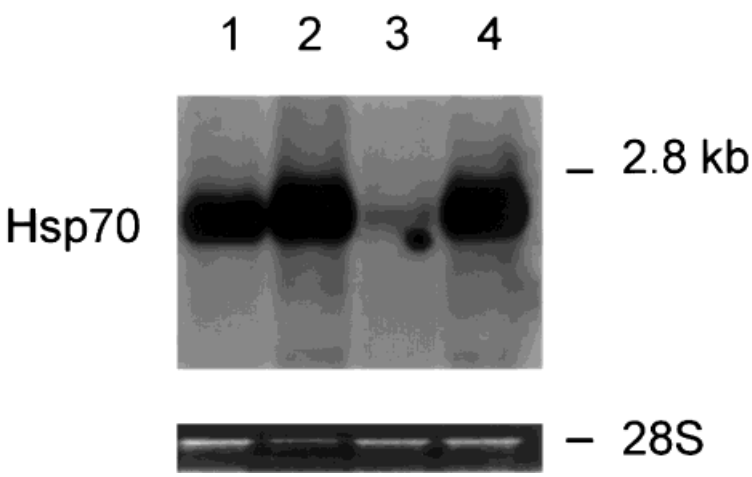

Fig. 6. Effect of aspirin on the expression of Hsp70 mRNA in mature chicken testis after testicular regression induced by DES treatment. Northern blotting analysis of Hsp70 mRNA obtained from the seminiferous tubules incubated at 39°C for 2h, without aspirin (**lane 1**) and in the presence of 6 mM aspirin (**lane 3**). Hsp70 mRNA was also prepared from seminiferous tubules exposed at 39°C for 1h followed by heat shock (44°C for 1h) in the absence of aspirin (**lane 2**) or in the presence of 6 mM aspirin (**lane 4**). Panels with ribosomal RNAs show the equalization of RNA samples used for analyses.

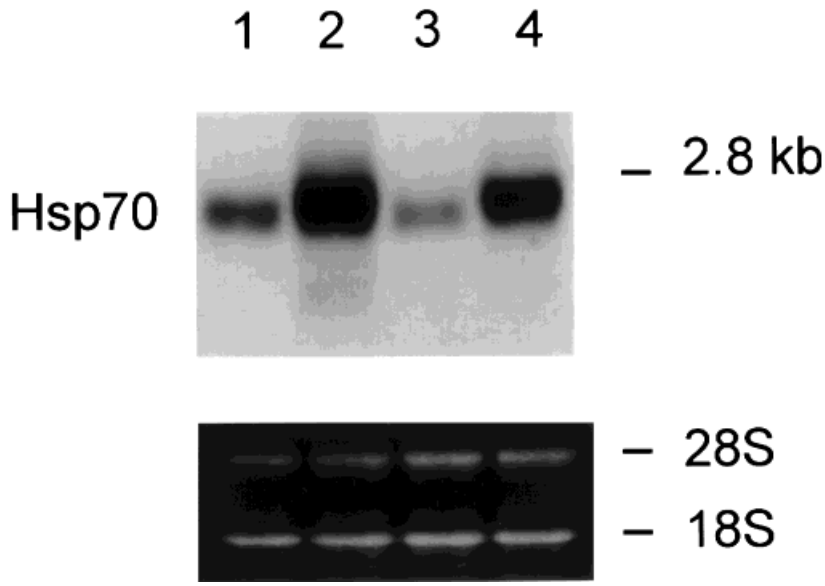

Fig. 7. Effect of aspirin on the expression of Hsp70 mRNA in mature chicken testis. Northern blotting analysis of Hsp70 mRNA obtained from the seminiferous tubules incubated at 39°C for 2 h without aspirin (**lane 1**) and in the presence of 10 mM aspirin (**lane 3**). Hsp70 mRNA extracted from seminiferous tubules exposed to heat shock (46°C for 2 h) in the absence of aspirin (**lane 2**) or in the presence of 10 mM aspirin (**lane 4**). Panels with ribosomal RNAs show the equalization of RNA samples used for analyses.

introns of the Hsc70 gene, the constitutive form of Hsp70 [Leverette et al., 1992; Liu and Maxwell, 1990]. Both, snoRNA U14 and Hsc70, are overexpressed in a heat shock resistant Chinese hamster cell line in normal growing conditions and after heat shock [Chen et al., 1996]. In Xenopus laevis, U14, localized within introns of the amphibian Hsc70 gene and ribosomal protein S13, is expressed during early oogenesis [Xia et al., 1995]. We do not know at present whether co-expression of Hsp70 mRNA containing 18S rRNA sequences and snoRNAs occurs during spermatogenesis or after heat shock. Further studies are necessary to shed light on the possible role that the expression of these molecules could play in the characteristic thermotolerance of avian spermatogenesis [Mezquita et al., 1998a].

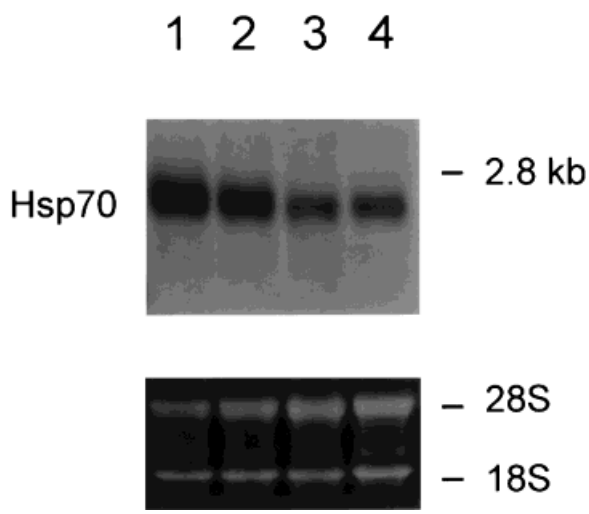

Fig. 8. Effect of aspirin on the expression of Hsp70 mRNA in mature chicken testis. Northern blotting analysis of Hsp70 mRNA obtained from the seminiferous tubules incubated at 46°C for 2h without aspirin (**lane 1**) and in the presence of 3 mM aspirin (**lane 2**), 6 mM aspirin (**lane 3**) and 10 mM aspirin (**lane 4**). Panels with ribosomal RNAs show the equalization of RNA samples used for analyses.

Expression of Hsp70 After Testicular Regression Induced by DES Treatment

Administration of the estrogen agonist DES to roosters induced a drastic atrophy of the testes similar to the changes described in Syrian hamsters upon DES treatment [Nonclercq et al., 1996]. In this model DNA fragmentation evolved in parallel with the reduction of testis weight and cell loss in seminiferous epithelium. Since Hsp70 plays an essential role interfering apoptotic pathways by several mechanisms, a decrease in the expression of the antiapoptotic protein Hsp70 upon DES treatment may increase the vulnerability of testicular cells to apoptosis. We have proposed that the expression and polyadenylation of Hsp70 induced by heat shock in adult chicken testis may contribute to the development of thermotolerance during avian spermatogenesis [Mezquita et al., 1998a].

Upon treatment with DES, in addition to a lower expression of Hsp70, we observed that the

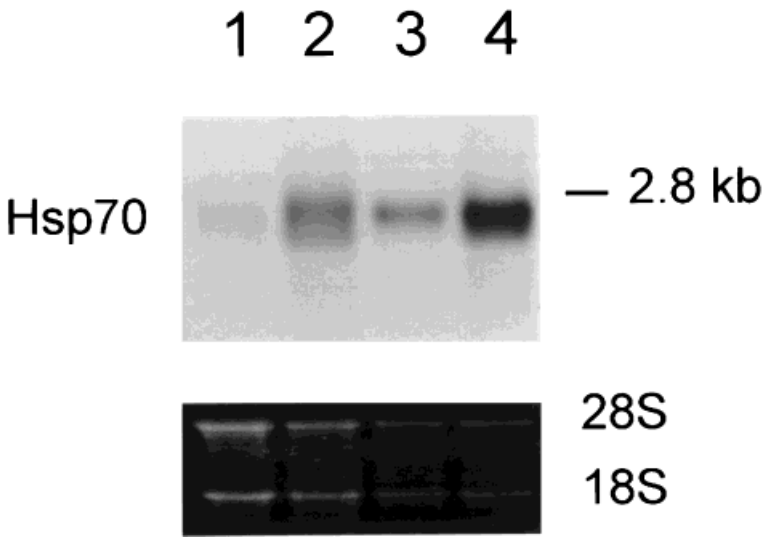

Fig. 9. Effect of aspirin on the expression of Hsp70 mRNA in chicken liver cells incubated in vitro at 39°C and 44°C for 2 h. Northern blotting analysis of Hsp70 mRNA obtained from liver cells incubated at 39°C without aspirin (**lane 1**) and in the presence of 10 mM aspirin (**lane 3**). Hsp70 mRNA extracted from liver cells incubated a 44°C in the absence (**lane 2**) or in the presence (**lane 4**) of 10 mM aspirin. Panels with ribosomal RNAs show the equalization of RNA samples used for analyses.

induction of Hsp70 occurred in vitro at lower temperatures (39°C) than in normal adult testis (44°C). A lower temperature of heat shock activation has been reported in mammalian male germ cells [Sarge, 1995] and also in the poikilotherm Rainbow trout [Le Goff and Michel, 1999] and this feature has been related to the particular vulnerability to heat shock of male germ cells. One possible explanation for the observed vulnerability is the arrest of spermatogenesis produced by the activation of the heat shock factor 1 (HSF1) [Nakai et al., 2000].

Effect of Aspirin on the Expression of Hsp70

Interestingly, the induction of Hsp70 at 39°C can be completely inhibited in the presence of 6 mM aspirin. Although it has been reported that aspirin treatment results in enhanced and prolonged transcription of Hsp70 in several tissues [Fawcett et al., 1997], as we have observed in chicken liver cells incubated in vitro at 39°C or 44°C, surprisingly, in other systems no evidence for increased Hsp70 gene transcription has been found [Giardina and Lis, 1995; Jurivich et al., 1995; Jurivich et al., 1992; Winegarden et al., 1996]. In these systems sodium salicylate induces the DNA binding activity of HSF1 but fails to stimulate the HSF1-mediated transcription. This is a similar situation to that observed in trout germ cells where the expression of the Hsp70 gene does not increase after HSF1 activation at low temperature [Le Goff and Michel, 1999]. Taking in consideration the proapoptotic effect of active HSF1 [Nakai et al., 2000] and the antiapoptotic effect of Hsp70, an increase in the relative proportion HSF1/Hsp70 would increase the vulnerability of male germ cells to apoptosis. Our results indicate that aspirin decreases the expression of Hsp70 in chicken testis cells incubated in vitro at 39°C or 46°C. This effect was particularly manifested when testicular cells exposed to DES treatment were incubated in vitro at 39°C. Our observations show for the first time that aspirin decreases the expression of Hsp70 in testicular cells and may increase the vulnerability of these cells to apoptosis. Evidence from the literature indicates that aspirin and salicylic acid exacerbated testis degeneration in vitamin E-deficient rats [Machlin et al., 1980].

Overexpression of Hsp70 during heat shock may protect cells from stress-induced apoptosis preventing the activation of pro-caspases 9 and 3 [Beere et al., 2000; Li et al., 2000]. In addition, release of cytochrome c from mitochondria was inhibited in cells expressing Hsp70 [Creagh et al., 2000; Mosser et al., 2000]. It has been shown in different cell lines that aspirin-induced apoptosis involves caspase activation through cytochrome c release from mitochondria [Pique et al., 2000]. The downregulation of Hsp70 expression in testicular cells by aspirin we describe here could contribute to a possible apoptotic effect of this molecule in testicular cells by increasing the release of cytochrome c from mitochondria. Further studies on this

mechanism are essential to establish a potential apoptotic effect of aspirin on the germinal cell line.

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