known[11, 12]. If extracellular CaM initiation of pollen germination and tube growth is the case, the activation of extracellular CaM may be an early step for the above processes, making the relationship between extracellular CaM activity and Ca^{2+} influx interesting. Our preliminary data show that extracellular CaM may mediate Ca^{2+} influx by controlling the G-protein coupled calcium channel activity (data not shown), and further studies are underway to investigate the signal transduction pathways for extracellular CaM in the above processes.

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Localization and expression of TR3 orphan receptor protein and its mRNA in rat

MU Xiaomin and LIU Yixun

State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100080, China

Abstract The cellular localization and specific expression of TR3 mRNA in rat testis were investigated by the method of immunohistochemistry and *in situ* hybridization. It was demonstrated that orphan receptor TR3 was expressed in a significant amount in rat testis and TR3 protein and mRNA were specifically localized in germ cells, suggesting that TR3 may have an important function in regulating rat germ cell development.

Keywords: orphan receptor TR3, immunohistochemistry, *in situ* hybridization, spermatogenesis.

ORPHAN receptor is a category of receptor whose cognate ligand is still unknown. It belongs to nuclear receptor superfamily[1] including steroid, thyroid and vitamin D3 receptor. Large numbers of orphan receptors have been found so far. TR3 (also known as NGFIB, Nur 77) was originally identified as an immediate-early gene product. It is rapidly induced by a variety of stimuli including growth factors[2, 3]. The physiological function of TR3 is not completely known today. But it has been known that TR3 is the me-

diator of hormonal and neurological responses in the hyptothalamic-pituitary-adrenocortical axis[4], and it plays a key role in the control of activation-induced apoptosis of T-cell[5, 6], and it regulates expression of the gene encoding steroid 21-hydroxylase[7, 8]. Research work on TR3 at present is focusing on its genomic structure and the mechanism by which TR3 exerts its biological function[9, 10]. Little is known about localization and expression of TR3 and its mRNA in tissues. We investigated localization and expression of TR3 in rat testis by immunohistochemistry and *in situ* hybridization. Our results show that TR3 orphan receptor protein and its mRNA were expressed in a significant amount in rat testis. The orphan receptor TR3 protein was specifically localized in germ cells. Its mRNA was specifically expressed in germ cells, indicating TR3 may play an important role during spermatogenesis in rat.

1 Materials and methods

(1) Animals and reagents. Adult SD rats were purchased from Animal Breeding Department of Institute of Zoology, Chinese Academy of Sciences. Rat TR3 polyclonal antibody and cDNA plasmid were provided by Prof. Chawnshang Chang (University of Wisconsin, Madison, USA). Immunohistochemical kit was purchased from SABS (Beijing). Dig RNA labeling reagents (Dig RNA labeling kit) and detection reagents were purchased from Boehringer Mannheim (Beijing). T7, T3 RNA polymerase and restriction enzyme were purchased from Promega (Beijing).

(2) Immunohistochemistry. Freshly dissected rat testis was snap-frozen in liquid nitrogen and balanced to -20℃. Cryosections were cut and thaw-mounted on APES (Beijing Zhongshan Biotechnology) coated slides. Slides were air-dried and fixed by 4% paraformaldehyde in PBS for 10 min. Endogenous peroxidase activity was blocked in tissue section by treatment with 0.3% H_2O_2 for 10 min. Slides were then rinsed and blocked with normal goat serum to reduce nonspecific staining, incubated for 30 min at room temperature with a rabbit polyclonal antibody against rat TR3 diluted 1∶100 in PBS. Reagents A and B in immunohistochemistry kit were diluted into a tube together in PBS, mixed well, and reacted for 30 min at room temperature, thus A and B complex was formed. Slides were reacted for 30 min at room temperature with A + B complex. Slides were then washed with 0.5% Triton X-100/PBS (slides were rinsed with PBS for 3×5 min after each treatment). Slides were stained with substrate solution of peroxidase.

(3) Dig cRNA probe preparation. TR3 cDNA plasmid was digested by appropriate restriction enzyme. TR3 anti-sense cRNA probe was transcribed by T7 RNA polymerase, and TR3 sense cRNA probe was transcribed by T3 RNA polymerase from linearized cDNA templates using the digoxigenin RNA labeling kit described above according to the manufacturer's recommendation.

(4) *In situ* hybridization and detection. Twelve micrometer cryosections were cut as described above and mounted onto microscope slides coated with APES. Slides were then air-dried at 55℃ for 10 min, fixed in 4% formaldehyde in PBS for 20 min. After washing in PBS for 3×5 min, slides were digested in 0.2 mol/L HCl for 25 min, incubated in 0.3% Triton X-100/PBS for 15 min to demembranized, washed in PBS for 3×5 mim, post-fixed in 4% paraformaldehyde in PBS for 5 min, washed in PBS for 3×5 min, slides were then acetylated in 0.25% acetic anhydride in 0.1 mol/L triethanolamine for 15 min. Sections were pre-hybridized in pre-hybridization buffer [$2\times$ SSC, 50% formamide, 250 μg/mL yeast tRNA, $2\times$ Denhardts solution, 10 mol/L Tris. HCl(pH 7.5)] for 2 h at 40℃, and hybridized in pre-hybridization buffer with 400 ng/mL anti-sense dig-cRNA probe or sense dig-cRNA probe for 20 h at 45℃. After hybridization, slides were washed in different strengths of SSC solution in the following order: $2\times$, $1\times$, $0.2\times$ for 0.5 h at room temperature, individually, $0.1\times$ SSC for 2×15 min at 40℃. The hybridized probes were detected according to the manufacturer's recommendation. Slides were examined and recorded in pictures by a brightfield light microscope. Black staining represents positive reaction[11, 12].

2 Results and discussion

2.1 Localization of TR3 receptor protein in rat testis

The immunohistochemistry result shows that TR3 orphan receptor was specifically localized to germ cells (fig. 1). It was not found in sertoli cells and leydig cells.

2.2 TR3 mRNA localization in rat testis

In situ hybridization result shows that TR3 mRNA was specifically expressed in germ cells (fig. 2),

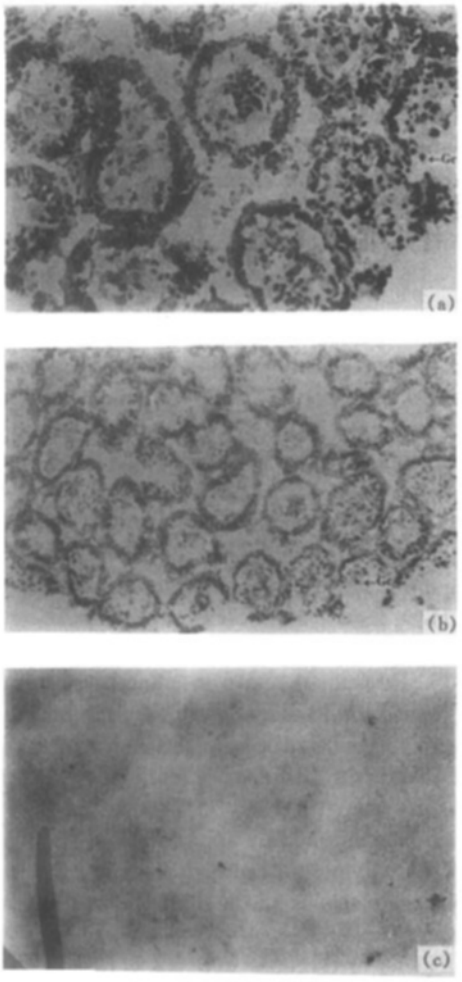

Fig. 1. Immunohistochemical localization of TR3 orphan receptor protein in rat testis. (a) Positive staining of TR3 receptor protein was specifically observed in germ cells (Gc, 100×); (b) difference in staining intensity was observed in different seminiferous tubules (40×); (c) positive staining was not observed with normal goat serum as antibody (control).

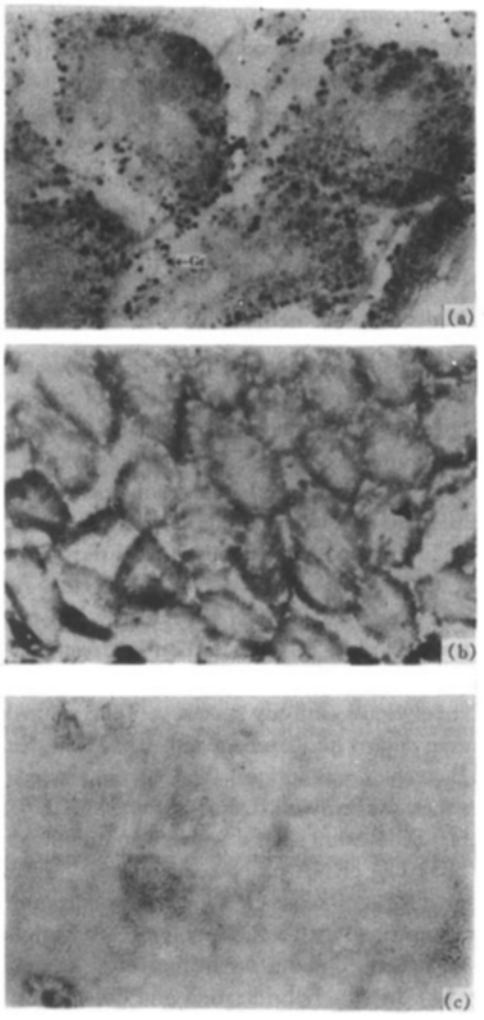

Fig. 2. *In situ* localization of TR3 mRNA in rat testis. (a) Positive staining for TR3 mRNA was observed specifically in germ cells (Gc, 100×); (b) difference in staining intensity was observed in different seminiferous tubules; (c) no detectable staining was observed when sense cRNA probe is used (control).

and TR3 mRNA was not found in sertoli cells and leydig cells.

In most mammals, spermatogenesis is a complicate cyclic physiological process. Spermatogenesis starts from the specific stage of individual development. It is a multiple cell differentiation process with multiple stages. Spermatogonia are transformed into spermatocytes through mitosis. Spermatocytes are transformed into round spermatids through meiosis. Round spermatids are transformed into mature spermatozoa through morphological change. Spermatogenesis is associated with a series of specific genes expression[13], it is of theoretical and practical importance to investigate the expression of these genes to demonstrate mechanism of spermatogenesis at molecular level. Orphan receptors are the highlight of the studying in biology of reproduction in recent years. They are members of nuclear receptor (steroid/thyroid hormone receptor) superfamily, and belong to transcription factor[1]. TR3 is an unusual member of orphan receptor since the expression of its gene is rapidly induced by diverse stimuli such as growth factor[2, 3]. It has been shown that TR3 is involved in many regulation process *in vivo*[9], especially in male

reproductive system. In this study we show that orphan receptor TR3 was expressed in a significant amount in rat testis at both protein and mRNA levels. TR3 receptor protein was specifically localized in germ cells, and its mRNA was specifically expressed in germ cells in rat. There are mainly three sorts of cells in testis: sertoli cells, leydig cells and germ cells. Sertoli cells support and nourish germ cells, leydig cells secrete testosterone and germ cells differentiate into spermatozoa. A series of genes which decide spermatogenesis mainly localize in germ cells and express in sequence. So the present study demonstrates that orphan receptor TR3 may play an important role during spermatogenesis.

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Extension of the rice DH population genetic map with microsatellite markers

XU Yunbi[1, 2, 3], SHEN Lishuang[1], Susan R. McCouch[2] and ZHU Lihuang[1]

1. Institute of Genetics, Chinese Academy of Sciences, Beijing 100101, China; 2. Department of Plant Breeding and Biometry, Cornell University, N. Y. 14853, USA; 3. Agronomy Department, Zhejiang Agricultural University, Hangzhou 310029, China

Abstract Genetic mapping of microsatellite markers was carried out in a rice DH population derived from a cross between Zaiyeqing 8 (indica) and Jingxi 17 (japonica). A total of 89 microsatellite markers, including 84 $(GA)_n$, 2$(TCT)_n$, 2$(ATT)_n$ and 1$(ATC)_n$ motifs, were integrated relatively evenly into the established genetic map of the DH population. This will facilitate the utilization of microsatillite markers in rice gene mapping and marker aided breeding.

Keywords: microsatellite marker, rice (*Oryza sativa* L.), doubled haploid population, genetic map.

MICROSATELLITE markers have been used extensively in the genetic mapping of humans and animals[1, 2].