A New Deletion of the Mouse Y Chromosome Long Arm Associated With the Loss of *Ssty* Expression, Abnormal Sperm Development and Sterility

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ABSTRACT

The mouse Y chromosome carries 10 distinct genes or gene families that have open reading frames suggestive of retained functionality; it has been assumed that many of these function in spermatogenesis. However, we have recently shown that only two Y genes, the testis determinant *Sry* and the translation initiation factor *Eif2s3y*, are essential for spermatogenesis to proceed to the round spermatid stage. Thus, any further substantive mouse Y-gene functions in spermatogenesis are likely to be during sperm differentiation. The complex *Ssty* gene family present on the mouse Y long arm (Yq) has been implicated in sperm development, with partial Yq deletions that reduce *Ssty* expression resulting in impaired fertilization efficiency. Here we report the identification of a more extensive Yq deletion that abolishes *Ssty* expression and results in severe sperm defects and sterility. This result establishes that genetic information (*Ssty*?) essential for normal sperm differentiation and function is present on mouse Yq.

THE male-specific region of the Y chromosome (MSY) is an inherently hostile environment for genes (Graves 1995) and it is assumed that genes still retained on the mammalian MSY must afford some form of male benefit, for example, by functioning in spermatogenesis (Lahn and Page 1997; Burgoyne 1998; Skaletsky *et al.* 2003). However, we have recently shown that in the mouse only two Y genes—*Sry* to trigger testis development and *Eif2s3y* that has an essential role during spermatogonial proliferation—are needed for spermatogenesis to complete meiosis, including both reduction divisions (Mazeyrat *et al.* 2001; P. S. Burgoyne, unpublished observations). Thus, if other genes on the mouse Y chromosome have substantive roles in the normal spermatogenetic process, it is likely to be during the haploid phase of sperm differentiation (spermiogenesis). Studies of mice with Yq deficiencies have implicated genetic information on MSYq [the Y long arm excluding the pseudoautosomal region (PAR)] as having an important role in spermiogenesis that impacts on sperm quality and function. Males with large interstitial Yq deletions exhibit an increased incidence of mild sperm-head anomalies with some impairment of sperm function and an intriguing distortion of the offspring sex ratio in favor of females (Moriwaki *et al.* 1988; Suh *et al.* 1989; Styrna *et al.* 1991a,b, 2002; Xian *et al.* 1992; Conway *et al.* 1994). X*Sxr*^{a}Y*^{X} males, in which the only MSY contribution is from the Yp-derived *Sxr*^{a} factor and that thus lack MSYq, have grossly abnormal sperm and are sterile (Burgoyne *et al.* 1992).

The mouse MSYq appears to be composed largely of repeats (Nishioka and Lamothe 1986; Eicher *et al.* 1989; Nishioka *et al.* 1992, 1993a,b; Fennelly *et al.* 1996; Navin *et al.* 1996; Bergstrom *et al.* 1997). Among these repeats is a complex gene family, *Ssty*, which is transcribed exclusively in the testis during the spermatid stages (Bishop *et al.* 1983; Prado *et al.* 1992; Conway *et al.* 1994). This gene family probably originated from a retroposed transcript of the autosomal gene *Spin* (Oh *et al.* 1997, 1998; Touré *et al.* 2004) and at least some *Ssty* transcripts retain an open reading frame that encodes a putative SPIN-like protein. Very recently we have identified an *Ssty* protein product and have shown that this derives from only a minor subset of the transcribed *Ssty* genes (Touré *et al.* 2004). It has been proposed that *Ssty* deficiency is responsible for the mild and severe sperm defects in mice with large interstitial Yq deletions and X*Sxr*^{a}Y*^{X} males, respectively (Burgoyne *et al.* 1992; Conway *et al.* 1994).

The problem with attributing the severe sperm abnormalities and sterility of X*Sxr*^{a}Y*^{X} males exclusively to the lack of MSYq is that these males are now also known to lack the majority of copies of *Rbmy*, a gene family on Yp close to the centromere that has also been implicated in maintaining sperm quality (Elliott *et al.* 1996; Mahadevaiah *et al.* 1998). Here we report the identification of a new mouse Yq deletion that removes all MSYq copies of *Ssty*, while leaving Yp (including all copies of *Rbmy*) intact. This deletion is also associated with severe sperm-head defects and sterility.

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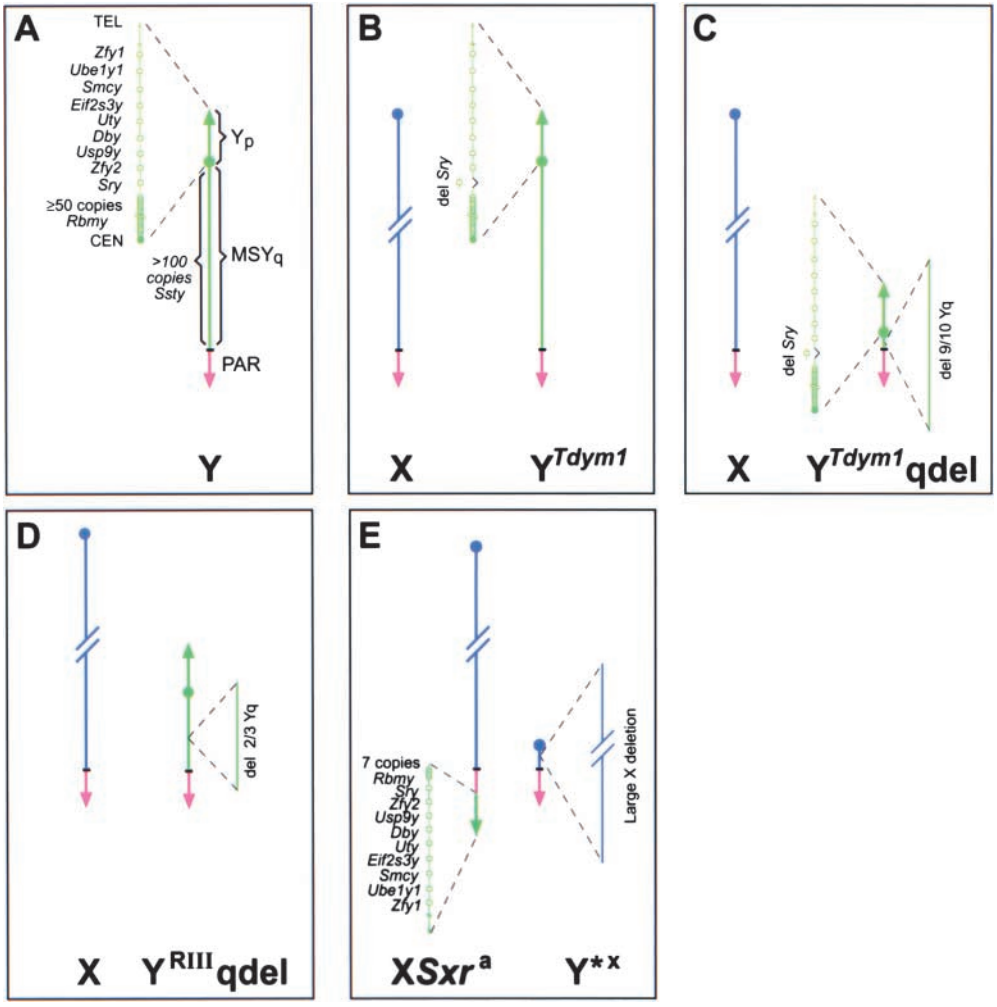

FIGURE 1.—Diagrams of the sex chromosome complement of variant mice, highlighting the MSY deficiencies. (A) The known gene content of the mouse MSY. Yp (shown expanded) carries seven single-copy genes, one duplicated gene (*Zfy*), and multiple copies of *Rbmy*. MSYq carries multiple copies of the *Ssty* gene family. (B) The variant Y^{Tdym1} has a complete Y-gene complement except that an 11-kb deletion has removed the testis determinant *Sry*. XY^{Tdym1} mice are female, but when the *Sry* deficiency is complemented by an *Sry* transgene, the mice are normal fertile males. (C) The new variant Y^{Tdym1}qdel, in addition to the deletion of *Sry*, has a large deletion removing most of MSYq. (D) The variant Y^{RIII}qdel is an RIII strain Y chromosome with a deletion removing about two-thirds of Yq. (E) $XSxr^aY^{*X}$ males are male because of the presence of the Y^{RIII}-derived sex-reversal factor Sxr^a attached distal to the X PAR. Sxr^a comprises most of Yp except for a marked reduction in copies of *Rbmy*. The Y^{*X} chromosome in effect is an X chromosome with a deletion from just proximal to *Amel* (close to the X PAR boundary) to within the *DXH XF34* sequence cluster adjacent to the centromere. It includes no MSY, but provides a second PAR, which is essential to avoid meiotic arrest. TEL, telomere; CEN, centromere; Yp, Y short arm; MSYq, male-specific region of the Y long arm; PAR, pseudoautosomal region of Yq.

MATERIALS AND METHODS

Mice: All the mice used in this study were produced on a random-bred albino MF1 strain (National Institute for Medical Research stock) background. The sex chromosome complement of the variant genotypes is illustrated in Figure 1. The new deletion was originally detected in male progeny of a cross of *Sry*-negative XXY^{Tdym1} females (LOVELL-BADGE and ROBERTSON 1990; GUBBAY *et al.* 1992; MAHADEVAIAH *et al.* 1993) with males carrying an autosomally located *Sry* transgene. The proband was a putative XXY^{Tdym1}*Sry* male, but proved to be either weakly positive or negative for a PCR that detects the *Ssty1* family, a subfamily of *Ssty* that is present in multiple copies throughout most of MSYq. This suggested that the Y^{Tdym1} of the proband carried an extensive Yq deletion; we call this Y variant Y^{Tdym1}qdel. The XXY^{Tdym1}qdel*Sry* male was sterile, as expected for an "XXY" male. However, the mother proved to carry the same chromosome and was used to establish a stock with this new Y variant.

To assess the effects of the new Yq deletion on spermatogenesis, it was necessary to produce XY^{Tdym1}qdel*Sry* males for comparison with XY^{Tdym1}*Sry* males (MAHADEVAIAH *et al.* 1998), which are the appropriate controls. XY^{Tdym1}qdel females were therefore mated to XY^{d1}*Sry* males (MAHADEVAIAH *et al.* 1998) to provide the same *Sry* transgene that is present in the XY^{Tdym1}-*Sry* males. The production of the XY^{Tdym1}qdel*Sry* males proved very difficult since the XY^{Tdym1}qdel females bred very poorly (*cf.* XY^{Tdym1} females; MAHADEVAIAH *et al.* 1993). The XY^{Tdym1}qdel*Sry* males were identified by PCR (see below) as being positive for the *Tdym1* deletion and negative for *Ssty1* and were initially distinguished from XXY^{Tdym1}*Sry* males by testis palpation on the assumption that they would have substantially larger testes (~100 mg *vs.* ~20–25 mg). The initial diagnosis was subsequently checked cytogenetically in air-dried bone marrow metaphases stained with pH 6.8-buffered Giemsa.

$XSxr^aY^{*X}$ males (BURGOYNE *et al.* 1992) used in comparisons with the new Yq deletion males were produced by mating XY^{*X} females (EICHER *et al.* 1991; BURGOYNE *et al.* 1998) to $XYSxr^a$ males (CATTANACH 1987). XY^{RIII}qdel males used to provide sperm and testis samples were derived from a stock originating from the mice described by CONWAY *et al.* (1994).

PCR assays: In defining the new Yq deletion, PCR assays were used that detect markers in Yp, Yq, and the PAR. These markers, together with the primers used, are listed in Table 1.

Southern blot analysis: The probes used for Southern blot analysis were the *Ssty1* cDNA clone pYMT2/B (BISHOP and HATAT 1987), an *Ssty1* intron probe (TOURÉ *et al.* 2004), the *Ssty2* cDNA clone pC11 (PRADO *et al.* 1992), and an *Rbmy* cDNA (MAHADEVAIAH *et al.* 1998). Genomic DNA was extracted from tail biopsies and 15 μg was digested with *Eco*RI, electropho-

TABLE 1

PCR assays for Y markers used in defining the Y^{Tdym1}qdel deletion

Marker/locus	Primer pairs (5′–3′)	Annealing temperature (°)	Product size (bp)	Reference
Y207 (*Yp* telomere)	207F: TGTA GACA GTCT TTCT GTGT G 207R: CACAGGCT CTCC TGAT TTTA G	60	200	King *et al.* (1994)
Ube1y1	oMJ52: CTCTG AGTAC ATCCG TGG oMJ54: GCAAT CCTGC TGAAC TGC	58	488	M. J. Mitchell (personal communication)
Dby	oMJ500: GGTAT ATGCA GAGTT GTTGG oMJ501: GCACT GTACT GACCA TCATG	58	614	M. J. Mitchell (personal communication)
Usp9y	oMJ324: CCAAA TCCAT TTGGT GACCC oMJ325: GCCAG GAGTC CTCAA ACAG	60	288	M. J. Mitchell (personal communication)
Tdym1 (*Sry* deletion)	MUTY3: GTGTC TCAAA GCCTG CTCTT C MUTYRP1: CATGT ACTGC TAGCA GCTAT C	63	204, Y^{Tdym1}; 435, Y or *Sry*tg	This article
Rbmy	RBMIF: CTCAA TCTTC TGGAA GGGCA G RBMIR: ATATT TACTC TGAAG AGACA T	58	250	This article
Ssty1	YMTFP1: CTGGAGCTCTACAGTGATGA YMTRP1: CAGTTACCAATCAACACATCAC	60	342	Turner *et al.* (2000)
Ssty2	PC11FP2: GTTTT TCCTC AGGTG AGGGA PC11RP2: CAGAG GGGTC TCTGG AATGT	58	237	This article
Sts	STSF: GCTCGCTGACATCATCCTC STSR: CACCGATGCCCAGGTCGTC	58	101	Salido *et al.* (1996)
Myog[a]	Om1a: TTACGTCCATCGTGGACAGCAT Om1b: TGGGCTGGGTGTTAGTCTTAT	60	245	Wright *et al.* (1989)
Sstx[b]	SSTXFP10: TCACA CAGAT AAGAG GGTAT TG SSTXRP13: GTTTT CCTAT CAGGC CATCC A	60	350	This article

[a] Autosomal; primers duplexed with *Y207*, *Usp9y*, *Ssty1*, and *Sts* primers as an amplification control.
[b] X-linked *Ssty*-related gene family (*DXHXF34* of Laval *et al.* 1997); primers duplexed with *Ssty2* primers as an amplification control.

resed through a 0.8% agarose gel, and transferred to a Hybond-N membrane (Amersham, Buckinghamshire, UK). After fixation, the membrane was hybridized overnight at 60° with ^{32}P-labeled probes in hybridization buffer (6× SSC, 5× Denhart's, 0.5% SDS, 100 μg/ml salmon sperm DNA). After two 60° washes (30 min 0.5× SSC, 0.1% SDS; 30 min 0.1× SSC, 0.1% SDS) the membrane was exposed to X-ray film or a phosphorimager screen overnight. An additional blot that was loaded with only 4 μg of *Eco*RI-digested control male DNA to allow longer exposures to X-ray film was prepared. This was hybridized to *Ssty* probes at very high stringency (hybridization at 65° and two 60-min washes at 65° with 0.1× SSC, 0.1% SDS) and was exposed to X-ray film for 2 weeks.

Northern blot analysis: The probes used for Northern analysis were the *Ssty1/2* cDNAs used for Southern analysis and an actin probe that recognizes α- and β-actin transcripts (Minty *et al.* 1981). Total RNA (20 μg) was electrophoresed in a 1.4% formaldehyde/agarose gel and transferred to Hybond-N membrane (Amersham) using 20× SSC buffer. The membrane was fixed for 2 hr at 80° and hybridized overnight at 60° with ^{32}P-labeled probes in hybridization buffer (6× SSC, 5× Denhart's, 0.1% SDS, 50 mm sodium phosphate, 100 μg/ml salmon sperm DNA). After two 60° washes (30 min 0.5× SSC, 0.1% SDS; 30 min 0.1× SSC, 0.1% SDS) the membrane was exposed to X-ray film for 5 hr and subsequently to a phosphorimager screen to allow quantitation of hybridization using ImageQuant software.

Western blot analysis: Testicular protein lysates were obtained by homogenization in liquid nitrogen and resuspension in Laemmli buffer at 10% w/v. Lysates were then denatured for 10 min at 95° and 5–10 μl were electrophoresed through an SDS/polyacrylamide minigel. Transfer to Hybond-C membrane was performed at 110 mA for 2 hr and the membrane was then processed for immunodetection. The membrane was blocked (PBS, 0.1% Tween, 5% milk powder) for 1 hr and incubated with first antibody diluted in the blocking solution for 2 hr at room temperature: rabbit anti-SSTY1 antibody, 1:500 (Touré *et al.* 2004); rabbit anti-RBMY antibody, 1:2000 (Turner *et al.* 2002); and mouse anti-ACTIN, 1:100 (sc-8432, Santa Cruz). After three washes (PBS, 0.1% Tween), the membrane was incubated with the secondary antibody for 45 min at room temperature (anti-rabbit or anti-mouse antibody coupled with HRP from Dako, Carpintaria, CA). Following three washes (PBS, 0.1% Tween), the signal was revealed by chemiluminescence (SuperSignal, Pierce, Rockford, IL) and recorded on X-ray film. Quantitation was carried out using National Institutes of Health Image 1.62 software.

Analysis of the XY^{Tdym1}qdel*Sry* males: The males were mated to two normal females for at least 3 weeks and in three cases the added females were checked for copulatory plugs as evidence of mating. When the males were killed, testes were weighed and sperm samples from the initial segment of the capita epididymides were used for sperm counts as previously described (Mahadevaiah *et al.* 1998) and/or for sperm smears (see below). One testis was fixed in Bouin and wax embedded and sections were stained with hematoxylin and eosin. XY^{Tdym1}*Sry* males were used to provide control material.

Comparisons of sperm-head morphology: Silver-stained sperm smears were produced using sperm from the initial segment of the capita epididymides (Mahadevaiah *et al.* 1998) of four XY^{Tdym1}qdel*Sry* males together with five XY^{Tdym1}*Sry* controls. The slides were coded and randomized along with

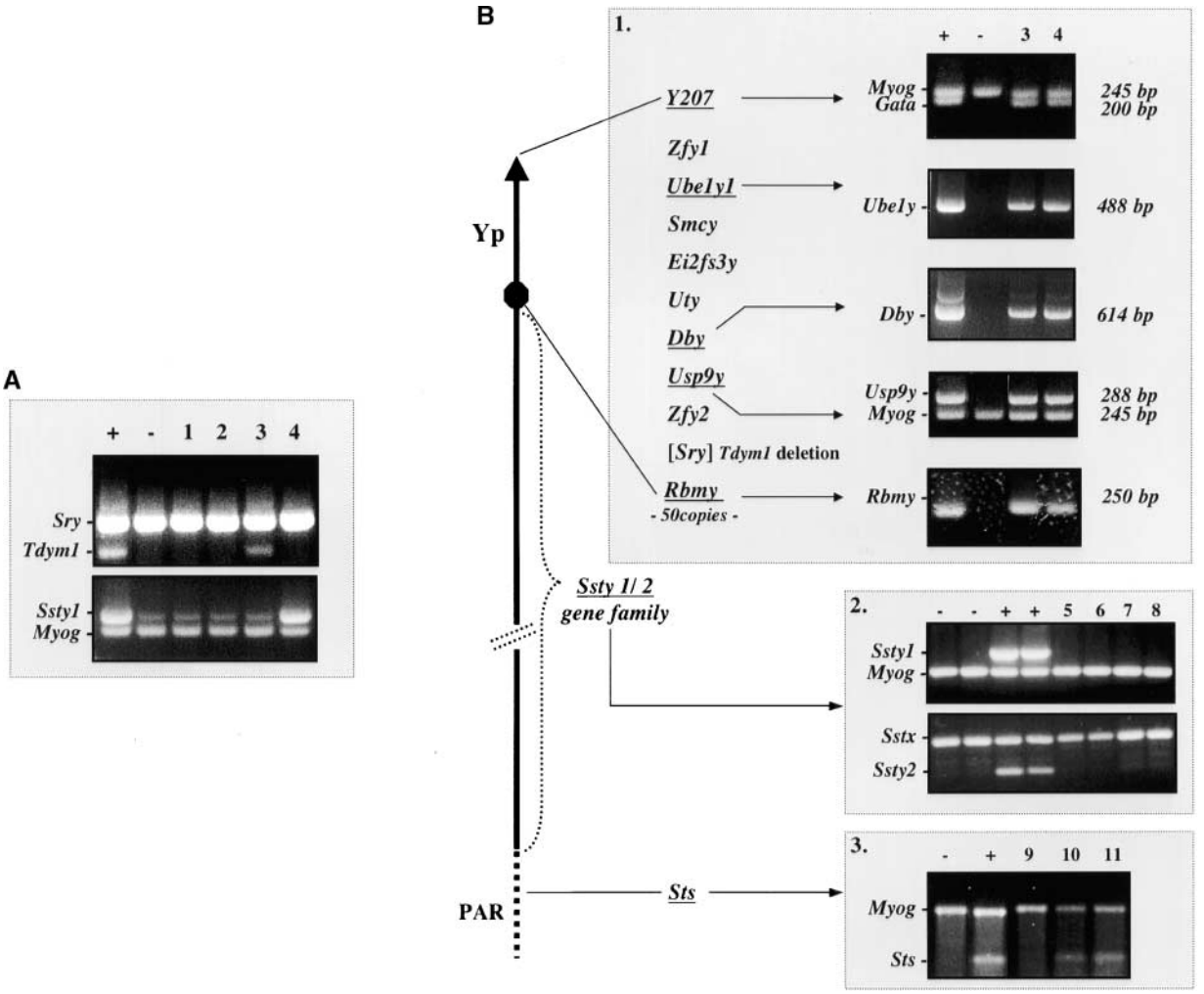

FIGURE 2.—PCR analysis of the new deletion (here denoted Y^{Tdym1}qdel). Positive and negative controls are indicated by "+" and "−" throughout. (A) Identification of the proband. Samples 1–4 are from brothers derived from the $XXY^{Tdym1} \times XYSry$ cross. Samples 1 and 2 are from XX*Sry* males and 4 is from an XY*Sry* male. Sample 3 is positive for the *Tdym1* deletion in Yp that has removed *Sry*, implying that Y^{Tdym1} is present; it should therefore also be positive for *Ssty1* located on Yq. However, only a faint *Ssty1* band is seen and a similar faint band is seen in the XX (−) negative control. This implies that most or all *Ssty1* copies on Yq have been deleted. (B) Evidence that the deletion is an interstitial deletion restricted to Yq. (B1) Analysis of Yp markers. The proband 3 and his brother 4 (XY*Sry*) are positive for all Yp markers tested. (B2) Analysis of *Ssty1* using more stringent conditions and of *Ssty2*. Samples 5 and 6 are from $XSxr^{a}Y^{*X}$ males that lack MSYq, and samples 7 and 8 are from XY^{Tdym1}qdel*Sry* males. Neither *Ssty1* nor *Ssty2* is detectable in the deletion males under stringent conditions. Thus the PCR results suggest that all *Ssty* copies have been deleted in Y^{Tdym1}qdel. (B3) Analysis of *Sts* located in the distal PAR. We have previously shown that the 129 strain has a variant *Sts* locus that is not detected by our standard PCR. The samples are 129 XY (−), MF1 XY (+), $X^{129}O$ (9), $X^{129}Y^{Tdym1}$qdel (10), and $X^{129}X^{MF1}$ (11). The deletion mouse is *Sts* positive and since the X is from the 129 strain, the PCR product must have been amplified from an *Sts* copy present on Y^{Tdym1}qdel.

slides from two XY^{RIII}qdel males and one $XSxr^{a}Y^{*X}$ male for comparative purposes, and 100 sperm from each of two to four slides from each male were classified as described by MAHADEVAIAH *et al.* (1998). Slides from two of the XY^{Tdym1}qdel*Sry* males were subsequently recoded and scored along with the slides from two $XSxr^{a}Y^{*X}$ males, this time including a new category of "extreme 1a" (see RESULTS and Figure 6C).

RESULTS

Origin of the Yq deletion: The proband was a putative $XXY^{Tdym1}Sry$ male (see MATERIALS AND METHODS), but, although shown by PCR analysis to be carrying the *Tdym1* deletion that has removed *Sry* from Yp, the male appeared to lack *Ssty1*, a multicopy gene distributed over most of MSYq (Figure 2A). This male had received this putative variant Y chromosome from his mother; she nevertheless had five sisters carrying the original *Ssty*-positive Y^{Tdym1} chromosome. We concluded that a Yq deletion event removing most or all copies of *Ssty1* had likely occurred during meiosis in the proband's XXY^{Tdym1} grandmother.

Characterization of the deletion: Once the initial PCR diagnosis on the proband was found to be replicated in the mother and a brother, more Y PCR assays were

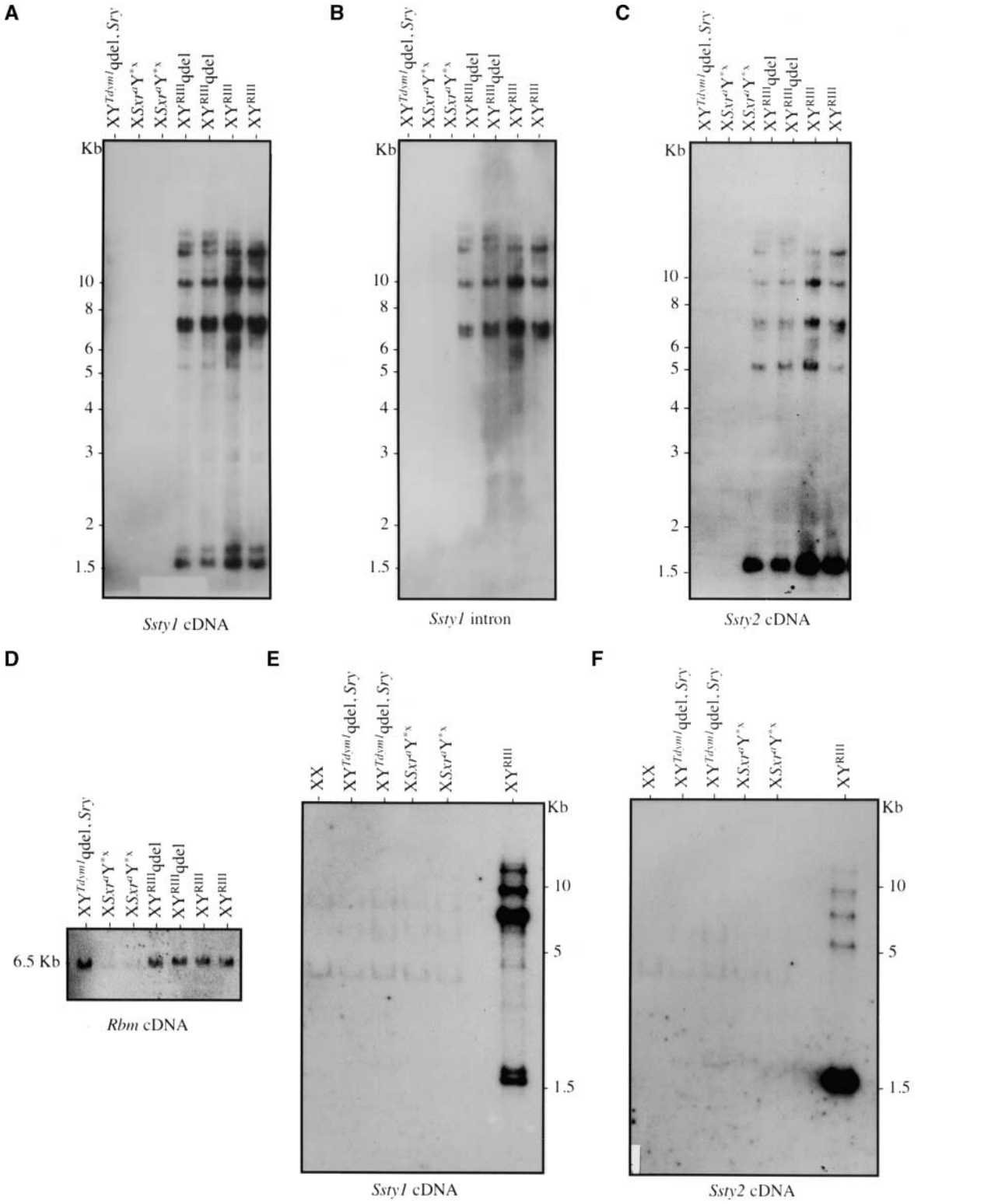

FIGURE 3.—Southern blot analysis of Y^{Tdym1}qdel. (A–D) A blot of *Eco*RI-digested XX, XY^{Tdym1}qdel*Sry*, X$Sxr^{a}Y^{*X}$, XY^{RIII}qdel, and XY^{RIII} DNA was consecutively hybridized with *Ssty1* cDNA, *Ssty1* intron, *Ssty2* cDNA, and *Rbmy* intron probes, which all detect fragments present in multiple copies in normal males. The XY^{Tdym1}qdel*Sry* male appears to be negative with all three *Ssty* probes, but is equivalent to the control male with respect to *Rbmy* hybridization. As expected, the X$Sxr^{a}Y^{*X}$ DNA samples show markedly reduced *Rbmy* hybridization, because the Yp-derived Sxr^{a} has only about one-eighth of the normal Yp complement of *Rbmy* copies. (E and F) Southern blot of *Eco*RI-digested XX, XY^{Tdym1}qdel*Sry*, X$Sxr^{a}Y^{*X}$, and XY^{RIII} DNA, for which only 4 μg (instead of 15 μg) of XY^{RIII} DNA was loaded. After exposure for 2 weeks, only two very faint *Ssty1/2*-hybridizing fragments are detected in the deletion males; these DNA fragments are not Y specific since they are also seen in XX female DNA.

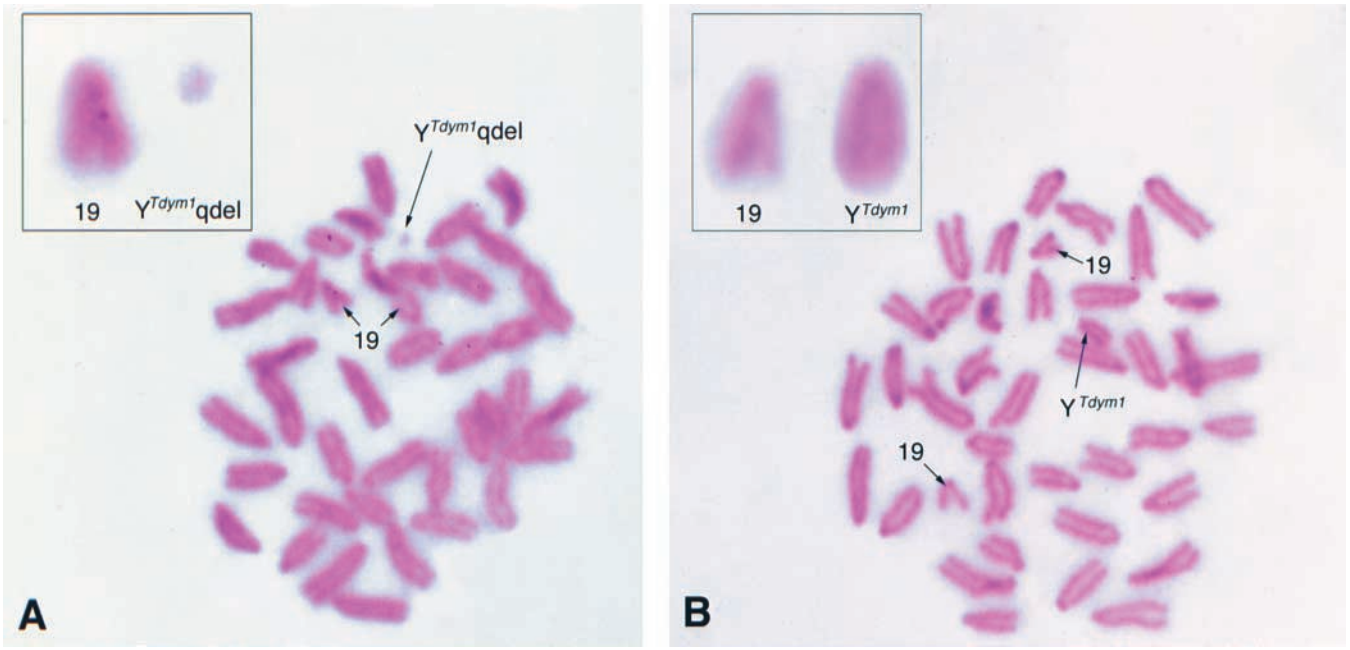

FIGURE 4.—The Y^{Tdym1}qdel chromosome in mitotic metaphase. (A) The Y^{Tdym1}qdel chromosome is minute compared to chromosome 19. (B) The Y^{Tdym1} from which the deleted chromosome arose is a little larger than chromosome 19.

carried out to assess whether the deletion was restricted to MSYq (Figure 2B). First, PCR assays that detect a GATA/GACA (*Y207*) repeat near the Yp telomere and the *Rbmy* gene cluster on Yp near the centromere were carried out. Both these PCRs were positive, suggesting that most, if not all, of Yp was present, except for the preexisting 11-kb *Tdym1* deletion that had removed *Sry*. Three other Yp-located genes were also shown to be present (Figure 2B1). We subsequently checked more mice with the new deletion for both *Ssty1* and *Ssty2*, using X*Sxr*^{a}Y*^{X} DNA as a control in which most of Yp is present but MSYq is absent. These two genotypes were indistinguishable, suggesting that all MSYq-located copies of *Ssty* have been deleted (Figure 2B2). To test for the PAR-located *Sts* gene, we introduced the strain 129 X chromosome, which has a variant *Sts* that is not detected by the standard *Sts* PCR assay (BURGOYNE *et al.* 1998), thus enabling the *Sts* status of the new Y variant to be assessed. This showed that *Sts* is present (Figure 2B3). These results strongly suggest that the deletion is an interstitial Yq deletion removing all *Ssty* copies but leaving at least the distal PAR intact.

We subsequently checked the extent of the *Ssty* deficiency by Southern analysis; DNA samples from Yq-deficient X*Sxr*^{a}Y*^{X} and XY^{RIII}qdel males were included for comparison (Figure 3). Using an *Ssty1* cDNA probe and an *Ssty1* intron probe that is present in a subset of *Ssty1* copies (TOURÉ *et al.* 2004), we could detect no hybridization in the male with the new deletion; furthermore, with an *Ssty2* cDNA probe, no hybridizing bands remained (Figure 3, A–C). This suggested that all MSYq-located *Ssty* copies are absent, as in X*Sxr*^{a}Y*^{X} males. Consistent with Yp being intact, a probe detecting the multicopy *Rbmy* gene family located on Yp hybridized with normal intensity (Figure 3D). As expected, *Rbmy* hybridization was markedly reduced in X*Sxr*^{a}Y*^{X} males because the Yp-derived *Sxr*^{a} factor has only approximately one-eighth of the normal Yp complement of *Rbmy* copies (MAHADEVAIAH *et al.* 1998). We produced a further blot loaded with much less control DNA to allow longer exposures of the filter with the *Ssty* probes (Figure 3, E and F). Two very faint bands were seen with the *Ssty* probes with a 2-week exposure, but these bands were also present in X*Sxr*^{a}Y*^{X} males that lack MSYq and in XX females. We presume these bands are due to hybridization to X or autosomally located *Ssty1*-related copies, since we have previously cloned and sequenced the PCR product intermittently obtained in XX females with *Ssty1* primers (see Figure 2A) and found two distinct *Ssty*-related sequences (P. S. BURGOYNE and S. H. LAVAL, unpublished results).

In summary, the deletion is an interstitial deletion within Yq that has occurred in a Y^{Tdym1} chromosome and that has removed all MSYq-located copies of *Ssty*. The extent of the Yq deletion is evident in bone marrow metaphases in which this chromosome is minute (Figure 4). The chromosome is here denoted "Y^{Tdym1}qdel."

XY^{Tdym1}qdel*Sry* males have gross sperm defects and are sterile: The XXY^{Tdym1}qdel*Sry* proband lacked spermatogenic cells as expected for any male with two X chromosomes. We therefore attempted to produce some XY^{Tdym1}qdel*Sry* transgenic males (see MATERIALS AND METHODS) to enable any effects of the deletion on spermatogenesis to be assessed. After a period of a few

TABLE 2

Testis weights and sperm counts for XY^{Tdym1}qdel*Sry* males and XY^{Tdym1}*Sry* controls

Mouse genotype	Age (wk)	Testis weight (mg)	Sperm per caput ($\times 10^{-3}$)[c]
XY^{Tdym1}qdel*Sry*[a]			
1	14	112.5	(480)
2	7	95.0	(520)
3	7	112.5	(315)
4	10	96.0	(245)
5	9	90.5	—
6	15	106.5	740
7	17	119.5	1050
8	16	89.5	540
9	14	96.0	532
	Mean ± SE:	102.0 ± 3.6	716 ± 121[d]
XY^{Tdym1}*Sry*[b]			
1	10	99.0	—
2	9	93.0	—
3	14	118.5	3090
4	14	97.5	2170
5	16	104.5	2825
	Mean ± SE:	102.5 ± 4.4	2695 ± 273

[a] All males of this genotype were found to be sterile after having been mated with two females for 3–6 weeks. For three males, mating was confirmed by the presence of copulatory plugs.

[b] Extensive breeding of XY^{Tdym1}*Sry* males has established that they are of normal fertility. Previously published testis weights and sperm counts for 10 males were in the ranges of 93.9–121.5 mg and 2287–3673, respectively (Mahadevaiah *et al.* 1998).

[c] Sperm counts for the first four males are underestimates because many detached abnormal heads were overlooked during the counting procedure. These sperm counts were excluded when calculating the mean.

[d] Significantly different from XY^{Tdym1}*Sry* ($t_{(5)} = 7.328$; $P < 0.001$).

months, a single putative XY^{Tdym1}qdel*Sry* male was produced with testes that palpation suggested were of normal size. However, when the male was mated to two females, they failed to become pregnant. The male was killed and the genotype confirmed by bone marrow metaphase analysis. Testis size (122 and 103 mg) fell within the range (94–122 mg) that we have previously observed for XY^{Tdym1}*Sry* males (Mahadevaiah *et al.* 1998); these latter males are of normal fertility. We have since test mated eight more XY^{Tdym1}qdel*Sry* males and for three of these males, mating was confirmed by the presence of copulatory plugs. Testis weights were again comparable to controls but all proved to be sterile (Table 2).

With Y chromosome deletions, the likely causes of sterility relate to impairment of sperm production and/or sperm quality. From the testis histology, sperm production appeared qualitatively normal. However, it was noted that there was a problem with sperm shedding, late spermatids (step 16) being retained along with step 9 spermatids in stage IX tubules and beyond, instead of being shed during stage VIII (Figure 5). Sperm counts for the initial segment of the caput epididymis for the first four males suggested a substantial reduction in sperm output (0.2–0.5 million *vs.* 2.2–3.7 million for controls). It was then realized that an unusually high number of detached abnormally shaped sperm heads were present in the sperm suspension (see examples in Figure 6C); these were being missed in the counting procedure. For four subsequent males for which care was taken to include the detached misshapen sperm heads, the sperm counts of 0.5–1.1 million were within the fertile range, although significantly reduced ($P < 0.001$) compared to controls (Table 2).

To assess the extent of the sperm abnormalities, silver-stained sperm smears were analyzed from four of the XY^{Tdym1}qdel*Sry* males together with four XY^{Tdym1}*Sry* controls. For comparative purposes, sperm smears were also included from two XY^{RIII}qdel males (predominantly mild sperm-head defects; Conway *et al.* 1994) and from one X*Sxr*^{a}Y*^{X} male (100% grossly abnormal sperm; Burgoyne *et al.* 1992). The results (Figure 6, C and F) show that all the sperm in XY^{Tdym1}qdel*Sry* males are abnormal, and the majority severely so. They are thus much more severely affected than the sperm of XY^{RIII} qdel males (Figure 6, D and F). Nevertheless, all four males had sperm in the "slightly abnormal" category (range 8–24%) and thus were less severely affected than the X*Sxr*^{a}Y*^{X} male analyzed, which had 100% grossly abnormal sperm (Figure 6, E and F), as did previous males of this genotype. In the course of scoring some of the slides (all of which proved to be from XY^{Tdym1}qdel*Sry*

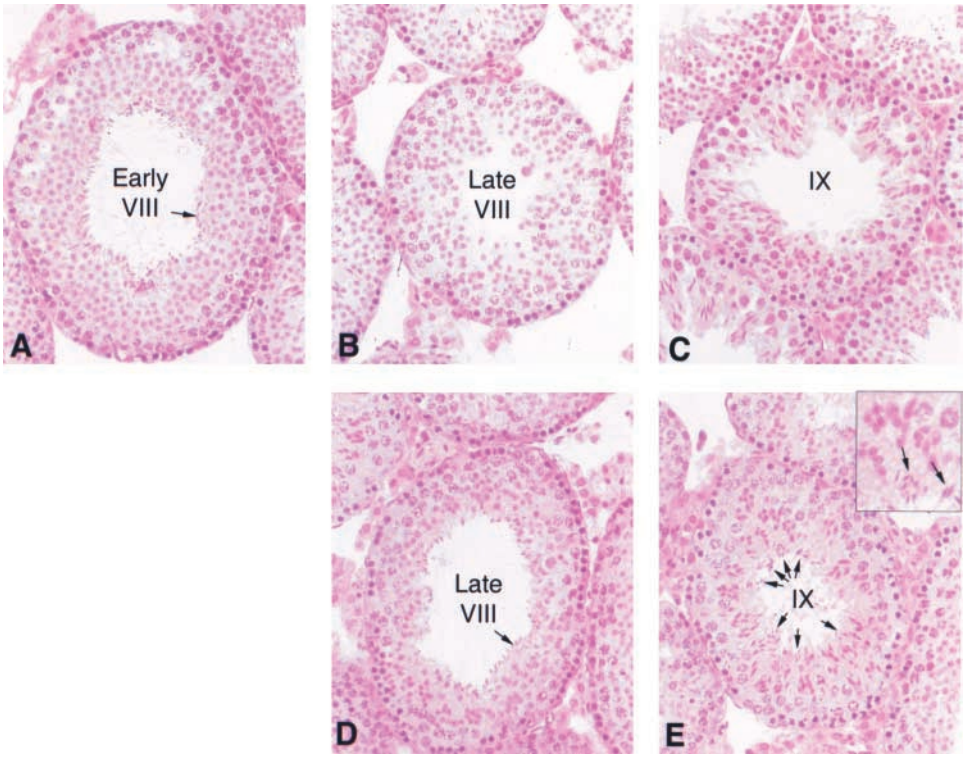

FIGURE 5.—Impaired sperm shedding in XY^{Tdym1}qdel*Sry* males. (A–C) Control XY^{Tdym1}*Sry* testis tubule sections. (A) Early stage VIII tubule with step 8 round spermatids together with a layer of mature sperm (arrow). (B) Late stage VIII tubule from which the mature sperm have been shed. (C) Late stage IX tubule with elongated stage 9 spermatids. (D) XY^{Tdym1} qdel*Sry* late stage VIII tubule with retention of a layer of mature sperm (arrow). (E) XY^{Tdym1}qdel*Sry* late stage IX testis tubule showing continued retention of pockets of mature sperm (arrows). Inset is a higher-power view of step 9 spermatids and a pocket of mature sperm that have not been shed.

males), difficulty was experienced in categorizing a prevalent sperm anomaly, which appeared to be an extreme form of the mild 1a anomaly characteristic of XY^{RIII}qdel males. Most of these extreme 1a sperm (see Figure 6C, e1a) were initially assigned to class 3, since they could not be classified as a mild anomaly. Rescoring coded slides from two of the XY^{Tdym1}qdel*Sry* males along with two X*Sxr*^{a}Y*^{X} males, while including a new category of extreme 1a, confirmed that this is a prevalent sperm anomaly in XY^{Tdym1}qdel*Sry* males and reinforced the conclusion that they are less severely affected than X*Sxr*^{a}Y*^{X} males (Table 3).

***Ssty* and *Rbmy* expression in relation to abnormal sperm development:** The more severe sperm anomalies in XY^{Tdym1}qdel*Sry* compared to XY^{RIII}qdel males parallels the increase in *Ssty* gene deficiency. However, with multicopy Y genes it is particularly important to establish whether the gene deficiencies are reflected in equivalent reductions at the level of RNA and/or protein. We therefore compared *Ssty* expression in these males with that of control males by Northern and Western analysis. As previously reported (CONWAY *et al.* 1994; TOURÉ *et al.* 2004), XY^{RIII}qdel males have a clear (54% by phosphorimager analysis) reduction in *Ssty2* transcripts, but *Ssty1* transcript levels are less affected (32% reduction; Figure 7A). Paradoxically, despite the modest reduction in *Ssty1* transcripts, SSTY1 protein appeared to be present at higher levels than in the XY^{RIII} control (Figure 7B). This apparent increase was confirmed in a further comparison of two XY^{RIII}qdel and two XY^{RIII} males; quantitation indicated a greater than twofold increase in SSTY1 expression (Figure 7C). Consistent with the Southern analysis, XY^{Tdym1}qdel*Sry* males had only very faint hybridization on Northerns with *Ssty1* and *Ssty2* probes (98% reduction in both cases); there was no detection of SSTY1 protein by Western analysis (Figure 7, A and B). We also assessed *Ssty* expression in X*Sxr*^{a}Y*^{X} males and found it to be indistinguishable from that in XY^{Tdym1}qdel*Sry* males (Figure 7, A and B).

Rbmy expression was checked by Western analysis. XY^{Tdym1}qdel*Sry* and XY^{RIII}qdel males had normal levels of RBMY protein. X*Sxr*^{a}Y*^{X} males, which have only a few copies of the multi-copy *Rbmy* gene family remaining in the Yp-derived *Sxr*^{a} factor, have a marked (~95%) reduction in RBMY protein expression (Figure 7D).

DISCUSSION

We have identified an extensive new interstitial mouse Yq deletion that results in male sterility, thus establishing that the mouse Y long arm carries genetic information essential for fertility. The only MSY genes known to be present on Yq are multiple copies of the complex *Ssty* gene family. The Southern analysis using *Ssty* probes at high stringency suggested that all Y-located copies of *Ssty* have been deleted. It has been previously reported that there are some Yp-located *Ssty* copies, but these are diverged noncoding copies that would probably not hybridize at high stringency (BOETTGER-TONG *et al.* 1998). In terms of *Ssty* expression, the new deletion males are indistinguishable from X*Sxr*^{a}Y*^{X} males that lack all of MSYq (BURGOYNE *et al.* 1992), there being

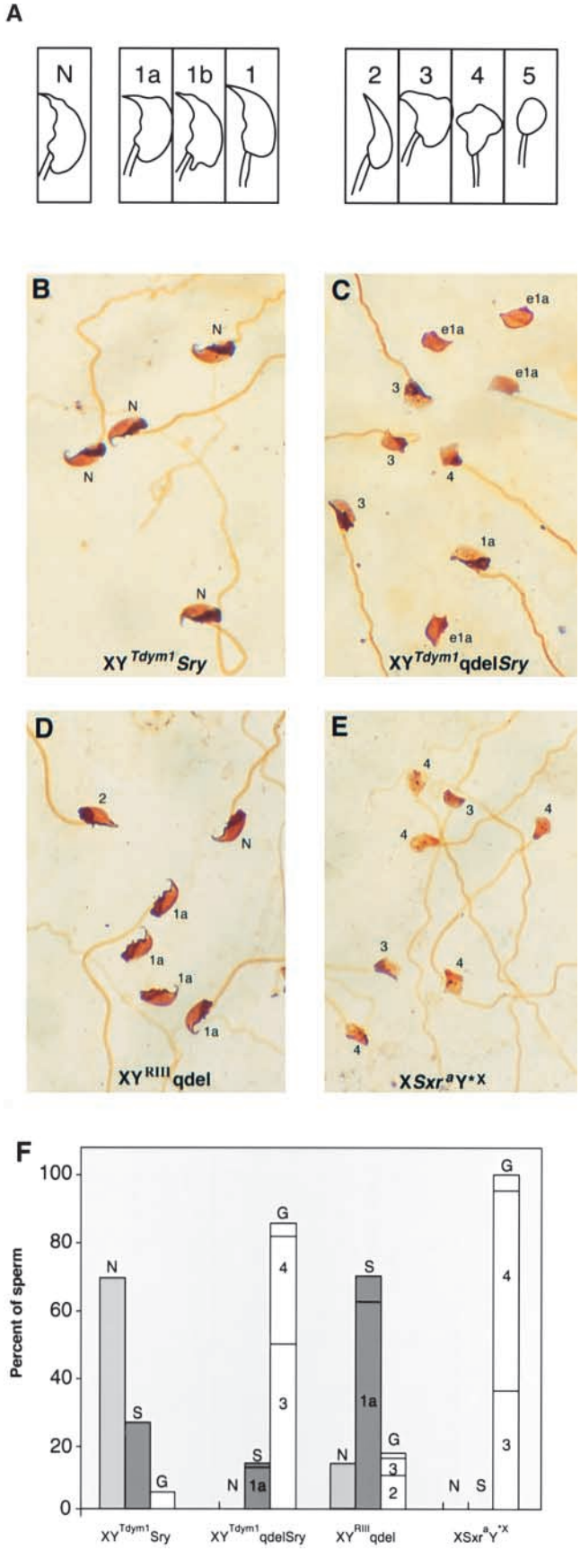

FIGURE 6.—Sperm abnormalities in XY^{Tdym1}qdel*Sry* males. (A) Diagram of our standard categories used in classifying abnormal sperm. (B–E) Examples of silver-stained sperm from control XY^{Tdym1}*Sry* males and the three genotypes with Yq deficiencies. In the XY^{Tdym1}qdel*Sry* males, many of the sperm fell into a category (extreme 1a) that was intermediate between abnormalities 1a and 3. The sperm abnormality classes 1a, extreme 1a, 3, and 4 can be viewed as showing progressively more severe acrosome abnormalities. (F) The percentage of normal, slightly abnormal, and grossly abnormal sperm, together with the proportions of the more prevalent sperm abnormalities, in XY^{Tdym1}qdel*Sry* males compared with those in XY^{Tdym1}*Sry* controls and the previously characterized genotypes XY^{RIII}qdel (predominantly slightly abnormal sperm) and X*Sxr*^{a}Y*^{X} (all grossly abnormal sperm). The sperm from XY^{Tdym1}qdel*Sry* males are much more severely affected than those from XY^{RIII}qdel males, but do not show the 100% gross abnormality characteristic of X*Sxr*^{a}Y*^{X} males. Numbers of animals and of sperm per genotype are: XY^{Tdym1}*Sry* 5, 2000; XY^{Tdym1}qdel*Sry* 4, 1500; XY^{RIII}qdel 2, 400; and X*Sxr*^{a}Y*^{X} 1, 400.

only trace levels of hybridization on Northerns and no expression of the only known *Ssty*-encoded protein, SSTY1.

The pedigree data indicate that the deletion originated in an XXY^{Tdym1} female. We have previously identified Y^{Tdym1} chromosomes with Yq deletions among the progeny of XXY^{Tdym1} females and have postulated that the frequent self-synapsis of the univalent Y^{Tdym1} in these females to form a "hairpin" structure may facilitate intrachromosomal events that result in deletion (MAHADEVAIAH *et al.* 1993). These in fact may be recombination events between runs of Yq repeats that are present in opposite orientations, as in the large palindromic repeats on the human Y (SKALETSKY *et al.* 2003).

In assessing the cause of the sterility of the XY^{Tdym1} qdel*Sry* males, it was established that they successfully mate with females. Sperm numbers assessed from the initial segment of the caput epididymis were substantially reduced compared to controls, perhaps because shedding of sperm from the epithelium was impaired. Nevertheless, the sperm counts were all higher than those expected to lead to sterility (RODRIGUEZ and BURGOYNE 2000, 2001). The sterility is therefore likely to be predominantly due to the defects of sperm differentiation reflected in the distortion of head shape. This is supported by some preliminary *in vitro* fertilization data: 0/62 eggs incubated with sperm from an XY^{Tdym1}qdel*Sry* male progressed to the two-cell stage compared with 40/94 and 42/83 for the controls.

The observed consequences of this new Yq deletion support the view (BURGOYNE *et al.* 1992) that the long arm of the mouse Y chromosome carries factors of crucial importance for sperm differentiation and fertility, with the severity of the phenotype being related to the extent of the deletion. Currently, members of the *Ssty*

TABLE 3

A comparison of the sperm-head abnormalities in X^{Tdym1}Yqdel*Sry* and X*Sxr*^{a}Y*^{X} males

	Frequency by category of sperm abnormality (%)								
Male	1a		1	2	3	4	5	Other	*n*
X^{Tdym1}Yqdel*Sry*									
1	12.3		0.0	0.3	48.7	33.0	0.0	5.7	300
2	10.0		0.2	0.2	52.2	36.5	0.0	0.8	400
3	7.8		0.0	0.0	49.0	42.0	0.2	1.0	400
4	24.2		0.0	0.5	52.2	21.0	0.0	2.0	400
Pooled	13.6		0.1	0.3	50.5	33.1	0.1	2.4	1500
X*Sxr*^{a}Y*^{X}									
1	0.0		0.0	0.0	36.0	59.5	3.0	1.5	400
Male	1a	ext.1a	1	2	3	4	5	Other	*n*
X^{Tdym1}Yqdel*Sry*									
1	4.0	17.5	0.0	0.0	39.5	36.0	1.0	2.0	200
4	12.0	33.5	0.0	0.0	32.0	20.5	2.0	0.0	200
Pooled	8.0	25.5	0.0	0.0	35.8	28.2	1.5	1.0	400
X*Sxr*^{a}Y*^{X}									
1	0.0	2.2	0.0	0.0	33.5	57.2	6.5	0.5	400
2	0.0	0.0	0.0	0.0	0.0	83.5	13.0	3.5	200
Pooled	0.0	1.5	0.0	0.0	22.3	66.0	8.7	1.5	600

gene family are the only genes known to be present in MSYq. Since *Ssty* is expressed in spermatids (Conway *et al.* 1994) and is present in multiple copies widely distributed on MSYq, it has the right credentials to explain the relationship between the size of Yq deletions and the severity of the sperm defects. However, interpretation of this putative relationship is not straightforward for two reasons. First, some uncertainties remain as to the protein-coding potential of *Ssty* transcripts. There is circumstantial evidence that the SSTY1 protein derives from only a subset of *Ssty1* genes that contain a 5′-intron that promotes subsequent transcription (Touré *et al.* 2004). No *Ssty2*-encoded protein has been identified, nor have we been able to identify any intron-containing members of this subfamily. Second, because partial Yq deletions lead to sex-ratio distortion in favor of females, we have long considered the possibility that the multicopy *Ssty* family may function to negate the effects of an X-linked meiotic driver that favors the transmission of the X chromosome (Conway *et al.* 1994). This role for *Ssty* could be mediated at the RNA level, as has been shown in the case for the *Stellate/Suppressor of Stellate* system in Drosophila (Aravin *et al.* 2001). The effects of Yq deletions may therefore depend not only on changes in the levels of *Ssty*-encoded protein(s), but also on changes in the profile of *Ssty* transcripts. It is noteworthy in this regard that SSTY1 expression increases in XY^{RIII}qdel males, despite the reduction in *Ssty1* transcripts; this could be due to an altered balance between protein-coding and noncoding *Ssty* transcripts, the latter acting to inhibit translation of SSTY1.

The XY^{Tdym1}qdel*Sry* males described here proved to be indistinguishable from X*Sxr*^{a}Y*^{X} males with respect to *Ssty* expression and both genotypes are sterile, yet the analysis of sperm-head morphology showed that the sperm-head defects in X*Sxr*^{a}Y*^{X} males are more severe. One possible explanation for this increased severity is that X*Sxr*^{a}Y*^{X} males have a marked reduction in the multicopy *Rbmy* gene family (Mahadevaiah *et al.* 1998), which we show here results in a 95% reduction in RBMY expression. RBMY deficiency has already been implicated as a cause of impaired spermiogenesis in other mice with a deletion removing most of the *Rbmy* gene cluster from Yp (Mahadevaiah *et al.* 1998).

The case for *Ssty* deficiency being responsible for the abnormal sperm development in XY^{Tdym1}qdel*Sry* males and the additional *Rbmy* deficiency for the more severe sperm defects in X*Sxr*^{a}Y*^{X} males would be strengthened if it could be established that no other genes map to the respective Yq and Yp regions. However, obtaining reliable and complete sequence information for large domains with extensive repeats is a daunting task (Kuroda-Kawaguchi *et al.* 2001). We are now using microarray analysis to identify transcriptional differences between mice with Yq deficiencies. In conjunction with the sequence information for the mouse genome, this should eventually allow the chromosomal assignment of all downregulated transcripts. However, proof that these gene deficiencies are responsible can probably derive only from transgene rescue approaches. We have already produced a transgenic line with an expressing *Rbmy* transgene, but for *Ssty* we need to generate new transgenic lines that, unlike previous lines (Touré *et al.* 2004), not only transcribe but also translate *Ssty*.

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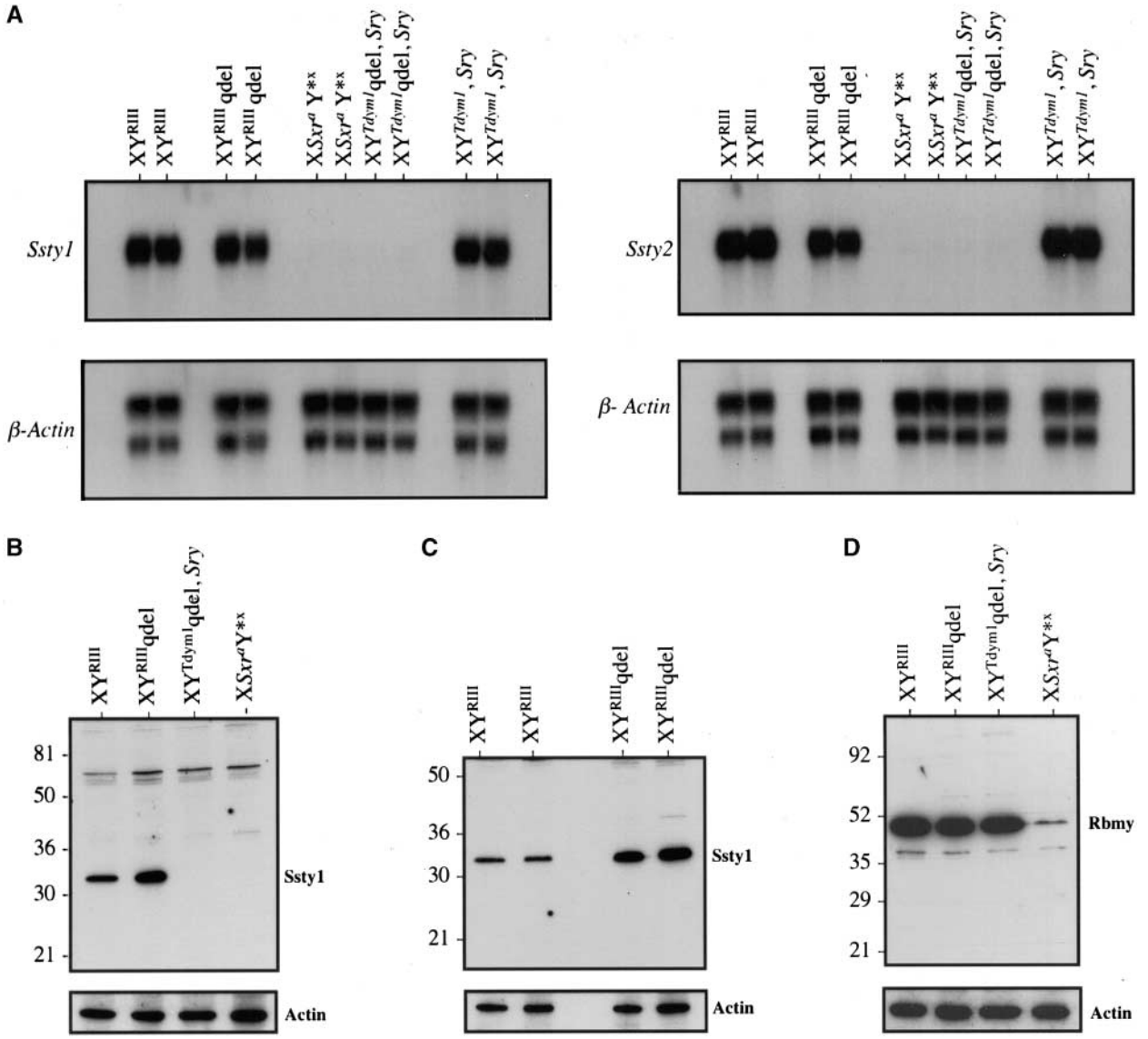

FIGURE 7.—A comparison of *Ssty* and *Rbmy* expression in XY^{RIII}qdel, X*Sxr*^{a}Y*^{X}, and XY^{Tdym1}qdel*Sry* males. (A) Two Northern blots of testis RNA probed with *Ssty1* and *Ssty2* cDNA probes with *actin* serving as a loading control. In XY^{RIII}qdel males, after allowing for loading, there is a 32% reduction in *Ssty1* transcripts relative to XY^{RIII} controls (*Ssty1/actin* ratios from phosphorimager analysis: XY^{RIII}qdel 56, 51; XY^{RIII} 37, 35) and a 54% reduction in *Ssty2* transcripts (*Ssty2/actin* ratios: XY^{RIII}qdel 74, 68; XY^{RIII} 33, 33). In X*Sxr*^{a}Y*^{X} and XY^{Tdym1}qdel*Sry* males, there was only a trace of hybridization with *Ssty1* or *Ssty2*. (B and C) Western analysis with an antibody specific for an *Ssty1*-encoded protein (SSTY1). There is an increase (more than twofold by quantitation) of SSTY1 protein in XY^{RIII}qdel males, but no SSTY1 protein is detected in X*Sxr*^{a}Y*^{X} and XY^{Tdym1}qdel*Sry* males. (D) Western analysis using an antibody that detects RBMY protein. XY^{RIII}qdel and XY^{Tdym1}qdel*Sry* males have normal levels of RBMY, which is consistent with their having a normal number of copies of *Rbmy*. By contrast, X*Sxr*^{a}Y*^{X} males, which have an ~80% reduction in *Rbmy* copies, have a markedly reduced amount of RBMY protein.

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