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RAPID COMMUNICATION

Postmeiotic Transcription of X and Y Chromosomal Genes during Spermatogenesis in the Mouse

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During the meiotic prophase of spermatogenesis, the X and Y chromosomes form the heterochromatic sex body, showing little transcriptional activity. It has been suggested that transcription of the *Xist* gene is involved in this inactivation. After completion of the meiotic divisions, at least two Y chromosomal genes, *Zfy* and *Sry*, are transcribed in haploid spermatids. In contrast, postmeiotic transcription of X chromosomal genes has not been demonstrated. Using highly purified preparations of mouse pachytene spermatocytes, round spermatids, and cytoplasmic fragments from elongated spermatids, the present experiments show differential postmeiotic expression of the Y chromosomal genes *Ubely* and *Sry*, with highest mRNA levels in round spermatids and cytoplasmic fragments, respectively. Postmeiotic transcription of the X chromosomal gene *Ube1x* is indicated by an increased level of *Ube1x* mRNA in round spermatids and cytoplasmic fragments. The X chromosomal gene *MHR6A* shows a marked temporary postmeiotic expression in round spermatids. This postmeiotic activity of the X chromosome is a novel finding, which may have implications for our understanding of X chromosome inactivation during spermatogenesis and paternal genome imprinting.

In meiotic prophase oocytes, the two X chromosomes are actively transcribed. In marked contrast, in pachytene spermatocytes both the X and Y chromosomes are heterochromatic, forming the so-called sex body. Incorporation studies using [^{3}H]uridine revealed very low, if any, transcriptional activity of sex body chromatin (reviewed by McKee and Handel, 1993). The long list of genes reported to be expressed postmeiotically in haploid spermatids does not include X chromosomal genes. In fact, it is known that the X chromosomal genes *phosphoglycerate kinase* (*Pgk-1*) and *pyruvate dehydrogenase E1α subunit* (*Pdha-1*) are silenced in spermatocytes and spermatids. The inactivation of these genes is followed by germ cell-specific expression of the autosomal genes *Pgk-2* (McCarrey *et al.*, 1992a) and *Pdha-2* (Dahl *et al.*, 1990). In contrast to X chromosomal genes, clear postmeiotic transcription has been reported for two Y chromosomal genes, *Zfy* (Nagamine *et al.*, 1990) and *Sry* (Capel *et al.*, 1993). In the present study we have analyzed the postmeiotic expression of five X chromosomal genes, *Ube1x*, *MHR6A*, *Fmr1*, *Pdha-1*, and *Xist*, and two Y chromosomal genes, *Ube1y* and *Sry*.

Spermatogenic cells were isolated from testes of mature Swiss CD-1 mice and highly purified using sedimentation at unit gravity (Staput procedure) followed by density gradient centrifugation (Percoll gradients) (Grootegoed and Den Boer, 1989, and references therein). The selected cell types were the meiotic prophase pachytene spermatocytes, postmeiotic round spermatids, and cytoplasmic fragments from elongated spermatids (these fragments are released during the cell isolation procedure and are different from residual bodies). RNA was isolated using the LiCl/urea procedure. Gene expression was studied by reverse transcriptase/polymerase chain reaction (RT-PCR) assay, and *MHR6A* was also studied by Northern blotting. For the RT-PCR assay, cDNA was synthesized using random hexamers. The nucleotide sequences of the primers used in the PCR are presented in Table 1. RT-PCR conditions were optimized to obtain semiquantitative results (Fig. 1). Three separate isolations of spermatogenic cells resulted in identical RT-PCR results.

As a control, RT-PCR was performed for the *follicle-stimulating hormone* (*FSH*) *receptor* gene, which is

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TABLE 1
PRIMERS USED IN THE RT-PCR ASSAYS

Gene	Primers used	Product size	
		cDNA	Genomic DNA
FSH receptor	GAATC CGTGG AGGTT TTCG CACCT TGCTA TCTTG GCAG	171 bp	171 bp
Activin receptor	CAGGG AACTG GATAT CTAGA GAGAA CTTCC TGGTC CTGGG TCTCG AGTAG GAACA AGTAC	596 bp	—
β-Actin	GCGGA CTGTT ACTGA GCTGC GT GAAGC AATGC TGTCA CCTTC CC	453 bp	453 bp
Ube1x	TGTCC ACACC CACTT ACT GCACT CTGCA ACTCC TGG	210 bp	340 bp
MHR6A	GACTG CTGAG CCCGC TAAAG GACTC CAACG GTTCT CGAAG	317 bp	—[a]
Fmr1	CTGGA GGTGC CAGAA GATTT ACG CTAGC TTGCT GAATA TTAGC ACC	344 bp	—
Pdha-1	CAAGT GTTGA AGAAT TAAAG TTCAA GCCTT TTTGT TGTCT G	286 bp	—
Xist	ACTGC CAGCA GCCTA TACAG GTTGA TCCTC GGGTC ATTTA	578 bp	—
Ube1y	GACCC CAAGT TCATG GAG CCTCC TAGTC CGTAT GTC	330 bp	1000 bp
Sry	GTGGT CCCGT GGTGA GAG TTTTG TTGAG GCAAC TGCAG	250 bp	250 bp

[a] Unpublished results. The nucleotide sequences were chosen to allow for amplification of X-encoded MHR6A mRNAs, but not of autosomally encoded MHR6B mRNAs.

known to be specifically expressed in Sertoli cells. *FSH receptor* mRNA was detected in RNA from total testis (which contains approximately 5% Sertoli cells), but of the three spermatogenic cell preparations only the pachytene spermatocytes (containing less than 0.5% Sertoli cells) yielded a weak signal. RT-PCR results of two autosomal genes, *activin type II receptor* and *β-actin*, demonstrate the integrity of the RNA preparations (Fig. 2A).

The mRNAs transcribed from the X chromosomal genes *Fmr1* and *Ube1x* were found in all germ cell types, but postmeiotic transcription of the *Ube1x* gene is indicated by an increased level of *Ube1x* mRNA in round spermatids and cytoplasmic fragments (Fig. 2B). The X chromosomal gene *MHR6A* shows a marked temporary

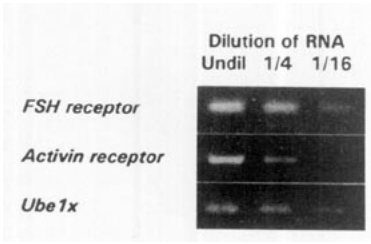

FIG. 1. The RT-PCR assay was optimized to yield semiquantitative results. The RT-PCR products of dilutions of total testis RNA are shown. Undil, undiluted.

postmeiotic expression in round spermatids (Fig. 2B). In contrast to *Ube1x* and *MHR6A*, postmeiotic transcription of *Fmr1* is not certain. *Fmr1* has been reported to be highly expressed in type A_1 spermatogonia (Bächner *et al.*, 1993), and it cannot be excluded that the *Fmr1* mRNA in spermatocytes and spermatids is derived from spermatogonia. The X chromosomal genes *Pdha-1* and *Xist*, for which testicular expression has been published (McCarrey *et al.*, 1992b; McCarrey and Dilworth, 1992), were included in the present experiments. mRNA representing *Pdha-1* was detected in total testis, and only a very low amount was detected in the pachytene spermatocyte preparation, but not in round spermatids and cytoplasmic fragments (Fig. 2B). The level of *Xist* mRNA found in round spermatids was lower than that in pachytene spermatocytes, while no *Xist* mRNA was detected in the cytoplasmic fragments (Fig. 2B). These results are in concordance with earlier published data (McCarrey *et al.*, 1992b; McCarrey and Dilworth, 1992). In addition, the *Pdha-1* mRNA expression data confirm the purity of the spermatocyte and spermatid preparations.

The postmeiotic expression of *MHR6A* as detected using RT-PCR was confirmed by Northern blotting. Using a mouse *MHR6A* cDNA probe, a mRNA with a size of approximately 0.8 kb was detected in round spermatids but not in pachytene spermatocytes (Fig. 3). A mRNA of

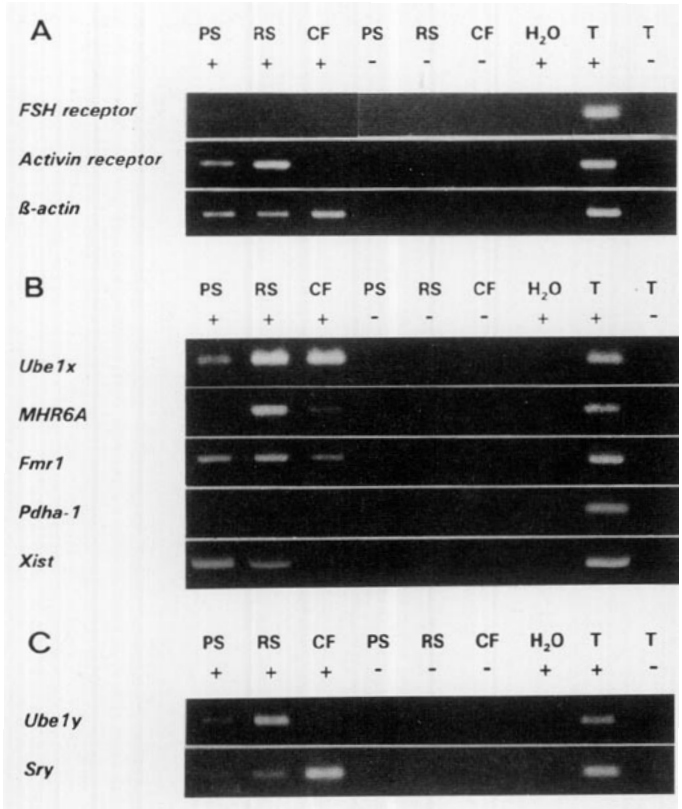

FIG. 2. RT-PCR analysis of mRNA expression in spermatogenic cell preparations. (A) Autosomal genes included as a control; (B) X chromosomal genes; and (C) Y chromosomal genes. The photomicrographs of ethidium bromide-stained gels are shown. PS, pachytene spermatocytes; RS, round spermatids; CF, cytoplasmic fragments from elongated spermatids; T, total testis; +/−, presence or absence of reverse transcriptase during cDNA synthesis.

that size can encode the MHR6A protein (Koken *et al.*, 1991).

As with *Ube1x* and *MHR6A*, the two Y chromosomal genes *Ube1y* and *Sry* also are expressed at higher mRNA levels in round spermatids than in pachytene spermatocytes (Fig. 2C). A remarkable accumulation of *Sry* mRNA was found at a late stage of spermatogenesis, in cytoplasmic fragments from elongated spermatids (Fig. 2C).

In spite of abundant postmeiotic transcription of the testis-determining gene *Sry*, this gene may not exert an essential function in spermatogenic cells (discussed in Capel *et al.*, 1993). *Sry* mRNA in adult mouse testes are circular molecules and probably are not translated (Capel *et al.*, 1993). The accumulation of *Sry* mRNA in elongated spermatids, as observed in the present experiments, may be a consequence of this lack of translation, since translation can lead to degradation of mRNA.

Mutation of the human *Fmr1* gene is involved in the fragile X syndrome, which is characterized by various symptoms including mental retardation and macroorchidism (Bakker *et al.*, 1994). Male transgenic mice in which the *Fmr1* gene is not functional are fertile (Bakker *et al.*, 1994). Therefore, the observed *Fmr1* expression in spermatogenic cells appears not to be essential for completion of spermatogenesis.

The protein products of *Ube1x* and *Ube1y* (Kay *et al.*, 1991) and *MHR6A* (Koken *et al.*, 1992) are all involved in ubiquitination; *Ube1x* and *Ube1y* are ubiquitin-activating enzymes, and MHR6A is a ubiquitin-conjugating enzyme. Ubiquitination might play a role in the turnover of histones (Jentsch, 1992). Therefore, it is tempting to suggest a role for *Ube1x*, *Ube1y*, and *MHR6A* gene products in the replacement of histone proteins by transition proteins and protamines during spermatogenesis.

The present results indicate that inactivation of both the X and Y chromosomes during spermatogenesis is limited to the period of meiosis. The main function of sex body formation in spermatogenic cells may be to prevent the initiation of potentially damaging recombination events between the sex chromosomes (McKee and Handel, 1993). This places the function of *Xist* expression in spermatogenic cells in another perspective. The level of *Xist* mRNA in testis is 1000- to 2000-fold lower than in female somatic tissues (Kay *et al.*, 1993), and *Xist* mRNA has been detected in developing germ cells from type A spermatogonia up to spermatids (McCarrey and Dilworth, 1992). Hence, the presence of *Xist* mRNA in spermatogenic cells might be unrelated to X chromosome inactivation. X and Y chromosome inactivation in spermatocytes might involve a mechanism other than X chromosome inactivation in female somatic cells. Possibly, *Xist* expression in spermatogenic cells is related to demethylation of the *Xist* gene during spermatogenesis, which recently was proposed to result in paternal imprinting of the X chromosome (Norris *et al.*, 1994).

The postmeiotic reactivation of the X chromosome raises the question of why the gene products of the X chromosomal genes *Pgk-1* and *Pdha-1* are replaced dur-

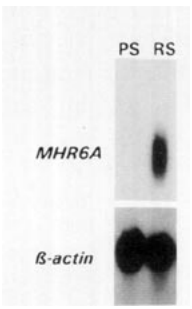

FIG. 3. Northern blot analysis of total RNA from pachytene spermatocytes (PS) and round spermatids (RS). Twenty micrograms of total RNA from each cell preparation was used. Blots were hybridized with a mouse *MHR6A* cDNA probe and, after washing, rehybridized with a mouse β-actin probe as a control for the integrity and amount of the RNAs.

ing spermatogenesis by isoenzymes encoded by the autosomal genes *Pgk-2* and *Pdha-2*. The cessation of *Pgk-1* transcription may involve the activity of a testis-specific transcription inhibitor, TIN-1 (Goto *et al.*, 1991), and might not be caused by a general X chromosome inactivation mechanism. Furthermore, during spermatogenesis, there is not only a switch from X-encoded genes to testis-specific autosomal genes, but also a switch from autosomal genes to testis-specific autosomal genes. Such testis-specific autosomal genes include *glyceraldehyde 3-phosphate dehydrogenase*$_S$, *lactate dehydrogenase C*, and *cytochrome* c_T genes (for a review, see Grootegoed and Den Boer, 1989, and Mori *et al.*, 1992). Therefore, the switch from X-encoded *Pgk-1* and *Pdha-1* to *Pgk-2* and *Pdha-1* may not be related to X chromosome inactivation, but may reflect a requirement of spermatozoa for specific isoenzymes.

Postmeiotic expression of X and Y chromosomal genes probably does not cause an inequality between X and Y chromosome-bearing spermatids, because sister spermatids can exchange gene products (Braun *et al.*, 1989). All Y chromosomal genes studied until now, *Sry*, *Zfy-1*, *Zfy-2*, and *Ube1y*, have been found to be transcribed in postmeiotic spermatogenic cells. Transcription of X chromosomal genes after meiosis shows differential regulation.

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