

**EXPRESSION OF HSP84 AND HSP86 IN CRYPTIC TESTES
OF BOTTLENOSE DOLPHIN, *TURSIOPS TRUNCATUS*
AND RISSO'S DOLPHIN, *GRAMPUS GRISEUS***

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Introduction

Cetaceans (whales, dolphins, and porpoises) have cryptic testes that lie within the abdominal cavity [13,16]. The abdominal position suggests that they are exposed to core body temperature [7,13,14]. In many species, testis temperature must be precisely maintained at a temperature significantly below that of tissues in the main body cavity [15]. In mice, testis temperature is approximately 30 °C, while tissues in the main body cavity are maintained at approximately 37 °C [6]. The importance of precise thermoregulation in testis is evidenced by the fact that even slight elevations in temperature can have a significant inhibitory effect on spermatogenic cell function, and are a major cause of male infertility [8,12,15,17]. Cetaceans lack a physical separation between the testes and the body core, and they lack a pampiniform plexus [6]. This suggests that cetaceans must either be immune to high-temperature effects on sperm viability like hyraxes and elephants [5,9] or they must possess a novel mechanism for controlling the temperature at the testis, as do some phocid seals [13]. It has remained a question as to how dolphins produce and store sperm with intraabdominal testes [13,14]. In this study, we have observed the hsp86 and hsp84 localization by immunohistochemistry. Hsps (Heat Shock Proteins) are known as stressed proteins and have been shown to be induced as a result of a moderate increase in the environmental temperature [2]. Sarge and Cullen (1997) have reported that the expression of heat shock proteins is a highly regulated event throughout the process of spermatogenesis[15]. In this study, we observed the significant expression of hsp86 and hsp84 in goat and dolphin testes. In addition, the function of hsp90 in the differentiation of spermatogenic cell is discussed.

Methods and Materials

Animals and Tissue Preparations:

We collect the samples from two different dolphin species. One is a bottlenose dolphin;

Tursiops truncatus and the other is a Risso's dolphin; *Grampus griseus*. The dolphin testes used in this study were taken at Taiji, Wakayama prefecture, Japan. Bottlenose dolphin's testes were fixed with Bouin's solution, while Risso's dolphin's testes were fixed with 10% formalin. Both of the fixed tissues were dehydrated in a graded series of ethanol, filtrated in xylene, and embedded in paraffin. They were sectioned at 4 μm . The goat testes used in this study were taken at Stock Farm, The University of Tokyo. Testes were fixed with Bouin's solution. As above mentioned, the fixed tissues were embedded in paraffin and sectioned at 4 μm .

Immunohistochemistry:

Deparaffinized sections were blocked with blocking solution containing 20% normal goat serum (Sigma; Louis, MO, USA), 1% BSA, and PBS for 1 hr at RT to minimize nonspecific staining. After rinsing with PBS, the sections were incubated for 1hr at RT with 10 $\mu\text{g/ml}$ rabbit anti-hsp84 (Neo Markers; Funakoshi, Japan). IgG suspended in PBS containing 0.1% BSA (BSA/PBS). They were then washed three times with PBS and incubated overnight at RT with 36 $\mu\text{g/ml}$ biotinylated goat anti-rabbit IgG (Tago; Burlingame, CA, USA) suspended in BSA/PBS. After washing with PBS, the sections were incubated with avidin-biotin-peroxidase complex (Vector, Burlingame, CA, USA) for 30 min. After washing with PBS, 0.05% 3,3'-diaminobenzidine tetrahydrochloride (Sigma) and 0.01% H_2O_2 in PBS were applied to the sections for 3min to develop the peroxidase reaction. The same procedures have been done in hsp86. HE staining have applied to observe the basic structure of the testis. The sections were observed by light microscopy.

Results

Goat:

Strong immunoreactivities with anti-hsp84 and anti-hsp86 was shown in the cytoplasm of spermatogenic cells. Hsp84 was expressed in the B spermatogonia. Hsp86 was expressed at high levels in spermatocytes, round spermatids, but not in elongate spermatids.

Bottlenosed dolphin:

Hsp86 was present throughout the germ cell lineage. Strong reaction was shown in type B spermatogonia, spermatocytes and round spermatids. The expression of hsp86 was found in leptotene and preleptotene spermatocytes but not in pachytene spermatocytes. On the other hand, hsp84 was strongly expressed in type B spermatogonia, whereas no reaction was detected in spermatids and Sertoli cells.

Risso's dolphin:

Hsp86 was expressed in pachytene and preleptotene spermatocytes. Hsp84 reacted in type B spermatogonia. Since Risso's dolphin's testis was fixed with 10% formalin, the reaction of immunohistochemistry was very weak and it was difficult to identify in detail.

Discussion

Prokaryotic and eukaryotic organisms synthesize a distinct set of proteins in response to exogenous stress. These proteins are known as Heat Shock Proteins. Hsps are also expressed in response to other stresses including cold shock, ionizing radiation, anoxia, heavy metals and viral infections [10,11,13,14]. In addition to their stress-related cytoprotective function, hsps are also involved in many normal cellular processes [15]. In this study, we used hsp86 and hsp84, because they are two main proteins belonging to the hsp90 family that are expressed in cells of the mouse testis [15]. Observation of expression of hsp86 and hsp84 in the dolphin testes were quite similar to that of mouse testes [4]. In mouse testes, hsp 86 was expressed at high levels in pachytene spermatocytes, round spermatids, cytoplasmic fragments and residual bodies [4]. In contrast to hsp86, hsp84 in mouse testes was expressed at highest levels in prepuberal testis suggesting that hsp84 was expressed primarily in somatic cells of the testis [4]. In recent studies, hsp90 has been shown to interact with steroid hormone receptors and to be important for the formation of the functional forms of these receptors [1,11,15]. Therefore, it is likely that regulated expression of hsp90 proteins in cells of the testis may be required for the proper regulation of testicular cell functions by steroid hormones such as testosterone [15]. It has also been demonstrated that expression of hsp90 itself can be modulated by steroid hormones such as estrogen [3]. Little is known about male reproductive organ of cetaceans, because of limited access to these animals. It remains unclear how they maintain their testes under these conditions. Slijper (1966) used a low body temperature of 35.5°C saying that spermatogenesis would not be blocked at the cryptic testis of cetaceans. Harrison (1948) suggested that cetaceans must be immune to high-temperature effects on sperm viability. Recent studies have shown the existence of a presumed countercurrent heat exchanger to maintain the testes under suitable conditions [3,4]. The reaction we found in this study may not give the straight answer to these questions, but the results we obtained here suggest that these proteins may play a role in the maintenance of cellular homeostasis. To find out, we need a further investigation.

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