Expression of the Spermatid-Specific Hsp70 Antigen is Conserved in Mammals Including Marsupials

Naoki TSUNEKAWA1,2, Takao NISHIDA3 and Hirokazu FUJIMOTO1,4

1Mitsubishi Kasei Institute of Life Sciences, 11 Minamiooya, Machida, Tokyo 194-8511 and 2Laboratory of Anatomy and Physiology, College of Bioresource Sciences, Nihon University, 1866 Kameino, Fujisawa, Kanagawa 252-8510, Japan

(Received 11 September 1998/Accepted 1 December 1998)

ABSTRACT. The anatomical location of testes in mammals ranges from a location close to that observed in the embryo to a lower position usually involving a scrotum. In scrotal mammals, the abdominal position of the cryptorchid testis, which elevates its temperature, is detrimental to spermatogenesis and causes infertility. Spermatocytes are sensitive but late spermatids are relatively resistant to thermal stress suggesting that the latter might be protected in some way. In general, most organisms express Hsp70 proteins, which play a crucial role in the protection of cells against thermal stress. We have found previously that the Hsc70 protein, a member of the Hsp70 family of proteins, is constitutively expressed in the late spermatids of mice. Here, we have utilized immunohistochemistry with anti-mouse Hsc70 antiserum to examine the expression of the spermatid-specific Hsp70 antigen in the testes of several mammalian species with different degrees of testes migration. Our data indicate that the antigen is conserved in the mammals including marsupials. We also examined whether antigens of Hsp70-related proteins were expressed in non-mammalian vertebrates including not only homoiothermal but also poikilothermal animals. The spermatid-specific Hsp70 antigens were not detectable in the testes of the animals examined. From results of immunohistochemistry with BRM22 monoclonal antibody which reacts broadly with Hsp70 family proteins, however, we revealed constitutive expression of antigens of Hsp70-related proteins in spermatogenic cells of the vertebrates. These results suggest that the expression of spermatid-specific Hsp70 protein may be involved in the developmental pathway during spermiogenesis in mammals rather than in thermotolerance.—KEY WORDS: Hsp70, immunohistochemistry, mammal, spermatogenesis, testis migration.


In many mammals, the testes migrate caudally to some extent during fetal life. Carrick and Setchell [6] classified arbitrarily the degree of testicular migration. In extreme cases such as in Primates, Ruminantia and Marsupialia, the testes descend into the scrotum. In scrotal mammals testicular temperature is usually 2 to 7°C cooler than body temperature [47, 49]. If the testicular temperature is elevated, spermatogenesis ceases [46]. This can be made to occur either by increasing the environmental temperature, or by moving the testes into the abdominal cavity (cryptorchidism). Moore and Chase [31] and Fukui [12] demonstrated experimentally that the higher temperature of the abdominal cavity is responsible for the changes observed in cryptorchid testes. Quantitative analysis of changes to the seminiferous epithelium of rats exposed to local testicular heating [7, 8] have shown that among germ cells primary spermatocytes are the most sensitive to damage by heat and that late spermatids are relatively resistant. The mechanism underlying heat damage in testes, however, remains obscure.

Cells exposed to elevated temperatures respond by synthesizing heat shock proteins [25]. Heat can stimulate active cellular processes resulting in transient resistance to subsequent heat challenge, a phenomenon that has been called thermotolerance. Among the proteins whose synthesis is stimulated during heat shock, the most prominent and evolutionary well conserved is the 70 kDa heat shock protein (Hsp70) [26]. Some role of the Hsp70 in cellular response to heat injury is suggested by the temporal correlation between Hsp70 synthesis and the appearance of thermotolerance [22, 23, 43] and the increased sensitivity to heat when intracellular Hsp70 levels are reduced by anti-Hsp70 antibodies [38] or by competitive inhibition of Hsp70 mRNA [18].

Most eukaryotes have a large family of at least five Hsp70-related proteins [15, 16, 32]. A unique pattern of expression of Hsp70 has been revealed during mouse and rat spermatogenesis [2, 19, 20]. Mouse Hsp70-2 has been shown to be expressed at high levels during meiosis [41]. Extensive analyses using knock-out mutant mice of the Hsp70-2 gene revealed that Hsp70-2 is required for synaptosomal complex desynapsis [1, 9, 10, 51]. We have showed that another kind of Hsp70-related protein, Hsc70, is expressed in late spermatids during spermiogenesis [27, 29, 45]. The amino acid sequences of mouse Hsc70t gene and human orthologous gene HSPA1L are highly similar to those of heat-inducible Hsp70 genes, but these genes differ in the regulation of their expression [17, 30]. It is an interesting idea that constitutive expression of the spermatid-specific Hsp70 protein might be related to the heat resistance of late spermatids, but role and/or function of this protein is not clear at this time.

In this study, we have utilized immunohistochemistry with anti-mouse Hsc70t antiserum to examine for a relationship between the expression of the spermatid-specific Hsp70 protein in testes and the location of the testes in...
several mammals. We also report an evolutionary conservation of antigens of the Hsp70-related protein expressed during spermatogenesis in vertebrates.

MATERIALS AND METHODS

Animals and tissue preparation: Animals derived from several sources were used for this study as shown in Table 1.

Although the testes of the different animals were processed differently, the basic procedure involved fixation in Bouin's fixative (Sigma, St. Louis, MO) and mounting in paraffin wax (Paraplast plus, Oxford labware, St. Louis, MO) for histological examination. The marmoset was an exception as the testes were fixed in 4% paraformaldehyde. The blue-white dolphin was another exception as the testis had been immersed for a long time in formalin fixative. To accommodate for this, a piece of the testis was dissected and fixed again in Bouin's fixative. The testes of these animals and habu-snake were transferred as paraffin blocks from other laboratories.

The testes of pig and horse were dissected and fixed by vascular perfusion of Bouin's fixative. The mouse, musk shrew and domestic fowl were perfused with Bouin's

<table>
<thead>
<tr>
<th>Class</th>
<th>Order</th>
<th>Species</th>
<th>Common name</th>
<th>Position of testes</th>
<th>Tissue preparation for paraffin blocks</th>
<th>Suppliers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammalia</td>
<td>Cetacea</td>
<td>Stenella coeruleoalba</td>
<td>Blue-white dolphin</td>
<td>2</td>
<td>P (1)</td>
<td>Dr. F. Sasaki and Mr. Y. Matsumoto</td>
</tr>
<tr>
<td></td>
<td>Insectivora</td>
<td>Suncus marinus</td>
<td>Musk shrew</td>
<td>4</td>
<td>A (5)</td>
<td>Dr. G. Iosomura</td>
</tr>
<tr>
<td></td>
<td>Rodentia</td>
<td>Mus musculus</td>
<td>Mouse</td>
<td>5</td>
<td>A (10)</td>
<td>Dr. B. F. Tesh</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Zygogonyx breviceae</td>
<td>Cane mouse</td>
<td>5</td>
<td>E (2)</td>
<td>Yale University</td>
</tr>
<tr>
<td></td>
<td>Chiroptera</td>
<td>Pteropus tonganus</td>
<td>Tongan flying fox (Fruit bat)</td>
<td>5</td>
<td>A (1)</td>
<td>Dr. P. Manuuelie</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Equus caballus</td>
<td>Horse</td>
<td>5</td>
<td>A (4)</td>
<td>Animal Health and Production Division, Ministry of Primary Industry, Fiji</td>
</tr>
<tr>
<td></td>
<td>Artiodactyla</td>
<td>Sus domestica</td>
<td>Pig</td>
<td>5</td>
<td>A (2)</td>
<td>Dr. T. Arai</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bos primigenius</td>
<td>Cattle</td>
<td>6</td>
<td>E (2)</td>
<td>Ibaraki Prefectural Experiment Station of Swine Industry</td>
</tr>
<tr>
<td></td>
<td>Primates</td>
<td>Callithrix jacchus</td>
<td>Marmoset</td>
<td>6</td>
<td>P (1)</td>
<td>Dr. K. Hamano</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Macaca fascata</td>
<td>Japanese monkey</td>
<td>6</td>
<td>E (1)</td>
<td>Livestock Improvement Association of Japan, Maebashi Institute</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Homo sapiens</td>
<td>Man</td>
<td>6</td>
<td>E (1)</td>
<td>Dr. Y. Kurata</td>
</tr>
<tr>
<td></td>
<td>Marsupialia</td>
<td>Trichosurus vulpecula</td>
<td>Brush-tailed possum</td>
<td>6</td>
<td>E (2)</td>
<td>Mitsubishi Chemical Safety Institute</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sminthopsis crassicaudata</td>
<td>Dunnart</td>
<td>6</td>
<td>E (2)</td>
<td>Drs. K. Shimizu</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Primate Research Institute, Kyoto University</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Drs. E. Kanazawa and M. Matsuno</td>
</tr>
<tr>
<td></td>
<td>Aves</td>
<td>Gallus domesticus</td>
<td>Domestic fowl</td>
<td>A (10)</td>
<td></td>
<td>Nihon University School of Dentistry at Matsudo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Coturnix coturnix Japanica</td>
<td>Japanese quail</td>
<td>A (2)</td>
<td></td>
<td>Dr. W. G. Breed University of Adelaide</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trimedius flavovoridis</td>
<td>Habu-snake</td>
<td>P (1)</td>
<td></td>
<td>Dr. W. G. Breed University of Adelaide</td>
</tr>
<tr>
<td></td>
<td>Lepidosauria</td>
<td>Squamata</td>
<td></td>
<td></td>
<td></td>
<td>Laboratory stock</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Laboratory stock</td>
</tr>
<tr>
<td></td>
<td>Reptilia</td>
<td>Pelodiscus sinensis</td>
<td>Chinese soft-shelled turtle</td>
<td>A (1)</td>
<td></td>
<td>Local breeder</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anura</td>
<td>African clawed frog</td>
<td>A (1)</td>
<td></td>
<td>Local breeder</td>
</tr>
<tr>
<td></td>
<td>Amphibia</td>
<td>Xenopus laevis</td>
<td>Medaka fish</td>
<td>A (3)</td>
<td></td>
<td>Laboratory stock</td>
</tr>
<tr>
<td></td>
<td>Osteichthyes</td>
<td>Cyprinodontida</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a) Types of testis position of mammals classified by Carrick and Setchell [6]
b) P: Paraffin blocks were transferred from other laboratories. A: Testes were freshly processed for fixation in our laboratory. E: Testes in 70% ethanol were transferred after fixation in other laboratory. Numbers in parentheses show a number of animals used for preparation of the testes.
c) We wish to thank persons for providing samples of the testes.
fixative when anesthetized with pentobarbital sodium solution. Then pieces of testes removed from these animals were kept in the same fixative for 4 hr. The testes dissected from Tongan flying fox, Japanese quail, Chinese soft-shelled turtle and African clawed frog were directly immersed in Bouin’s fixative for 4 hr. In the case of medaka fish, the whole body was immersed in Bouin’s fixative after ventral dissection. These fixed samples were rinsed in 70% ethanol overnight and processed for embedding in paraffin wax. In the case of man, Japanese monkey, cattle, cane mouse, brush-tailed possum and dunnart, the testes in 70% ethanol were transferred to our laboratory after fixation in Bouin’s fixative.

Antibody: The rabbit anti-mouse Hsc70t antiserum was prepared as described in our previous study [28, 45]. Amino acid sequences of different Hsc70 proteins were closely similar, but vary much more in the carboxyl terminal than the amino terminal part [32]. Thus, the carboxyl terminal fragment of the Hsc70t protein was used as antigens for preparation of antisera. A 1.1 kb Pst I fragment of the pHS2 cDNA clone [27], which encodes a part of the carboxyl terminal region (377-641) of mouse Hsc70t, was subcloned in frame into the pPUR292 lacZ expression vector. The electrophoretically purified fusion protein was injected subcutaneously into rabbits every two weeks. After five immunizations, the blood was collected for preparation of anti-mouse Hsc70t antisera. Two dimensional polyacrylamide gel electrophoresis and Western blot analyses revealed that this antiserum reacted with the Hsc70t strongly and also weakly with Hsp70-2 and Hsc70. Immunohistochemical analyses using this antiserum indicated that the antigen was present in the elongated spermatids of the testes of mice.

Mouse monoclonal antibody BRM-22 (Sigma, St. Louis, MO) was used to recognize members of the Hsp70 family of proteins. The BRM-22 antibody recognizes at least five Hsp70-related proteins including both Hsp70-2 and Hsc70 [1]. Our previous study has shown that this antibody also recognizes the Hsc70t protein on Western blot analyses [45].

Immunohistochemistry: Immunohistochemical staining was carried out by the avidin-biotin technique on 6-7 μm paraffin sections. Deparaffinized serial sections were treated with 0.3% hydrogen peroxide (H₂O₂) for 30 min to eliminate endogenous peroxidase activity. After washing in phosphate-buffered saline (PBS; pH 7.4), the sections were incubated with blocking solution containing 3% bovine serum albumin in PBS for 90 min. The sections were incubated overnight at 4°C with anti-Hsc70t antiserum at a dilution of 1:1,500 or with anti-Hsp70 monoclonal antibody (BRM-22) at a dilution of 1:1,000. They were then washed five times with PBS and incubated for 90 min with biotinylated goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA) or goat anti-mouse IgG antibody (Tago, Burlingame, CA) at a dilution of 1:200. After washing with PBS, the sections were incubated with avidin-biotin-peroxidase complex (Vector Laboratories, Burlingame, CA) for 40 min. Peroxidase activity was visualized following a 5 to 10 min incubation in medium containing 0.05% 3,3'-diaminobenzidine, 0.01% H₂O₂ in PBS. Samples were rinsed in distilled water and counterstained with hematoxylin, dehydrated, and mounted in Eukitt (O. Kindler, Germany).

In the case of sections not showing positive signals by the ordinary method, a specific antigen retrieval protocol based on heating sections in 0.01 M citrate buffer (pH 6.0) in a microwave oven was performed before H₂O₂ treatment for elimination of the endogenous peroxidase activity [42].

Generation of images: All photographs were taken on an Olympus AX80 photomicroscope. Images were captured on photographic prints, scanned and stored on a computer. All images were processed by Adobe Photoshop 4.0 software package (Adobe Systems, San Jose, CA) and reflected the original image as closely as possible.

RESULTS

Hsc70t antigen was detected in the spermatids of mammals including marsupials: The development of spermatids in the mouse can be divided into 16 steps based on acrosome and nuclear morphology [35]. Clear immunological reactivity with the anti-Hsc70t antiserum appeared between steps 11 and 12 of spermatid development and strong reactivity of the cytoplasm of the spermatids persisted through step 13 to the final step 16 [45]. As shown in Fig. 1, the immunological reaction pattern in all the mammalian testes examined was basically the same as that observed in the mouse testis, that is, major signals were observed in the late spermatid layer.

According to the classification of Carrick and Setchell [6], the mammalian testes can be divided into 6 types according to their position in the body as follows. Type 1: testes that remain just behind the kidneys. Type 2: testes that migrate to lie at the posterior end of the abdominal cavity. Type 3: testes that migrate to, or just through the ventral abdominal wall. Type 4: testes that migrate near the base of the tail. Type 5: testes that descend into a non-pendulous scrotum underneath the anus. Type 6: testes that descend into an obvious pendulous scrotum with a distinct neck. On the basis of these anatomical classifications, several mammalian species were selected to examine the expression pattern of the Hsc70t antigen.

As examples of type 5 testes such as observed in the mouse, we selected cane mouse, pig, Tongan flying fox and horse. In the testes of these animals, the antigen was detected in the cytoplasm of the elongated spermatids (Fig. 1a-d). Cattle, marmoset, Japanese monkey and man were selected as examples of animals having type 6 testes. In these cases as well, strong immuno-staining was also detected in the cytoplasm of the elongated spermatids (Fig. 1e-h).

As an example of a type 4 testes, the testis of a musk shrew was stained with the anti-Hsc70t antiserum. The development of the spermatid of the musk shrew can be divided into 13 steps [21]. In this species, the cytoplasm of
Fig. 1.

Fig. 2.
the spermatocytes was also stained in addition to that of the elongated spermatids (Fig. 1f). The expression of the antigen was precisely examined with reference to this staging table. Strong immunostaining was observed in step 8–13 spermatids. Weak signals were detected in step 6 and 7 spermatids. Clear staining was also detected in late pachytene spermatocytes.

In the blue-white dolphin selected as an animal with type 2 tests, no part of the testis section could be stained by the normal process of immunohistochemistry. The antigen, however, became detectable in the elongated spermatid layer after the antigen retrieval procedure (Fig. 1g). There is a possibility that some of the antigen epitopes were masked by the prolonged immersion in formalin fixative. Although the signal was week, the cytoplasm of the late spermatids of the blue-white dolphin was nonetheless positively stained by anti-Hsc70t antisera.

Carrick and Setchell [6] classified the position of the tests of marsupials as type 6. Thus, we selected brush-tailed possum and dunnart as examples of animals with this type of tests which are located in the scrotum. In the tests of these animals, the elongated spermatids were strongly stained as well as weak staining of the round spermatids (Fig. 1k-l). The signal in the round spermatid was weak, but stronger compared to that of other mammals, except for the musk shrew. The nuclei and acrosomes of germ cells were stained in some sections, but these reactions were also detected when normal rabbit serum was used as the first antibody (data not shown). These non-specific stainings may have been caused by prolonged immersion of the tests in 70% ethanol during sample transportation.

**Non-mammalian vertebrates did not express the Hsc70t antigen, but expressed antigens of other Hsp70 family proteins in their spermatogenic cells:** The tests of non-mammalian vertebrates (domestic fowl, Japanese quail, habu-snake, Chinese soft-shelled turtle, African clawed frog and medaka fish) were also immunostained with the anti-Hsc70t antisera to examine the distribution of the antigen. We could not detect any immunostaining of sections from these tests at a 1:1,500 dilution of the anti-Hsc70t antisera (Fig. 1m-p). Only weak staining of the spermatogenic cells could be detected using high concentrations of the antisera, suggesting that such staining might be a cross reaction with other Hsp70 family proteins (data not shown). The use of the antigen retrieval protocol, however, did not further enhance the staining intensity. Thus, the Hsc70t antigen could not be detected in non-mammalian testes examined.

We used a mouse anti-Hsp70 monoclonal antibody, BRM-22, which reacts broadly with Hsp70 family proteins to examine whether other proteins of the Hsp70 family were expressed in the testes of non-mammals. Figure 2a and b show testes of mouse and pig immunostained with BRM-22, respectively. In contrast with the anti-Hsc70t antisera, this antibody stained most of spermatogenic cells in addition to the late spermatogenic cells, but not interstitial cells. This staining pattern was also observed in the testes of other mammalian species including marsupials (data not shown). Figure 2c and d show testes of domestic fowl and Japanese quail immunostained with BRM-22, respectively. Spermatogenic cell types in sections of the testes of birds were classified according to the description of Tiba et al. [44] and Lin et al. [24] for domestic fowl and Japanese quail, respectively. We found that immunoreactive signals with this antibody were detected in the cytoplasm of all the kinds of spermatogenic cells in both birds, but not in the interstitial cells.

In the other non-mammalian vertebrates, habu-snake, Chinese soft-shelled turtle, African clawed frog and medaka fish, the spermatogenic cells were also immunoreactive. In reptiles, habu-snake and Chinese soft-shelled turtle, antigens were detected in the cytoplasm of their spermatogenic cells like in birds (Fig. 2e-f). In the amphibian, the African clawed frog, antigens were detected strongly in the cytoplasm of the round and elongated spermatids (Fig. 2g). Positive signals were also observed in the cysts of spermatocytes and spermatogonia, but these signals were weaker than those of spermatids. In the fish, medaka fish, signals were observed in the cytoplasm of the spermatogenic cells of each cyst (Fig. 2h).

**DISCUSSION**

We have demonstrated that the cytoplasm of elongated spermatids of mammalian testes is immunoreactive for antibodies raised against the mouse Hsc70t protein. This suggests that orthologous genes of the mouse Hsc70t gene are widely conserved in mammals including marsupials. The mouse Hsc70t gene and orthologous genes of the rat
and man have been physically mapped to the MHC class III region with linkage to two heat-inducible Hsp70 genes, suggesting that the Hsp70 gene expressed in spermatids may be the result of gene duplication of the heat-inducible Hsp70 gene [17, 30, 48]. The swine MHC region also comprises three closely linked Hsp70 loci which have been mapped to the class III region [34, 35, 50]. Linkage of the Hsp70 gene with MHC has also been reported in the goat and cattle [5, 13]. Thus, a degree of conservation of the Hsp70 genes located in MHC class III region is apparent among mammalian species and one of the Hsp70 genes could express specifically in the spermatid.

From an anatomical point of view, Carrick and Setchell [6] classified the position of the testes in mammals into 6 types. The temperature is appreciably lower than body temperature only in species with type 5 and type 6 testes, that is, rat, rabbit, dog, pig, sheep, cattle, man, monkey and marsupials. Carrick and Setchell [6] cited data that the body/testis temperature differential was 0.7°C for the European hedgehog with type 3 testes. During spermatogenesis, however, the testicular temperature of hedgehogs was significantly lower than body temperature and similar to that of many scrotal mammals [11]. Anatomical evidence has shown that cetaceans such as dolphins with cryptic testes possess a vascular counter current heat exchanger [37, 39, 40]. Spermatic arteries in the posterior abdomen are juxtaposed to veins returning cooled blood from the surfaces of the dorsal fin and tail flukes. Colonic temperatures adjacent to the countercurrent heat exchanger were maximally 1.3°C cooler than temperatures measured outside this region. Cooled blood may be introduced into the deep abdominal cavity and function specifically to regulate the temperature of the arterial blood flow to the dolphin testes. Another case of an ascrctal mammal is the musk shrew with type 3 testes, which lacks a pampiniform plexus, a structure of the countercurrent heat exchanger in scrotal mammals. In this species, testicular temperature is 1.3°C lower than body temperature [14]. The data presented here clearly showed that the spermatid-specific Hsp70 antigen was expressed in the cytoplasm of the testes of the dolphin and the musk shrew, and was similar to that seen in scrotal species. An interesting exception to scrotal mammals is provided by the cane mouse living in the tropics. All of the scrotal mammals tested to date have been rendered infertile by cryptorchidism [3]. In contrast, the cane mouse is the first scrotal mammal found to be little affected by cryptorchidism [4]. This exception seems to be a necessary adaptation developed to maintain fertility in the tropics, where heat resistance must be a critical survival factor. In this species, our study showed that the expression pattern of the Hsp70 antigen detected by anti-Hsc70t antiserum was not different from that of the other scrotal species.

Different expression pattern of the Hsp70 antigen in the testis of the mammals examined was observed in the musk shrew, blushed-tailed possum and dunnart. We detected the Hsp70 antigen in the late pachytene spermatocyte and in the round spermatid in addition to the elongated spermatid of the musk shrew. We also detected the antigen in the round spermatids in addition to the elongated spermatids of the brush-tailed possum and dunnart. The musk shrew is of special interest as an example of a primitive eutherian mammal. Our findings suggest that marsupials and primitive eutherian mammals continue to express the Hsp70 during spermatogenesis in a primitive style different from the expression pattern of other mammals. Our previous study, however, indicated that the anti-Hsc70t antiserum reacts faintly with the Hsp70-2 protein expressed in spermatocytes during mouse spermatogenesis [45]. Thus, there is a possibility that different pattern of the Hsc70t antigen expression in these species may be caused by masking of epitopes of the different Hsp70-related molecules. This possibility remains to be examined using immunoblotting analyses of proteins isolated from testicular germ cells of these animals.

We also examined the evolutionary conservation of the Hsc70t antigen in the testes of other vertebrates. The antigen recognized by the anti-Hsc70t antiserum was not detectable in the testes of non-mammalian vertebrates examined. We can not exclude a possibility that epitopes of Hsp70 antigens are masked in the testes of these species. The present results with the BRM-22 monoclonal antibody, however, add to our knowledge about the kinds of Hsp70 family proteins constitutively expressed in the spermatogenic cells of the testis of non-mammals, including not only homoiothermal but also poikilothermal animals. In the testes of the mouse, spermatocytes and spermatids which express Hsp70-2 and Hsc70t, respectively were immunohistochemically stained by the BRM-22. Although our study has not made clear the precise identity of the Hsp70 family proteins expressed in the testes of non-mammalian vertebrates, the germ cells of vertebrates require expression of the Hsp70 family of proteins during spermatogenesis.

Thus, we could detect no obvious correlation between the degree of testis migration and the expression of the antigen detectable by anti-Hsc70t antiserum. Our finding suggests that the expression of spermatid-specific Hsp70 protein may be involved in the developmental pathway during spermiogenesis in mammals rather than in thermotolerance.

ACKNOWLEDGMENTS. We wish to thank the people for providing samples of animal testes. We are also grateful to Ms. A. Tokumasu for her technical assistance.

REFERENCES


