Expression Pattern of the Mitochondrial Capsule Selenoprotein mRNA in the Mouse Testis after Puberty; in situ Hybridization Study

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ABSTRACT. Mitochondrial capsule selenoprotein (MCS) has been known as a structural protein of the mitochondrial sheath in spermatozoa. In this study, to determine the expression pattern of MCS mRNA in the mouse testis after puberty, in situ hybridization using digoxigenin-labeled RNA probes for MCS was performed in the testes of 8- and 20-week-old ICR mice. In the testes of both ages, MCS mRNA first appeared in step 3 round spermatids, gradually increased during early spermiogenesis, and persisted a high level until step 14 spermatids. After the step 14 spermatids, the signal began to decline and was weakly detected in steps 15–16 spermatids. On the other hand, compared with that in the testes of 8-week-old mice, MCS mRNA level in the testes of 20-week-old mice increased over 2-fold at stages VI-III, while it slightly increased at stages IV-V. These findings suggest that MCS gene transcription may be up-regulated after puberty in the mouse testis. — KEY WORDS: in situ hybridization, mitochondrial capsule selenoprotein, puberty, testis.

Selenium (Se) is an essential micronutrient for mammals. Since it was first demonstrated that Se is a structural component of the active center of the enzyme glutathione peroxidase (GSH-Px; EC 1.11.1.9.) [19], other selenoproteins have been identified using radiolabeled Se [2]. Among these, only 5 have been characterized in mammalian tissues as selenoproteins containing selenocysteine which is encoded by an in-frame UGA codon, formerly known as a stop codon [3, 8, 15, 20, 23].

Se has been known to play a part in the male reproductive system. In studies of animals fed a Se-deficient diet, the Se retention was highest in the male reproductive organs. Abnormal spermatozoa with impaired motility due to characteristic midpiece damage were frequently observed in the animals [4, 24, 25]. Tracer studies using 75Se demonstrated that Se is localized within the midpiece of spermatozoa and that it is bound to a polypeptide of 15,000 to 17,000 daltons confined to the mitochondrial sheath [5, 6, 14]. This molecule has been shown in the bull and rat to be a major component of the keratinous mitochondrial capsule [7, 18] and it has been characterized as a cysteine- and proline-rich selenoprotein [6, 17]. Recently, it was reported that the mitochondrial capsule selenoprotein (MCS) cloned from mouse testes is a structural protein of the mitochondrial sheath of spermatozoa [11, 12, 22]. Although it has been suggested that MCS is related to the formation of the mitochondrial sheath in spermatozoa, the exact role of MCS in testes is still controversial.

In our previous study using Northern blot analysis, we demonstrated that the MCS mRNA greatly increases after puberty in the mouse testis. The level in the testis of 80-week-old mouse increased about 6-fold as compared with that of 3-week-old mouse [16]. In this study, to determine the expression pattern of MCS mRNA in the mouse testis after puberty, we performed in situ hybridization using digoxigenin (DIG)-labeled RNA probes for MCS in the testes of 8- and 20-week-old mice.

MATERIALS AND METHODS

Experimental animals: Male ICR mice (3 weeks old) were purchased from Charles River, Japan Inc. (Yokohama). All animals were housed in the room where the temperature (22 ± 2°C), relative humidity (55 ± 10%) and light/dark cycles (14L:10D) were controlled. They received food pellets (CLEA Ltd., Tokyo, Japan) and water ad libitum. At 8 and 20 weeks of age, animals were sacrificed under pentobarbital anesthesia. For in situ hybridization studies, the testes were perfused with Bouin’s fixative, immersed in the same fixative for 12 to 24 hr and subsequently processed for sectioning.

In situ hybridization: RNA probes were synthesized by transcription of a pCRII plasmid (Invitrogen, Tokyo, Japan) containing the MCS cDNA (592 bp) [16] with Sp6 or T7 RNA polymerase after linearization with Hind III or Xho I.

Hybridization was performed on the 5 μm sections of testes. Deparaffinized sections were incubated in 10 μg/ml of proteinase K in phosphate buffered saline (PBS) at 37°C for 5 to 30 min and treated with 4% paraformaldehyde in PBS for 10 min, 0.2 M HCl for 10 min, and 0.25% acetic anhydride in 0.1 M triethanolamine (pH 8.0) for 15 min. Sections were prehybridized in 50% formamide (FA) plus 2-strength standard saline citrate (SSC) at 42°C for 30 min and hybridized at 42°C for 16 hr by adding DIG-labeled riboprobes in the following solution: 50% FA, 10 mM Tris-HCl (pH 7.6), 200 μg/ml yeast tRNA, 1-strength Denhardt’s reagent (the following component diluted with 500 μl of H2O: 0.1 g of Ficoll [Type 400; Pharmacia, Tokyo, Japan], 0.1 g of polyvinylpyrrolidone, and 0.1 g of bovine serum albumin), 10% dextran sulfate, 600 mM NaCl, 0.25% SDS,

and 1 mM EDTA (pH 8.0) in DEPC-treated water. These sections were rinsed in 50% FA/2-strength SSC at 42°C for 20 min 3 times and treated with 10 µg/ml of RNase A in buffer (10 mM Tris-HCl [pH 7.6], 500 mM NaCl, and 1 mM EDTA) at 37°C for 30 min. Sections were then washed in 2-strength SSC at 42°C for 20 min, followed by washing in 0.2-strength SSC at 42°C for 20 min twice, and processed with immunohistochemical procedures. Each was incubated with an anti-DIG alkaline phosphatase-conjugated antibody (Boehringer Mannheim, Tokyo, Japan) and developed with 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium (Boehringer Mannheim, Tokyo, Japan) in 0.1 M Tris buffer for 1 hr, respectively.

Signal intensity analysis: To determine the relative level of MCS mRNA in the testes of 8- and 20-week-old mice, the signal intensity was measured with a laser densitometer (Scanning Densitometer; BIO-RAD, Tokyo, Japan) from black and white negative films photographed under the same conditions on both of in situ analysis slides. An average of 6 seminiferous tubules in each stage grouping was randomly selected from the testes of both ages. The signal intensity in the seminiferous tubules was analyzed from the spermatocytes where the signal was not detected.

In the testes of 8- and 20-week-old mice, the analysis using a DIG-labeled sense riboprobe for MCS showed no signals over haploid cells or specific stages of tubules (data not shown).

RESULTS

In the testes of 8- and 20-week-old mice, the in situ analysis using a DIG-labeled antisense riboprobe for MCS showed that the MCS mRNA was concentrated in the upper region of the seminiferous epithelium (spermatids) and that the amount of signal varied greatly depending on the stage of the tubules (Fig. 1). At stage I, the MCS mRNA was not detected in the cytoplasm of step 1 spermatids, but was intensely expressed in the cytoplasm of step 13 spermatids (Fig. 2A). At stages II–III, the signal first appeared in the cytoplasm of step 3 spermatids and was intensely expressed in the cytoplasm of step 14 spermatids. At stages IV–VIII, the signal began to increase during early spermiogenesis and greatly increased in the cytoplasm of step 8 spermatids (Figs. 2B and 2C). At stages IX–XII, the signal attained a high level in the cytoplasm of steps 9–12 spermatids (Fig. 2D). No signal was observed in spermatogonia, spermatocytes, Sertoli cells, peritubular myoid cells, and interstitial cells. On the other hand, the signal in the testes of 20-week-old mice remarkably increased as compared with that in the testes of 8-week-old mice (Figs. 1 and 2).

To quantify the stage-dependent changes of MCS mRNA at both ages, the signal intensities were measured with a laser densitometer (see Materials and Methods). Compared with that in the testes of 8-week-old mice, the MCS mRNA level in 20-week-old mouse testes increased over 2-fold at stages VI–III, while it increased slightly at stages IV–V (Fig. 3).

In addition, the testes of both ages hybridized with a DIG-labeled sense riboprobe for MCS showed no signals over haploid cells or specific stages of tubules (data not shown).

DISCUSSION

Se is necessary for spermatogenesis in mammals and its concentration considerably increases in the testes during puberal maturation [1]. In tissues, Se has been known to be incorporated into a selenocysteine which recognizes specific UGA codons in selenoprotein [8]. Previously, we demonstrated that MCS mRNA greatly increases after puberty in the mouse testis [16]. In the present study, the expression of MCS mRNA changed stage-dependently and increased in the seminiferous epithelium of 20 weeks old as compared with that of 8 weeks old.

Previous studies showed that during spermatogenesis, Se is present in the form of selenoprotein and is localized in the outer membrane of spermatozoa mitochondria [5, 18]. Calvin et al. [7] reported that in rats, incorporation of 75Se into a selenoprotein begins in late primary spermatocytes, increases steadily during step 1–6 spermatids, is highest in steps 7–12, declines during steps 13–15, and is undetectable during steps 16–19. In the present study using a DIG-labeled riboprobe, MCS mRNA was expressed in step 3–16 spermatids of mouse testes, which was consistent with the results using a radiolabeled riboprobe [22]. These findings indicate that Se and MCS are necessary for spermatogenesis.

On the other hand, many studies [9, 10] have demonstrated that aging results from imperfect protection against tissue damages, particularly mitochondrial injuries, which were brought about by free radicals produced during normal aerobic metabolism. It has been also known that mitochondrial DNA is more susceptible to oxidative damage than nuclear DNA due to the lack of histones protecting the DNA and DNA repair enzymes, and the proximity of mitochondrial DNA to oxidants generated during oxidative phosphorylation [21]. Lee et al. [13] found that unlike muscle and liver, the deletion of mitochondrial DNA induced by aging in humans is undetectable in the testes until age 60. Furthermore, previous studies [1, 4, 25] have shown that the Se content in testes is especially high in Se-deficient animals as compared with that in main storage tissues such as muscle and liver, and considerably increases in maturing animals after the initiation of spermatogenesis. Previously, we demonstrated that MCS mRNA begins to increase in mouse testes according to aging and the level in the testes of 80-week-old mouse increases about 6-fold as compared with that of 3-week-old mouse. Additionally, its expression in the testes remarkably decreases by Se deficiency [16]. In this in situ analysis, the MCS mRNA was expressed in the cytoplasm of steps 3–16 spermatids.

During the period, remarkable changes of mitochondrial morphology occurs. Moreover, the expression level of MCS...
Fig. 1. *In situ* hybridization using a DIG-labeled antisense riboprobe for MCS in testes. The signals of MCS mRNA are concentrated in the upper region of the seminiferous epithelium (spermatids). Compared with that in the testes of 8-week-old mouse, MCS mRNA is highly expressed in the testes of 20-week-old mouse. A) 8 weeks old, B) 20 weeks old. × 80.
Fig. 2. *In situ* analysis using a DIG-labeled MCS riboprobe in 8- and 20-week-old mice testes. Compared with that in 8-week-old mouse testes, the expression of MCS mRNA in 20-week-old mouse testes greatly increases at stages I, VIII, and XII, while it slightly increases at stage V. A) stage I, B) stage V, C) stage VIII, D) stage XII. × 640.
INCREASE OF MCS mRNA IN TESTIS AFTER PUBERTY

mRNA in mouse testes differed considerably between 8- and 20-week-old mice. Compared with that in the testes of 8-week-old mice, the MCS mRNA level in the testes of 20-week-old mice increased over 2-fold at stages VI-III, while it slightly increased at stages IV-V. From these findings, it is conceivable that besides its involvement in the formation of the mitochondrial sheath in spermatozoa, the MCS may have an additional function (for example, antioxidant defense system) in mouse testes according to aging, as in other selenoproteins.

In conclusion, these findings suggest that MCS gene transcription may be up-regulated after puberty in the mouse spermaticogenic cells. To investigate the exact role of MCS in mammalian testes, more experiments would be necessary.

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REFERENCES


