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Heterogeneity in the *Sxr* (*sex-reversal*) locus of the mouse as revealed by synthetic GA$^{T}_{C}$A probes

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Summary

The sex-reversal mutation, *Sxr* and a variant form, *Sxr′* have been established on the inbred C57BL/6Mcl background by repeated backcrossing to form the CB and CB′ strains, respectively. DNAs of normal *XY*, *XX Sxr* and *XX Sxr′* as well as *XY Sxr* and *XY Sxr′* carrier male mice have been digested with the restriction enzymes *Hae* III and *Hinf* I and electrophoresed. The DNAs show many common but also differing hybridization bands with synthetic GA$^{T}_{C}$A oligonucleotide probes. In *XY Sxr* (and *XY Sxr′*) carrier males, the hybridization patterns of normal *XY* and those of *XX Sxr* (and *XX Sxr′*) males are simply superimposed. Individual differing bands can be categorized by their differential hybridization behaviour to the $(GATA)_4$, $(GACA)_4$, $(GATA)_2$ GACA $(GATA)_2$ and $(GATA)_3$ $(GACA)_2$ probes. In general, the hybridization patterns are regularly inherited. In addition to the predominant pattern in each strain, one additional *XX Sxr* and one additional *XX Sxr′* hybridization pattern was observed: the additional pattern in the CB strain was transmitted (via variant *XY Sxr* carriers) while the secondary *XX Sxr′* pattern in the CB′ strain could only be observed once. Thus 'DNA finger printing' with GA$^{T}_{C}$A oligonucleotide probes can successfully be used to discriminate the DNAs of normal *Y* chromosomes, *XX Sxr* and *XX Sxr′* variants as well as *XY Sxr* and *XY Sxr′* carrier mice. Implications of the comparatively high unequal recombination rate involving the murine *Y* chromosome are discussed, as well as possible mechanisms.

1. Introduction

In mammals, one or more genes on the *Y* chromosome play a pivotal role in male primary sex determination. Mammals with *XY* sex chromosomes are male, while *XX* individuals are female. Whenever a *Y* chromosome is present, the undifferentiated gonad develops as a testis, while in the absence of a *Y* chromosome ovaries are induced and the female phenotype develops (Welshons & Russell, 1959). Human patients as well as animal models with aberrant sex chromosome constitutions have been used to assign male-specific genes to subregions of the *Y* chromosomes. An operationally defined locus encoding the *t*estis *d*etermining *f*actor (*TDF*) has been provisionally placed on the short arm of the human *Y* (see e.g. Arnemann *et al.* 1987) and the pericentromeric region of the mouse *Y* chromosome (for review see Eicher & Washburn, 1986).

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Sxr (Tp(Y)1Ct) is a *Y* chromosome transposition in which a testis determining region which is normally located proximally in the chromosome has been moved to the distal end (Cattanach, Pollard & Hawkes, 1971). In this position, as a result of obligatory *X*–*Y* crossing over at meiosis in *XY* carrier males, it is transferred to one chromatid of the *X*. Half the *XX* progeny therefore receive the *Sxr* factor and develop as males (Evans, Burtenshaw & Cattanach, 1982). These cytological findings had been anticipated on the basis of *in situ* hybridization data (Singh & Jones, 1982), using a hybridization probe isolated from a snake: the *b*anded *k*rait *m*inor satellite (*Bkm*; Singh, Purdom & Jones, 1981) DNA. Major constituents of this initially uncloned Bkm DNA were shown to be the GA$^{T}_{C}$A simple repeats (Epplen *et al.* 1982, 1983; Singh, Phillips & Jones, 1984). The GA$^{T}_{C}$A sequences are therefore valuable tools to analyse *Y* chromosomal DNA in the mouse (Schäfer, Ali & Epplen, 1986; Schäfer *et al.* 1986). In a preliminary study, a variety of hybridization patterns had been

observed in DNAs from outbred *XX Sxr* individuals which had been received from independent animal facilities (Epplen, unpublished results). We have therefore decided to carry out a survey of the variation in hybridization pattern among *XX Sxr*, *XY* and *XY Sxr* mice from an inbred stock. An *Sxr* variant exists, termed *Sxr′* (McLaren *et al.* 1984), which retains the ability to induce testis formation but no longer codes for the expression of H–Y antigen. Since the transition from *Sxr* to *Sxr′* may have involved a chromosomal deletion or rearrangement, we have also examined mice from a second inbred stock in which *Sxr′* is segregating.

2. Materials and methods

Mice

Mice were kept and bred at the MRC Mammalian Development Unit. In the CB strain (Fig. 3) *XY Sxr* males have been continuously backcrossed onto C57BL/6Mcl female mice. The *Sxr′* variant was first demonstrated in the daughter of an *X Sxr Y Sxr* fertile male mated to a female carrying the T(X; 16)16H translocation (McLaren & Monk, 1982). This *Sxr′* female unexpectedly typed negative for the H–Y transplantation antigen and so did her *XX Sxr′* male progeny (McLaren *et al.* 1984). *XY Sxr′* carrier males were backcrossed in successive generations onto C57BL/6Mcl female mice (CB′; Fig. 4).

Oligonucleotide hybridizations

DNA was isolated according to Blin & Stafford (1976) from livers, kidneys and/or tails of mice which had been sent coded to Freiburg. Then 10 μg DNA of each mouse was digested with the restriction enzymes *Hae* III and *Hinf* I according to the manufacturers' recommendations. Electrophoresis was carried out for 48 h at 45 V in 0·7% agarose gels in TAE buffer (40 mM-Tris, 12 mM sodium acetate, 2 mM-EDTA; pH 8·3). The gels were dry-blotted on a vacuum-gel dryer for 1 h at ambient temperature and 1 h at 60 °C (Tsao, Brunk & Pearlman, 1983). Prior to hybridization gels were denatured in 0·5 M-NaOH/0·15 M-NaCl for 30 min, neutralized in 0·5 M-Tris/0·15 M-NaCl for 30 min at room temperature under gentle agitation and equilibrated in 6 × SSC (20 × SSC: 175·3 g NaCl; 88·2 g sodium citrate per litre).

Oligonucleotides were synthesized on an automated DNA synthesizer (Applied Biosystems 381A); the composition has been described (Ali, Müller & Epplen, 1986). In addition the $(GATA)_3(GACA)_2$ probe was used. Oligonucleotides were labelled with [^{32}P]-γ-ATP (Amersham, Frankfurt, FRG) in a T4 kinase reaction and purified on a DE52 column (Whatman) according to Maniatis, Fritsch & Sambrook (1982). Hybridizations were carried out at the temperatures indicated below for 3–4 h in 5 × SSPE (1 × SSPE = 180 mM-NaCl, 10 mM-$Na_{1.5}PO_4$, 1 mM-EDTA; pH 8·0), 0·1% sodium dodecyl sulphate (SDS), 10 μg/ml sonicated and denatured *E. coli* DNA and 1×10^6 cpm/ml of the probes: $(GACA)_4$, at 43 °C; $(GATA)_4$, 35 °C; $(GATA)_2$ GACA $(GATA)_2$, 47 °C; $(GATA)_3$ $(GACA)_2$, 49 °C. After hybridization gels were washed three times for 30 min at room temperature in 6 × SSC followed by a 1 min wash at the hybridization temperatures, respectively. Gels were exposed to Kodak XAR-5 X-ray films at room temperature. For control exposures and before reprobing gels were washed twice for 30 min each in 5 mM-EDTA (pH 7) at 60 °C and finally re-equilibrated with 6 × SSC (20 min) at room temperature.

3. Results

Approximately 100 coded tissue samples of the CB and CB′ back-cross strains were examined for their genomic DNA hybridization patterns with variations of the GA^T_CA oligonucleotide probes. In Figs. 1*a*, 2*a* and 5*a* representative composite autoradiographic exposures of *Hae* III- or *Hinf* I-digested DNA samples from the CB strain are shown after consecutive in-gel hybridizations with the $(GATA)_4$, $(GACA)_4$, $(GATA)_2$ GACA $(GATA)_2$ and $(GATA)_3$ $(GACA)_2$ probes. It is obvious that most bands are shared by the different DNA samples after hybridization with the respective oligonucleotide. Normal *XY* individuals always exhibited the assigned pattern 1. Among *XX Sxr* males, two different hybridization patterns were detected. Eight *XX Sxr* mice descended from one male in the third generation of the pedigree shown in Fig. 3 had one pattern, termed pattern 2; five *XX Sxr* mice descended from his brother showed a different pattern, termed pattern 4. In *XY Sxr* carrier male DNA, the normal *Y*-chromosomal pattern 1 is superimposed either onto the *XX Sxr* pattern 2, resulting in pattern 3, or onto the variant *XX Sxr* pattern 4, resulting in pattern 5. It is mainly one GATA-positive band (left arrow; ~ 6 kilobases (kb) long) that varies in the different DNAs after *Hae* III-digestion. This particular band does not hybridize with the chemically pure $(GACA)_4$ nor the $(GATA)_3(GACA)_2$ probes. After prolonged exposure the same positions are marked by the labelled $(GATA)_2$ GACA $(GATA)_2$ oligonucleotide. These results indicate that in this particular DNA fragment, only GACA *monomers* are interspersed among many GATA-elements. The latter are each at least 20–25 bases in length as evidenced by additional pure GATA probes of up to 23 nucleotides (data not shown).

In Figs. 1*b* and 2*b* the hybridization patterns of selected DNA samples of the CB′ mice (Fig. 4) are depicted after *Hae* III and *Hinf* I digestion. Using different probes most hybridization bands are of equivalent molecular weight. Yet again in the range of 5·5–6·5 kb one band varies when probed with the $(GATA)_4$ oligonucleotide. This particular band does

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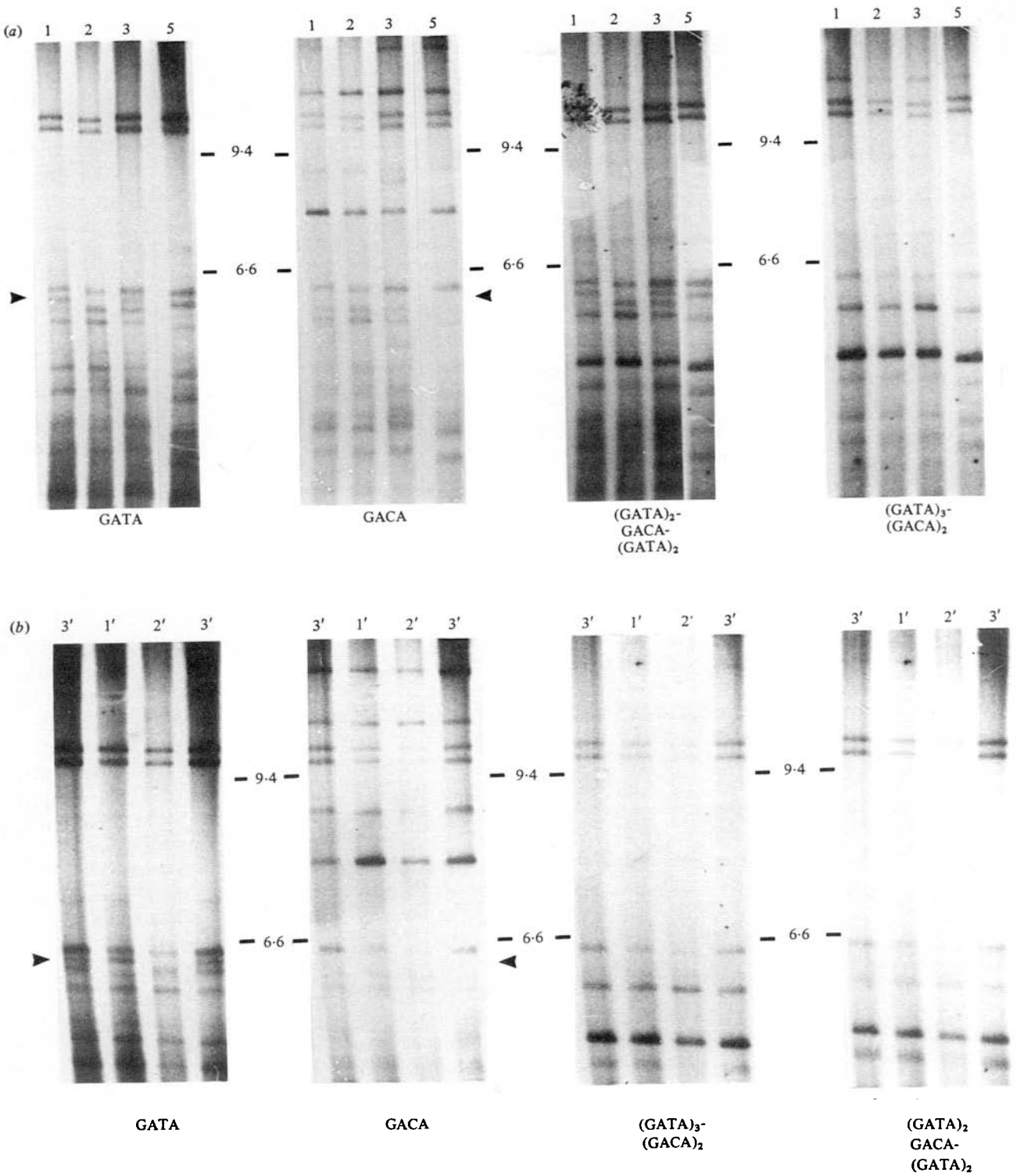

Fig. 1(*a*) and (*b*). GA^T_CA sequences are distributed differently in *XY*, *Sxr* and *Sxr′* DNAs. Liver or tail DNA of *XX Sxr*, *XX Sxr′*, *XY Sxr*, *XY Sxr′* and *XY* male mice was digested with the restriction enzyme *Hae* III to completion; after electrophoresis the gel was consecutively hybridized with the probes indicated as described in the Materials and methods section. Numbers on top of the lanes identify the hybridization patterns observed. 1 and 1′, *XY* of the CB and CB′ strains; 2 and 2′, *XX Sxr* and *XX Sxr′*; 3 and 3′, *XY Sxr* and *XY Sxr′* carriers; 5, *XY Sxr* variant carrier. Arrows point to GATA-positive, GACA-negative bands that distinguish the hybridization patterns of *XY*, *Sxr* and *Sxr′* mice. Fragment lengths are indicated in kilobases.

not hybridize to the pure $(GACA)_4$ probe. Pattern 1′ is clearly distinguishable from pattern 1 of the CB strain (Fig. 5*b*). The polymorphic band is not surprising since the sources for the *Y* chromosomes are different: the *Y* of the CB strain stems from the original *Sxr* mutation (Cattanach, Pollard & Hawkes, 1971), but in the CB′ strain a *Y* chromosome from a tabby stock was introduced before continuous backcrossing (Fig. 4). Patterns 2 and 4, identified in *XX Sxr* males, were not seen in any of the CB′ DNA

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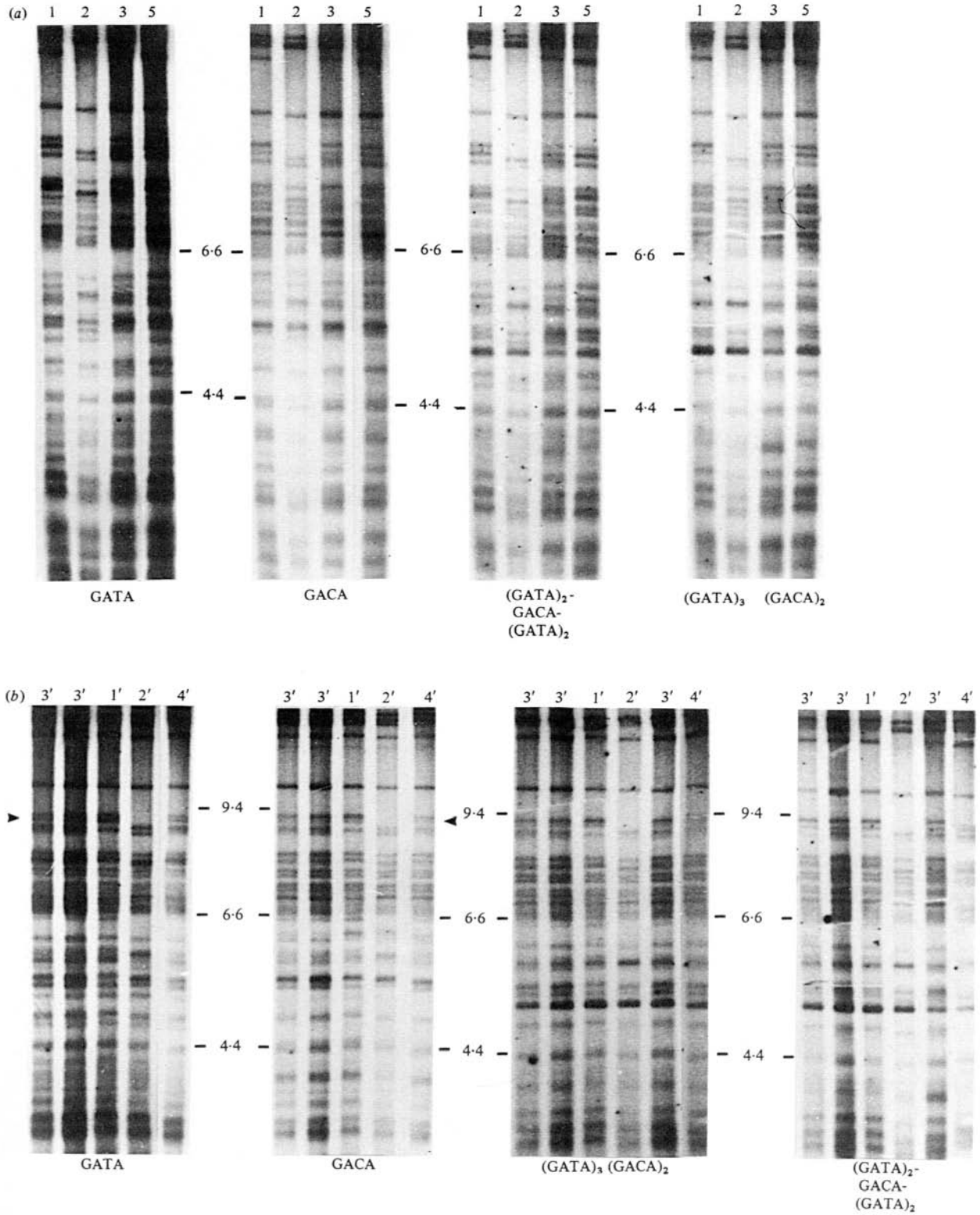

Fig. 2 (*a*) and (*b*). *Hinf* I-digested mouse DNA from *XY*, *XX Sxr*, *XX Sxr′* and *XY Sxr* males can be distinguished by $GA^{T}_{C}A$ oligonucleotide probes. For methodological details see Materials and methods. Numbers on top of the lanes identify the patterns observed. 2 and 2′, *XX Sxr* and *XX Sxr′*; 3 and 3′, *XY Sxr* and *XY Sxr′* carrier; 4′, *XX Sxr′* variant; 5, *XY Sxr* variant carrier. Fragment lengths are indicated in kilobases. Arrows point to differences in the hybridization patterns in the 9·2 kb range.

samples. Most of the *XX Sxr′* males showed the same pattern (termed pattern 2′), but one variant individual was observed (pattern 4′). *XY* pattern 1′ superimposed on *XX Sxr′* pattern 2′ yields pattern 3′ in *XY Sxr′* males. Since the father of the single pattern-4′ *XX Sxr′* male showed the 3′ pattern, the variant pattern presumably arose during the previous meiosis, and would not have been transmitted to subsequent generations since *XX* males are sterile.

Within each of the pedigrees illustrated in Figs. 3

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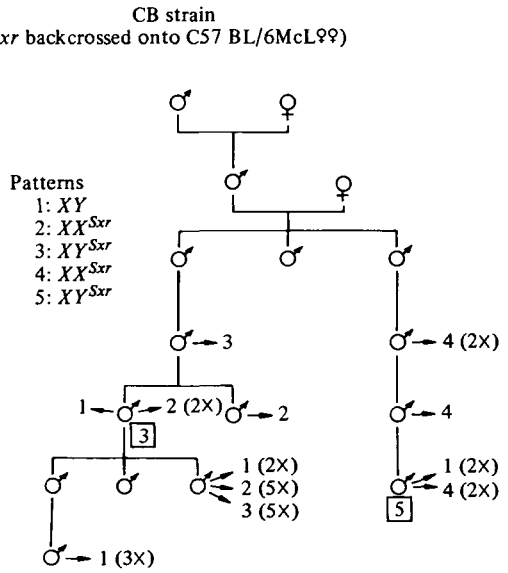

Fig. 3. Pedigree of the CB strain, in which *XY Sxr* males are continuously backcrossed onto C57BL/6 female mice. Only the male *XY Sxr* carriers are indicated after the first two backcrosses. Boxed numbers mean that the *XY Sxr* carrier status (pattern 3) was confirmed by hybridization analysis; numbers at the tip of arrows identify hybridization patterns observed in the progeny of the respective carrier male (numbers in parentheses indicate in how many progeny the patterns were observed). Note that *XX Sxr* pattern 2 was regularly inherited in the left-hand section of the pedigree while the variant pattern 4 is seen only in the right-hand section.

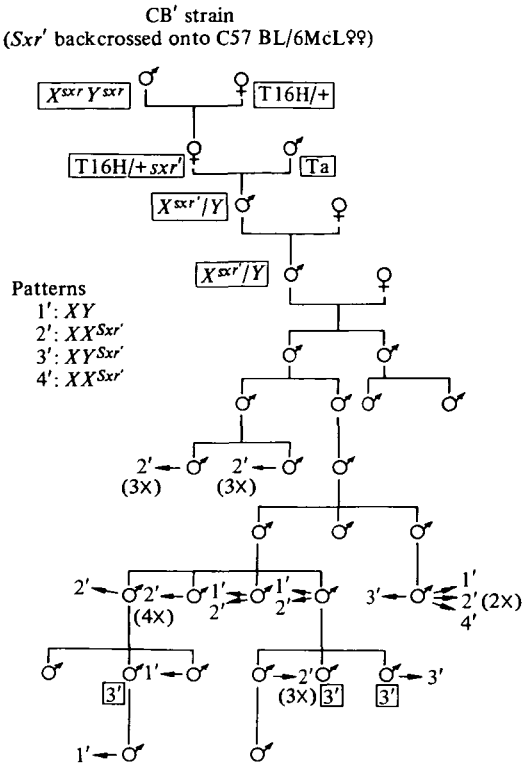

Fig. 4. Pedigree of the CB′ strain, where the *Sxr′* mutation is backcrossed onto C57BL/6 female mice. The *Sxr′* variant was first demonstrated in the daughter of an *X Sxr Y Sxr* fertile male which had been mated to a female carrying the T(X;16)16H translocation. This fertile *Sxr*-carrying female (McLaren & Monk, 1982) typed unexpectedly negative for the H–Y antigen and so did her *XX Sxr′* male progeny. Mating the female to a male with the tabby (*Ta*) coat colour mutation resulted in a fertile male carrying the *Sxr′* factor. Successive backcrossing onto C57BL/6 female mice resulted in the pedigree shown (in the lower part only the *XY Sxr′* carrier males are depicted). 3′ means that the *XY Sxr′* carrier status has been confirmed by hybridization pattern analysis. Numbers indicated by arrows identify hybridization patterns observed in the progeny of the respective carrier male (numbers in brackets indicate in how many offspring the patterns were actually observed). Note that the *XX Sxr′* pattern 4′ was only observed once and no further generations of the respective carrier male could be examined.

and 4, we can estimate how many meiotic events, i.e. how many opportunities for recombination resulting in a new pattern, were interposed between the different individuals whose DNA we analysed. The results are shown in Table 1. The 29 *XY Sxr* and *XY Sxr′* males analysed in the two strains tested a total of 46 meiotic events, but no variants of the respective *Y*-chromosome patterns were observed. On the other hand the 63 meiotic events tested by the 46 *XX Sxr*, *XY Sxr*, *XX Sxr′* and *XY Sxr′* mice whose DNA was analysed gave rise to two variations (pattern 2 *vs.* pattern 4; pattern 2′ *vs.* pattern 4′).

4. Discussion

Gene order is a stable characteristic of all organisms analysed so far (for review see Borst & Greaves, 1987). DNA rearrangements can be either programmed or incidental (mutational). In both the CB and CB′ strains (Figs. 3 and 4), new and unexpected hybridization patterns (4 and 4′) arose that involved the *Sxr*- and *Sxr′*-specific (GATA-positive, GACA-negative) DNA fragments. The DNA mutation was inherited in a regular manner in the CB strain, while in the CB′ strain the mutant pattern was seen in a single *XX Sxr′* mouse. In both strains the mutations affect the critical band that differs between *XY*, *XX Sxr* and *XX Sxr′* males.

A hotspot for unequal recombination is a possible explanation for these results. Actual candidates for recombinational hotspots in mammals have been identified in the *m*ajor *h*istocompatibility *c*omplex

Table 1. *Mutations observed in DNA fragments carrying* GA^T_CA *simple repetitive elements in the backcross strains CB and CB′*

Strain	Investigated meioses involving	*n*	Mutations observed
CB	Normal *Y* chromosome	24	0
	Sxr mutation	28	1
CB′	Normal *Y* chromosome	22	0
	Sxr′ mutation	35	1

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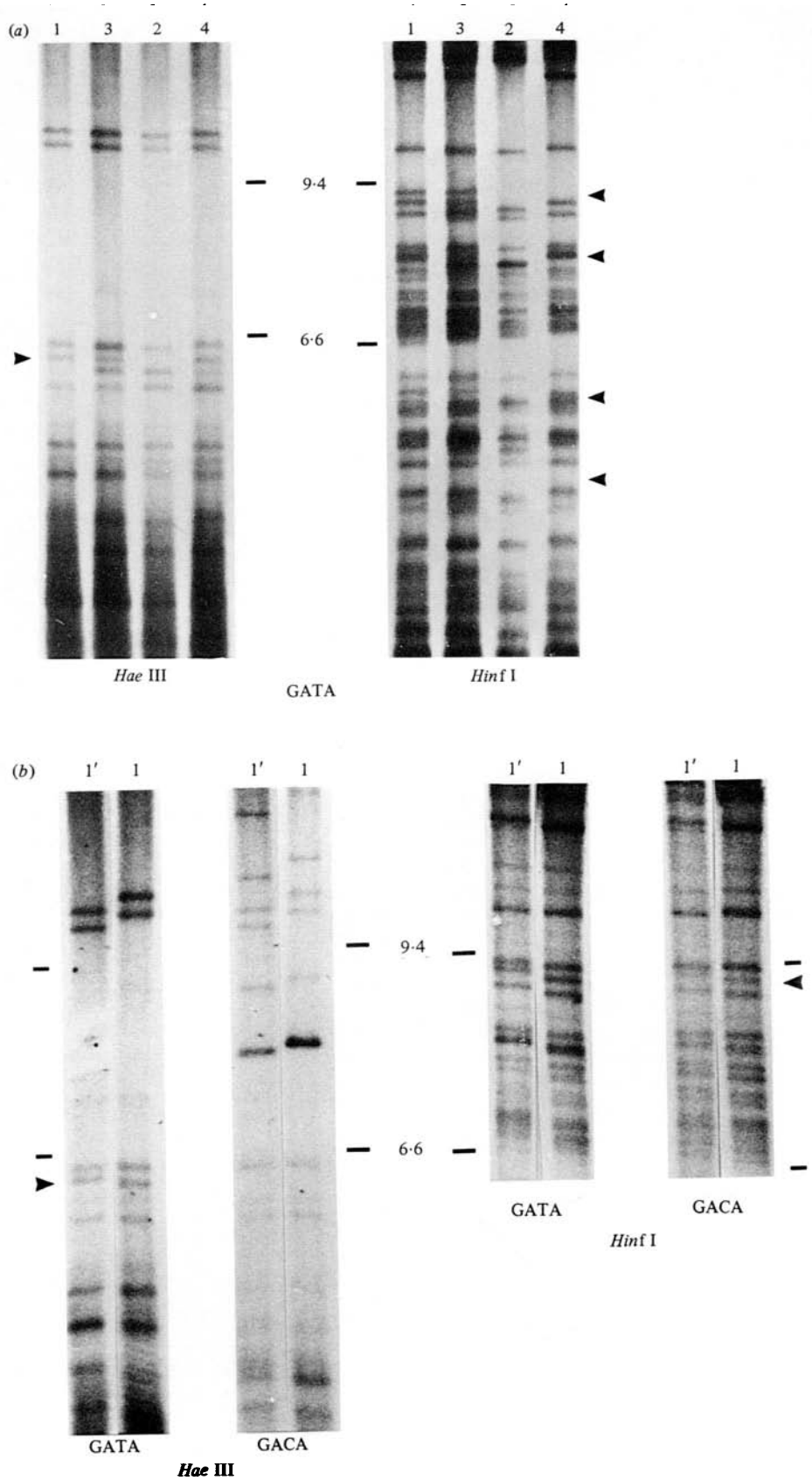

Fig. 5 (*a*). The oligonucleotide probe $(GATA)_4$ hybridizes differently to *XY*, *XY Sxr* and *XX Sxr* DNA. Numbers on top of the lanes identify the hybridization patterns observed. 1, *XY*; 3, *XY Sxr*; 2 and 4, *XX Sxr*. Arrows indicate differences in the patterns; the restriction enzymes used are depicted below the lanes. (*b*) GA^T_CA oligonucleotide probes hybridize differently to *Y*-chromosomal DNA of the CB- and CB′-strains. Numbers on top of the lanes identify the hybridization patterns observed. 1, *XY* of the CB strain; 1′, *XY* of the CB′ strain. Probes and restriction enzymes used are indicated below the lanes. For methodological details see Materials and methods. Fragment lengths are indicated in kilobases.

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(*MHC*; Brégégère, 1983; Kobori *et al.* 1986; for review see Steinmetz, Uematsu & Fischer-Lindahl, 1987). In the immediate vicinity of one hotspot the simple quadruplet repeat $(CAGG)_{7-9}$ is found, while close to another hotspot the sequence $(CAGA)_6$ is present. By shifting the register the identity with $(GACA)_n$ is obvious. In our hybridization studies we identified many GATA copies in the DNA fragment involved in the recombination event, while the presence of a few GACA simple repeats has not yet been excluded. The specific 'GATA-positive, GACA-negative' bands are currently being isolated from genomic DNA libraries containing 6–7 kb–*Hae* III fragments from *XY Sxr* and *XY Sxr'* carriers, as well as from an *XX Sxr'* mouse typing positive for the H–Y transplantation antigen (A. McLaren and E. Simpson, unpublished observations). The latter mouse appears to be especially informative on the mechanism of unequal exchange, for which apparently a *Y Sxr* chromosome may be necessary.

The *Y* chromosome is unique in the genome of virtually all mammalian species in that it is perpetually monosomic. Normally recombination is only possible in the small region that pairs with the *X* chromosome during meiosis. The *Sxr* mutations give us a unique opportunity to study the obligatory crossover between the meiotic *X* and *Y* chromosomes by virtue of the GA^T_CA simple repeats. In mammals, there is so far only one situation in which a completely unpaired mammalian chromosome is seen. In *Ellobius lutescens* (Microtinae, Rodentia), both sexes carry a cytologically identical karyotype with $2n = 17$ chromosomes (Matthey, 1953, 1958). We have identified the unpaired chromosome 9 as the *X* chromosome. While GA^T_CA sequences are intensively interspersed over the whole autosomal chromosome complement, these simple repeats are very significantly reduced or even absent on the unpaired *X* chromosome (Vogel *et al.* in press).

The significance for recombination of simple GA^T_CA repeats and their variants remains open to speculation. In our system only unequal recombination events can be detected. So far no indication of unequal recombination has been observed in the normal *Y* chromosomes of different inbred mouse strains using the GA^T_CA probes. Though the exact number of meioses tested in inbred mice cannot be precisely evaluated, the observed rate of unequal recombination involving GA^T_CA repeat bands is certainly below 0·3%. Yet the unequal recombination rate at one specific locus in our CB and CB' strains is unexpectedly high, approximately 3% (Table 1). An even higher rate of unequal recombination (7%) has been reported for the pseudoautosomal region of the mouse *Y* chromosome, and involvement of uncharacterized repetitive DNA has been suggested (Harbers *et al.* 1987).

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