Development 101 Supplement, 177–183 (1987)
Printed in Great Britain © The Company of Biologists Limited 1987

Testis-specific transcripts detected by a human Y-DNA-derived probe

P. LEROY[1], E. SEBOUN[1], M. G. MATTEI[2], M. FELLOUS[1] and C. E. BISHOP[1]

[1]*Unité d'Immunogénétique Humaine, INSERM U. 276, Institut Pasteur, 25 rue du Docteur Roux 75724, Paris Cedex 15, France*
[2]*Centre de Génétique Médicale, INSERM U. 242, Hôpital d'Enfants de la Timone 13385 Marseille Cedex 5, France*

Summary

A genomic sequence (12f3), derived from the long arm of the human Y chromosome, detects a 1·6 kb mRNA, expressed in human and mouse testis, but not in other tissues tested by Northern blot analysis. Using 12f3 as a probe, a mouse cDNA, designated PL5, was isolated from an adult mouse testis cDNA library. The profile obtained by Southern blot analysis using PL5 as probe under high-stringency conditions, reveals that 12f3 probably represents a Y-located pseudogene which was derived from an autosomally located gene. Southern blot analysis of different vertebrate species, using probe PL5, shows that this gene has been highly conserved during evolution. Preliminary *in situ* hybridizations on testis tissue sections indicate that PL5 is expressed during the postmeiotic stages of male germ cell differentiation and thus may play a role during spermatogenesis. A second cDNA, also obtained from the testis cDNA library, weakly cross-reacts with 12f3. This cDNA, designated PL10, detects a mRNA of approximately 4 kb which is highly expressed in mouse testis, but not in male or female mouse liver. The gene corresponding to this cDNA is also well conserved among vertebrates.

Key words: Y chromosome, autosomes, testis-specific transcripts, human, DNA-derived probe.

Introduction

The putative sex-determining gene(s) on the human Y (*TDF*) is localized on Yp (Goodfellow, Darling & Wolfe, 1985), but in the mouse the corresponding gene *TDY* is localized to the pericentric region of the chromosome (Eicher & Washburn, 1986). In both man and mouse, evidence for the presence of additional male-specific genes has been reported, such as genes involved in spermatogenesis (Tiepolo & Zuffardi, 1976; Levy & Burgoyne, 1986). In an attempt to find such genes on the human Y-chromosome, random DNA sequences obtained from a human Y-specific cosmid library (Bishop *et al.* 1983) were studied by Northern blot analysis of human RNA. Two probes, derived from the long arm of the Y chromosome, were found to detect mRNAs specifically expressed in the testis: probe 49F previously described (Seboun *et al.* 1986) and probe 12f3 reported here. These probes are probably derived from pseudogenes on the Y chromosome. Because mRNAs detected by them may be involved in spermatogenesis, they may represent useful tools for studying this poorly defined process at a molecular level. They may also be of use for understanding the evolution of the Y chromosome.

Results

Isolation of the genomic probe 12f3

Approximately 50 random Y genomic sequences, isolated from a human Y-chromosome library (Bishop *et al.* 1983), were used to probe Northern blots of human testis poly(A)$^+$ RNA and several other male and female human tissues. In this way, probe 12f3 was shown to detect an mRNA expressed in human testis but not in other tissues tested.

Probe 12f3 is a 1·7 kb *Bgl*II fragment which with the flanking sequences 12f1 and 12f2 was subcloned from the genomic probe 12f (Fig. 1A), a 5·1 kb *Eco*RI fragment derived from cosmid 12 (Fig. 1B).

Characterization of probe 12f3

Southern blot analysis with human DNA digested by *Eco*RI (Fig. 2A) reveals that, under either stringent or nonstringent conditions, 12f3 detects the 5·1 kb cognate fragment which can be assigned to the Y chromosome due to its increased hybridization intensity on the 49X,YYYY line. In addition, the

probe detects several homologous sequences which cannot be localized to the Y or the X (as shown by no increased hybridization signal on the 49XXXX,Y line

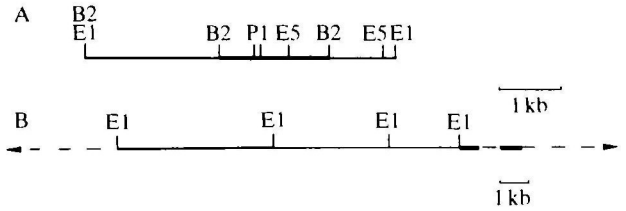

Fig. 1. (A) Restriction map of the genomic sequence 12f, a 5·1 kb *Eco*RI fragment. The 1·7 kb *Bgl*II fragment, 12f3, is drawn as a heavy line. 12f1 is located to the right and 12f2 to the left. (B) Localization of 12f in cosmid 12, from which it was subcloned. 12f is represented as a heavy line (the orientation of 12f is not known); at right, the cosmid vector is represented by a heavy broken line B2, *Bgl*II; E1, *Eco*RI; E5, *Eco*RV; P1, *Pst*I.

(Fig. 2B)) and are therefore presumed to be autosomal. Using a Y-deletion panel (data not shown) 12f3 has been mapped to the long arm of the human Y chromosome, between Yq 11.22 and Yq 11.23.

In the chimpanzee, the Southern blot profile (using DNA digested by *Eco*RI) is very similar to the human (Fig. 3). A male-specific band of 5·1 kb can be detected and as this band has the same size as the Y-located band of the human, it is probably also a Y-specific band.

Human Northern blot analysis with 12f3 detects a 1·6 kb mRNA in human testis, but not in other tissues tested, including ovary (Fig. 4). Occasionally, a 3·8 kb transcript which is not testis specific can be weakly detected in several tissues. Using mouse mRNA, the same specificity is also obtained but Southern analysis reveals only one hybridization

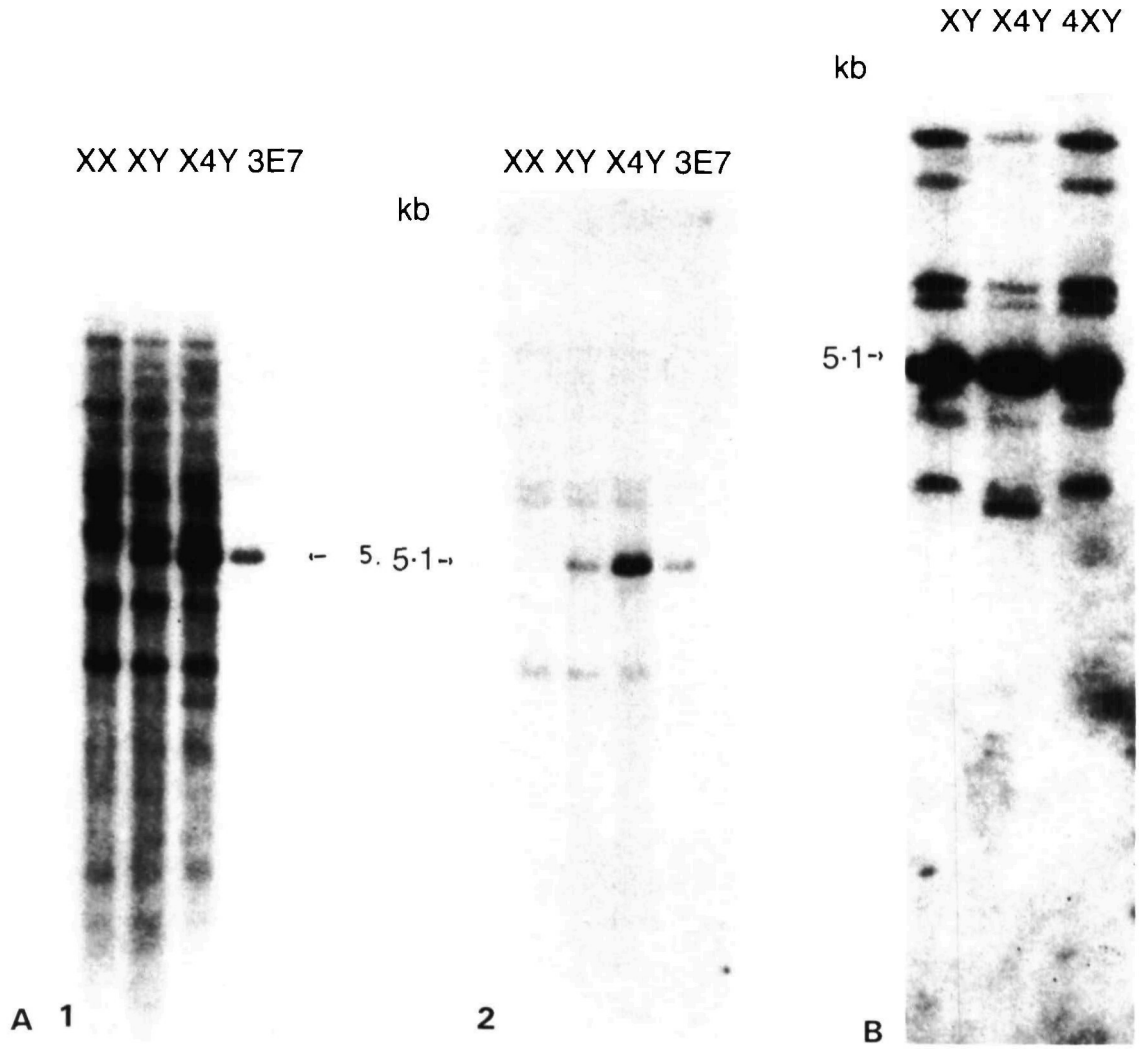

Fig. 2. Southern blots of human genomic DNA digested with *Eco*RI, and hybridized with probe 12f3 Approximately 10 μg DNA was hybridized with ^{32}P-labelled probe 12f3. XX, female DNA; XY, male DNA; X4Y, 49 X,YYYY DNA; 4XY, 49 XXXX,Y DNA; 3E7, mouse/human hybrid contains multiple Y chromosomes as its only detectable human material. (A) The same blot was washed under (1) nonstringent conditions (2 × SSC, 65°C); (2) stringent conditions (0·1 × SSC, 65°C). (B) A second blot washed under stringent conditions.

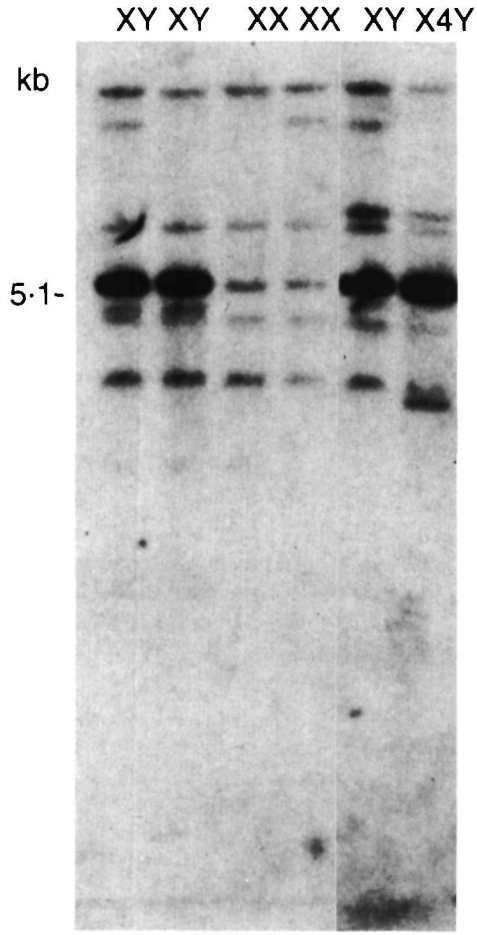

Fig. 3. Southern blots of DNA digested by *Eco*RI and hybridized with 12f3. Washing was performed under nonstringent conditions. The first four lanes contain chimpanzee DNA; the last two lanes contain human DNA. XX, female DNA; XY, male DNA; X4Y, 49 X,YYYY DNA.

band of approximately 7·2 kb on both male and female mouse DNA digested by *Eco*RI.

Isolation of mouse cDNAs

Due to the fact that 12f3 cross-reacts with the mouse and the ready availability of these tissues, we chose to investigate the function of 12f3 in this species. Approximately 7×10^4 plaques from an amplified adult mouse testis cDNA library (Bishop & Hatat, 1987) were screened with 12f3 and eight positive plaques were purified. The longest cDNA obtained, designated PL7, is 1·4 kb and contains a repetitive sequence at one of its extremities (probably the 3′ end). Further analyses were carried out using the shorter overlapping clone PL5, a 0·8 kb cDNA which is included in the restriction map of PL7 and which does not carry the repeated element (Fig. 5). We have neither determined the relationship between the two transcripts detected by 12f3 nor from which of them these cDNAs have been synthesized.

Characterization of the cDNA PL5

The restriction maps of PL7 and PL5 are shown in Fig. 5. The repetitive sequence present in PL7 has been localized to the left end of the map. Fig. 6 shows the hybridization pattern observed on a Southern blot of human DNA, hybridized consecutively with the human genomic probe 12f3 and the mouse cDNA PL5. Under low-stringency conditions, 12f3 and PL5 detect the same bands but with different intensities; the very low intensity of the 5·1 kb Y-specific band observed with PL5 suggests that the mRNA is probably expressed from an autosomally located gene.

Probing *Eco*RI-digested mouse DNA with PL5 (Fig. 7A) detects, under either nonstringent or stringent conditions, only one band of 7·2 kb present on both male and female (the same profile that is obtained using human probe 12f3). No mouse signal is obtained with a hybrid cell line containing only the mouse chromosomes X and 16 on a Chinese hamster background. Thus, we can exclude the possibility of an X–Y common sequence. Southern blot analysis of DNA from several vertebrate species shows that PL5 corresponds to a putative gene which is highly conserved from reptiles to mammals (with the possible exception of the viper) (Fig. 8).

By Northern blot analysis (Fig. 7B), PL5 detects (like 12f3) a 1·6 kb mRNA highly expressed in testis (both mouse and human) but not in numerous other tissues tested, including ovary. In contrast, the 3·8 kb mRNA is present in all tissues tested but apparently at different levels. Partial sequence analysis of PL7 has revealed an open reading frame of at least 500 bp. Searching the Los Alamos data bank with 50 bp of this sequence did not detect any significant homologies at the 72 % level.

Isolation of the cDNA PL10

A second category of cDNA (represented by PL10), which only hybridizes weakly to the probe 12f3, was obtained from the adult mouse testis cDNA library. When this 2·1 kb cDNA is used to probe mouse DNA restricted by *Eco*RI, a single hybridization band of 6·8 kb can be seen on both male and female (Fig. 9A).

By Northern blot analysis, PL10 detects an approximately 4 kb mRNA which is highly expressed in mouse testis but not in male or female liver (Fig. 9B). Occasionally, a 4·3 kb mRNA can be detected in testis and liver.

PL10, like PL5, is highly conserved among vertebrates (data not shown) suggesting a role of fundamental importance. At present, we do not know the exact relationship between PL10 and PL5.

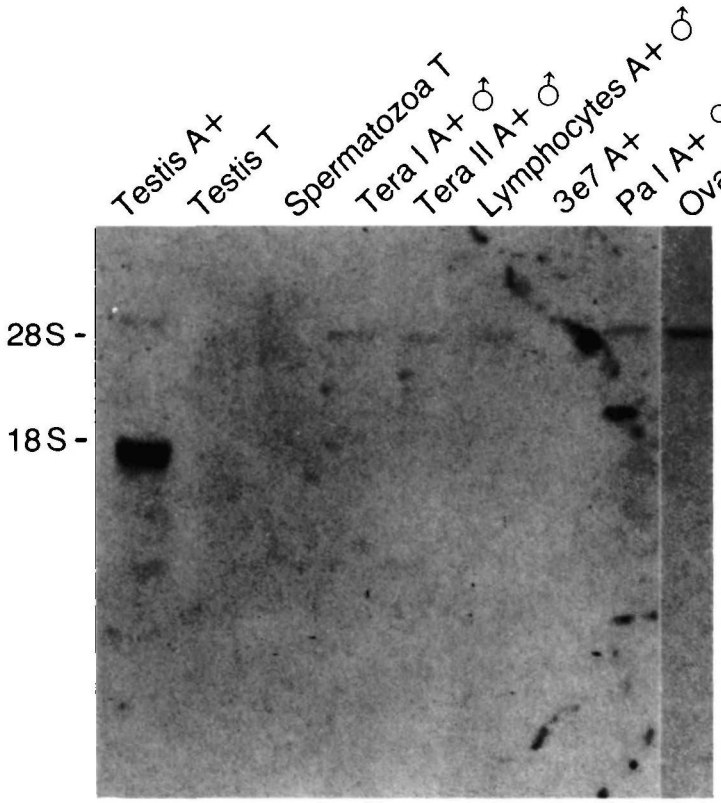

Fig. 4. Northern blot of RNA from various human tissues, hybridized with probe 12f3. About 5 μg of poly(A)$^+$ RNA (or total RNA for second and third lanes) were separated on a methyl mercury agarose gel.

Fig. 5. Restriction map of PL10 (a) and PL7 (b). PL5 is included in PL7 and represented as a heavy line. The hatched line shows the position of the repetitive sequence. The restriction maps of PL10 and PL7 are different (B2, *Bgl*II; E5, *Eco*RV; H2, *Hinc*II; P1, *Pst*I).

In situ *hybridization on mouse chromosomes*

Initial results from *in situ* hybridization to mouse metaphase chromosomes (unpublished observations) indicates that PL5 is located in the central region of chromosome 13 and PL10 in the telomeric region of chromosome 1. Under nonstringent conditions, a secondary hybridization site is detected with PL10 on the X chromosome.

In situ *hybridization to testis tissue sections*

Preliminary *in situ* hybridizations to sheep testis tissue sections, using probe 12f3, shows a high expression at late spermatid stage. These preliminary results are consistent with the RNA playing a role during the postmeiotic stages of spermatogenesis.

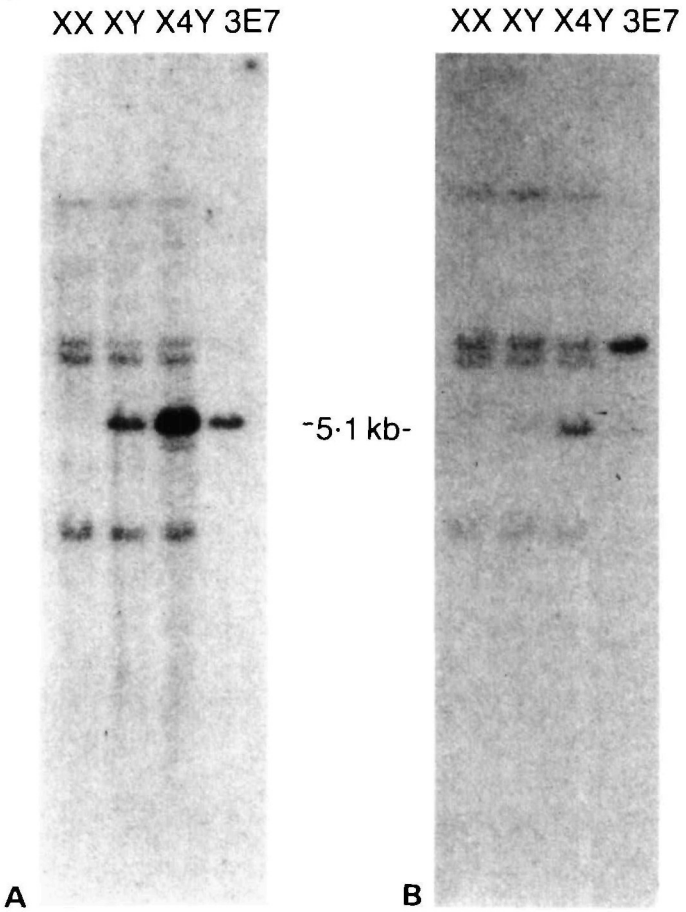

Fig. 6. Human Southern blot analysis using DNA digested by *Eco*RI. Washing was performed under stringent conditions. (A) Blot hybridized with 12f3. (B) Blot hybridized with PL5.

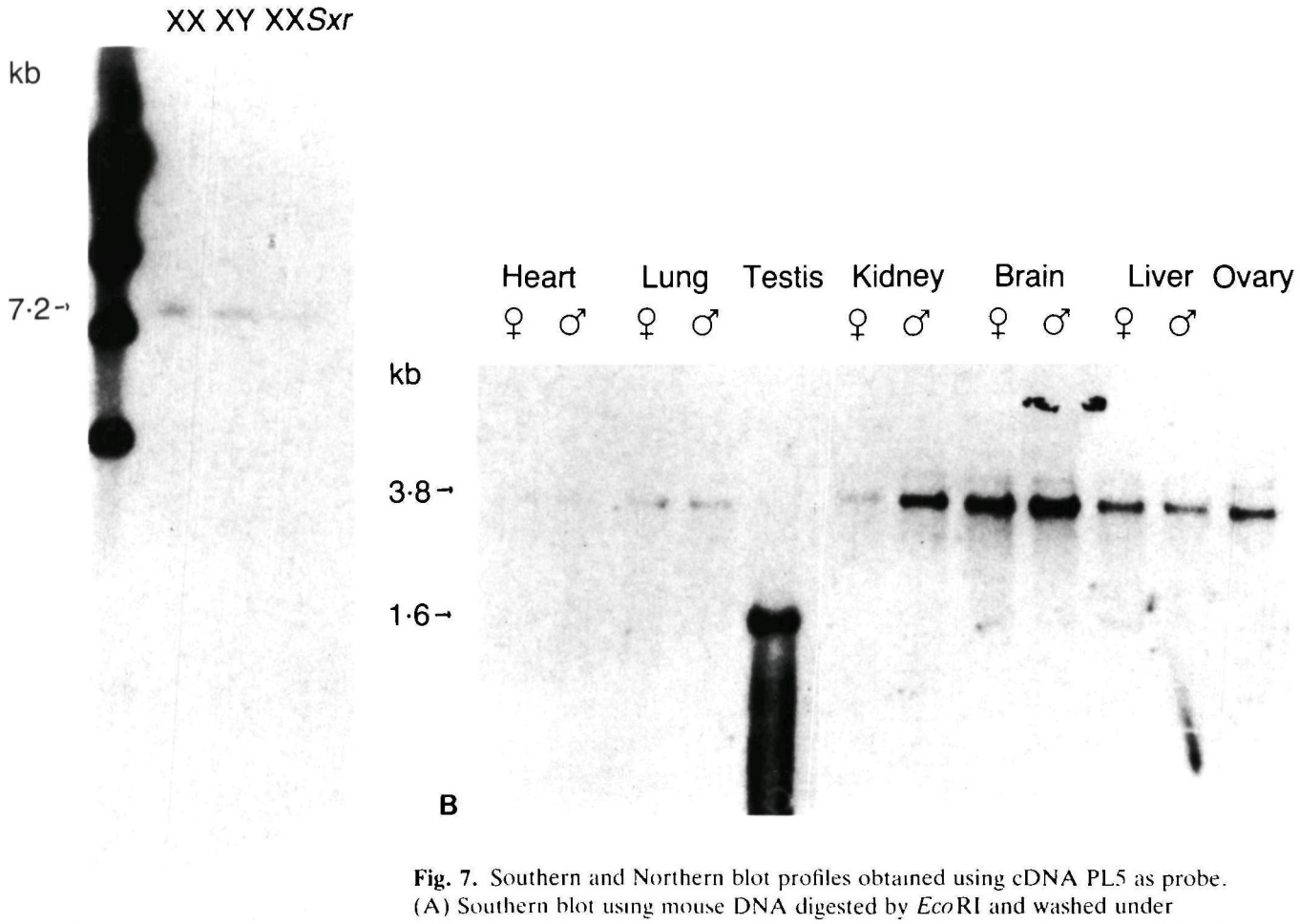

Fig. 7. Southern and Northern blot profiles obtained using cDNA PL5 as probe. (A) Southern blot using mouse DNA digested by *Eco*RI and washed under stringent conditions (the same profile is obtained under nonstringent conditions). XX, female DNA; XY, male DNA; XX*Sxr*, sex reversed mouse DNA. (B) Northern blot using poly(A)$^+$ RNA isolated from various mouse tissues.

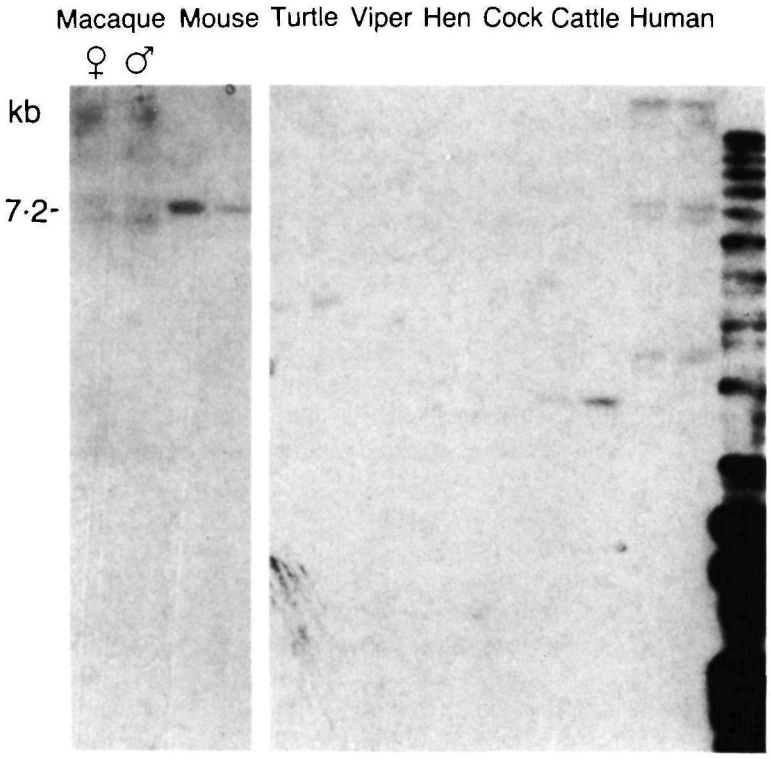

Fig. 8. Conservation among vertebrates. Southern blot analysis using DNA from various vertebrate species digested by *Eco*RI and probed with PL5 (washed under stringent conditions).

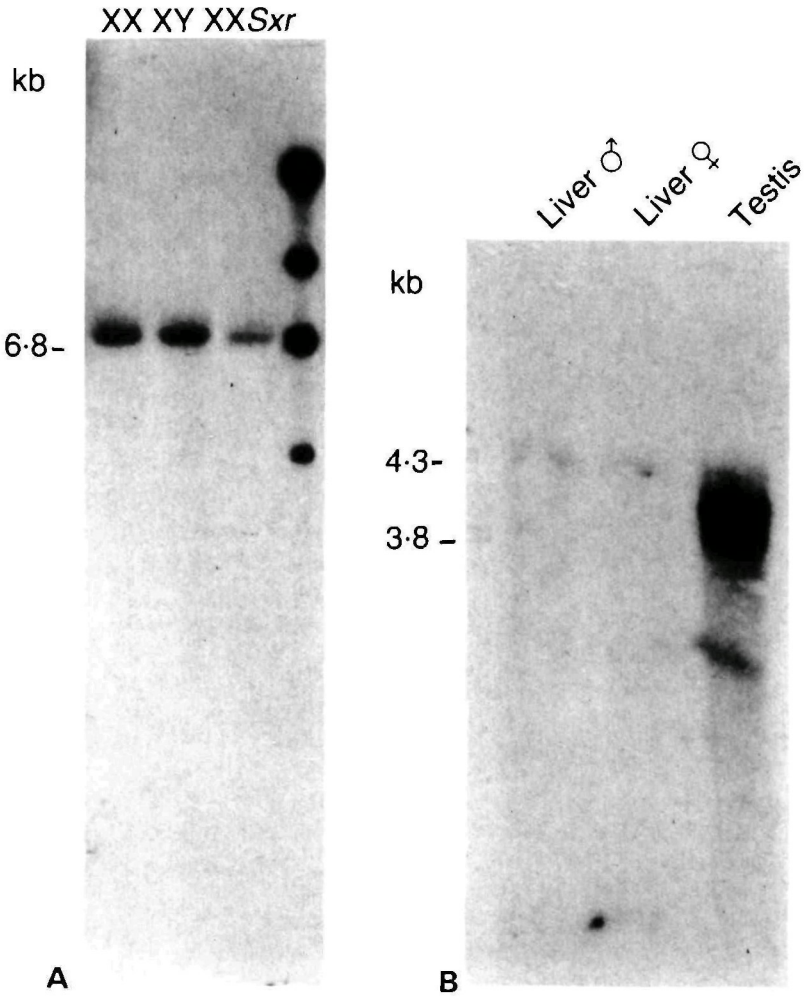

Fig. 9. Southern and Northern blot profiles obtained using cDNA PL10 as probe. (A) Southern blot using DNA digested by *Eco*RI and washed under nonstringent conditions. XX, female DNA; XY, male DNA; XX*Sxr*, sex reversed mouse. (B) Northern blot using poly(A)$^+$ RNA isolated from mouse testes and male and female liver (washed under nonstringent conditions).

Discussion

Origin of the sequence 12f3

A random human sequence, 12f3, derived from the long arm of the Y chromosome, detects an abundant testis-specific transcript of 1·6 kb in both man and mouse. Using the mouse cDNA PL5, isolated with 12f3, we conclude that the 12f3 sequence does not represent the active gene, which is probably autosomally located. This conclusion is drawn from a comparison of human Southern blot patterns obtained with 12f3 and PL5. Under stringent conditions, in contrast to the genomic probe 12f3, the intensity of the Y-located band is drastically reduced. We cannot rule out the possibility, however, that PL5 is derived from the non-testis-specific transcript (3·8 kb) and thus that the 1·6 kb transcript could be expressed from the human Y chromosome. But, in mouse, *in situ* hybridizations to metaphase chromosomes indicate that these transcripts originate from a single region of mouse chromosome 13. In addition, results obtained by Southern blot analysis using mouse DNA suggest that these transcripts are synthesized from a single-copy gene. Possibly the 1·6 kb mRNA is obtained by a testis-specific splicing. Moreover, in the different vertebrates species tested (except for primates and man), a single band common to both male and female is detected on Southern blotting. Thus, we propose that, in lower vertebrates, there is one coding gene, autosomally located, which has spread into both autosomes and the Y before the separation of the chimpanzee and human but after rodent and primate divergence. These results are similar to those obtained with the human Y genomic probe 49F, which has been localized to the same region of the Y chromosome as 12f3 (Seboun *et al.* 1986).

We do not know the mechanism of the genomic dispersion. DNA sequences 12f1 and 12f2 which flank 12f3 (Fig. 1A) do not detect transcripts (Seboun *et al.* 1986). Moreover, in cosmid 12 (the cosmid from which 12f was subcloned and in which 12f is at least 5 kb from one end (Fig. 1B), PL5 detects no homologous sequences other than 12f3. As the RNA is expressed during spermatogenesis, reintegration by a mechanism of retroposition in the germ line is possible and would give rise to such hereditary events. Several examples of pseudogenes found in the human Y chromosome have been described, such as the housekeeping genes arginosuccinate synthetase and actin (Daiger, Wildin & Su, 1982; Heilig *et al.*

1984). The essentially nonfunctional nature of the Y chromosome may explain the easy reintegration of sequences in this chromosome.

Significance of the transcripts

The two cDNAs are both highly conserved among vertebrates; although, no signal was observed with the viper. We have not yet tested amphibian, fish or invertebrates. The conservation observed suggests an important role for these genes among higher vertebrates. Preliminary histological localization of the transcripts to the germ line using 12f3 suggests a role during the haploid stages of spermatogenesis. Northern blot analysis, using ovarian poly(A)$^+$ RNA, failed to detect the 1·6 kb transcript but the number of germ cells is much lower than in the testis and perhaps this type of analysis is not sensitive enough. In addition, the stage during which transcripts are detected in the male germ line would correspond to a stage reached by the oocytes after fertilization and thus not possible to detect in an ovary. This ambiguity could possibly be resolved by *in situ* studies on ovarian sections of fertilized oocytes. Finally, we do not know the exact relationship between PL5 and PL10. Although both were identified using the same genomic probe, PL5 reacts much more strongly with 12f3 than PL10. In addition, PL5 and PL10 are only weakly cross-reactive. Neither the genomic sequence nor the two cDNAs cross-react with the 49F sequence which also detects a germ line transcript (Seboun *et al.* unpublished result).

We wish to thank Drs Andreas Weith and David Sassoon for their helpful comments in preparing this manuscript.

References

Bishop, C. E., Guellaen, G., Geldwerth, D., Voss, R., Fellous, M. & Weissenbach, J. (1983). Single copy DNA sequences specific for the human Y chromosome. *Nature, Lond.* **303**, 831–832.

Bishop, C. E. & Hatat, D. (1987). Molecular cloning and sequence analysis of a mouse Y chromosome RNA transcript expressed in the testis. *Nucleic Acid. Res.* (in press).

Daiger, S. P., Wildin, R. S. & Su, T. S. (1982). Sequences on the human Y chromosome homologous to the autosomal gene for arginosuccinate synthetase. *Nature, Lond.* **298**, 682–684.

Eicher, E. M. & Washburn, L. L. (1986). Genetic control of primary sex determination in mice. *A. Rev. Genet.* **20**, 327–360.

Goodfellow, P., Darling, S. & Wolfe, J. (1985). The human Y chromosome. *J. Med. Genet.* **22**, 329–344.

Heilig, R., Hanauer, A., Grzeschik, K. H., Hors-Cayla, M. C. & Mandel, J. L. (1984). Actin like sequences are present on the X and Y chromosomes. *EMBO J.* **3**, 1803–1807.

Levy, E. R. & Burgoyne, P. S. (1986). 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.

Seboun, E., LeRoy, P., Casanova, M., Magenis, E., Bouccekine, C., Disteche, C., Bishop, C. E. & Fellous, M. (1986). A molecular approach to the study of the human Y chromosome and anomalies of sex determination in man. *Cold Spring Harbor Symposium: Molecular Biology of* Homo Sapiens.

Tiepolo, L. & Zuffardi, O. (1976). Localisation of factors controlling spermatogenesis in the non-fluorescent portion of the human Y-chromosome long arm. *Genet.* **34**, 119–124.