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TAF4b, a TBP associated factor, is required for oocyte development and function

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Abstract

Development ′of a fertilizable oocyte is a complex process that relies on the precise temporal and spatial expression of specific genes in germ cells and in surrounding somatic cells. Since female mice null for *Taf4b*, a TBP associated factor, are sterile, we sought to determine when during follicular development this phenotype was first observed. At postnatal day 3, ovaries of *Taf4b* null females contained fewer ($P < 0.01$) oocytes than ovaries of wild type and heterozygous *Taf4b* mice. However, expression of only one somatic cell marker *Foxl2* was reduced in ovaries at day 15. Despite the reduced number of follicles, many proceed to the antral stage, multiple genes associated with granulosa cell differentiation and oocyte maturation were expressed in a normal pattern, and immature *Taf4b* null females could be hormonally primed to ovulate and mate. However, the ovulated cumulus oocyte complexes from the *Taf4b* null mice had fewer ($P < 0.01$) cumulus cells, and the oocytes were functionally abnormal. GVBD and polar body extrusion were reduced significantly ($P < 0.01$). The few oocytes that were fertilized failed to progress beyond the two-cell stage of development. Thus, infertility in *Taf4b* null female mice is associated with defects in early follicle formation, oocyte maturation, and zygotic cleavage following ovulation and fertilization.

Keywords: TAF4b; Oocyte; Primordial follicle; Foxl2; Granulosa cells; Cumulus oocyte complex; Fertilization; Zygotic genome activation; Ovary; TBP

Introduction

Ovarian follicle development is a complicated process that requires the transcription of many genes that must be expressed in specific temporal and spatial locations within the gonadal somatic cells and germ cells (Richards et al., 2002b). Expression of each gene is controlled by combinatorial transcription factor binding sites within promoters. Recruitment of transcription factors to these binding sites can sequester co-activators or co-repressors to facilitate the recruitment of the RNA polymerase II holoenzyme. The pre-initiation complex (PIC) that recruits RNA polymerase II to promoters consists of a variety of subunits including TFIID. TFIID contains TBP (the TATA-binding protein) and several TAFs (TBP associated factors) (Hochheimer and Tjian, 2003).

One of these, TAF4b (originally $TAF_{II}105$), was found be expressed at high levels in human B cells (Dikstein et al., 1996). However, analysis of mice null for *Taf4b* indicated that this factor is required for gonadal development but not immune function (Falender et al., 2005; Freiman et al., 2001, 2002). Specifically, we have shown that TAF4b is essential in male mice for the proliferation of spermatogonia in the neonatal testis (Falender et al., 2005). *Taf4b* null male mice are initially fertile, but then seminiferous tubules become devoid of sperm resulting in complete loss of fertility by 11 weeks of age. The developmental basis of this phenotype was traced to a defect occurring between postnatal day 0 and day 3. TAF4b appears to be required for the expression of spermatogonial genes including stimulated by retinoic acid gene 8 (*Stra8*), PLZF (*Zbtb1*), and the Ret oncogene (*Ret*) (Falender et al., 2005). In contrast to males, *Taf4b* null females are completely infertile and present a complex phenotype (Freiman et al., 2001). To identify specific stage(s) of follicle development at which TAF4b is critical and to determine if female germ cell development might be defective in the female as in the male, additional analyses have been done with particular focus on key stages of early postnatal ovarian development and oocyte maturation.

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In the embryonic gonad, primordial germ cells proliferate and migrate to the gonad where they enter meiosis around 13.5 days post-coitum (d.p.c.) and arrest in prophase I of meiosis by 16.5 d.p.c. (McLaren, 2003). At birth, meiotically arrested oocytes are organized into structures called cysts that consist of several conjoined germ cells and surrounded by somatic cells (Pepling et al., 1999). Between postnatal day 0 and postnatal day 3, these germ cell cysts breakdown as primordial follicles form (Pepling and Spradling, 2001). During primordial follicle formation, oocytes grow in size and become surrounded by a single layer of squamous granulosa cells. Between postnatal day 3 and day 8, some primordial follicles initiate growth and become primary follicles that are characterized by further increases in oocyte size and granulosa cells that become cuboidal in shape (Kezele et al., 2005).

Primary follicles respond to pituitary and intra-ovarian hormones to become secondary follicles and then pre-ovulatory follicles. Around the time of ovulation, the oocyte undergoes the process of meiotic maturation: germinal vesicle breakdown (GVBD) occurs, the oocyte progresses from the dictyate stage of prophase I to metaphase I of meiosis, chromosomes become condensed, the spindle body forms as microtubules reorganize, and polar body emission occurs (Wasserman and Albertini, 1994). Following fertilization, the haploid maternal and paternal pronuclei each undergo one round of DNA replication leading to the development of the 2-cell stage zygote (Hamatani et al., 2004). By this time, 90% of the maternal RNA has degraded setting the stage for zygotic genome activation (ZGA) (Schultz, 2002). During ZGA which occurs between the 1-cell and 2-cell stage of development in mice, the zygotic (both paternal and maternal) genome begins active transcription, and the translation of RNA is initiated (Nothias et al., 1995).

The studies described herein show that loss of *Taf4b* causes complete infertility in the female due to developmental defects observable as early as postnatal day 3 when the number of oocytes is markedly reduced. Although follicular development can proceed and ovulation can be hormonally stimulated, many *Taf4b* null oocytes fail to release a polar body, and the few oocytes that are fertilized fail to develop beyond the 2-cell stage. These results suggest that defects in somatic cell–oocyte interactions during the follicle formation and oocyte maturation, including ZGA, cause the infertility in *Taf4b* null females.

Materials and methods

Animals

Mice null for *Taf4b* were generated by an insertion of a neomycin resistance cassette in the reverse orientation into the 6th exon of the *Taf4b* gene as previously described (Freiman et al., 2001). Heterozygous mice were back-crossed to the inbred C57BL/6 strain (Harlan, Indianapolis, IN). Mice were maintained on a 14L:10D cycle with free access to food and water in the vivarium at Baylor College of Medicine. Mice were maintained in accordance with the NIH *Guide for the Care and Use of Laboratory Animals* with Institutional oversight by Baylor College of Medicine.

Hormone treatments and fertilization experiments

Immature female C57BL/6 (d25) mice were obtained from Harlan Sprague–Dawley, Inc. (Indianapolis, IN) or from heterozygous crossings and injected with 5 IU of pregnant mare serum gonadotropin (PMSG, Gestyl) purchased from Professional Compounding Center of America (Houston, TX) to stimulate follicle growth followed 48 h later with 5 IU of human chorionic gonadotropin (hCG, Pregnyl) from Organon Special Chemicals (West Orange, NJ) to stimulate ovulation and luteinization.

Females used for hormone treatment studies were treated with the same regime listed above (5 IU PMSG followed by 5 IU of hCG) and sacrificed at various time points. For fertilization experiments, wild type, heterozygous, and *Taf4b* null females were treated with PMSG followed by hCG, and then each female was placed in a cage with an individual wild type male. On the following morning, vaginal plugs were observed, and, immediately or 24 h later, COCs were collected from the oviducts, and oocytes were isolated. Oocytes (unfertilized and fertilized) were stained with the DNA dye Drac55 (generously provided by M. Mancini, Baylor College of Medicine) and imaged using the confocal microscope (Zeiss LSM 510 Laser Confocal Microscopy, Carl Zeiss Inc., Thronwood, NY). Other oocytes were fixed with 4% paraformaldehyde and immunostained for tubulin as described below.

Histology and immunohistochemistry

Ovaries were collected and fixed in 4% paraformaldehyde or Bouin's fixative overnight, dehydrated in 70% ethanol, and embedded in paraffin. Sectioned tissues were dehydrated and stained with hematoxylin and eosin then re-dehydrated and mounted. The cellular and subcellular localization of germ cell nuclear antigen 1 (GCNA1, dilution 1:1) (generously provided by George C. Enders, University of Kansas; Enders and May, 1994) and TAF4b (1:500) (generously provided by Ken Geles, University of California, Berkeley; Falender et al., 2005) were analyzed by immunostaining. Bouin's fixed sections (7 μm) embedded in paraffin were deparaffinized in xylene washes and quenched with 3% hydrogen peroxide in methanol. Following re-hydration, sections were incubated with 10 mM sodium citrate (pH 6.0) at 90°C for 20 min and cooled to room temperature. Sections were then incubated with nonimmune goat serum (20% for GCNA1 and 5% for TAF4b) to block nonspecific sites followed by incubation with the GCNA1 monoclonal antibody at 33°C for 90 min or with polyclonal antibodies against TAF4b overnight at 4°C. For GCNA1, horseradish-peroxidase-conjugated anti-rat IgMμ diluted to a concentration of 1:450 was applied for 60 min at room temperature (Pierce Biotechnology, Inc., Rockford, IL). For TAF4b, the Vectastain ABC kit (Vector Labs, Burlingame, CA) and manufacturer's instructions were followed. Finally, sections were incubated with DAB substrate (3,3′-diaminobenzidine) (Vector Labs, Burlingame, CA), counter-stained with hematoxylin, dehydrated, and mounted.

Cumulus oocyte complex isolation

For isolation of unexpanded cumulus oocyte complexes, females were sacrificed 48 h after treatment with PMSG. Ovaries were punctured using 26-gauge needles, and unexpanded COCs were isolated manually. Unexpanded COCs were placed in expansion medium (MEM with Earle's salts supplemented with 25 mM HEPES, 0.25 mM sodium pyruvate, 3 mM L-glutamine, 1 mg/ml BSA, plus 1% FBS) and cultured overnight with or without FSH (100 ng/ml) at 37°C in a humidified incubator (95% air, 5% CO_2). On the following morning, COCs were observed and scored as "good" expansion (10 or more layers of cumulus cells), "poor" expansion (2–9 layers of cumulus cells), or no expansion (denuded oocyte or primary follicle). COCs were viewed using standard light microscopy.

For isolation of expanded COCs, PMSG-hCG primed females were sacrificed 24 h after treatment with hCG, and ovulated COCs were collected from the oviducts, immediately frozen, and stored at −80°C in preparation for RNA isolation.

Germ cell counting

To determine the relative number of oocytes present in the ovaries of neonatal mice at D0, D3, and D8, paraffin-embedded tissues were serially sectioned at a thickness of 5 μm and stained with hematoxylin and eosin. Oocytes from every fifth serial section were counted and classified as primordial or primary. For each group, 6 ovaries were counted (Rajkovic et al., 2004).