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Utp14b: A unique retrogene within a gene that has acquired multiple promoters and a specific function in spermatogenesis

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Abstract

The mouse retrogene *Utp14b* is essential for male fertility, and a mutation in its sequence results in the sterile juvenile spermatogonial depletion (*jsd*) phenotype. It is a retrotransposed copy of the *Utp14a* gene, which is located on the X chromosome, and is inserted within an intron of the autosomal acyl-CoA synthetase long-chain family member 3 (*Acsl3*) gene. To elucidate the roles of the *Utp14* genes in normal spermatogenic cell development as a basis for understanding the defects that result in the *jsd* phenotype, we analyzed the various mRNAs produced from the *Utp14b* retrogene and their expression in different cell types. Two classes of transcripts were identified: variant 1, a transcript driven by the host gene promoter, that is predominantly found in germ cells but is ubiquitously expressed at low levels; and variants 2–5, a group of alternatively spliced transcripts containing some unique untranslated exons that are transcribed from a novel promoter that is germ-cell-specific. *Utp14b* (predominantly variant 1) is expressed at moderately high levels in pachytene spermatocytes, the developmental stage at which the expression of the X-linked *Utp14a* is suppressed. The levels of both classes of *Utp14b* transcripts were highest in round spermatids despite the transcription of *Utp14a* in these cells. We propose that when *Utp14b* initially inserted into *Acsl3,* it utilized the *Acsl3* promoter to drive expression in pachytene spermatocytes to compensate for inactivation of *Utp14a* expression. The novel cell-type-specific promoter for *Utp14b* likely evolved later, as the protein may have acquired a germ cell-specific function in spermatid development.

Keywords: Spermatogenesis; Retroposons; Retrotransposition; Utp14; Juvenile spermatogonial depletion; X-chromosome; Spermatocytes; Spermatogonia; Male germ cells

Introduction

Spermatogenesis is a precisely regulated sequence of events during which daughter cells of the stem spermatogonia undergo a series of mitotic divisions to eventually form spermatocytes. In the spermatocytes, chromosome pairing and genetic recombination occur, during which the sex chromosomes form the transcriptionally inactive sex body (Ayoub et al., 1997), followed by chromosome desynapsis and the meiotic divisions resulting in haploid spermatids. The spermatids express numerous germ-cell-specific genes and, despite the cessation of transcription midway through their development, many messages are stored for later translation, which contribute to the unique morphological and functional characteristics of spermatozoa.

Although retrotransposed copies of genes are widely distributed throughout mammalian genomes, these gene copies, which often have arisen via an mRNA intermediate, generally do not possess promoters, are intronless, carry remnants of 3′ polyadenylation sequences, and therefore are generally not active (Boer et al., 1987). However, a few retrotransposition events have resulted in new functional genes, designated

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retrogenes. Among the functional retrogenes expressed during spermatogenesis, there is a disproportionately high frequency of ones originating from X-linked progenitors (Emerson et al., 2004; Wang, 2004). It has been proposed that developmental processes during mammalian spermatogenesis are dependent on such autosomal retrogenes to compensate for X-chromosome silencing during meiosis (McCarrey and Thomas, 1987). Alternative hypotheses for the prevalence of testis-biased expression of such retrogenes (Wang, 2004) are that syncytial connections between X- and Y-bearing spermatids do not allow the latter to get sufficient levels of an X-chromosomal gene product, or that these genes have evolved additional functions to meet the special needs of germ cells. The latter alternative, however, does not explain the selectivity for X-chromosome progenitors.

Spontaneous and induced mutations in many genes in mice and humans are known to specifically disrupt spermatogenesis (Matzuk and Lamb, 2002). Mutations in several genes, including *Kit*, *Kitl*, *Eif2s3y*, *Sohlh1*, and *Utp14b* (Ballow et al., 2006; Bedell and Mahakali Zama, 2004; Mazeyrat et al., 2001; Rohozinski and Bishop, 2004), cause blocks in spermatogonial differentiation. A frameshift mutation in coding sequence of *Utp14b*, which introduces a stop codon that truncates the predicted UTP14B protein (Bradley et al., 2004; Rohozinski and Bishop, 2004), results in the juvenile spermatogonial depletion (*jsd*) phenotype. The only defect in *jsd* mutant mice is male sterility, characterized by several waves of spermatogenesis in young animals, followed by the progressive failure of type A spermatogonia to differentiate. As a consequence, differentiated germ cells are absent and only type A spermatogonia and Sertoli cells remain in the seminiferous tubules. Reciprocal stem spermatogonial transplantation experiments have shown that the defect is confined to the germ cells themselves rather than the supporting cell lineages (Boettger-Tong et al., 2000; Ohta et al., 2001).

Utp14b is a retrotransposed copy of the X-linked *Utp14a* gene; whereas the coding region of mouse *Utp14a* consists of 15 exons, *Utp14b* is encoded within a single exon. *Utp14a* and *Utp14b* are mouse homologs of the yeast UTP14 gene (Bradley et al., 2004; Rohozinski and Bishop, 2004). In yeast, UTP14 is part of the pre-18S–rRNA-processing complex and is required for viability (Dragon et al., 2002). The *Utp14b* gene is the first protein-coding retrogene in mammals, to which a phenotype has been ascribed.

Although X-derived retrogenes are important in male reproductive physiology and biology, so far there is only one example, that of *Pgk2* in the mouse, of a pathway by which testis-specific expression of these novel genes is acquired. Here the original mRNA transcript of the X-linked *Pgk1* is believed to have been retrotransposed onto an autosome, carrying the proximal portion of the progenitor gene's promoter sequence with it (McCarrey, 1990; McCarrey and Thomas, 1987). Transcription of the retrogene was likely initially regulated like *Pgk1*, with ubiquitous expression. Later the promoter region of *Pgk2* appears to have diverged, losing the CpG-island and subsequently acquiring testis-specific expression (McCarrey, 1990; McCarrey et al., 1992, 2005).

A different mechanism for acquisition of testis-specific expression must be involved in the case of *Utp14b*. There is no evidence that the *Utp14b* retrogene contains the progenitor gene's 5′UTR and promoter. Unlike *Pgk2*, which is located within a gene-less expanse of chromosomal DNA, *Utp14b* is located within an intron of a host gene, acyl-CoA synthetase long-chain family member 3 (*Acsl3*) on mouse chromosome 1. It inserted 3′ of the existing promoter element, which drives ubiquitous expression of *Acsl3* (Bradley et al., 2004; Rohozinski and Bishop, 2004).

To elucidate the roles of the *Utp14* genes in spermatogenic cell development, the selection pressures for high levels of germ cell expression, and to understand their relationship with the *jsd* phenotype, we analyzed the sequences of the various mRNAs produced from this retrogene and its progenitor and their expression in specific germ and somatic cells of the testis and in other tissues. We report here that the *Utp14b* gene has multiple transcripts, with the production of one transcript being controlled by the host gene's (*Acsl3*) promoter and that of another set of transcripts being regulated by a unique germ cell-specific promoter. Furthermore, we found that *Utp14a* expression is greatly reduced in pachytene spermatocytes; thus we postulate that *Utp14b* expression was selected to compensate for sex chromosome inactivation during meiosis. We have also shown that expression of the testis-specific *Utp14b* transcripts is highest in round spermatids, indicating that UTP14B may have developed a novel germ cell-specific function, and during evolution the second promoter may have been selected for spermatid expression.

Materials and methods

5′-rapid amplification of cDNA ends (RACE) PCR

RACE-Ready mouse testis cDNA was obtained from Ambion (Austin, TX) and amplified with gene-specific and 5′ RACE-specific primer pairs using a FirstChoice RLM-RACE Kit (Ambion). First-round touchdown PCR was performed with 400 nM 5′ RACE outer primer (supplied in the kit), 400 nM *Utp14b*- or *Acsl3*-specific reverse outer primers, 200 mM dNTPs, and 0.025 unit/μl of Super Taq™ DNA polymerase (Ambion). The reverse outer primers were Utp14bO, specific for *Utp14b* exon 3, or Acsl3O for *Acsl3* exon 4 (Table 1, Fig. 1). The samples were denatured for 3 min at 94 °C, followed by 35 cycles of amplification (94 °C 30 s, 60 °C 45 s, 72 °C 1 min) and a final elongation for 7 min at 72 °C. From the first-round PCR, 2 μl of product was amplified with the 5′ RACE inner primer (supplied in kit), and one of the following specific reverse inner primers: Utp14bI3 for *Utp14b* exon 3, Utp14bI2 for *Utp14b* exon 2, Utp14bI15 for *Utp14b* exon 1.5, or Acsl3I for *Acsl3* exon 3 (Table 1, Fig. 1), using the same PCR conditions as in the first round. Fresh PCR products were run on 3% agarose gels, recovered, cloned into the pCR4-TOPO vector (Invitrogen, Carlsbad, CA), and subsequently sequenced with ABI Big Dye kit (Applied Biosystems, Foster City, CA). The nucleotide sequence data were analyzed by DNAStar software (DNASTAR, Madison, WI).

Preparation of spermatogenic cells

Male C57BL/Law mice, bred-in house, and *W/W*^{v} mice, whose testes only contain somatic cells and a very few undifferentiated spermatogonia (Ohta et al., 2003), purchased from the Jackson Laboratory (Bar Harbor, ME), were maintained and the M. D. Anderson Cancer Center. Oct4-EGFP transgenic mice (Szabo et al., 2002) were bred and maintained at the University of Washington. All mice were housed in animal facilities approved by the American Association