Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 6446–6449, September 1988
Genetics

Molecular and cytogenetic evidence for the location of *Tdy* and *Hya* on the mouse Y chromosome short arm

(sex-reversed mutation/DNA analysis/*in situ* mapping)

C. ROBERTS*, A. WEITH*, E. PASSAGE†, J. L. MICHOT*, M. G. MATTEI†, AND C. E. BISHOP*‡

*Unité d'Immunogénétique Humaine, Institut National de la Santé et de la Recherche Médicale U.276, Institut Pasteur, 25 rue du Dr. Roux, 75724 Paris Cedex 15, France; and †Groupe Hospitalier de la Timone, Institut National de la Santé et de la Recherche Médicale U.242, 13385 Marseille Cedex 5, France

Communicated by Alfred Jost, May 2, 1988

ABSTRACT **Using a combination of *in situ* mapping and DNA analysis with recombinant DNA probes specific for the *Sxr* region of the mouse Y chromosome, we show that both the gene(s) controlling sex determination and the expression of the male-specific antigen H-Y (*Tdy* and *Hya*, respectively) are located on the minute short arm of the mouse Y chromosome. We demonstrate that the H-Y$^-$ variant of *Sxr* (*Sxr'*) arose by a partial deletion within the *Sxr* region. Also, we show that intrachromosomal recombination between the Y short arm and *Sxr'* can sometimes occur during male meiosis, restoring the deleted DNA sequences and resulting in an H-Y$^+$ mouse (male 719 in this paper). Based on these results, we propose a model for the generation of the original *Sxr* region and the *Sxr'* and *Sxr*^{719} variants.**

Sex reversed (*Sxr*)-[Tp(Y)1Ct] is a small fragment of the mouse Y chromosome that has transposed to the distal pairing/recombination region (the pseudoautosomal region) of the Y chromosome in XY*Sxr* mutant mice (1–4). During male meiosis, this fragment is regularly transferred by recombination to the paternal X chromosome, giving rise to sterile XX*Sxr* (sex-reversed) males. It has been shown that *Sxr* contains the genes controlling primary sex determination (*Tdy*) and the H-Y transplantation antigen (*Hya*) as defined by graft rejection and by the cytotoxic T-cell assay (5). An H-Y$^-$ variant (*Sxr'*) has also been described (6), and it has been suggested that H-Y expression may play a role in spermatogenesis (7). *Sxr* contains a high concentration of simple Bkm-related repeated sequences, which, when used to probe the Y*Sxr* chromosome *in situ*, shows a heavy concentration of grains in the pericentric region and another peak around the telomere (1). This led to the conclusion that *Sxr* represented a fragment transposed from the subcentromeric region of the Y chromosome distal to the pseudoautosomal region. Hence, both *Tdy* and *Hya* were mapped to a region just below the centromere of the normal Y. We present here direct ctyological and molecular data showing that *Sxr* is, in fact, derived from the normal Y chromosome short arm and that occasionally during meiosis in XY*Sxr'* mice *Sxr'* can recombine with the homologous region on the Y chromosome short arm.

MATERIALS AND METHODS

Origin of Mice and a Note on Nomenclature. Mice were obtained from colonies maintained either at the Institut Pasteur (Paris) or at the Mammalian Development Unit (London) as indicated in the text. Sex-reversed male mouse 719 was a H-Y$^+$ variant that arose in a stock segregating Sxr', which is normally negative for H-Y antigen expression (see ref. 8). In this paper, we have used the terms *Sxr'* and *Sxr*^{719} to refer to the variant forms of *Sxr* studied. This nomenclature is nonstandard, and we favor the proposition that such variants can be regarded as *Sxr* haplotypes and, therefore, in the future should take superscript letters—i.e., *Sxr*^{a}, *Sxr*^{b}, *Sxr*^{c}, etc.

Southern Blot Analysis. High molecular weight DNA was extracted from single mouse livers by standard procedures. Fifteen micrograms was digested with restriction enzymes according to the manufacturer's instructions (Amersham), separated on 0.8% agarose gels, transferred to Hybond-N membranes (Amersham), and fixed by UV irradiation. Membranes were hybridized to DNA probes labeled with ^{32}P by random priming (9) (specific activity $>5 \times 10^8$ cpm/μg). The blots were then washed stringently (0.1× SSC at 68°C; 1× SSC = 0.15 M NaCl/0.015 M sodium citrate) and exposed moist to XAR-5 film (Kodak) with an intensifying screen at −80°C for 18–24 hr.

Recombinant DNA Probes. pY8/B is a single-copy 2-kilobase (kb) *Eco*RI DNA fragment of the Y chromosome that specifically detects the *Sxr* region of the mouse Y (9). pY291/B is a Y-specific 2.6-kb *Eco*RI DNA fragment (derived from a Y chromosome microcloned library) that detects both *Sxr* and non-*Sxr* located sequences.

***In Situ* Hybridization.** The entire pY8/B plasmid was ^{3}H-labeled by nick-translation to a specific activity of $\approx 0.7 \times 10^8$ dpm/μg. Concanavalin A-stimulated lymphocytes from C57BL/6/Pas, WMP/Pas, and XYSxr/Pas male mice were cultured at 37°C for 72 hr and 5-bromodeoxyuridine was added for the final 5.5 hr of culture to ensure good posthybridization chromosomal R banding and optimal visualization of the Y short arm. Metaphase cells were hybridized with a probe concentration of 10 ng/ml in the hybridization mixture as described (18). Slides were covered with Kodak NTB2 nuclear track emulsion and exposed for 15 days at 4°C. After development, the chromosome spreads were first stained with buffered Giemsa solution and the metaphase chromosomes were photographed. R banding was then performed by the fluorochrome/photolysis/Giemsa method and the metaphase spreads were rephotographed prior to analysis. At least 100 metaphase spreads were examined for each analysis.

RESULTS

Recently, we have isolated random DNA probes recognizing sequences within the *Sxr* region of the mouse and have used them to analyze this mutation at the molecular level (10). One probe (pY8/B) is a 2-kb *Eco*RI fragment isolated from a Y chromosome-enriched library (11). Fig. 1*a* shows that, on genomic blots of *Taq* I-digested DNA, pY8 detects four bands in XY and XY*Sxr* males but fails to react with female DNA, showing them to be Y located. All bands can be found in DNA from the XX*Sxr* male, demonstrating that they are located within the *Sxr* region. In an analysis of >100

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

‡To whom reprint requests should be addressed.

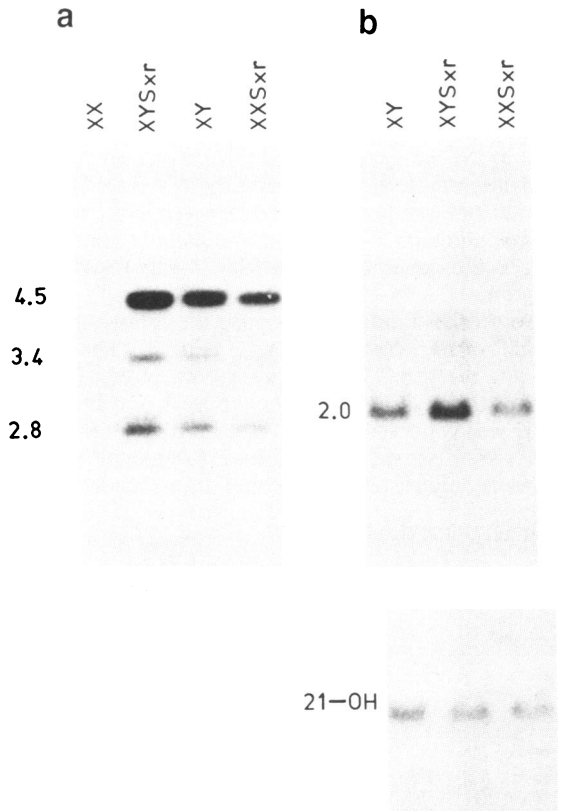

FIG. 1. (*a*) Southern blot analysis of *Taq* I-digested DNA from an XX female, XY*Sxr* male, XY male, and an XX*Sxr* male using pY8. (*b*) Southern blot analysis of the same DNAs as in *a* digested with *Eco*RI. (*Lower*) Result of reprobing the same blot with the autosomal 21-hydroxylase probe to quantitate the amount of DNA present. All mice were from a stock maintained at the Institut Pasteur.

backcross mice, co-segregation of *Sxr* and pY8 was always observed (12). Fig. 1*b* shows the result of probing *Eco*RI-digested RNA from XY, XY*Sxr*, and XX*Sxr* males. pY8 detects the 2-kb cognate sequence and, in addition, two homologous bands of 2.6 and 2.8 kb, all of which map to the *Sxr* region. Densitometric scanning of the autoradiographs revealed that all three bands were twice as intense in the XY*Sxr* carrier male as in the normal XY male or the XX*Sxr* male. The amount of DNA in each lane was quantitated by reprobing with a steroid 21-hydroxylase probe located on chromosome 17 (13). Further dosage analysis showed the cognate band to be present as a single copy in normal male C57BL/6 XY DNA (data not shown). Due to the unique nature of this probe, we were able to use it to accurately define the region of the Y that has been duplicated in the Y*Sxr* chromosome. Fig. 2 shows the results of *in situ* hybridization of pY8 to mouse metaphase chromosomes: a normal C57BL/6 (Fig. 2*a*) male, a WMP male (Fig. 2*b*), and an XY*Sxr* carrier male (Fig. 2*c*). Contrary to expectations, the peak of hybridization on the normal Y was not seen proximally on the long arm but in the short arm. The silver grains in fact define the short arm of the Y. In the Y*Sxr* chromosome in addition to the short arm peak, an equally intense hybridization to the telomeric region of the long arm was seen, consistent with the presence of *Sxr* in the pseudoautosomal region. Although the existence of a Y short arm is not well documented, it was first described by Ford (14) and is frequently used to identify the Y in cytogenetic studies. This immediately suggests a simpler explanation for the origin of *Sxr* involving the relocation of the entire (or a portion) of the Y short arm containing *Tdy* and *Hya* to the pseudoautosomal region. This has the advantage of involving a less complicated

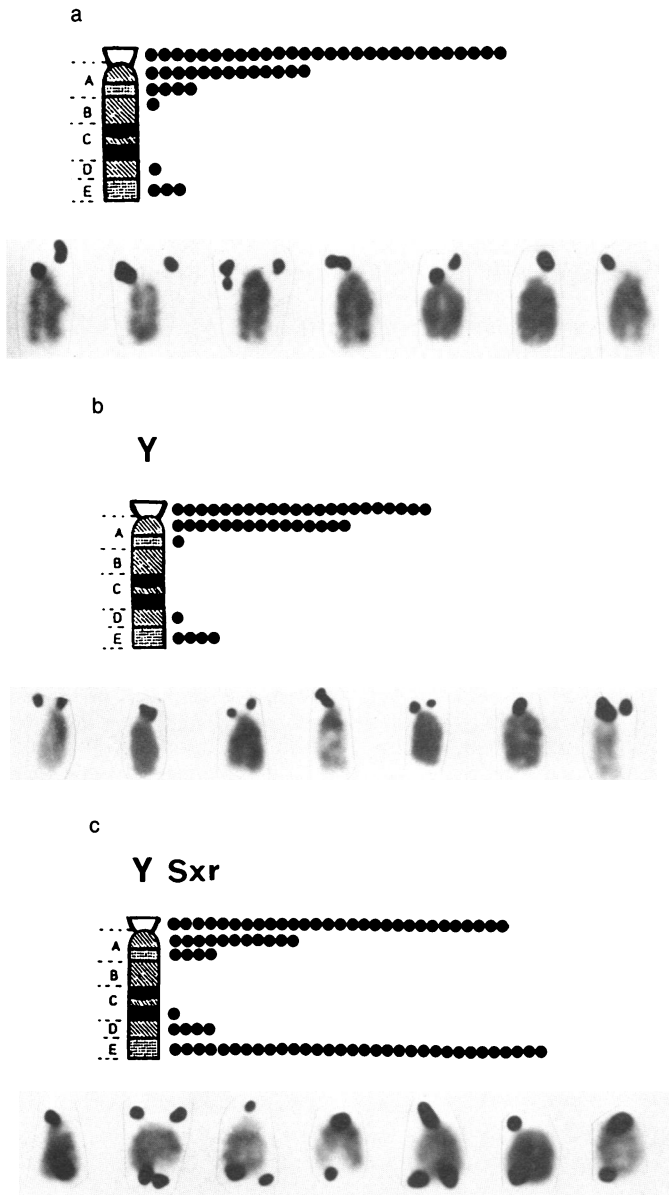

FIG. 2. Hybridization *in situ* of probe pY8 to the Y chromosomes of C57BL/6 (*a*), WMP (*b*), and XY*Sxr* (*c*) male mice from stocks maintained at the Institut Pasteur. Because of the demonstrated Y specificity of the probe on Southern blot analysis, only the grain distribution on the Y was analyzed. For C57BL/6 in the 120 metaphase cells examined there were 312 silver grains associated with chromosomes and 50 of these (16%) were located on the Y. The distribution of grains on this chromosome was not random: 82% (41/50) of them mapped to the pericentromeric region of the Y with a maximum on the short arm. The four grains associated with the long arm telomere are not statistically significant. Similarly, in the 120 WMP metaphase cells analyzed, 282 silver grains were counted, 43 (15.2%) of which mapped to the Y; 86% of these grains (37/43) mapped to the pericentromeric region with again a maximum on the short arm. The five grains associated with the long arm telomere are not statistically significant. One hundred XY*Sxr* metaphase cells were analyzed and 28.4% of grains counted (81/285) were Y located; 49.3% of these grains (40/81) mapped to the pericentromeric region with a maximum on the short arm and 44.4% of them mapped to the telomeric region of the long arm.

rearrangement and the mobilization of the short arm telomere.

With this model in mind, we investigated the generation of the H-Y$^-$ XX*Sxr'* mouse (6) and the recently discovered H-Y$^+$ XX*Sxr*^{719} variant male originating from the XX*Sxr'* strain (see ref. 8). Probing the DNA of these mice with pY8

revealed no differences in the hybridization profiles. Fig. 3, however, shows the result of probing *Bam*HI-digested DNA from an XY male (CB′ strain), XX female (C57BL/6 strain), XX*Sxr*, XX*Sxr′*, and XX*Sxr*^{719} (latter three males from N7-8 generations backcrossed onto C57BL/6) with the probe pY291. Four Y located bands are detected in the normal XY male from the CB′ strain at ≈12.0 kb (band A), 9.0 kb (band B), 6.0 kb (band C), and 3.0 kb (band D). These bands are not present in female DNA. All four are present in XX*Sxr*, although 291B and 291C are reduced in intensity. This suggests that 291B and -C are repeated on the Y but only a limited fraction of them map within *Sxr*. A comparison of the banding pattern of XX*Sxr* with XX*Sxr′* reveals a clear deletion of band 291A, whereas the other bands remain unchanged. In the XX*Sxr*^{719} DNA band 291A reappears at the same position and approximately the same intensity as that found in XX*Sxr* and XY DNA. In addition, the intensity of band 291C has increased to an intensity intermediate between that found in the XY DNA and that of XX*Sxr* or XX*Sxr′*. Probing similar blots digested with six different enzymes (*Sac* I, *Hin*dIII, *Pvu* II, *Pst* I, *Msp* I, and *Eco*RI) yielded similar results, giving no evidence for gross rearrangements.

DISCUSSION

These data clearly show that the generation of the *Sxr′* (H-Y$^-$) mouse from *Sxr* (H-Y$^+$) was not merely due to a point mutation affecting the *Hya* gene (leading to loss of H-Y expression) but involved a partial deletion event within *Sxr* itself. Furthermore, the generation of the *Sxr*^{719} (H-Y$^+$) variant from *Sxr′* is accompanied by the complete reappearance of the deleted band. To verify that this latter event did not simply involve the insertion of a large fragment of the Y into the XX*Sxr* genome, the blots were dehybridized and reprobed with pY353/B (15), which detects Y located repetitive (approximately 200–250) sequences, which are not present in *Sxr* but are dispersed throughout the Y. A normal hybridization pattern was seen on the XY male DNA but no Y chromosomal sequences were detected in the other DNAs (data not shown). We therefore conclude that the event leading to the generation of XX*Sxr*^{719} was restricted to the *Sxr* region.

Based on these data, we propose the following model for the origin of *Sxr*, *Sxr′*, and *Sxr*^{719} (Fig. 4). The *Sxr* region containing the genes (structural or controlling) for *Tdy* and *Hya* maps to the short arm of the mouse Y chromosome (Fig. 4*A*). *Sxr* was generated by the relocation of the whole or part of this region to the distal pseudoautosomal region. The relocation could result either from a translocation or a single

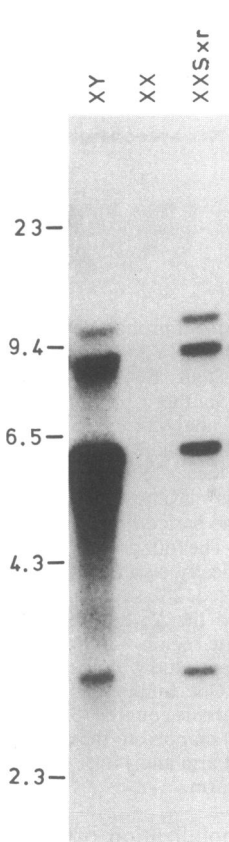

FIG. 3. Southern blot analysis of *Bam*HI-digested DNA from XY male (CB′ strain), XX female (C57BL/6 strain), and XX*Sxr*, XX*Sxr′*, and XX*Sxr*^{719} males with pY291 (XXSxr″ = XXSxr^{719}). With the exception of the XX female, which was from the Institut Pasteur stock, all mice were from stocks maintained by A. McLaren.

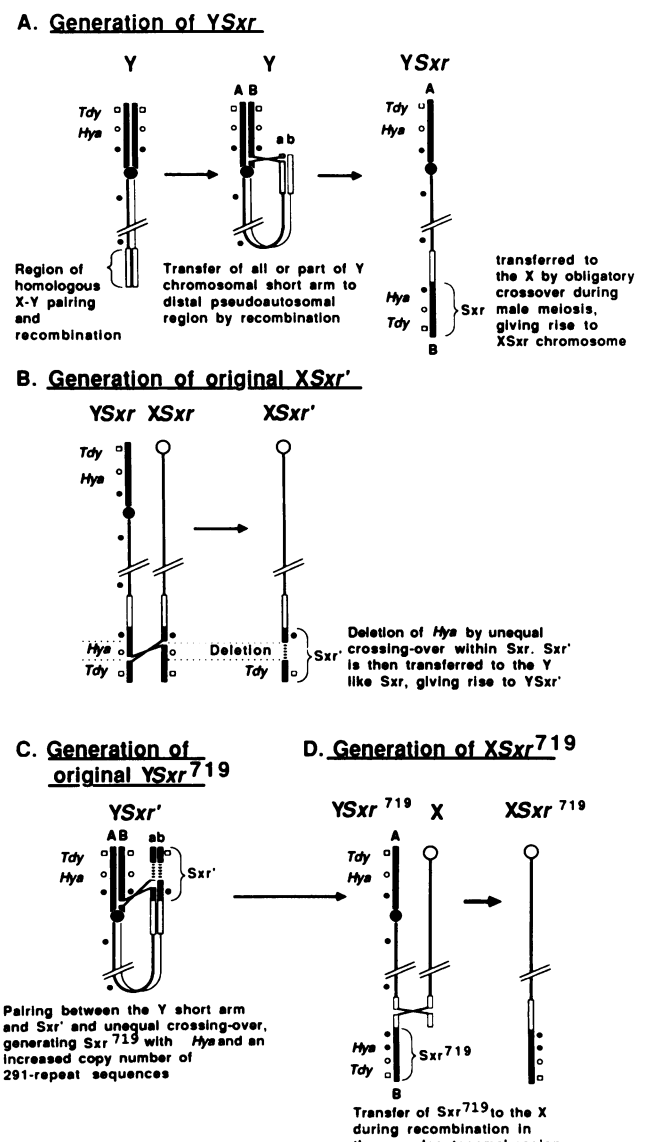

FIG. 4. Proposed origin of *Sxr*, *Sxr′*, and *Sxr*^{719}. □, Unique *Sxr*-specific sequence recognized by pY8; ○, unique sequence detected by pY291, which is deleted in *Sxr′*; ●, repeated sequences detected with this probe. The exact location of these sequences relative to each other has yet to be determined. Similarly, the order of *Tdy* and *Hya* is arbitrary. For simplicity, the generation of XX*Sxr*^{719} is shown in two stages—the generation of Y*Sxr*^{719} followed by the transfer of *Sxr*^{719} to the X. In reality, these two recombination events probably occur in the same meiotic figure.

recombinational level. If it were due to a translocation, two chromosomal breaks would be required—one in the short arm and the other near the long arm telomere. A recombination event would involve the transfer of the short arm from one chromatid to the distal pairing region of the sister chromatid by nonhomologous exchange, and the short arm telomere would then become the telomere of the Y*Sxr* chromosome long arm. We favor this latter possibility for the origin of the original Y*Sxr* chromosome because of its simplicity.

At the DNA level, this *Sxr* fragment would contain *Sxr*-specific sequences recognized by pY8 and pY291 (open box and open circle, respectively, in Fig. 4), a concentration of simple repeats (GATA/GACA), and several Y-specific (but not *Sxr*-specific) repeats recognized by pY291 (solid circle in Fig. 4). The placing of the ancestral *Sxr* region on the short arm of the Y is not in conflict with the results of Bkm hybridization *in situ* as this latter probe is too highly repeated on the Y to resolve the short arm. The generation of *Sxr'* from *Sxr* was a relatively simple event, bearing in mind that the original father was carrying *Sxr* on both the X and the Y chromosomes (X*Sxr*/Y*Sxr*) (6). An unequal recombination event could have occurred between the two *Sxr* regions during male meiosis, leading to the deletion of DNA carrying all or part of the H-Y gene *Hya* (Fig. 4*B*). At the DNA level, this would involve the loss of *Sxr*-specific unique sequences recognized by pY291. Indeed, surprisingly high rates of unequal crossover have recently been reported by Harbers *et al.* (16) in the mouse pseudoautosomal region. We propose that Sxr^{719} arose from *Sxr'* by an intrachromosomal recombination event in the Y*Sxr'* chromosome involving sister chromatids (Fig. 4 *C* and *D*). On this carrier Y, there is one copy of the original unaffected H-Y^+ region on the short arm and *Sxr'* (H-Y^-) at the telomere of the long arm. If during male meiosis these regions were to back-pair on themselves (facilitated by sequence homology) and recombine, the previously deleted region could be cleanly regained. A simultaneous second crossover with the X could then have generated the XXSxr^{719} mouse, which would be H-Y^+. The observed DNA banding pattern, however, shows that more pY291 repeated sequences are present in XXSxr^{719} than in either XX*Sxr* or XX*Sxr'*. This can be most simply explained by postulating a misalignment of these repeated sequences during pairing, leading to a homologous but unequal crossover and the gain of repeats by Sxr^{719}.

In a recent electron microscope study of meiotic pairing in XY*Sxr* males (17), a minority of prophase cells showed the Y paired both autologously and with a subterminal region of the X (probably representing the normal pairing region). This self-pairing of the Y is consistent with the model proposed for the generation of Sxr^{719} from *Sxr'* and suggests that both the exchange between the short arm and the *Sxr'* fragment and the subsequent transfer of Sxr^{719} to the X during normal X–Y recombination occurred in the same meitoic configuration. Finally, it should be emphasized that the mapping of the ancestral *Sxr* region to the short arm of the Y is crucial to this model. If the region were located subcentromerically on the long arm, any intrachromosomal recombination event would inevitably lead to an unstable dicentric chromosome, which would be lost.

We would like to acknowledge the continuous interest and support of Drs. M. Fellous, C. Rijnders, and D. Traud during the course of this work; J. Weissenbach and M. Mitchell for critical comments; and J.-L. Guenet for the WMP strain. We acknowledge the financial support of the Fondation pour la Recherche Médicale (C.R.), the Deutsche Forschungsgemeinschaft (A.W.), and the Institut National de la Santé et de la Recherche Médicale.

1. Singh, L. & Jones, K. W. (1982) *Cell* **28**, 205–216.
2. Evans, E. P., Burtenshaw, M. D. & Cattanach, B. M. (1982) *Nature (London)* **300**, 443–445.
3. Hansmann, I. (1982) *Cell* **30**, 331–332.
4. Eicher, E. M. & Washburn, L. L. (1986) *Annu. Rev. Genet.* **20**, 327–360.
5. Simpson, E., McLaren, A., Chandler, P. & Tomonari, K. (1984) *Transplantation* **37**, 17–25.
6. McLaren, A., Simpson, E., Tomonari, K., Chandler, P. & Hogg, H. (1984) *Nature (London)* **312**, 552–555.
7. Burgoyne, P., Levy, E. & McLaren, A. (1986) *Nature (London)* **320**, 170–173.
8. McLaren, A., Simpson, E., Epplen, J. T., Studer, R., Koopman, P., Evans, E. P. & Burgoyne, P. S. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 6442–6445.
9. Feinberg, A. P. & Vogelstein, B. (1984) *Anal. Biochem.* **137**, 266–267.
10. Bishop, C. E., Roberts, C., Michot, J.-L., Nagamine, C., Winking, H., Guenet, J.-L. & Weith, A. (1987) *Development* **101**, Suppl., 167–175.
11. Baron, B., Metezeau, P., Hatat, D., Roberts, C., Goldberg, M. & Bishop, C. E. (1986) *Somat. Cell Mol. Genet.* **12**, 289–293.
12. Nagamine, C. M., Michot, J.-L., Roberts, C., Guenet, J.-L. & Bishop, C. E. (1987) *Nucleic Acids Res.* **15**, 9227–9238.
13. Amor, M., Tosi, M., Duponchel, C., Steinmetz, M. & Meo, T. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4453–4457.
14. Ford, C. E. (1966) *Transplantation* **4**, 333–335.
15. Bishop, C. E. & Hatat, D. (1987) *Nucleic Acids Res.* **15**, 2959–2969.
16. Harbers, K., Soriano, P., Muller, U. & Jaenisch, R. (1986) *Nature (London)* **324**, 682–685.
17. Chandley, A. C. & Speed, R. M. (1987) *Chromosoma (Berlin)* **95**, 345–349.
18. Dautigny, A., Mattei, M. G., Morello, D., Alliel, P. M., Pham-Dinh, D., Amar, L., Arnaud, D., Simon, D., Mattei, J.-F., Guenet, J.-L., Jolles, P. & Auner, P. (1986) *Nature (London)* **321**, 867–869.