

## Serological and cytological evidence for increased Y-chromosome related material in *Sxr*,XY (sex-reversed carrier, male) mice

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In order to define the nature of the genetic lesion which gave rise to *Sxr*, the sex-limited autosomal dominant sex reversal condition in the mouse, cytological and serological studies were carried out comparing *Sxr*,XY (males carrying the sex-reversal gene) and normal XY male mice. Cytotoxic H-Y antisera were absorbed by splenocytes or sperm from both *Sxr*,XY and normal XY male mice. Our results indicate that for both types of tissue, *Sxr*,XY cells absorbed consistently greater amounts of cytotoxic activity than did normal XY cells. Whole-mount electron micrographs as well as light micrographs of silver-stained spermatocytes suggest that during meiotic prophase in *Sxr*,XY males, the paracentromeric region of the normal Y chromosome pairs with a supernumerary Y chromosomal fragment. This fragment can be identified as one of Y chromosomal origin by its thickened axial core. Taken together, our findings support the notion that the *Sxr* syndrome in the mouse can be due to a supernumerary Y chromosomal fragment containing male determining factors (including one or more H-Y structural or regulatory genes) rather than to a constitutive autosomal mutation.

### Introduction

It has been suggested that differentiation of the totipotent gonad to a testis in mammals is associated with the presence of histocompatibility-Y (H-Y) antigen on the surfaces of an organism's cells. Support for this is derived from our knowledge of normal sexual development as well as most instances of mammalian 'sex-reversal' (see Wachtel, 1979). One such mammalian sex-reversal syndrome is the *Sxr* mutation in the mouse. This autosomal dominant, sex-limited condition causes XX mice to appear as phenotypic males. These 'males' have external and internal male organs normal in every respect except for the existence of complete azoospermia (Cattanach, 1975). *Sxr*,XY mice are fertile males whose only phenotypic aberration is slightly reduced testis size. Although the precise genetic event that gave rise to the *Sxr* condition has thus far eluded definition, two hypotheses are presently favored: (1) a constitutive mutation at an autosomal locus normally dependent upon the presence of the Y chromosome for expression, and (2) the presence at an autosomal

location of a region of the Y chromosome containing male determining factors (including, quite possibly, one or more H-Y genes).

We provide here two independent lines of evidence that additional Y chromosome-associated material is present in *Sxr,XY* mice relative to normal XY males. The first is a serological study comparing *Sxr,XY* mice with normal XY male littermates as to the level of H-Y antigen they express on splenocytes and sperm. Although the weight of evidence tends to support the notion that the cells of both *Sxr,XX* and wild-type males are quantitatively equivalent with respect to H-Y antigen, it has been reported that *Sxr,XY* males express 'near normal' levels of H-Y (Bennett et al., 1977). We report here serological evidence that H-Y antigen is present on the cells of *Sxr,XY* mice in greater amounts than on those of either *Sxr,XX* or wild-type XY males. Our evidence is consistent with the notion that the cells of *Sxr,XY* males contain two-fold the levels of H-Y antigen as those of either *Sxr,XX* or wild-type XY males. The second comprises cytological studies of meiotic preparations using both whole-mount electron microscopy and light microscopy of silver-stained preparations. The results obtained in this study suggest that compared to normal XY littermates (co-isogenic controls), *Sxr,XY* male mice possess a supernumerary centromeric Y fragment (presumably containing either the H-Y structural gene, or an associated positive regulatory element) in addition to the normal Y chromosome.

Considered together, our studies provide both serological and cytological evidence that the *Sxr* syndrome in the mouse can be due to a supernumerary Y chromosomal fragment containing male determining factors (including one or more H-Y genes) rather than to a constitutive autosomal mutation.

## Materials and Methods

### *Animals*

C57Bl/6J (B6) mice were bred from a stock obtained from the Jackson Laboratories, Bar Harbor, Maine. *Sxr* mice were maintained in the 129/SvPA background as supplied by Dr. Jean-Louis Guenet, Institut Pasteur, Paris. *Sxr,XX* 'males' were distinguished by use of the X-linked coat color marker, tabby, whereas *Sxr,XY* males were so classified by progeny testing.

### *Preparation of epidermal cells*

Epidermal cells were prepared by the method of Scheid et al. (1972). Mature male B6 mice were killed and the entire piece of skin covering the tail removed. The tail skin was then incubated in Puck's Saline F (PSF) containing 1% Trypsin (2× crystallized — Gibco) for 50 min at 37°C in order to separate dermis and epidermis. The dermis was then discarded and the epidermis placed in Medium 199 (GIBCO) containing 10% heat-inactivated fetal calf serum (FCS). Epidermal cell clumps were then teased free of the epidermis by gentle scraping with the tip of a bent, fire-polished Pasteur pipette. These clumps were then incubated in PSF containing 0.1% Trypsin for 15–20 min at 37°C in order to yield a suspension of single cells.

After this incubation, a two-fold excess of Medium 199 with 10% FCS was added to the incubation mixture. The resultant cells were pelleted by centrifugation at  $100 \times g$  at  $4^{\circ}\text{C}$  and then washed three times in Medium 199 containing 10% FCS. Residual multicell clumps were then removed by centrifugation at  $10\text{--}20 \times g$ . The final suspensions of epidermal cells were then adjusted to a concentration of approximately  $3 \times 10^6$  cells per ml for use in the direct cytotoxicity test.

#### *Preparation of sperm*

Mature male mice were sacrificed and their epididymides removed. The epididymides were placed in Beatty's salt solution, minced with a sharp scalpel, then passed through a screen in order to remove large pieces of tissue. The resultant suspension was then left for 10 min at  $1 \times g$  to enable large-sized clumps of debris to settle out. Sperm were then pelleted by centrifugation at  $800 \times g$  and the pellet washed three times in Beatty's salt solution.

#### *Preparation of splenocytes*

Mature mice were killed and their spleens removed. The spleens were then placed in Dulbecco's phosphate-buffered saline (PBS), minced, and teased through a screen. The resultant suspension was left for 10 min at  $1 \times g$  to enable large-sized stromal elements to settle out. Splenocytes were pelleted at  $400 \times g$  and the pellet washed 3 times in PBS.

#### *Direct cytotoxicity test*

The procedure used is similar to that of Scheid et al. (1972). Equal volumes (20  $\mu\text{l}$ ) of H-Y antiserum, epidermal cells ( $3 \times 10^6$  per ml) and guinea-pig complement (Gibco) diluted 1/8 were incubated at  $37^{\circ}\text{C}$  for 50 min. The dye Trypan blue (20  $\mu\text{l}$  of 0.2% dye) was added for the final 5 min of incubation to stain dead epidermal cells. Cell killing was measured by mean cytotoxic index defined as

$$\frac{\text{No. dead cells} - \text{No. cells killed by complement alone}}{\text{Total No. cells} - \text{No. cells killed by complement alone}}$$

#### *Statistics*

Statistical significance was assessed using the Mann-Whitney *U*-test on values of the cytotoxic index as defined above.

#### *Absorptions*

Aliquots of H-Y antiserum (50  $\mu\text{l}$ ) diluted 1/4 were added to known numbers of splenocytes or sperm. This suspension was incubated on ice for one hour. After the period of incubation, the absorbing cells were then removed from the absorbed antiserum by centrifugation at the centrifugal force appropriate for pelleting them (above). The resultant antiserum was then tested for residual direct cytotoxicity against isolated male murine epidermal cells.

### *Preparation of antisera*

Antisera were raised according to the method of Krco and Goldberg (1976) in (B6) female mice against (B6) male splenocytes. An antiserum was judged as H-Y specific if (1) it had cytotoxic activity against isolated male, but not female (B6) epidermal cells, and (2) this activity could be absorbed by male, but not female (B6) splenocytes.

### *Whole-mount electron microscopy*

A technique based on the method of Miller and co-workers (Miller and Beatty, 1969; Miller and Bakken, 1972) and adapted for the study of structural and functional aspects of testicular genomes (Kierzenbaum and Tres, 1974; Tres, 1977) was used. Briefly, testes from control XY littermates and *Sxr,XY* mice were removed under ether anesthesia. The seminiferous tubules were cut into small segments, suspended in PBS and the cells from the seminiferous epithelium dissociated by shearing forces with a syringe. Isolated cells were transferred with a pipette to a 0.3% solution of Nonidet P-40 (BDH Chemicals Ltd., Poole, U.K.) (pH 8.6). Cells were allowed to disperse for 5 min at 4°C and the nuclear contents transferred into a plastic trough containing carbon-coated electron microscope grids and a solution of 0.1 M sucrose with 10% formaldehyde (pH 8.6). After centrifugation at 3000 rev./min for 5 min at 4°C, grids were rinsed in 0.4% Kodak Photo-flo and air-dried. Preparations were stained with 1% phosphotungstic acid in 95% ethanol (pH 2.5 unadjusted), rinsed in 95% ethanol and air-dried. Electron micrographs were taken with a JEM 100B electron microscope operated at 60 kV.

### *Silver-staining technique*

Seminiferous tubules were minced and dissociated with a syringe in PBS (pH 7.0). The isolated testicular cells in suspension were centrifuged at 1000 rev./min for 5 min. The pellet was resuspended in fresh PBS and 2–3 drops of the sample were allowed to disperse in a solution of 0.3% Nonidet P-40 for 10 min. After dispersal of nuclear contents, two drops of the resulting sample were deposited on a large drop of 0.1 M sucrose in 10% formaldehyde solution placed on a microscope slide. Cells were allowed to sediment for 10 min in a moist chamber with formaldehyde vapors. The preparations were dried for 3 h in an oven at 45°C. After rinsing with distilled water, the slides were air-dried and then stained in 50% aqueous solution of silver nitrate at 60°C for 1 h. Preparations were rinsed with water, air-dried and examined with  $\times 63$  to  $\times 100$  oil-immersion objectives using bright illumination.

## **Results**

We have quantitated the amount of H-Y antigen in *Sxr,XY* mice by absorption studies. Uniform aliquots of H-Y antiserum were absorbed with known numbers of cells. The resulting absorbed sera were then tested for direct cytotoxicity on isolated male murine epidermal cells (Scheid et al., 1972). Since it is the experience of our laboratory that the absolute amount of H-Y specific cytotoxic activity absorbed by a

single cell sample is subject to some day-to-day variation, we endeavored to employ three types of internal standards within any one day's experiments. These standards were (1) a control for cytolysis by complement alone, (2) a control for cytolysis by complement plus unabsorbed H-Y antiserum, and (3) the quantitative comparison of experimental cell samples in a pairwise fashion as to their absorption of H-Y specific cytotoxic activity. The pairwise comparison of experimental samples renders our cytotoxicity data especially amenable to statistical analysis.

As seen in Fig. 1A,  $2.5 \times 10^6$ ,  $5 \times 10^6$  and  $10^7$  *Sxr,XX* spleen cells absorb the same level of cytotoxic activity as equal numbers of wild-type XY cells. This result is in agreement with similar studies previously reported (Bennett et al., 1977). Fig. 1B shows the result of direct cytotoxicity tests using sera absorbed with different numbers of spleen cells isolated from *Sxr,XY* and wild-type XY males. These data demonstrate that  $5 \times 10^6$  and  $10^7$  *Sxr,XY* spleen cells absorb consistently more cytotoxic activity than  $5 \times 10^6$ , and  $10^7$  cells from wild-type males, respectively. These results suggest that splenocytes isolated from *Sxr,XY* male mice contain more, perhaps two-fold, the H-Y antigen on their plasma membranes than do the same cells derived from wild-type males of the same genetic background. Since these two genotypes of mice are identical at all loci with the exception of the chromosomal site bearing the *Sxr* mutation, it is probable that the *Sxr* gene provides the additional quantity of H-Y antigen on the surfaces of *Sxr,XY* splenocytes.

The same quantitative immunoabsorption procedure was carried out using sperm. Fig. 1C shows the cytotoxic profiles of H-Y antisera absorbed with different numbers of sperm from *Sxr,XY* and wild-type XY males. The data shown indicate that  $5 \times 10^5$  and  $10^6$  *Sxr,XY* sperm repeatedly absorb amounts of cytotoxic activity equivalent to that of  $10^6$  and  $2 \times 10^6$  wild-type XY sperm, respectively. These results, much like those for splenocytes in Fig. 1B, are consistent with the notion that *Sxr,XY* sperm contain more, perhaps twice as much, H-Y antigen on their surfaces as do the sperm from wild-type XY animals.

Although not all the data which comprises our serological studies were analyzed as coded samples, representative experiments were carried out blind. These experiments yielded results equivalent in their significance to those presented in this report.

Meiotic prophase spermatocytes from five *Sxr,XY* carrier male mice as well as 3 littermates were studied in whole-mount electron microscopic preparations (Table I). In normal mouse spermatocytes, X and Y chromosomes are identified at early pachytene in whole-mount electron microscopic preparations as two associated chromosomes of unequal length, paired with a synaptonemal complex over a portion at one end (Fig. 2). The length of the paired segment approaches the length of the Y chromosome at late zygotene-early pachytene and decreases progressively (Fig. 3) until it completely disappears at diplotene (Tres, 1977). When X and Y chromosomes display maximal pairing a small terminal segment of the Y chromosome (comprising about 10% of the Y chromosome's total length) diverges from the X chromosomal core. In the mouse, this unpaired Y segment corresponds to the paracentric region of the Y chromosome (Schnedl, 1972; Tres, 1977).

390 *Sxr,XY* (zygotene, 60; pachytene, 285; diploplotene, 116) spermatocytes were

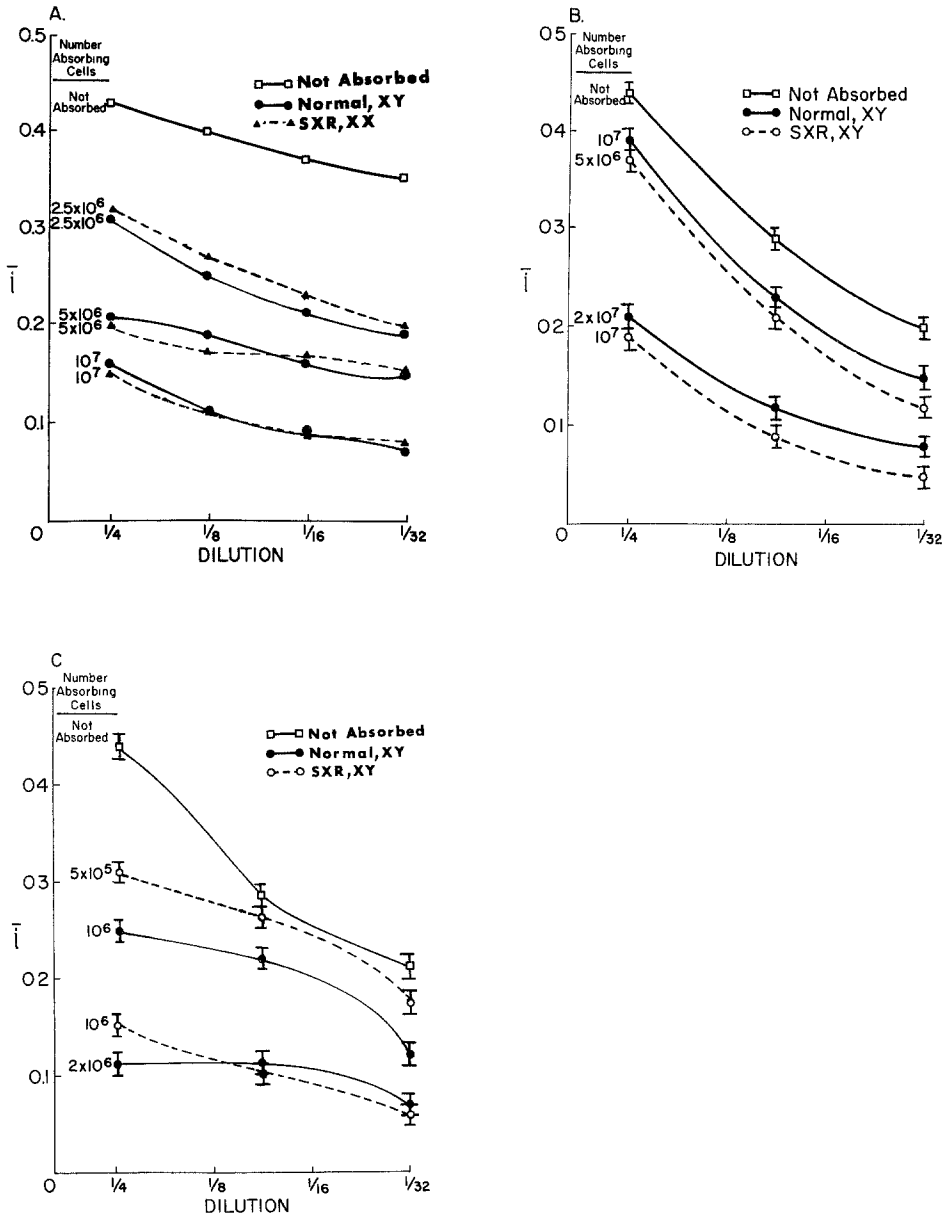


Fig. 1. A. H-Y antisera absorbed with different numbers of spleen cells from *Sxr,XX* and wild-type XY (129-SV) animals. Numbers of cells used to absorb each aliquot of serum (50  $\mu$ l) are indicated on the figure. Complement control was 25%. B. H-Y antisera absorbed with different numbers of spleen cells from *Sxr,XY* and wild-type XY (129-SV) animals. Complement control was 31%. Each point represents the mean of three observations with standard errors included. C. H-Y antisera absorbed with different numbers of sperm from *Sxr,XY* and wild-type XY (129-SV) animals. Complement control was 32%. Each point represents the mean of three observations with standard errors included.

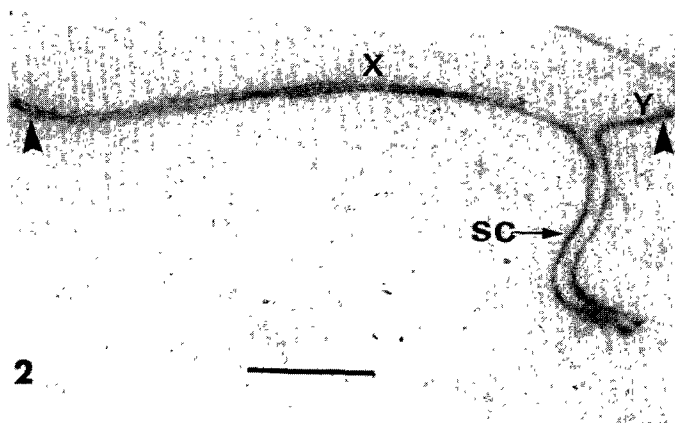


Fig. 2. Sex bivalent (XY) of a normal mouse spermatocyte at early pachytene displaying an extensive synaptonemal complex (SC). The free ends (arrowheads) of the X and Y chromosomes correspond to the paracentromeric regions. Whole-mount electron microscopy. Scale mark:  $2\ \mu\text{m}$ .

examined. Three combined structural features appeared characteristic of the *Sxr,XY* males. First, X and Y chromosomes appear to be separated from each other more often than in normal XY males. This separation, attributable to early disjunction of the XY pair, was observed in 35% (84 out of 240) of pachytene spermatocytes from *Sxr,XY* males whereas it was only detectable in 3.2% (9 out of 285) of the controls (Table 1). In *Sxr,XY*, unpaired XY chromosomes are more frequently seen during diplotene (65%, Table 1). Furthermore, in 18.3% (44 out of 240, Table 2) of pachytene spermatocytes examined from *Sxr,XY* males, sex chromosomes displayed a

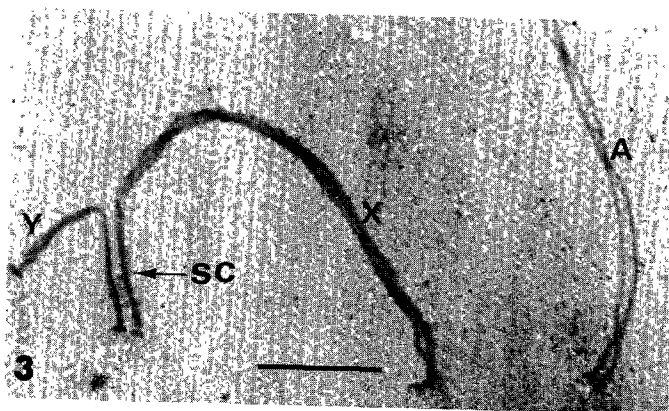


Fig. 3. Sex bivalent (XY) of a normal mouse spermatocyte at mid-pachytene. The pairing segment has decreased in length compared to that in Fig. 1. SC, synaptonemal complex. An autosomal (A) bivalent displaying complete pairing is also observed in the micrograph. Note the difference in thickness of the axial chromosomal cores of the X and Y chromosomes relative to the lateral elements of the autosomal synaptonemal complex. Whole-mount electron microscopy. Scale mark:  $2\ \mu\text{m}$ .

TABLE 1

Whole-mount electron microscopic analysis of the pairing of X and Y chromosomes in *Sxr,XY* carriers and control littermates during pachytene and diplotene stages of meiotic prophase.

Stage	No. mice	Paired XY		Unpaired XY		Total cells analyzed
		No. cells	%	No. cells	%	
<i>Pachytene</i>						
<i>Sxr,XY</i>	5	156	65	84	35	240
Control	3	276	96.8	9	3.2	285
<i>Diplotene</i>						
<i>Sxr,XY</i>	5	31	34.5	59	65.5	90
Control	3	112	96.5	4	3.5	116

ring-shaped configuration (as reported by Chandley and Fletcher, 1980, and Evans et al., 1980) which was not present in control spermatocytes (Table 2).

Second, a chromosomal fragment was present which appeared to pair preferentially with the centromeric end of the normal Y chromosome (Fig. 4). This morphological feature was observed during the early stages of meiotic prophase (late zygotene-pachytene) and was unique to the *Sxr,XY* carrier males. In addition, this fragment was identified as Y chromosome-derived by its characteristically thickened chromosomal core. Hence, we shall refer to this as the Y-fragment ( $Y^f$ ).

Third, a free supernumerary  $Y^f$  was present in close proximity to the unpaired X and Y chromosome (Fig. 5), another structural feature unique to the *Sxr,XY* male mouse. At pachytene,  $Y^f$  has a length of  $1.12 \mu\text{m}$  ( $\pm .37 \mu\text{m}$ ). As meiotic prophase progresses towards metaphase I, bivalents condense and decrease in length. Therefore, the length of  $Y^f$  is reduced.

Fig. 4 shows the X and Y chromosomes of the *Sxr,XY* mouse held together by a short synaptonemal complex. At the opposite end, the paracentromeric region of the Y chromosome is associated by means of a very short synaptonemal complex with a chromosomal fragment,  $Y^f$ , displaying a chromosomal core characteristic of the

TABLE 2

Whole-mount electron microscopic analysis of XY bivalents with ring configuration in *Sxr,XY* carriers and control littermates during pachytene stage of meiotic prophase

Type of mouse	No. mice	Pachytene		Diplotene	
		Total cells	XY ring shape	Total cells	XY ring shape
<i>Sxr,XY</i>	5	240	44 (18.3%)	90	17 (18.8%)
Control	3	285	0	116	0



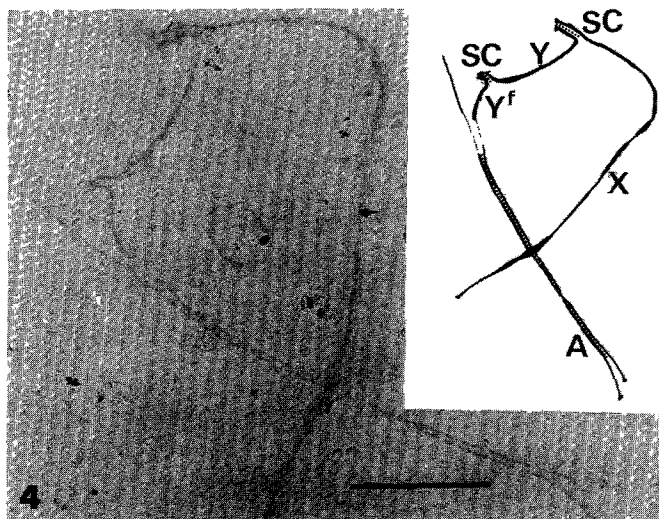


Fig. 4. Mid-pachytene spermatocyte of an *Sxr,XY* carrier, male mouse. The long arm of the X chromosome is paired with the long arm of the Y chromosome by means of a short synaptonemal complex (SC). The opposite centromeric end of the complete Y chromosome is associated through a synaptonemal complex (SC) with an extra Y fragment ( $Y^f$ ). Note that the X and Y chromosomes and the  $Y^f$  fragment have thicker axial chromosomal cores than the autosomal bivalent. Whole-mount electron microscopy. Scale mark:  $2\ \mu\text{m}$ .

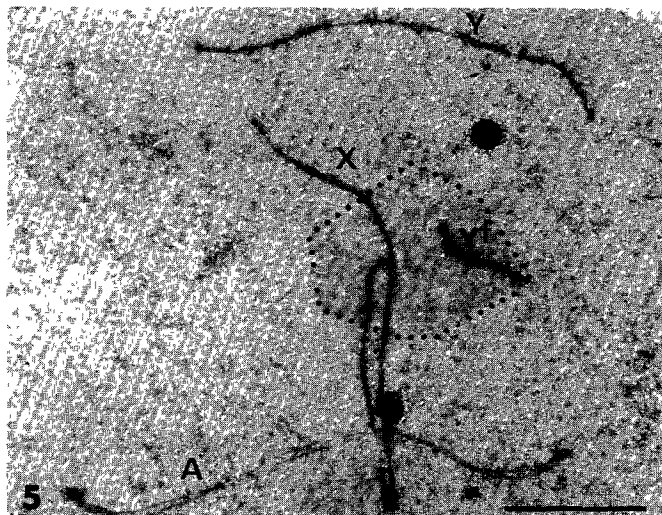


Fig. 5. Late pachytene spermatocyte of an *Sxr,XY* carrier, male mouse. X and Y chromosomes are separated from each other. A thick axial core of a Y-chromosome fragment ( $Y^f$ ) is located close to the condensed paracentromeric region (dotted line) of the X chromosome (partially folded on itself). The synaptonemal complex of an autosome (A) is also present in the micrograph. Note that the axial cores of the X and Y chromosomes and of the Y-chromosome fragment are thicker than the lateral elements of the autosomal synaptonemal complex. Whole-mount electron microscopy. Scale mark:  $2\ \mu\text{m}$ .

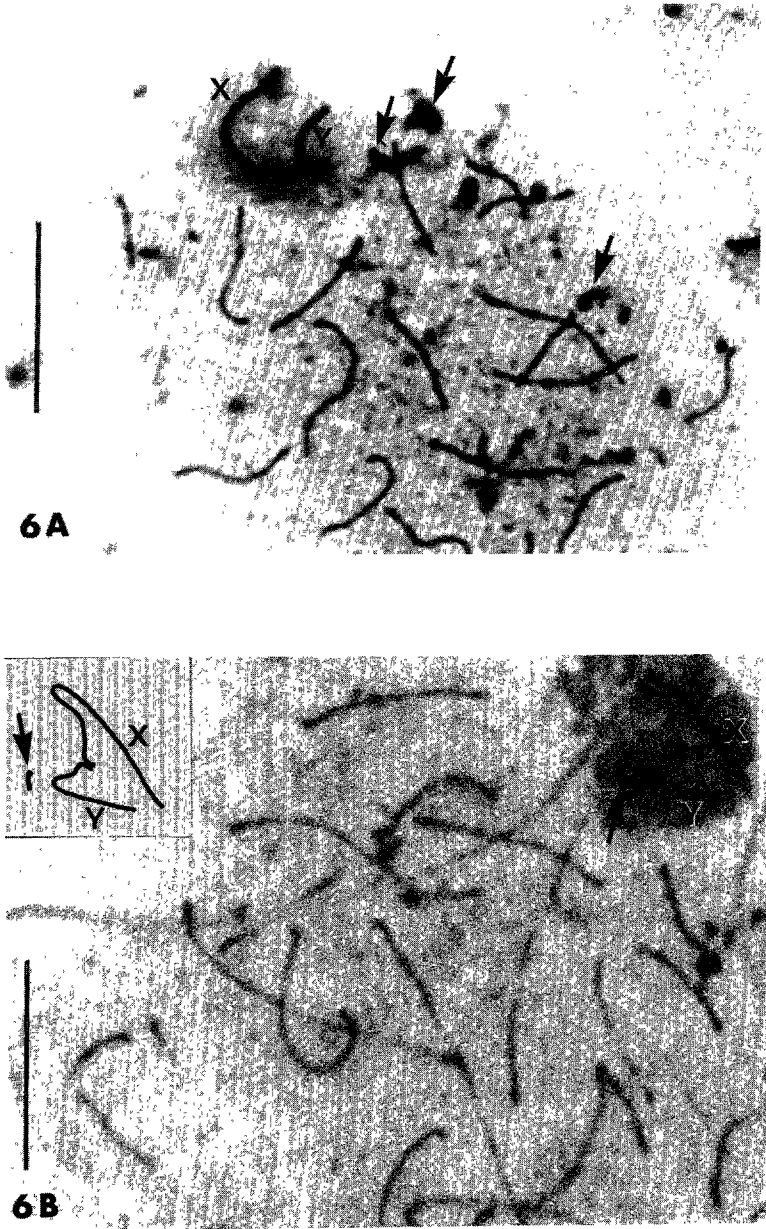


Fig. 6. Light microscopic photographs showing partial chromosomal complements of pachytene spermatocytes stained with the silver technique. A. Control. Compare the increased chromatin condensation of the XY bivalent with autosomal bivalents. Arrows indicate nucleolar masses associated with autosomes. B. *Sxr,XY*. The arrow points to a small chromosomal core fragment within the condensed chromatin mass of the sex bivalent. X and Y chromosomes are associated by a short pairing segment (inset). Scale mark: 10  $\mu$ m.

normal Y chromosome.  $Y^f$  is in close proximity to an autosomal bivalent. The canonical interpretation of this micrograph is that the autosomal bivalent contains material of Y chromosomal origin with which  $Y^f$  has paired non-randomly.

The pairing behavior of meiotic chromosomes in normal and *Sxr,XY* carrier male mouse spermatocytes was also studied in silver-stained preparation with the light microscope. This technique allows the study of a large number of spermatocytes but unfortunately does not facilitate a clear distinction among close proximity, pairing and association of autosomal bivalents with sex chromosomes owing to limitations in the resolving power of the light microscope. Three *Sxr,XY* carrier male mice as well as two littermate controls were studied. Mouse spermatocytes at middle-late pachytene and diplotene were analyzed. We have found that  $Y^f$  was present as a free chromosomal segment in 16% of middle-late pachytene spermatocytes (in 24 of 147 scored pachytene) as opposed to 0 controls. At diplotene,  $Y^f$  was observed in about 18% of *Sxr,XY* nuclei (18 of 98 scored diplotenes) as opposed to 0 of controls. Fig. 6 illustrates the XY bivalent at pachytene in a control (6a) and *Sxr,XY* spermatocyte (6b). A free  $Y^f$  within the condensed chromatin mass of the sex chromosomes and in close proximity to X and Y chromosomal cores in *Sxr,XY* is illustrated (Fig. 6b). These data further support our contention that *Sxr,XY* nuclei contain a supernumerary fragment of Y chromosomal origin.

## Discussion

This report provides evidence that additional Y chromosome-associated material is present in *Sxr,XY* mice relative to normal XY males. Cytological studies of meiotic preparations, using both whole-mount electron microscopy and light microscopy of silver-stained preparations, suggest that *Sxr,XY* male mice possess a supernumerary Y chromosomal segment,  $Y^f$ , which is not seen in normal XY littermates (co-isogenic controls). The chromosomal segment was identified by its pairing with the homologous paracentromeric region of the Y chromosome during late zygotene-pachytene and by structural features of its axial core. This fragment presumably carries a region of the Y chromosome containing either the H-Y structural gene or an associated positive regulatory element. This inference is also compatible with our serological data which, using both splenocytes and sperm, suggest that *Sxr,XY* cells absorb roughly twice as much H-Y specific cytotoxic activity as do the equivalent wild-type XY cells. The simplest interpretation of our findings is that the *Sxr* condition is due to the presence of a  $Y^f$  which comprises a major portion of the differential segment of the Y chromosome.

The murine *Sxr* condition is thought to have arisen as the result of either (1) a constitutive mutation at an autosomal locus normally dependent upon the Y chromosome for expression, or (2) the presence at an autosomal location of a Y chromosomal region containing male determining factors (such as one or more H-Y genes). Experimental determination of how *Sxr* originated has hinged upon the search for a Y-autosome rearrangement. Previous light microscope studies of silver-stained spread preparations of spermatocytes from *Sxr,XO* (Chandley and Fletcher,

1980) and *Sxr,XY* (Chandley and Fletcher, 1980; Evans et al., 1980) mice have not detected such a rearrangement. However, our finding of a considerable number (35%) of late pachytene–early diplotene spermatocytes with unpaired or early disjoined X and Y chromosomes in *Sxr,XY* are not incompatible with the observations of Chandley and Fletcher (1980), Evans et al. (1980) and others (Winsor et al., 1978).

Evidence in support of a Y-autosome rearrangement in *Sxr* mice has been provided by two laboratories. One study has involved the use of satellite DNA isolated from female *Elapid* snakes containing sequences quantitatively derived from the W chromosome which is thought to function in this snake much like the Y chromosome in the mouse (Singh, Purdom and Jones, 1980; Jones and Singh, 1981). In the mouse, these sequences are arranged in a sex-specific pattern as seen in Southern blots and preferentially hybridize to the proximal tip of the Y chromosome in situ. Hybridization of these sequences to Southern blots of *Sxr,XX* DNA reveal an arrangement of these sequences similar to that of normal murine male DNA, compatible with the interpretation that a Y-autosome rearrangement has taken place in the *Sxr,XX* mouse. A second study revealed the presence of a Y chromosomal fragment at an autosomal location in *Sxr,X0* and *Sxr,XY* mouse spermatocytes at diakinesis (Winsor et al., 1978). This fragment is also visualized in our whole-mount electron microscopic preparations of pachytene spermatocytes as a dense chromosomal core measuring 0.75–1.50  $\mu\text{m}$  in length and surrounded by condensed chromatin. The paracentric region of sex and autosomal bivalents can be identified in electron and light microscopic whole-mount preparations as characteristically condensed chromatin masses ('basal knobs') (Schnedl, 1972; Tres, 1977). Since  $Y^f$  displays comparable chromatin condensation and, in addition, pairs with the paracentromeric region of the Y chromosome, it is possible to conclude that  $Y^f$  is homologous to the Y-paracentromeric region.

Two possible interpretations of the chromosomal arrangement illustrated in Fig. 4 can be made (1) a free  $Y^f$  pairs with part of the Y chromosome, and (2) the  $Y^f$  is associated with an autosomal bivalent and behaves as a segment homologous to the corresponding segment of the Y chromosome. It could be argued that synaptonemal complexes can be observed between non-homologous chromosomes or chromosomal segments (Gillies et al., 1973; Holm et al., 1979). Therefore a synaptonemal complex by itself may not be sufficient to establish homology (Chandley and Fletcher, 1980). However, Rasmussen and Holm (1980) have suggested that early meiotic prophase (zygotene) is the period of homologous chromosome pairing. During pachytene, a second round of synaptonemal complex formation can account for non-homologous pairing.

The more consistent association of  $Y^f$  with an autosome in our material may be due to the uniform genetic background on which *Sxr* has been placed. It is also possible, as pointed out by Chandley and Fletcher (1980), that if the  $Y^f$  translocated to an autosome is too short, then the  $Y^f$ -Y chromosome pairing could be unstable and irregular. It is also possible that  $Y^f$ -Y chromosomal pairing takes place during early meiotic prophase stages. Cattanaach et al. (1971) proposed that in the original *Sxr,XY* male mouse, a chromatid, rather than a chromosome, exchange had oc-

curred. The original male could then have carried a complete Y in addition to the male-determining region of the Y attached to an autosome. Another possible interpretation is that the Y<sup>f</sup>-autosomal association is non-specific in nature (Chandleley and Fletcher, 1980). However, the serological evidence showing increased H-Y antigen bolsters the contention of extra Y material in *Sxr,XY*.

In a previous report, Bennett and her collaborators (1977) had concluded that liver cells isolated from *Sxr,XY* animals absorb the same level of cytotoxic activity from H-Y antisera as do wild-type XY liver cells. Data presented in their report, however, show that in a comparative epidermal cell cytotoxicity test similar to the one we use here, H-Y antiserum in the presence of complement lysed *Sxr,XY* epidermal cells to a markedly greater extent than either *Sxr,XX* or normal XY cells. Using splenocytes and sperm, we find that *Sxr,XY* cells absorb roughly twice as much cytotoxic activity as do the equivalent wild-type XY cells, a result compatible with the epidermal cytotoxicity test data presented by Bennett et al. (1977). This result, along with the cytogenetic evidence presented here lends favor to the hypothesis that the *Sxr* mutation is the result of the presence of a supernumerary fragment of Y chromosomal origin containing specific male determinants. However, does this supernumerary Y material contain the H-Y structural apparatus, or only a regulatory element necessary for its expression?

Four models have been proposed to explain the expression of the H-Y gene product: (1) Y-linkage of an H-Y structural complex, (2) autosomal linkage of structural elements under androgen-modified control, (3) autosomal linkage of structural elements under the control of a Y-linked regulator and (4) multiple locations for the H-Y structural gene (Wachtel, 1980). Evidence in favor of the Y-linked regulator hypothesis includes the observation that XX goats homozygous for the autosomal mutation polled (which causes hornlessness in its heterozygous state) develop as phenotypic males that are H-Y positive (Wachtel et al., 1978). This observation suggests that the putative Y-linked H-Y regulator can be replaced by a regulator which acts in an autosomal recessive fashion. Another piece of evidence in favor of the Y-linked regulator scheme is the report that X0 mole-voles can develop as H-Y positive phenotypic males (Nagai and Ohno, 1977).

The androgen-modified expression model for H-Y antigen has been reviewed (Erickson, 1977). Although this model has a certain aesthetic appeal, clear evidence for its applicability to mammalian systems has yet to be presented. However, it has been reported that the cells of ZZ male chickens can express histocompatibility-W antigen (the avian analog to H-Y) when treated with estradiol at the fourth day of embryonic development (Müller et al., 1979).

The evidence to date for Y-linkage of an H-Y structural complex is more correlative than demonstrative. It has been suggested that in mammals testicular differentiation is absolutely correlated with the presence of H-Y antigen on the male cell surface (Wachtel, 1979). Usually the presence of H-Y antigen on the cell surface correlated with the presence of a Y chromosome. In those cases where H-Y positive cells do not appear to contain an intact Y chromosome, undetected translocations of Y-derived genetic material have been invoked to explain the expression of H-Y antigen.

Evidence for a supernumerary Y chromosome fragment in *Sxr* carrier males has now been presented. In light of this, we believe that the difference in the level of H-Y antigen seen in *Sxr,XY* over wild-type XY animals in the present study is due to the presence of additional H-Y specific genetic material of Y chromosomal origin. This genetic material consists of either an H-Y structural element or an unusual regulator that generates approximately a one-fold increase in the level of its associated structural gene product detected by immunoabsorption. Although one would imagine that this degree of increase in the level of H-Y antigen is more likely to be due to a doubling in the number of H-Y structural genes, genetic factors other than an H-Y structural element can affect the level of H-Y (Kralová and Lengerová, 1979). This locus, however, should not have allelic variation in our *Sxr* material which is maintained on a 129/SvPa background.

The present results also cast a new light on the finding of oocytes in *Sxr,XX* 'males' (McLaren, 1980). The presence of oocytes near the rete testis in these 'males' may be due to an occasional loss of the Y-chromosome fragment or be analogous to the occasional induction of XY oogenesis in  $XX \leftrightarrow XY$  chimeric ovaries. Thus, germ cell sex may or may not be determined separately from somatic cell sex in mammals: it is in fact separately determined in *Drosophila* (Marsh and Weischaus, 1978). Even though XO, *Sxr* mice produce spermatozoa, they are non-functional. The sterility of *XX, Sxr* is therefore probably not due to the presence of an extra X chromosome alone, but also to the absence of Y-linked genes necessary for sperm development and function in mammals, as is the case in *Drosophila* (Meyer, 1972). We believe the Y-chromosome fragment in *XX, Sxr* may well have lost these genes but retained the H-Y determining locus.

**Note added in proof** (*Received 14 June 1982*)

Since the submission of this paper, Singh and Jones (Cell 28, 205–216, 1982) have reported evidence for additional Y chromosomal material in *Sxr,XY* mice by in situ hybridization with a *Drosophila* genomic clone which cross-hybridizes to the *Elapid* probe discussed in the text. This material was attached to the distal terminus of a large mouse chromosome which they speculate is the X.

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