CHAPTER 1

THE ROLES OF MOUSE Y CHROMOSOME GENES IN SPERMATOGENESIS

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Because the mammalian Y chromosome is largely isolated from the reciprocal recombination with a paired homolog that has underpinned the mapping and positional cloning of genes elsewhere in the genome, the identification of Y gene functions separable from that of testis determination has derived primarily from deletion mapping. Evidence for a Y gene function in spermatogonial proliferation mapping to the mouse Y chromosome short arm was first obtained in 1986, and functions in spermiogenesis to the long arm in 1988 and to a second deletion interval on the short arm in 1998. Defining the gene content of the deletion intervals has been a major task. The first short arm deletion interval proved to be >900 kb, and it was not until 1998 that the eight genes lying partially or completely within the interval were identified. The other two deletion intervals are much larger, and comprise regions of repetitive DNA within which there are multi-copy Y genes. Assigning the functions defined by the deletions to specific genes has proved extremely difficult because gene targeting is not an option for multi-copy genes, and has been unsuccessful for single-copy Y genes. Here, we review attempts to use transgene rescue to identify the Y gene(s) that underlies each of the deletion phenotypes. The only success has been in assigning the Y short arm gene function in spermatogonial proliferation to *Eif2s3y*. There is a closely related X-linked copy, *Eif2s3x*, which encodes a virtually identical protein (a subunit of the translation initiation factor EIF2); we have now shown that an *Eif2s3x* transgene also

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rescues the spermatogonial proliferation failure. Since *Eif2s3x* escapes X inactivation and is thus expressed from both X copies, the normal expressed *Eif2* dosage is two in males and females; this suggests that the spermatogonial block in the deletion mice is due to haploinsufficiency for the EIF2 subunit. However, this still begs the question why spermatogonia are the only cells that are overtly compromised by this haploinsufficiency. Although the transgene rescue approach has so far only achieved this single success, analysis of the *Eif2* transgene rescue mice has pointed to two further functions for genes mapping to the same deletion interval as *Eif2s3y*: one in relation to the spindle checkpoint at the first meiotic metaphase, and the second at the stage when sperm head elongation and tail development begin. Hopefully, it will not be long before the Y genes matching these two new functions are identified.

Keywords: Mouse; Y chromosome; infertility; spermatogonial proliferation; meiotic checkpoints; spermiogenesis.

Introduction

The first evidence that the mammalian Y chromosome encodes information essential for spermatogenesis that is separable from the testis determinant was published for the human Y chromosome in 1976 (the azoospermia factor, AZF), and evidence for a spermatogenesis gene on the mouse Y chromosome 10 years later (Burgoyne *et al.*, 1986; Levy and Burgoyne, 1986a; Tiepolo and Zuffardi, 1976). In the ensuing years, the study of men and mice with deletions of the Y chromosome has increased the number of separable Y-encoded functions in spermatogenesis, and one or a few candidate Y chromosomal genes have been identified within each of the deletion intervals (Mitchell, 2000; Vogt, 2005). Yet, progress in assigning the spermatogenic functions to specific Y genes has been painfully slow. This reflects the fact that the Y chromosome, aside from the pseudoautosomal region (PAR), is isolated from the reciprocal recombination with a paired homolog during meiosis that has underpinned the fine mapping of functions and positional cloning of genes elsewhere in the genome. Furthermore, despite extensive attempts, there have been no reports of the production of mice with targeted mutations of mouse Y genes, despite an encouraging report 3 years ago (Rohozinski *et al.*, 2002). Here, we review attempts to match mouse Y genes to their spermatogenic functions by identifying candidate genes in defined deletion intervals, and then adding back the candidate genes singly to see whether they correct the deletion phenotype.

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The Structure and Gene Content of the mouse Y Chromosome

The mouse Y chromosome (Fig. 1A) has been estimated to contain ∼78 megabases (Mb) of DNA (Gregory *et al.*, 2002), of which 0.7 Mb lies within the single PAR at the end of the long arm (Burgoyne, 1982; Perry *et al.*,

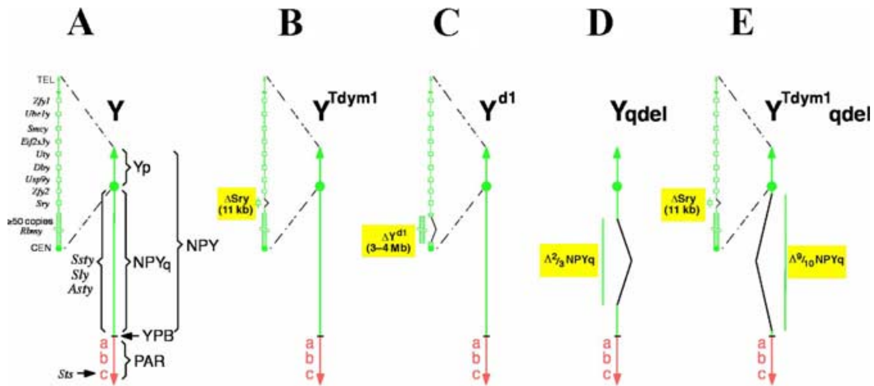

Fig. 1. Diagrammatic representations of the mouse X and Y chromosomes and of variant sex chromosomes relevant to the present review. A. The mouse Y chromosome comprises a short arm (Yp) with seven mapped single-copy genes including the testis determinant *Sry*, one duplicated gene (*Zfy1/2*), and one multi-copy gene *Rbmy*; a long arm (Yq) that can be divided into the distal pseudoautosomal region (PAR) with a single known gene *Sts*; and the remaining non-PAR (NPYq) that is known to consist of highly repeated DNA including multi-copy genes *Ssty*, *Sly*, and *Asty*. **B.** The Y^{Tdym1} variant has a 11 kb deletion removing the testis determinant *Sry* (Gubbay *et al.*, 1992). **C.** The Y^{d1} variant has a deletion of at least 3–4 Mb that removes the majority of the copies of *Rbmy*. XY^{d1} mice are female because *Sry*, although present, is transcriptionally repressed (Capel *et al.*, 1993). To study spermatogenesis in mice with this deletion, it was necessary to complement the *Sry* deficiency with an *Sry* transgene (Mahadevaiah *et al.*, 1998). **D.** Two Yqdel deletions have been described that remove $\sim^2/_3$ of NPYq (Conway *et al.*, 1994; Styrna *et al.*, 1991a). **E.** A larger deletion of $\sim^9/_{10}$ of NPYq occurs in the context of a Y^{Tdym1} chromosome (Touré *et al.*, 2004c). **F.** The mouse X is acrocentric with a distal PAR. **G.** The Y^{*X} chromosome is one of two recombinant chromosomes produced by XY* males (Eicher *et al.*, 1991) (see **J**). It is in essence an X chromosome with a huge deletion removing most of NPX (Burgoyne *et al.*, 1998). **H.** The Sxr^a factor, officially denoted $Tp(Y)1Ct^{Sxr\text{-}a}$ (Cattanach *et al.*, 1971), comprises most of Yp attached distal to the PAR, where it can cross over and thus be present on the X chromosome as shown or on the Y chromosome. In 1984, the Sxr^b variant was discovered, which proved to have >900 kb removing six single-copy Y genes and creating a *Zfy1*/2 fusion gene from the two copies of *Zfy* (Mazeyrat *et al.*, 1998; McLaren *et al.*, 1984; Simpson and Page, 1991). **I.** The X^Y chromosome is the second recombinant chromosome generated by XY* males, and consists of an X chromosome and a Y chromosome joined end to end via their PARs with deletion of both copies of *Sts* (Burgoyne *et al.*, 1998).

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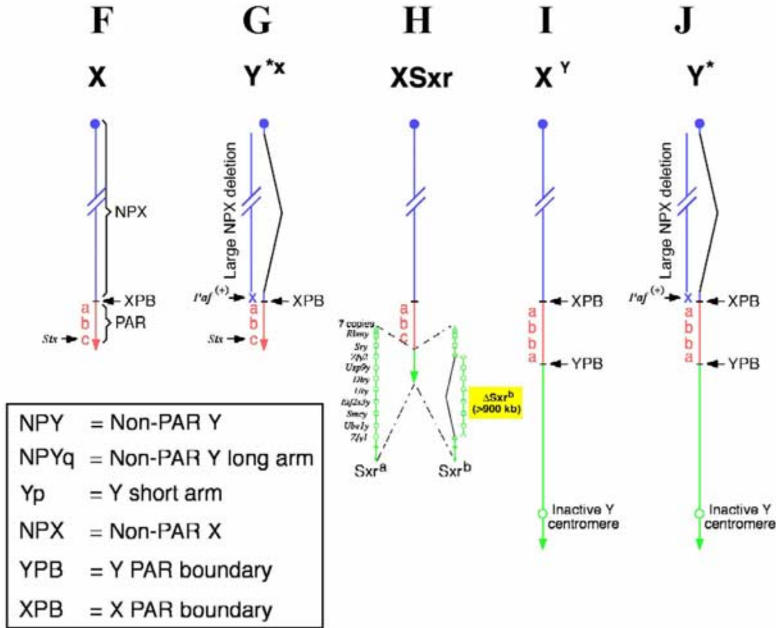

Fig. 1. (*Continued*)

2001); the PAR is homologous to and recombines with the X PAR. The functional genes so far reported to be present on the mouse Y short arm (Yp) are relatively long-term "residents" on the Y of eutherian mammals, and all have X homologs. All but *Rbmy* have been shown to be present on the Y chromosome of the pig and/or the cat, which are placed in a separate superordinal clade to mouse and man (Murphy *et al.*, 2001). *Rbmy* is considered to be one of the oldest residents (Lahn and Page, 1999; Sandstedt and Tucker, 2004), and — together with *Sry*, *Ube1y*, and *Smcy* — is present on the metatherian Y chromosome (Agulnik *et al.*, 1999; Delbridge *et al.*, 1997; Foster and Graves, 1994; Mitchell *et al.*, 1992). The human Y is unusual in that it lacks *Ube1y* and *Eif2s3y*, which were lost during the evolution of the primate lineage (Ehrmann *et al.*, 1998; Mitchell *et al.*, 1998), but X copies of these genes have of course been retained.

The male-specific region of the mouse Y long arm (non-PAR Y long arm, NPYq), which represents about 90% of the total NPY, contains highly repetitive DNA within which lie multiple copies of at least four distinct

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genes that are expressed in spermatids (Bishop and Hatat, 1987; Conway *et al.*, 1994; Prado *et al.*, 1992; Touré *et al.*, 2004a; Touré *et al.*, 2005). These NPYq genes may be restricted to the muridae, since no related Y chromosomal sequences have been detected in the rat by Southern analysis.

The Y^{d1} Deletion Causes an Increase in Sperm Defects and *Rbmy* Deficiency

As will become apparent in the course of this review, the mouse Yp-derived sex reversal factor *Sxr*^{a} (Fig. 1H) — officially denoted Tp(Y)1Ct$^{Sxr\text{-}a}$ (Cattanach *et al.*, 1971) — has been of critical importance in delineating mouse Y functions in spermatogenesis. Carrier males have an additional copy of most of Yp (from the telomere to within the *Rbmy* cluster close to the centromere) located on Yq distal to the PAR, and this can consequently be transferred to the X chromosome by PAR recombination. *Sxr*^{a} can also recombine with Yp (McLaren *et al.*, 1988); this, by unequal crossing-over within the *Rbmy* repeat, very occasionally deletes multiple copies of the repeat (Laval *et al.*, 1995). The Y^{d1} deletion (Δ^{d1}) is the most extensive (at least 3–4 Mb), with a >10-fold reduction in *Rbmy* copies (Fig. 1C) and an even greater reduction in *Rbmy* transcription (Mahadevaiah *et al.*, 1998). Males with this deletion have an increased incidence of sperm with sperm head defects (Fig. 2). *Rbmy* encodes a nuclear RNA-binding protein that is implicated in splicing (Elliott, 2000; Elliott *et al.*, 1996). From their expression analysis, Mahadevaiah *et al.* (1998) concluded that *Rbmy* is expressed in spermatogonia, is shut down in pachytene spermatocytes (in which the X and Y chromosomes are transcriptionally repressed — see chapter 2), and is then reactivated in spermatids with the remaining protein expressed in elongating spermatid stages. This pattern of expression suggested that reduced expression of *Rbmy* in Δ^{d1} males during spermiogenesis might be responsible for the increased incidence of abnormal sperm heads. However, the Δ^{d1} sperm phenotype was not "rescued" by the provision of an *Rbmy* transgene comprising an *Rbmy* cDNA driven by the spermatid-specific mouse protamine 1 (mP1) promoter, even though transcription and translation of *Rbmy* from the transgene was confirmed (Szot *et al.*, 2003).

Perplexingly, the antibody used in this study failed to detect any RBMY protein in spermatids in the control males; this is in agreement with the

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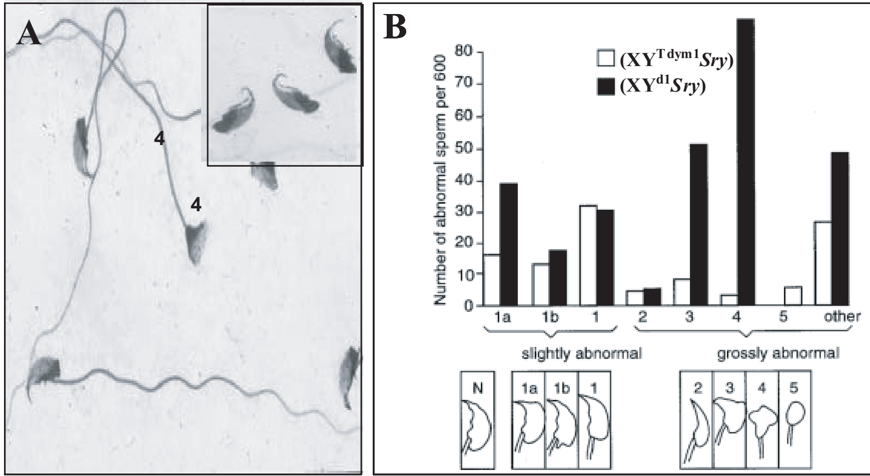

Fig. 2. The Y^{d1} deletion is associated with an increased frequency of abnormal sperm, although males with this deletion are fertile. A. Sperm from an XY^{d1}*Sry* male, showing two normal sperm and two sperm with the most prevalent head abnormality (4) (cf. sperm from control XY^{Tdym1}*Sry* male, inset). **B.** Histogram showing the overall increase in frequency of abnormal sperm in XY^{d1}*Sry* males as compared to controls (data from Mahadevaiah *et al.*, 1998).

recent findings by another group (Lee *et al.*, 2004), who failed to detect either *Rbmy* RNA (by *in situ* hybridization) or RBMY protein in spermatids. Nevertheless, we feel it is premature to conclude that the original evidence of expression in spermatids is incorrect. There is no question, in our view, that the *Rbmy* cDNA probe used by Mahadevaiah *et al.* (1998) detected transcripts in round spermatids, so it is not clear why the *Rbmy* cDNA used by Lee *et al.* (2004) to provide a probe for *in situ* hybridization failed to show specific localization to round spermatids. With respect to the conflicting antibody results, it remains possible that spermatids transcribe an *Rbmy* splice variant encoding a protein that lacks the epitope recognized by the later antibodies. The *Rbmy* cDNA used for the mP1-*Rbmy* transgene construct that did produce a protein detected by our later antibody originated from a 17.5 dpp testis library, and was thus not derived from a spermatid transcript.

If *Rbmy* is not expressed in spermatids, it seems perhaps unlikely that the reduced expression in spermatogonia and early spermatocytes in Δ^{d1} males would lead to an increased incidence of sperm head abnormalities. It is of

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course possible that there is another gene(s) mapping to this deletion interval that is expressed in spermatids, but no other genes have yet been identified in this interval. Another possibility is that the deletion leads to the repression of a gene lying outside the deletion. We already know that Δ^{d1} leads to the repression of *Sry* transcription; indeed, Δ^{d1} mice develop as females (Capel *et al.*, 1993), and the identification of the effects of the deletion on spermatogenesis required the addition of an *Sry* transgene (Mahadevaiah *et al.*, 1998). The repression of *Sry* is thought likely to be due to the gene having been brought close to centromeric heterochromatin. Very recently, a Y-encoded transcript was identified that almost certainly derives from spermatids (Ellis *et al.*, 2005) and that matched a Y transcript (XM_358268) predicted from the mouse Y sequence; the gene(s) encoding this sequence apparently maps somewhere between *Zfy2* and the Yd1 deletion. However, by microarray testis transcriptome analysis of Δ^{d1} males, we have been unable to detect a reduction of XM_358268 or any other Y-encoded transcript that would constitute alternative candidates to *Rbmy*.

NPYq Deletions Affect Sperm Development, with Larger Deletions Causing Sterility

Two deletions removing $\sim^2/_3$ of NPYq ($\Delta^2/_3$NPYq) have been described: one involving a C57BL/10 strain Y chromosome, and the other an RIII strain Y chromosome (Fig. 1D). Both deletions have been shown to cause minor changes in sperm head shape, notably a flattening of the acrosomal cap (Fig. 3B); and although the mice are of good fertility, *in vitro* tests showed that the sperm have impaired fertilizing ability and that there is an intriguing distortion of the offspring sex ratio in favor of females (Conway *et al.*, 1994; Moriwaki *et al.*, 1988; Siruntawineti *et al.*, 2002; Styrna *et al.*, 2002; Styrna *et al.*, 1991a; Styrna *et al.*, 2003; Styrna *et al.*, 1991b; Touré *et al.*, 2004b; Xian *et al.*, 1992). Analysis of mice with the B10 Y deletion also found increased aromatase activity in the testis with a consequent increase in levels of estradiol relative to testosterone (Kotula-Balak *et al.*, 2004).

Two, more extensive, NPYq deficiencies have been described that cause sterility. Total absence of NPYq (NPYq$^-$) occurs in mice with the exotic genotype X*Sxr*^{a}Y^{*X} (Burgoyne *et al.*, 1992), in which the only NPY region present is the Yp-derived *Sxr*^{a} (Fig. 1H). The minute Y^{*X} chromosome

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(Eicher *et al.*, 1991) is in effect a highly deleted X chromosome (Burgoyne and Evans, 2000; Burgoyne *et al.*, 1998), and serves to provide a second PAR (Fig. 1G). The second is a recently described deletion removing $\sim^9/_{10}$ of NPYq ($\Delta^9/_{10}$NPYq) that occurs in the context of an *Sry*-negative Y chromosome (Fig. 1E), necessitating the complementation of the *Sry* deficiency with an *Sry* transgene (Touré *et al.*, 2004b). In both cases, all the sperm have grossly misshapen sperm heads (Figs. 3C and 3D), which are presumably the cause of the infertility.

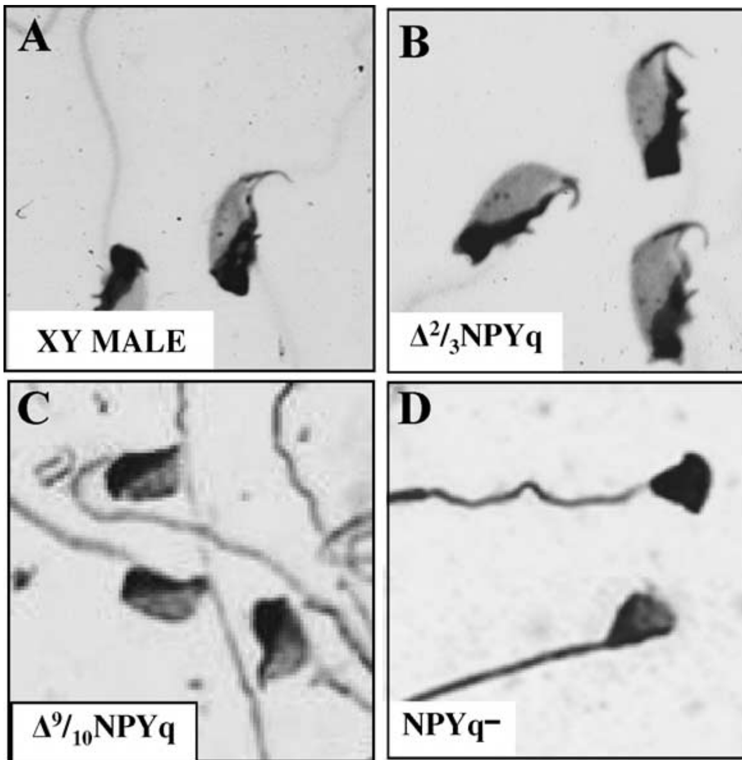

Fig. 3. Sperm abnormalities in mice with NPYq deficiencies. A. Sperm from a control male. **B.** Sperm from a $\Delta^2/_3$NPYq male, in which the majority of sperm have a broadening of the acrosomal region and a flattening of the acrosome anteriorly. Although the sperm perform poorly *in vitro*, the males are fertile. **C.** Sperm from a $\Delta^9/_{10}$NPYq male showing the more severely affected sperm, again with a flattened acrosome anteriorly. These males are almost invariably sterile. **D.** Grossly abnormal sperm from an X*Sxr*^{a}Y^{*X} (NPYq$^-$) male, in which the Yp-derived *Sxr*^{a} factor provides the only NPY material. All males tested are sterile.

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In considering what might be the genetic basis of the spermiogenic defects in mice with NPYq deficiencies, it is simpler to summarize what we now know about the NPYq-encoded testis transcriptome rather than to give a historical perspective. We sought to determine this directly by generating a mouse testis cDNA microarray enriched for spermatogenic cell transcripts (Ellis *et al.*, 2004), and then identifying NPYq-encoded transcripts among those that are dramatically reduced or absent in $\Delta^9/_{10}$NPYq and NPYq$^-$ males (Touré *et al.*, 2005). This study was complemented by the Mouse Y Sequencing Program, which has been tackling the herculean task of sequencing NPYq [Mouse Chromosome Y Mapping Project (Jessica E. Alfoldi, Helen Skaletsky, Steve Rozen, and David C. Page at the Whitehead Institute for Biomedical Research, Cambridge, MA, and the Washington University Genome Sequencing Center, St. Louis, MO)]. The testis-expressed NPYq genes identified so far (*Ssty1/2*, *Sly*, and *Asty*) are multi-copy, are expressed in spermatids, and show a progressive reduction in transcript levels with increasing NPYq deficiency; thus, they are all candidates for contributing to the sperm defects associated with NPYq deletions. All have multi-copy X homologs.

Based on the microarray data and Northern analysis, *Ssty2* appears to be the most abundant transcript, but no protein product has yet been identified. *Ssty1* is much less abundant, but does produce a protein (Touré *et al.*, 2004a) that has substantial homology to the autosomally encoded protein SPIN (Oh *et al.*, 1998; Oh *et al.*, 1997). Genes encoding SPIN are now widely found in vertebrates including man, chicken, the frog *Xenopus tropicalis*, and fish (Itoh *et al.*, 2001; Wang *et al.*, 2005). In the mouse, SPIN is found in oocytes and early embryos, where it locates to the metaphase spindle (Oh *et al.*, 1998). *Sly* is also a very abundant transcript in spermatids, but it is not yet known whether the transcripts are translated. If *Sly* does make a protein, it would have substantial homology to the chromatin-associated proteins XLR (46% amino acid identity) and XMR (48% identity) that are encoded by the related multi-copy *Xlr/Xmr* gene family on the X chromosome. These related X and Y genes in turn have homology — particularly in the COR 1 domain (pFAM accession PF04803) — with the protein SYCP3, which is also chromatin-associated in that it is a component of the synaptonemal complex of paired homologous chromosomes in meiosis. *Asty* seems to be transcribed at a very low level, but there are transcripts derived from *Asty*

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recombinant loci [*Asty*(rec)] lacking the first *Asty* exon and driven by an *Ssty1* promoter that seem to be more abundant. At present, no strong case can be made for the translation of the *Asty* or *Asty/rec* transcripts.

On and off over the past 12 years, we have attempted transgene rescue of the MSYq deletion phenotypes. Initially, we used *Ssty1* and *Ssty2* cDNAs driven by the spermatid-specific mP1 promoter (which was used for the *Rbmy* cDNA transgene construct). These *Ssty1* and *Ssty2* transgenes were expressed at levels close to endogenous levels, but neither singly nor in combination had any detectable effect on the flattened acrosome phenotype or offspring sex ratio distortion of $\Delta^2/_3$NPYq males, or on the more severe sperm defects of $\Delta^9/_{10}$NPYq and NPYq$^-$ males (unpublished data). However, we subsequently found that the mP1-*Ssty1* transgene is not translated (supplemental information in Touré *et al.*, 2004a). Since then, we have made transgenic lines with partially characterized genomic BAC clones containing *Ssty1* and/or *Ssty2*, but these failed to transcribe. Most recently, we have tried a sequenced *Sly* BAC (kindly provided by Jessica Alfoldi of the Mouse Y Sequencing Consortium), but again this failed to rescue the MSYq-deletion phenotypes. However, the level of expression from the *Sly* transgene was markedly lower than the endogenous level, so this needs to be repeated with a more highly expressing *Sly* transgene. An alternative approach for these multi-copy NPYq genes would be to reduce transcript levels in normal males for each gene separately by using an RNAi approach, either by injecting the siRNAs directly into the testis (Shoji *et al.*, 2005) or by transgenic delivery of short hairpin RNAs that are converted into siRNAs (Rubinson *et al.*, 2003).

Evidence That an NPYq Gene(s) Represses X Gene Expression in Spermatids

The microarray analysis of the testis transcriptome of the males with NPYq deficiencies, in addition to identifying NPYq genes expressed in the testis, also identified some downstream transcriptional consequences of the deficiencies. The most dramatic finding was that a number of X-linked genes expressed in spermatids were markedly upregulated, among which were *Xmr* and some *Xmr*-related family members. As we have seen, *Xmr* shows significant homology to the NPYq multi-copy gene *Sly* and is a member of the complex X-linked *Xmr/Xlr* gene family of chromatin-associated

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proteins. It has previously been argued that the amplification of X and Y genes could be a consequence of a past "genomic conflict" between sex-linked meiotic drivers and suppressors (Hurst, 1992). Briefly, if the X acquires a gene (or a mutation) that promotes transmission of X-bearing gametes, thus distorting the sex ratio in favor of females, then there will be selection for a mechanism that will counteract this sex ratio distortion; this could be the acquisition or mutation of a Y gene that acts to rebalance the sex ratio. Duplication of the X gene may then distort the sex ratio again, leading to selection for duplication of the Y gene; over time, this may lead to multiple copies of the X and Y genes, which are in balance with respect to their effects on the sex ratio. Deletion of some of the Y genes is predicted to lead to offspring sex ratio distortion in favor of females, just as we see with $\Delta^2/_3$NPYq. Support for this genomic conflict model is provided by *Drosophila Stellate* (multi-copy gene on the X encoding a subunit of casein kinase II) and *Suppressor of Stellate* (multi-copy suppressor on the Y) (Hurst, 1992; Hurst, 1996). Recent evidence suggests that *Suppressor of Stellate* keeps *Stellate* in check by generating small interfering RNAs that target the *Stellate* RNA (Aravin *et al.*, 2001). We feel that the situation in the mouse is likely to be more complicated because not only are several X genes upregulated in spermatids, but so are some spermatid-expressed Yp genes (Ellis *et al.*, 2005). Solving this puzzle will be a fascinating task for the future.

The Consequences of Sex Chromosome Univalence at the First Meiotic Metaphase

Evidence from yeast to man has established that when mitotic cells reach metaphase of the cell division cycle, the cell checks to make sure that all the chromosomes have a bipolar attachment of their centromeres (kinetochores) to the mitotic spindle, and that anaphase onset (chromatid separation) is prevented if this is not achieved (Tan *et al.*, 2005). It is widely assumed that a similar checkpoint operates at the first and second meiotic metaphases. The mechanics of the second meiotic division are exactly comparable to mitosis, but the first meiotic metaphase (MI) is unusual in that it is the pairs of homologous chromosomes (each pair is termed a bivalent) that must align on the metaphase plate and separate into their constituent chromosomes at

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anaphase. This requires that the two kinetochores (of the two chromatids) of one chromosome must mono-orient towards one spindle pole, and the two kinetochores of the second chromosome must mono-orient towards the opposite spindle pole; following attachment to the spindle, the two chromosomes are then drawn to opposite poles. While the molecular basis of the spindle checkpoint at the first meiotic metaphase has been defined in some detail in the yeast and is closely related to that operating at mitosis, the details are less clear for mammals (Petronczki *et al.*, 2003). Furthermore, there seem to be differences in the efficiency of the spindle checkpoint between male and female mammals (Eaker *et al.*, 2002; Eaker *et al.*, 2001; Homer *et al.*, 2005; LeMaire-Adkins *et al.*, 1997; Wassmann *et al.*, 2003).

For the XY pair in mouse spermatogenesis, it is the obligate chiasma between the X and Y PARs that holds the X-Y bivalent together at the first meiotic metaphase, and it is the pairs of X and Y kinetochores that orient towards opposite poles; thus, the X and Y separate at anaphase I (Fig. 4A). In the X*Sxr*^{a}Y^{*X} (NPYq^{-}) mouse described in the previous section, the obligate chiasma is in fact between two X PARs and it is two X centromeres that attach to opposite spindle poles. Nevertheless, this minimal sex bivalent satisfies the requirements of the spindle checkpoint and, as described in the previous section, these mice have active spermiogenesis (although the sperm produced are grossly abnormal). The crucial importance of maintaining a sex bivalent association by means of this PAR-located chiasma can be illustrated by considering the consequences of removing the Y^{*X} chromosome to produce an X*Sxr*^{a}O male. With only a single univalent sex chromosome present, spermatogenesis arrests at MI and the arrested cells are eliminated by apoptosis (Cattanach *et al.*, 1971; Kot and Handel, 1990; Sutcliffe *et al.*, 1991). The same MI arrest is seen in males with a single X-attached-Y chromosome (X^{Y}, Fig. 1I), but importantly, in this case the addition of a Y^{*X} chromosome (to give X^{Y}Y^{*X}) does not overcome the arrest (Burgoyne *et al.*, 1992). The crucial difference between the two genotypes is that X*Sxr*^{a}Y^{*X} males form a sex bivalent; but in X^{Y}Y^{*X} males, the interstitial positioning of the X^{Y} PAR is incompatible with synapsis, and thus the X^{Y} and Y^{*X} are present as univalents at MI. Although in 1992 we favored the view that the MI arrest reflected a requirement for PAR synapsis *per se*, we now believe the MI arrest and subsequent apoptosis are due to the operation of an MI checkpoint which, as in yeast, detects the presence of univalent chromosomes that are only attached to a single spindle pole

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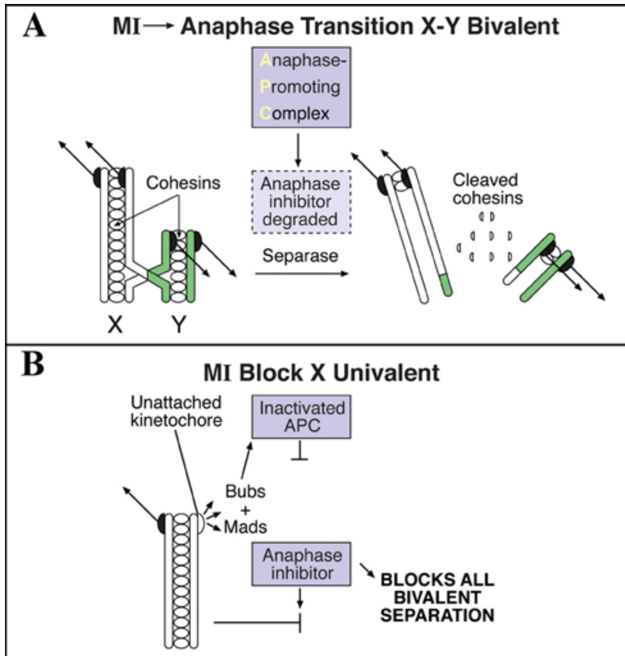

Fig. 4. The disjunction of the X and Y at the first meiotic division, and the postulated spindle checkpoint response in the face of sex chromosome univalence. A. All the paired chromosomes (bivalents) have to align on the metaphase plate before disjunction of homologs takes place. In the case of the X-Y bivalent, this requires that the two kinetochores of the sister X chromatids mono-orient towards one pole and attach to spindle microtubules originating from that pole (syntelic attachment), while the sister Y chromatids achieve syntelic attachment to the opposite pole. Once all bivalents are attached in this way to the spindle, the anaphase-promoting complex (APC) triggers the degradation of an inhibitor of the enzyme separase, which then cleaves the cohesins between the chromatid arms (but not at the centromeres) of all the bivalents. This releases the link between homologs provided by chiasmata (the obligatory PAR chiasma in the case of the X-Y bivalent), allowing homologs to disjoin (Petronczki *et al.*, 2003). **B.** If any univalent chromosomes are present (for example, the X of X*Sxr*^{a}O males), this is thought to inhibit mono-orientation, and either attachment to the spindle fails to take place or (as illustrated) a single kinetochore attachment is made; bipolar attachment is rarely achieved. If kinetochores that are not attached to the spindle are present, spindle checkpoint proteins of the Bub and Mad families maintain the APC in an inactive state and the anaphase inhibitor prevents activation of separase, thus preventing disjunction of all bivalents. In the rare case when bipolar attachment to the spindle is achieved, this may avoid the spindle checkpoint and lead to separation of the chromatids of the univalent at MI. This model is based principally on molecular analysis of the checkpoint in budding yeast, but the molecular details of the checkpoint at MI in male mammals is ill-defined. Indeed, one of the spindle checkpoint proteins, Mad2, which is shed from the kinetochores during mitosis when spindle attachments are completed, is apparently retained at the kinetochores throughout the first meiotic division in male rats and mice (Kallio *et al.*, 2000).

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(Fig. 4B). The important message in the current context is that sex chromosome univalence will cause MI arrest, even if there is a full complement of Y genes.

Eif2s3y from the *Sxr^b* Interval on Yp Has a Role in Spermatogonial Proliferation

An important milestone in defining the functions of the mouse Y chromosome was the discovery by McLaren *et al.* (1984) of the variant *Sxr^b* (Fig. 1H), officially Tp(Y)1Ct$^{Sxr\text{-}b}$, which had lost genetic information required for H-Y antigen expression. Subsequent comparison of X*Sxr^b*O with X*Sxr^a*O males revealed that this variant had also lost information needed for normal spermatogonial proliferation (Burgoyne *et al.*, 1986; Sutcliffe and Burgoyne, 1989). The *Sxr^b* variant arose in a male that was carrying two copies of *Sxr^a*, one attached to the X PAR and the other to the Y PAR, and it was suspected that unequal crossing-over had resulted in the deletion of a gene(s) encoding H-Y. This was subsequently substantiated by Simpson and Page (1991), who showed that there was indeed a deletion with breakpoints lying within the zinc finger genes *Zfy1* and *Zfy2*. It was concluded that *Zfy1* from one *Sxr^a* copy had aligned and recombined with *Zfy2* from the second *Sxr^a* copy, and the result was the generation of a *Zfy2*/*Zfy1* fusion gene (which is still transcribed) and the loss of the >900 kb of DNA lying between the two loci. The first gene to be identified from within the deletion was *Ube1y*, which — like its X homolog *Ube1x* — is presumed to produce the ubiquitinating enzyme E1 (Kay *et al.*, 1991; Mitchell *et al.*, 1991), but it was a further 7 years before the full (we hope!) gene content of the deletion interval was determined (Mazeyrat *et al.*, 1998).

Attempts to rescue the spermatogonial block in X*Sxr^b*O mice by adding back genes from the *Sxr^b* deletion interval, as transgenes followed along in the wake of the gene discovery (Agulnik *et al.*, 2001; Mazeyrat *et al.*, 2001), eventually established that the spermatogonial proliferation factor was *Eif2s3y* (Mazeyrat *et al.*, 2001). *Eif2s3y* is a Y chromosomal homolog of the X-linked *Eif2s3x* that encodes subunit 3 of the translation elongation and initiation factor EIF2. Since making proteins is an essential cellular function, it is not surprising that these genes are widely (presumably ubiquitously) expressed (Ehrmann *et al.*, 1998). However, given the near

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identity (97%) of the predicted X- and Y-encoded proteins, why has a functional copy been retained on the majority of eutherian Y chromosomes? *Eif2s3x* is one of only a handful of mouse X genes that are known to escape X inactivation (Ehrmann *et al.*, 1998) and that have ubiquitously expressed Y homologs. It is argued that these genes encode functions for which expressed dosage is critical; and that this has required the retention of functional Y copies, together with X copies, which escape X inactivation. We have formally tested the underlying assumption that the *Eif2s3y* and *Eif2s3x* serve equivalent functions by seeing if an *Eif2s3x* transgene also rescued the spermatogonial block in X*Sxr*^{b}O males; this proved to be the case (unpublished). This still begs the question as to why differentiating A spermatogonia are the only cells for which EIF2S3 dosage is critical. As we observed earlier, the human Y does not have a copy of *Eif2s3y*, but it does have an autosomal copy originating from retroposition of an X transcript, which may have allowed the loss of the Y copy (Ehrmann *et al.*, 1998).

Are there any genes remaining in *Sxr*^{b} that are essential for spermatogenesis prior to the block seen in X*Sxr*^{b}O males? *Sxr*^{b} includes the testis determinant *Sry*, which is required for obvious reasons. But, evidence that there are no other mouse Y genes with essential early spermatogenic functions comes from our finding that XO,*Sry* transgenic males have a block in spermatogonial proliferation indistinguishable from that in X*Sxr*^{b}O mice, and that addition of *Eif2s3y* to XO,*Sry* transgenic males similarly overcomes this block (Mazeyrat *et al.*, 2001).

Evidence for Further Spermatogenic Functions on Yp Mapping to Δ*Sxr*^{b}

It is our belief that the majority of the current mouse Yp gene complements, particularly those that have a testis-specific pattern of expression (e.g. *Zfy1/2*, *Ube1y*, *Usp9y*), will prove to potentiate the spermatogenic process, even if they are not essential. We have therefore analyzed the spermatogenic phenotype of the X*Sxr*^{b}O males and XO,*Sry Eif2s3y* transgene rescue males (hereafter referred to as "*Eif2* rescue males"), seeking to obtain evidence for functions that can be linked by transgene rescue to further Δ*Sxr*^{b} genes. If the addition of *Eif2s3y* achieved full rescue of the Δ*Sxr*^{b} phenotype, the mice would be expected to show the checkpoint-initiated MI arrest and apoptosis that are seen in X*Sxr*^{a}O males. If so, then provision of the Y^{*X} to

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generate a minimal sex bivalent, and thus avoid the MI checkpoint, should allow assessment of any postmeiotic functions of ΔSxr^b.

As already pointed out by Mazeyrat *et al.* (2001), the spermatogenic phenotype of the *Eif* rescue males is not equivalent to that seen in XSxr^a O males. Intriguingly, spermatogenesis proceeds a little further, with the majority of spermatocytes completing the first meiotic division to form diploid secondary spermatocytes, but failing to undergo the second meiotic division (Fig. 6A). This spermatogenic phenotype suggests two possible ΔSxr^b gene functions: one needed for the efficient functioning of the MI spindle checkpoint, and one to allow progression through the second meiotic division. We have so far added back to the *Eif2* rescue males the ΔSxr^b genes *Ube1y*, *Dby*, *Smcy*, and *Usp9y*; individually, none of these transgenes have reinstated the MI checkpoint. The only other candidate gene we know of within the interval is *Uty*, for which we now have transgenic lines established. However, it also remains possible that the disruption of the MI checkpoint is a consequence of *Zfy* deficiency, since only a single *Zfy2/1* fusion gene remains in the deletion mice. Alternatively, it could be that the simultaneous reintroduction of two ΔSxr^b genes is required to reinstate MI checkpoint function.

Although we have as yet been unable to reinstate the MI checkpoint function, we have nevertheless gone ahead and added back Y^{*X} to *Eif2* rescue males in order to recreate a minimal sex bivalent and thus avoid any checkpoint response to sex chromosome univalence. If the failure to progress through the second meiotic division is due to a ΔSxr^b gene deficiency, the addition of Y^{*X} should make no difference, but in fact we found that the majority of secondary spermatocytes now complete the second division to form haploid round spermatids (Fig. 5A). This implies that the failure to complete the second meiotic division is a consequence of sex chromosome univalence (or PAR dosage deficiency) rather than the lack of a ΔSxr^b gene.

In the absence of any further ΔSxr^b gene functions, we would expect the spermatogenic phenotype of the *Eif2* rescue males after the addition of Y^{*X} to equate to that in X$Sxr^a Y^{*X}$ males; namely, active spermiogenesis with the production of near normal numbers of sperm, albeit with grossly abnormal heads (Burgoyne *et al.*, 1992). However, the haploid spermatids formed in these *Eif2* rescue + Y^{*X} males fail to elongate or form an axoneme (Fig. 5B), thus encouraging our view that there will be further identifiable

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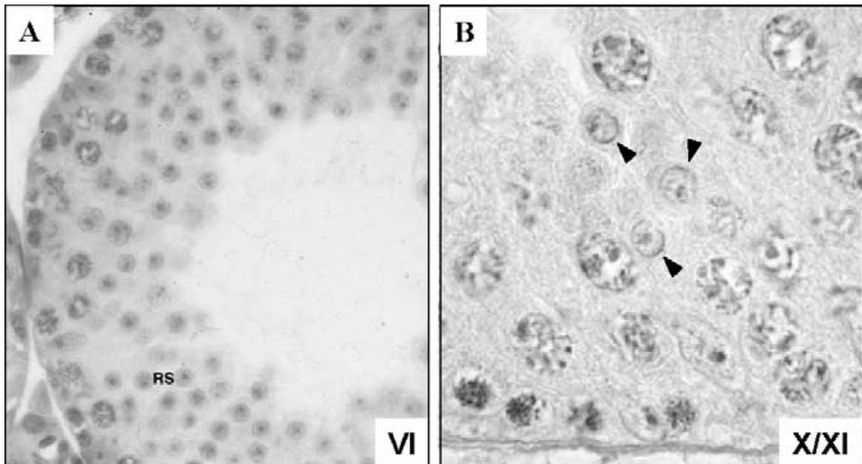

Fig. 5. A gene mapping to the Δ*Sxr*^{b} interval on mouse Yp has a role in spermatid development. X*Sxr*^{b}O,*Eif2s3y* males, in which the spermatogonial block due to Δ*Sxr*^{b} has been rescued by an *Eif2s3y* transgene, have spermatogenic arrest during the meiotic divisions (see Fig. 6A) (Mazeyrat *et al.*, 2001). This arrest can be avoided by providing the tiny Y^{*X} chromosome (Fig. 1G) which, through PAR recombination with the X*Sxr*^{b} chromosome, forms a sex bivalent. **A.** At tubule stage VI in these X*Sxr*^{b}Y^{*X},*Eif2s3y* males, it is apparent that spermatogenesis has progressed to the round spermatid stage (RS). **B.** At tubule stage X/XI, spermatids of control males or X*Sxr*^{a}Y^{*X} males have elongated heads with a developing axoneme (not shown); but in these X*Sxr*^{b}Y^{*X},*Eif2s3y* males, the sperm head fails to elongate and there is no axoneme (step 7 arrest — arrows).

functions for Δ*Sxr*^{b} genes. Once again, we have tried transgene rescue to overcome the block in spermatid differentiation; frustratingly, *Ube1y*, *Dby*, *Smcy*, and *Usp9y* have each again failed to rescue the block. *Usp9y* was our strong favorite, but because this is a very large gene with 47 exons spanning ~80 kb, we had to create a minigene for the transgene rescue. We know the minigene is transcribing appropriately in spermatids, but we have yet to show that the transgene RNA is translated.

Secondary Changes in the Testicular Phenotype in Mice with Y Deficiencies

Because the mice with Y deficiencies that we have studied can be generated at will (although often requiring complex mouse crosses), we have the luxury of being able to study the spermatogenic phenotype from birth

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through to adulthood. Such longitudinal studies have previously suggested that genetic defects which initially lead to arrest at specific spermatogenic stages may subsequently manifest with a more complex phenotype as a consequence of secondary changes. Thus, in X*Sxr*^{b}O males, studies of the first spermatogenic wave revealed a uniform block in the proliferation of differentiating A spermatogonia; but very occasionally, groups of cells that reached the pachytene stage were seen in older mice (Burgoyne *et al.*, 1986; Sutcliffe and Burgoyne, 1989). Similarly, in young X*Sxr*^{a}O males, there is almost total arrest at MI followed by apoptotic elimination of all the arrested cells; but in older males, some cells complete one or both meiotic divisions and may progress through spermiogenesis to form sperm with abnormal sperm heads (Levy and Burgoyne, 1986b; Sutcliffe *et al.*, 1991).

We have documented the secondary changes a little more thoroughly in *Eif2* rescue males (Mazeyrat *et al.*, 2001). In the mouse, the first wave of spermatogenesis is complete by about 1 month. At this age, the *Eif2* rescue males show a uniform phenotype of spermatocyte arrest prior to the second meiotic metaphase, with the arrested cells remaining for one or more spermatogenic cycles before forming multinucleate bodies (apoptotic?) that are presumably in the process of being phagocytosed by the Sertoli cells (Fig. 6B). By 2 months (mice are mature at 6 weeks), holes begin to appear at the base of the seminiferous epithelium, perhaps due to the death of Sertoli cells that have been overwhelmed by the apoptotic load (Fig. 6C). This undoubtedly destroys the patency of the Sertoli cell tight-junctional blood-testis barrier, which separates premeiotic from meiotic cells (Dym and Fawcett, 1970; Xia *et al.*, 2005). By 3 months, there is usually a mosaic phenotype: some tubule sections show severe germ cell loss [and may even appear to be Sertoli cell-only (SCO)]; but paradoxically, some others have elongating spermatids present, albeit with abnormal heads (Fig. 6D). This is very reminiscent of the testicular phenotype of many men with AZFb or AZFc deletions. By 6 months, some *Eif2* rescue males have such severe spermatogenic damage that it would be easy to mistake them for males without the *Eif2s3y* transgene (Fig. 6E).

It is important to note that although in some tubules the spermatogenesis gets progressively worse, in others spermatogenesis may proceed beyond the stage of the initial block for a while. In this context, it is important to remember that all mouse Y genes identified so far have related homologs on

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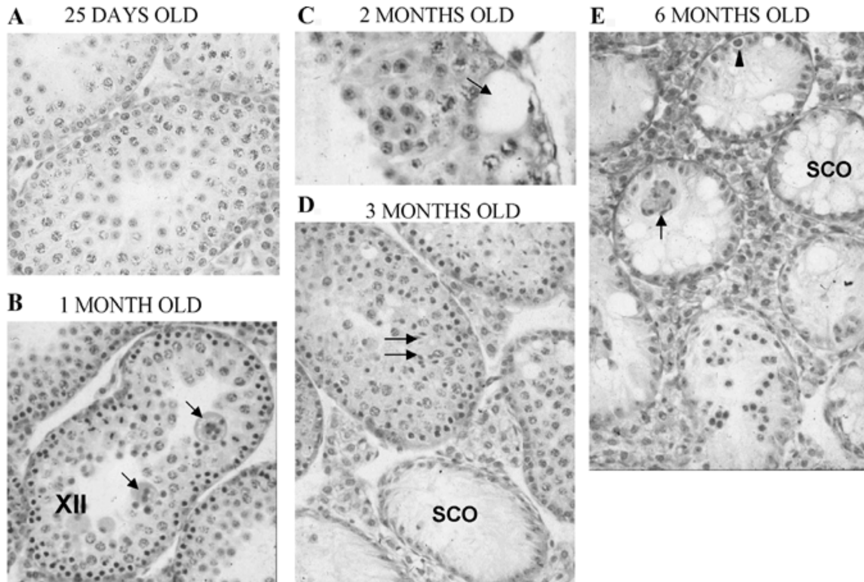

Fig. 6. Secondary changes in the testes of X*Sxr*^{b}O,*Eif2s3y* males. A. At 25 days, there is a uniform arrest — spermatocytes complete the first meiotic division, but fail to progress through the second meiotic division. **B.** At 1 month, the arrested cells from the previous spermatogenic cycle are seen being removed in stage XII tubules, as evidenced by the formation of multinucleate bodies (arrows). **C.** By 2 months, large vacuoles (arrow) begin to appear that span from the basal lamina to the pachytene spermatocyte layer; this suggests that they may breach the Sertoli cell tight-junctional blood-testis barrier. **D.** At 3 months, a mosaic phenotype begins to appear, with spermatogenesis in some tubules now progressing to elongating spermatid stages (arrows), while in other tubules there is spermatogenic failure with only Sertoli cells remaining (SCO). **E.** Although the rate at which secondary damage progresses is variable, by 6 months some testes have very little spermatogenic activity remaining. The lower tubule has what appear to be pycnotic spermatocytes; other tubules have only Sertoli cells and spermatogonia (arrowhead) or only Sertoli cells (SCO), while in some the Sertoli cells are beginning to slough off (arrow).

the X chromosome, and in several cases (e.g. *Eif2s3y*) the proteins encoded by the X and Y genes are sufficiently similar to be expected to have near identical properties. We suspect that it is the abrogation of Sertoli responses to arrested cells usually ensuring their prompt removal, together with the accumulation of X-encoded proteins, that may allow progression beyond the initial block. In light of these observations in the mouse, the spermatogenic phenotype in men with Y deletions when they first present at a fertility

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clinic may be far removed from that present years earlier, when the Y gene deficiency first impacts on spermatogenesis.

It is a sobering thought for those battling to document mammalian Y gene functions in spermatogenesis that mice spermatogenic cells with only two Y genes, *Sry* and *Eif2s3y*, can in some circumstances complete spermatogenesis and produce sperm, although they do have distorted heads. This supports the view that many Y genes have been retained on the Y because they potentiate the spermatogenic process rather than because they provide an essential spermatogenic function (Burgoyne, 1998). However, despite the delaying tactics afforded by gene conversion in palindromic Y chromosomal repeats (Rozen *et al.*, 2003), the mammalian Y is probably on an evolutionary road to oblivion (Graves, 2004); in at least one mammal, the creeping vole *Ellobius lutescens*, the Y has already reached the end of the road (Just *et al.*, 1995).

Acknowledgments

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References

Agulnik, A. I., Harrison, W. R., and Bishop, C. E. (2001). Smcy transgene does not rescue spermatogenesis in sex-reversed mice. *Mamm Genome* **12**, 112–116.

Agulnik, A. I., Longepied, G., Ty, M. T., Bishop, C. E., and Mitchell, M. (1999). Mouse H-Y encoding *Smcy* gene and its X chromosomal homologue *Smcx*. *Mamm Genome* **10**, 926–929.

Aravin, A. A., Naumova, N. M., Tulin, A. V., Vagin, V. V., Rozovsky, Y. M., and Gvozdev, V. A. (2001). Double-stranded RNA-mediated silencing of genomic tandem repeats and transposable elements in the D. melanogaster germline. *Curr Biol* **11**, 1017–1027.

Bishop, C. E., and Hatat, D. (1987). Molecular cloning and sequence analysis of a mouse Y chromosome RNA transcript expressed in the testis. *Nucleic Acids Res* **15**, 2959–2969.

Burgoyne, P. S. (1982). Genetic homology and crossing over in the X and Y chromosomes of mammals. *Hum Genet* **61**, 85–90.

Burgoyne, P. S. (1998). The mammalian Y chromosome: a new perspective. *Bioessays* **20**, 363–366.

Burgoyne, P. S., and Evans, E. P. (2000). A high frequency of XO offspring from X*Paf*Y* male mice: evidence that the *Paf* mutation involves an inversion spanning the X PAR boundary. *Cytogenet Cell Genet* **91**, 57–61.

Burgoyne, P. S., Levy, E. R., and McLaren, A. (1986). Spermatogenic failure in male mice lacking H-Y antigen. *Nature* **320**, 170–172.

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Burgoyne, P. S., Mahadevaiah, S. K., Perry, J., Palmer, S. J., and Ashworth, A. (1998). The Y* rearrangement in mice: new insights into a perplexing PAR. *Cytogenet Cell Genet* **80**, 37–40.

Burgoyne, P. S., Mahadevaiah, S. K., Sutcliffe, M. J., and Palmer, S. J. (1992). Fertility in mice requires X-Y pairing and a Y-chromosomal 'spermiogenesis' gene mapping to the long arm. *Cell* **71**, 391–398.

Capel, B., Rasberry, C., Dyson, J., Bishop, C. E., Simpson, E., Vivian, N., Lovell-Badge, R., Rastan, S., and Cattanach, B. M. (1993). Deletion of Y chromosome sequences located outside the testis determining region can cause XY female sex reversal. *Nat Genet* **5**, 301–307.

Cattanach, B. M., Pollard, C. E., and Hawkes, S. G. (1971). Sex reversed mice: XX and XO males. *Cytogenetics* **10**, 318–337.

Conway, S. J., Mahadevaiah, S. K., Darling, S. M., Capel, B., Rattigan, Á. M., and Burgoyne, P. S. (1994). Y353/B: a candidate multiple-copy spermiogenesis gene on the mouse Y chromosome. *Mamm Genome* **5**, 203–210.

Delbridge, M. L., Harry, J. L., Toder, R., O'Neill, R. J. W., Ma, K., Chandley, A. C., and Graves, J. A. M. (1997). A human candidate spermatogenesis gene, *RBM1*, is conserved and amplified on the marsupial Y chromosome. *Nat Genet* **15**, 131–136.

Dym, M., and Fawcett, D. W. (1970). The blood-testis barrier in the rat and the physiological compartmentation of the seminiferous epithelium. *Biol Reprod* **3**, 308–326.

Eaker, S., Cobb, J., Pyle, A., and Handel, M. A. (2002). Meiotic prophase abnormalities and metaphase cell death in MLH1-deficient mouse spermatocytes: insights into regulation of spermatogenic progress. *Dev Biol* **249**, 85–95.

Eaker, S., Pyle, A., Cobb, J., and Handel, M. A. (2001). Evidence for meiotic spindle checkpoint from analysis of spermatocytes from Robertsonian-chromosome heterozygous mice. *J Cell Sci* **114**, 2953–2965.

Ehrmann, I. E., Ellis, P. S., Mazeyrat, S., Duthie, S., Brockdorff, N., Mattei, M. G., Gavin, M. A., Affara, N. A., Brown, G. M., Simpson, E., *et al.* (1998). Characterization of genes encoding translation initiation factor eIF-2gamma in mouse and human: sex chromosome localization, escape from X-inactivation and evolution. *Hum Mol Genet* **7**, 1725–1737.

Eicher, E. M., Hale, D. W., Hunt, P. A., Lee, B. K., Tucker, P. K., King, T. R., Eppig, J. T., and Washburn, L. L. (1991). The mouse Y* chromosome involves a complex rearrangement, including interstitial positioning of the pseudoautosomal region. *Cytogenet Cell Genet* **57**, 221–230.

Elliott, D. J. (2000). RBMY genes and AZFb deletions. *J Endocrinol Invest* **23**, 652–658.

Elliott, D. J., Ma, K., Kerr, S. M., Thakrar, R., Speed, R., Chandley, A. C., and Cooke, H. (1996). An *RBM* homologue maps to the mouse Y chromosome and is expressed in germ cells. *Hum Mol Genet* **5**, 869–874.

Ellis, P. J., Clemente, E. J., Ball, P., Toure, A., Ferguson, L., Turner, J. M., Loveland, K. L., Affara, N. A., and Burgoyne, P. S. (2005). Deletions on mouse Yq lead to upregulation of multiple X- and Y-linked transcripts in spermatids. *Hum Mol Genet* **14**, 2705–2715.

Ellis, P. J., Furlong, R. A., Wilson, A., Morris, S., Carter, D., Oliver, G., Print, C., Burgoyne, P. S., Loveland, K. L., and Affara, N. A. (2004). Modulation of the mouse testis transcriptome during postnatal development and in selected models of male infertility. *Mol Hum Reprod* **10**, 271–281.

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Foster, J. W., and Graves, J. A. M. (1994). An *SRY*-related sequence on the marsupial X chromosome: implications for the evolution of the mammalian testis-determining gene. *Proc Natl Acad Sci U S A* **91**, 1927–1931.

Graves, J. A. (2004). The degenerate Y chromosome — can conversion save it? *Reprod Fertil Dev* **16**, 527–534.

Gregory, S. G., Sekhon, M., Schein, J., Zhao, S., Osoegawa, K., Scott, C. E., Evans, R. S., Burridge, P. W., Cox, T. V., Fox, C. A., *et al.* (2002). A physical map of the mouse genome. *Nature* **418**, 743–750.

Gubbay, J., Vivian, N., Economou, A., Jackson, D., Goodfellow, P., and Lovell-Badge, R. (1992). Inverted repeat structure of the *Sry* locus in mice. *Proc Natl Acad Sci U S A* **89**, 7953–7957.

Homer, H. A., McDougall, A., Levasseur, M., and Herbert, M. (2005). Restaging the spindle assembly checkpoint in female mammalian meiosis I. *Cell Cycle* **4**, 650–653.

Hurst, L. D. (1992). Is *Stellate* a relict meiotic driver? *Genetics* **130**, 229–230.

Hurst, L. D. (1996). Further evidence consistent with *Stellate*'s involvement in meiotic drive. *Genetics* **142**, 641–643.

Itoh, Y., Hori, T., Saitoh, H., and Mizuno, S. (2001). Chicken spindlin genes on W and Z chromosomes: transcriptional expression of both genes and dynamic behavior of spindlin in interphase and mitotic cells. *Chromosome Res* **9**, 283–299.

Just, W., Rau, W., Vogel, W., Akhverdian, M., Fredga, K., Graves, J. A. M., and Lyapunova, E. (1995). Absence of *Sry* in species of the vole *Ellobius. Nat Genet* **11**, 117–118.

Kallio, M., Eriksson, J. E., and Gorbsky, G. J. (2000). Differences in spindle association of the mitotic checkpoint protein Mad2 in mammalian spermatogenesis and oogenesis. *Dev Biol* **225**, 112–123.

Kay, G. F., Ashworth, A., Penny, G. D., Dunlop, M., Swift, S., Brockdorff, N., and Rastan, S. (1991). A candidate spermatogenesis gene on the mouse Y chromosome is homologous to ubiquitin-activating enzyme E1. *Nature* **354**, 486–489.

Kot, M. C., and Handel, M. A. (1990). Spermatogenesis in XO,*Sxr* mice: role of the Y chromosome. *J Exp Zool* **256**, 92–105.

Kotula-Balak, M., Grzmil, P., Styrna, J., and Bilinska, B. (2004). Immunodetection of aromatase in mice with a partial deletion in the long arm of the Y chromosome. *Acta Histochem* **106**, 55–64.

Lahn, B. T., and Page, D. C. (1999). Four evolutionary strata on the human X chromosome. *Science* **286**, 964–967.

Laval, S. H., Glenister, P. H., Rasberry, C., Thornton, C. E., Mahadevaiah, S. K., Cooke, H. J., Burgoyne, P. S., and Cattanach, B. M. (1995). Y chromosome short arm-*Sxr* recombination in X*Sxr*/Y males causes deletion of *Rbm* and XY female sex reversal. *Proc Natl Acad Sci U S A* **92**, 10403–10407.

Lee, J., Hong, J., Kim, E., Kim, K., Kim, S. W., Krishnamurthy, H., Chung, S. S., Wolgemuth, D. J., and Rhee, K. (2004). Developmental stage-specific expression of Rbm suggests its involvement in early phases of spermatogenesis. *Mol Hum Reprod* **10**, 259–264.

LeMaire-Adkins, R., Radke, K., and Hunt, P. A. (1997). Lack of checkpoint control at the metaphase/anaphase transition: a mechanism of meiotic nondisjunction in mammalian females. *J Cell Biol* **139**, 1611–1619.

Levy, E., and Burgoyne, P. S. (1986a). The fate of XO germ cells in the testes of XO/XY and XO/XY/XYY mouse mosaics: evidence for a spermatogenesis gene on the mouse Y chromosome. *Cytogenet Cell Genet* **42**, 208–213.

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Levy, E. R., and Burgoyne, P. S. (1986b). Diploid spermatids: a manifestation of spermatogenic impairment in XOSxr and T31H/+ male mice. *Cytogenet Cell Genet* **42**, 159–163.

Mahadevaiah, S. K., Odorisio, T., Elliott, D. J., Rattigan, A., Szot, M., Laval, S. H., Washburn, L. L., McCarrey, J. R., Cattanach, B. M., Lovell-Badge, R., and Burgoyne, P. S. (1998). Mouse homologues of the human *AZF* candidate gene *RBM* are expressed in spermatogonia and spermatids, and map to a Y deletion interval associated with a high incidence of sperm abnormalities. *Hum Mol Genet* **7**, 715–727.

Mazeyrat, S., Saut, N., Grigoriev, V., Mahadevaiah, S. K. M., Ojarikre, O. A., Rattigan, A., Bishop, C., Eicher, E. M., Mitchell, M. J., and Burgoyne, P. S. (2001). A Y-encoded subunit of the translation initiation factor Eif2 is essential for mouse spermatogenesis. *Nat Genet* **29**, 49–53.

Mazeyrat, S., Saut, N., Sargent, C. A., Grimmond, S., Longepied, G., Ehrmann, I. E., Ellis, P. S., Greenfield, A., Affara, N. A., and Mitchell, M. J. (1998). The mouse Y chromosome interval necessary for spermatogonial proliferation is gene dense with syntenic homology to the human *AZFa* region. *Hum Mol Genet* **7**, 1713–1724.

McLaren, A., Simpson, E., Epplen, J. T., Studer, R., Koopman, P., Evans, E. P., and Burgoyne, P. S. (1988). Location of the genes controlling H-Y antigen expression and testis determination on the mouse Y chromosome. *Proc Natl Acad Sci U S A* **85**, 6442–6445.

McLaren, A., Simpson, E., Tomonari, K., Chandler, P., and Hogg, H. (1984). Male sexual differentiation in mice lacking H-Y antigen. *Nature* **312**, 552–555.

Mitchell, M., Woods, D. R., Wilcox, S. A., Graves, J. A. M., and Bishop, C. E. (1992). Marsupial Y chromosome encodes a homologue of the mouse Y-linked candidate spermatogenesis gene *Ube1y*. *Nature* **359**, 528–531.

Mitchell, M. J. (2000). Spermatogenesis and the mouse Y chromosome: specialisation out of decay. In: *The Genetic Basis of Male Infertility*, K. McElreavy, ed., Springer-Verlag, Berlin & Heidelberg, pp. 233–270.

Mitchell, M. J., Wilcox, S. A., Watson, J. M., Lerner, J. L., Woods, D. R., Scheffler, J., Hearn, J. P., Bishop, C. E., and Graves, J. A. M. (1998). The origin and loss of the ubiquitin activating enzyme gene on the mammalian Y chromosome. *Hum Mol Genet* **7**, 429–434.

Mitchell, M. J., Woods, D. R., Tucker, P. K., Opp, J. S., and Bishop, C. E. (1991). Homology of a candidate spermatogenic gene from the mouse Y chromosome to the ubiquitin-activating enzyme E1. *Nature* **354**, 483–486.

Moriwaki, K., Suh, D.-S., and Styrna, J. (1988). Genetic factors effecting sperm morphology in the mouse. *Mouse News Letter* **82**, 138.

Murphy, W. J., Eizirik, E., Johnson, W. E., Zhang, Y. P., Ryder, O. A., and O'Brien, S. J. (2001). Molecular phylogenetics and the origins of placental mammals. *Nature* **409**, 614–618.

Oh, B., Hampl, A., Eppig, J. J., Solter, D., and Knowles, B. B. (1998). SPIN, a substrate in the MAP kinase pathway in mouse oocytes. *Mol Reprod Dev* **50**, 240–249.

Oh, B., Hwang, S.-Y., Solter, D., and Knowles, B. (1997). Spindlin, a major maternal transcript expressed in the mouse during the transition from oocyte to embryo. *Development* **124**, 493–503.

Perry, J., Palmer, S., Gabriel, A., and Ashworth, A. (2001). A short pseudoautosomal region in laboratory mice. *Genome Res* **11**, 1826–1832.

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Petronczki, M., Siomos, M. F., and Nasmyth, K. (2003). Un menage a quatre: the molecular biology of chromosome segregation in meiosis. *Cell* **112**, 423–440.

Prado, V. F., Lee, C.-H., Zahed, L., Vekemans, M., and Nishioka, Y. (1992). Molecular characterization of a mouse Y chromosomal repetitive sequence that detects transcripts in the testis. *Cytogenet Cell Genet* **61**, 87–90.

Rohozinski, J., Agoulnik, A. I., Boettger-Tong, H. L., and Bishop, C. E. (2002). Successful targeting of mouse Y chromosome genes using a site-directed insertion vector. *Genesis* **32**, 1–7.

Rozen, S., Skaletsky, H., Marszalek, J. D., Minx, P. J., Cordum, H. S., Waterston, R. H., Wilson, R. K., and Page, D. C. (2003). Abundant gene conversion between arms of palindromes in human and ape Y chromosomes. *Nature* **423**, 873–876.

Rubinson, D. A., Dillon, C. P., Kwiatkowski, A. V., Sievers, C., Yang, L., Kopinja, J., Rooney, D. L., Ihrig, M. M., McManus, M. T., Gertler, F. B., *et al.* (2003). A lentivirus-based system to functionally silence genes in primary mammalian cells, stem cells and transgenic mice by RNA interference. *Nat Genet* **33**, 401–406.

Sandstedt, S. A., and Tucker, P. K. (2004). Evolutionary strata on the mouse X chromosome correspond to strata on the human X chromosome. *Genome Res* **14**, 267–272.

Shoji, M., Chuma, S., Yoshida, K., Morita, T., and Nakatsuji, N. (2005). RNA interference during spermatogenesis in mice. *Dev Biol* **282**, 524–534.

Simpson, E. M., and Page, D. C. (1991). An interstitial deletion in mouse Y chromosomal DNA created a transcribed Zfy fusion gene. *Genomics* **11**, 601–608.

Siruntawineti, J., Yamagata, K., Nakanishi, T., and Baba, T. (2002). Occurrence of small, round vesicles in the acrosome of elongating spermatids from a mouse mutant line with partial deletion of the Y chromosome. *J Reprod Dev* **48**, 513–521.

Styrna, J., Bilinska, B., and Krzanowskaa, H. (2002). The effect of a partial Y chromosome deletion in B10.BR-Ydel mice on testis morphology, sperm quality and efficiency of fertilization. *Reprod Fertil Dev* **14**, 101–108.

Styrna, J., Imai, H. T., and Moriwaki, K. (1991a). An increased level of sperm abnormalities in mice with a partial deletion of the Y chromosome. *Genet Res* **57**, 195–199.

Styrna, J., Kilarski, W., and Krzanowska, H. (2003). Influence of the CBA genetic background on sperm morphology and fertilization efficiency in mice with a partial Y chromosome deletion. *Reproduction* **126**, 579–588.

Styrna, J., Klag, J., and Moriwaki, K. (1991b). Influence of partial deletion of the Y chromosome on mouse sperm phenotype. *J Reprod Fertil* **92**, 187–195.

Sutcliffe, M. J., and Burgoyne, P. S. (1989). Analysis of the testes of H-Y negative $XOSxr^b$ mice suggests that the spermatogenesis gene (*Spy*) acts during the differentiation of the A spermatogonia. *Development* **107**, 373–380.

Sutcliffe, M. J., Darling, S. M., and Burgoyne, P. S. (1991). Spermatogenesis in XY, $XYSxr^a$ and $XOSxr^a$ mice: a quantitative analysis of spermatogenesis throughout puberty. *Mol Reprod Dev* **30**, 81–89.

Szot, M., Grigoriev, V., Mahadevaiah, S. K., Ojarikre, O. A., Toure, A., von Glasenapp, E., Rattigan, A., Turner, J. M., Elliott, D. J., and Burgoyne, P. S. (2003). Does Rbmy have a role in sperm development in mice? *Cytogenet Genome Res* **103**, 330–336.

Tan, A. L., Rida, P. C., and Surana, U. (2005). Essential tension and constructive destruction: the spindle checkpoint and its regulatory links with mitotic exit. *Biochem J* **386**, 1–13.

THE Y CHROMOSOME AND MALE GERM CELL BIOLOGY IN HEALTH AND DISEASES
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http://www.worldscibooks.com/lifesci/6303.html

Tiepolo, L., and Zuffardi, O. (1976). Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. *Hum Genet* **34**, 119–124.

Touré A., Clemente, E. J., Ellis., P., Mahadevaiah, S. K., Ojarikre, O. A., Ball, P. A., Reynard, L., Loveland, K. L., Burgoyne, P. S., and Affara, N. A. (2005). Identification of novel Y chromosome encoded transcripts by testis transcriptome analysis of mice with deletions of the Y chromosome long arm. *Genome Biol* **6**, R102.

Touré, A., Grigoriev, V., Mahadevaiah, S. K., Rattigan, A., Ojarikre, O. A., and Burgoyne, P. S. (2004a). A protein encoded by a member of the multicopy Ssty gene family located on the long arm of the mouse Y chromosome is expressed during sperm development. *Genomics* **83**, 140–147.

Touré, A., Szot, M., Mahadevaiah, S. K., Rattigan, A., Ojarikre, O. A., and Burgoyne, P. S. (2004b). A new deletion of the mouse Y chromosome long arm associated with loss of *Ssty* expression, abnormal sperm development and sterility. *Genetics* **166**, 901–912.

Vogt, P. H. (2005). Azoospermia factor (AZF) in Yq11: towards a molecular understanding of its function for human male fertility and spermatogenesis. *Reprod Biomed Online* **10**, 81–93.

Wang, X. L., Mei, J., Sun, M., Hong, Y. H., and Gui, J. F. (2005). Identification of three duplicated Spin genes in medaka (Oryzias latipes). *Gene* **350**, 99–106.

Wassmann, K., Niault, T., and Maro, B. (2003). Metaphase I arrest upon activation of the Mad2-dependent spindle checkpoint in mouse oocytes. *Curr Biol* **13**, 1596–1608.

Xia, W., Mruk, D. D., Lee, W. M., and Cheng, C. Y. (2005). Cytokines and junction restructuring during spermatogenesis — a lesson to learn from the testis. *Cytokine Growth Factor Rev* **16**, 469–493.

Xian, M., Azuma, S., Naito, K., Kunieda, T., Moriwaki, K., and Toyoda, Y. (1992). Effect of a partial deletion of Y chromosome on in vitro fertilizing ability of mouse spermatozoa. *Biol Reprod* **47**, 549–553.

Note Added in Proof

Further analysis of older XSxr^bO,*Eif2s3y* and *XOSry,Eif2s3y* males has revealed that, while both show the progressive damage leading to the presence of increasing numbers of Sertoli cell-only tubules, it is only the former males that show clear evidence of the "leak" in the spermatogenic block that allows some elongated spermatids with abnormal heads to form. This indicates that genetic information remaining in Sxr^b potentiates this progression through to elongated spermatids.

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