
A human Y-chromosomal DNA sequence expressed in testicular tissue

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Abstract

Clone pJA36B(DYS14) was isolated from a human Y chromosome enriched cosmid library. Southern blot analysis revealed a male-specific hybridization pattern. Deletion mapping with patients' DNA localized pJA36B to the median region of Yp, being present in the DNA of nine of fifteen XX-males tested so far and therefore localized in the region neighbouring the TDF-locus. Northern blot analysis showed a transcription signal in poly(A)+RNA of human testis. Sequence analysis of the genomic DNA sequence revealed an open reading frame of 522 basepairs in the absence of control or signal sequences for the regulation of transcription or polyadenylation. This suggests that only one exon of a translatable sequence is present in clone pJA36B. A computer aided search revealed no significant homologies with known DNA or protein sequences.

Introduction

Male determining DNA sequences have been claimed to exist on the human Y chromosome as a consequence of studies on patients with abnormal sex chromosome constitution. For this reason the Y chromosome is of primary interest for the analysis of male sex determination and sex differentiation. According to a classical cytogenetical model the human Y chromosome is defined into a meiotic pairing region, a pericentric region and the heterochromatic region of the long arm (1, 2). Karyotype-phenotype correlations have been used to assign male specific genes to certain regions of this chromosome: The locus encoding the testis determining factor (TDF) has been provisionally placed in the median region of the short arm (Yp) and genes involved in spermiogenesis on the long arm, Yq (3).

The map of the human Y chromosome was improved by molecular biological techniques employing Y chromosome-derived DNA probes.

We distinguish: a) a "pseudoautosomal" homologous pairing region distal on Yp with highly homologous DNA sequences on the distal parts of Xp (4, 5, 6); b) a pericentric non-homologous pairing region, where DNA sequences of different degrees of sequence homology can be found, ranging from homology between Y and X or Y and autosomes to Y specificity (7, 8, 9, 10, 11); c) a meiotic non-pairing region including most of the long arm .

The DNA linkage map of the human Y chromosome and its correlation with defined cytogenetical regions is steadily improving due to accumulating data from DNA of patients with abnormal sex chromosomes hybridized with Y-specific DNA probes (10-13). Nothing is known, however about the molecular structure and function of the operationally defined TDF, which plays an essential role in sex determination. Several far-reaching hypotheses on the nature of the TDF and on the possible involvement of the H-Y antigen have been put forward. As yet none of the models can be verified, since no DNA sequence has been found which is present in all XX-males (10-13); until now no human Y-specific, expressed DNA sequences have been reported. We describe here a new Y chromosomal DNA sequence, which is transcribed in human testis tissue.

Materials and Methods

Genomic DNA preparation and hybridization analyses

DNA was prepared from peripheral blood cells or cultured skin fibroblasts by standard methods (14). Restriction enzyme digestion, gel electrophoresis, Southern blotting, hybridization and washing procedures were done as described (15).

RNA preparation and Northern blot analyses

Total RNA was isolated from adult human testis, thymus, lung and liver tissue by the guanidinium thiocyanate/cesium chloride gradient procedure (16). Poly (A)+ RNA was prepared by oligo- (dT) chromatography (14). RNA samples were denatured with formamide, electrophoresed in 1% agarose gels containing formaldehyde (31), ethidium bromide stained, photographed under UV light and then blotted onto Genescreen membranes (NEN). Hybridization was done at 42°C in the presence of formamide (14).

Radioactive labelling of probes

Plasmid clones were nick-translated to an approximate specific activity of 10^8 cpm/ug (17).

Subcloning, restriction mapping and DNA sequence analyses

Subcloning was done in the plasmid vectors pUC8 (pJA36B, pJA36B2) or M13 mp9 and M13 mp18 (J8, J16, J17, J 22). Restriction mapping was done by single and double digestions with the indicated restriction enzymes. Dideoxysequencing was carried out according to the procedure of Sanger (18) using universal sequencing primers.

Results

Clone pJA36B is localized on the short arm of the human Y chromosome

A cosmid clone (cos36) was isolated from a human Y-chromosome enriched cosmid library (4), constructed from DNA of the mouse-human cell hybrid 3E7 (19). This cell hybrid has retained the Y chromosome as the only cytogenetically detectable human chromosome. The cosmid library was screened for human sequences by hybridization with labelled total human DNA and subsequent identification of single or low copy segments within cosmid clones by restriction enzyme mapping. In this way, subclone pJA36B (DYS 14; 20) was established. Southern blot hybridization of total male and female DNA with probe pJA36B gave a male-specific banding pattern with all restriction enzymes tested (Figure 1a). Male-specificity was observed even under low stringency washing conditions (65°C , $2\times\text{SSC}$; Figure 1b). pJA36B and cos 36 were mapped with restriction enzymes and by subsequent hybridization with various subclones of cos 36 (Figure 2). pJA36B hybridises to Hind III genomic fragments, 6.9 kb and 2.4 kb of length, respectively, the intensity of the 6.9 kb band being much stronger than that of the 2.4 kb band (Fig. 1c). When Taq I-digested DNA is probed with pJA36B, a predominant 2.8 kb band appears in addition to two bands of weaker intensity (Fig. 1d). pJA36B was further subcloned into pJA36B1 and pJA36B2. pJA36B1 is a 6.7 kilobase Eco RI/Hind III fragment. It is homologous to the predominant bands in Hind III and Taq I genomic digests; it is estimated that there exist at least 10 copies on the human Y chromosome. pJA36B2 is a 1.6 kb

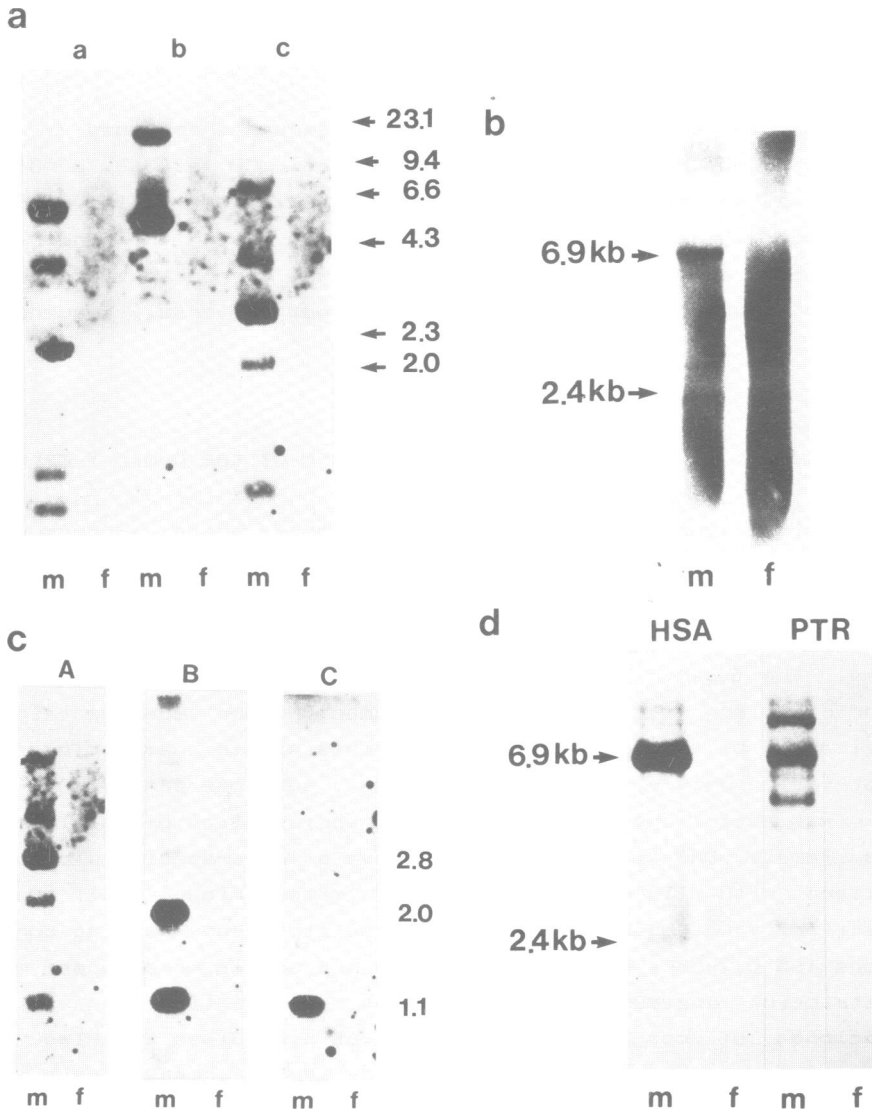


Figure 1a: Southern blot hybridization of male (m) and female (f) genomic DNA samples digested with Pst I (a), Bam HI (b) and Taq I (c). The blot was probed with pJA36B and washed at 65°C (0.1xSSC, 0.1% SDS). 1b: Male (m) and female (f) genomic DNA digested with Hind III and probed with pJA36B at low stringency (65°C, 2xSSC). 1c: Male (m) and female (f) genomic DNA of man (HSA) and chimpanzee (PTR) digested with Hind III and probed with pJA36B at high stringency (65°C, 0.1xSSC). 1d: Male (m) and female (f) genomic DNA digested with Taq I and probed with pJA36B(A), pJA36B2(B) and J16(C).

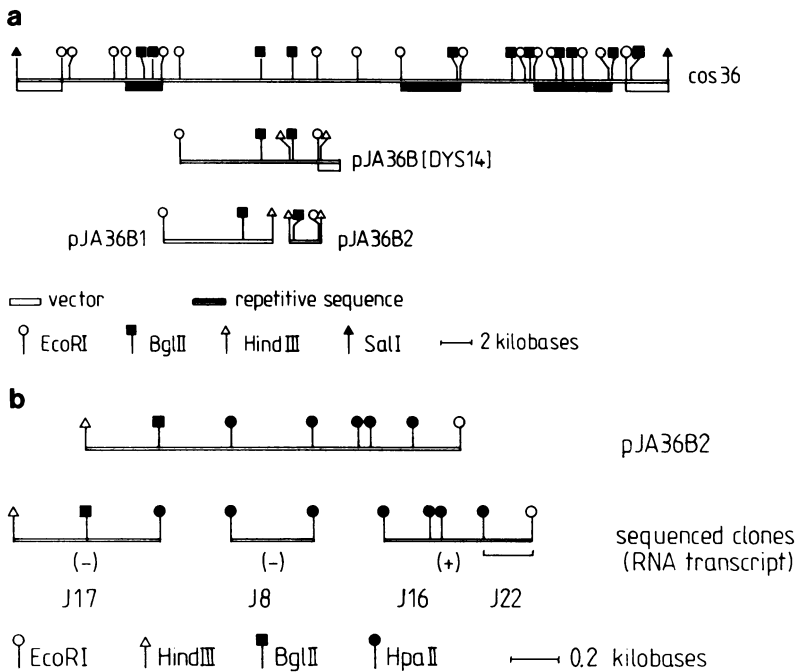


Figure 2a: Restriction map of cosmid clone cos 36 and derived subclones. 2b: M13 Sequencing strategy

Hind III/Eco RI fragment; it hybridizes in a single copy manner to the 2.4 kb genomic Hind III fragment or to two Taq I genomic fragments, 2.0 and 1.1 kb of length, respectively (Figure 1d). Subclone pJA36B consists of a 6.9 kilobase Eco RI/Hind III fragment (subclone pJA36B1), which hybridizes with the properties of a low-copy DNA sequence. It is estimated that there exist at least 10 copies on the human Y chromosome. A 1.6 kb Hind III/Eco RI fragment (subclone pJA36B2) hybridizes in a single copy manner to a 2.4 kb genomic Hind III fragment (Figure 1c,d). The hybridization data show that no more repeated sequences corresponding to subclone pJA36B1 are present on the cosmid cos36. A clustered organization of this repeated element could not be confirmed from the mapping data.

In order to localize this particular sequence chromosomally, DNA samples of different patients with aberrant sex chromosomes were tested for the presence or absence of clone pJA36B1 and pJA36B2.

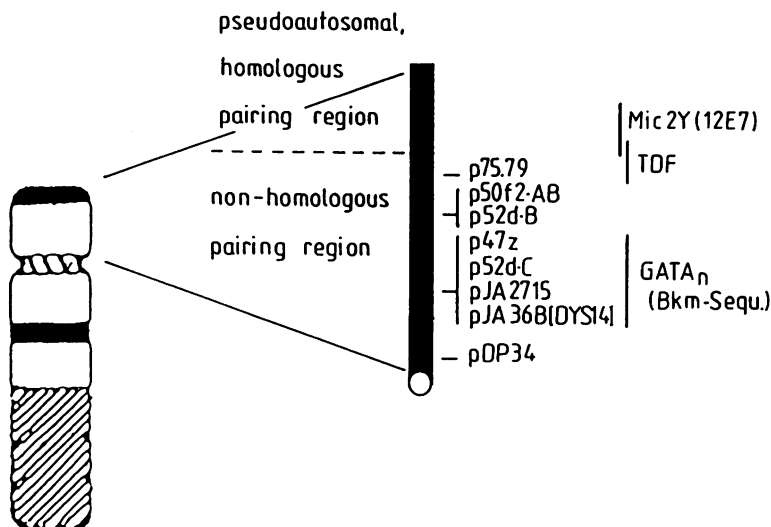


Figure 3: Localization of pJA36B(DYS14) on the short arm of the Y chromosome, based on hybridization data with patient's DNA.

The patients' DNAs were also hybridized with the probes p47z(DXYS5), p50f2, p52d (7, 9, 16), p75.79 (22) and pDP34 (23), which were used as well defined reference probes on the human Y chromosome (12, 24). From the data presented in table 1 a localization of DYS14 in the median region of the short arm Yp is derived (Figure 3). This is confirmed by hybridization in situ with human prometaphase chromosomes (unpublished data, Arnemann et al.). To test for a Y-linked evolutionary conservation of DYS14 Southern blots of DNA from different species, including chimpanzee, bull, rat and mouse were hybridized with pJA36B and washed under stringent (65°C, 0.1xSSC) and moderately stringent conditions (60°C, 1xSSC). A hybridization signal was obtained only in DNA of the chimpanzee. Also in this species the hybridization pattern was male-specific (Figure 1c), but was different from the human pattern. In chimpanzee DNA the number of the repeated elements is reduced and the size of the single copy Hind III fragment is slightly increased (Human: 2.4kb; Chimpanzee: 2.6 kb).

JJA36B is transcribed in human testis tissue

In search for Y chromosomal transcribed DNA sequences Northern

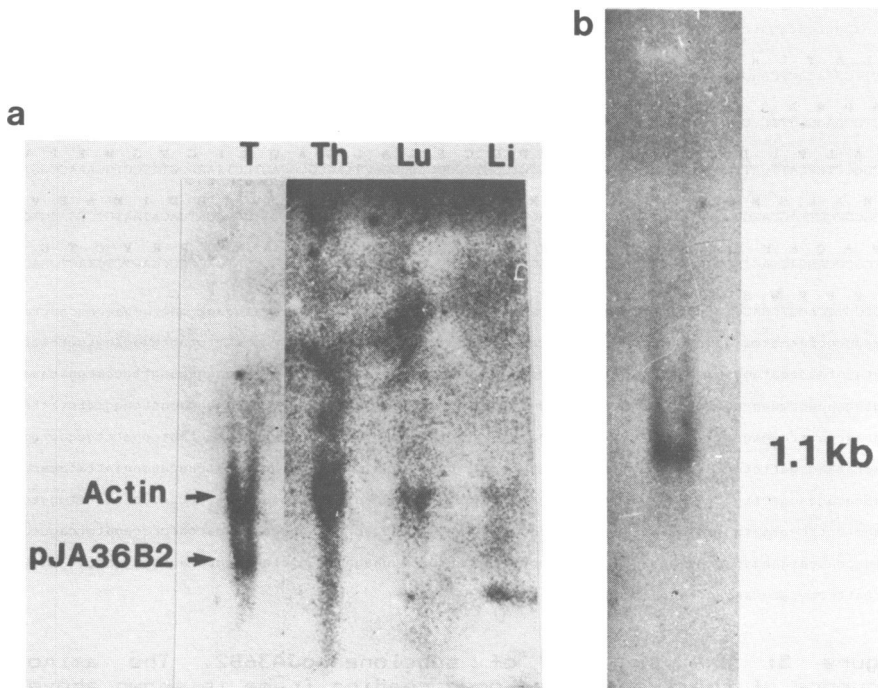


Figure 4a: Northern blot of total RNA from different human tissues probed with pJA36B and actin-cDNA. T= Testis, Th= Thymus, Lu= Lung, Li= Liver.

Figure 4b: Northern blot of total RNA of human testis probed with subclone J16 containing the open reading frame.

blots of poly(A)⁺ RNA of adult human testis tissue were hybridized with 18 different Y chromosome probes derived from the cosmid library. Only pJA36B gave a distinct hybridization signal of approximately 1.1 Kb in length (Figure 4a). To test for tissue-specific expression preliminary Northern blot hybridization experiments with RNA samples of human liver, lung, thymus and testis were done, using the probe pJA36B2. No hybridization signals were observed with liver, lung and thymus RNA but with testis RNA, indicating that either the transcription of the pJA36B sequence is of low abundance in somatic tissues, or restricted to certain tissues or even limited to testis tissue.

Sequence analysis of the transcribed region

The 8.5 kb EcoRI insert of pJA36B was mapped with different restriction enzymes and subcloned into pUC8 (Figure 2). The re-

Dayhoff and EMBL Data libraries revealed no significant homologies with protein and DNA sequences described so far.

Discussion:

It is to be expected that the human Y chromosome will soon be thoroughly mapped on the molecular level. Several groups have established Y-specific DNA sequences and attempted to construct a linear order by deletion mapping the DNA of patients with aberrant Y chromosomes (10-13). The comparison of the karyotype-phenotype relations has led to an assignment of functions to various subregions of this chromosome (1,3). Very little is known, however, about Y-linked expressed genes. Here we describe a DNA sequence, which is Y-specific and transcribed into poly(A)+ RNA in human testis tissue.

Southern blot analysis with probe pJA36B(DYS14) demonstrated Y-specificity not only with different enzymes, but also under nonstringent hybridization and washing conditions(Figure 1). Restriction mapping and Southern blot hybridization identified a low-copy repeat element (subclone pJA36B1) and a single copy element (subclone pJA36B2). The analysis of a male specific evolutionary conservation showed homology with chimpanzee, but not with bull, rat and mouse DNA. In the chimpanzee, the banding pattern was also Y-specific, but the bands corresponding to the repeated element were of weaker intensity and of different mobility (Figure 1c), indicating perhaps rapid evolutionary change. In order to map DYS14 regionally, Southern blots of DNA of patients with different deletions of the Y chromosome were analyzed in combination with p47z(DXYS5), p50f2, p52d, p75.79 and pDP34 (DXYS1), used as regionally allocated reference probes (12,19). From the data in Table 1 a localization in the median region of Yp is postulated. pJA36B gave positive hybridization also in the DNA of 9 of 15 XX-males. Since other observations (11-13, 24) indicate, that around 90% of the XX-males carry DNA sequences detectable with Y-chromosomal probes, these findings suggest that DYS14 is not very closely linked with TDF. In the same DNA linkage group Bkm related sequences are mapped as well (25), but absent from cos36. Bkm (banded krait minor) related DNA is preferentially present on the heterogametic sex chromosome

Patients	cytoget.	Diagnosis	p75.79	p52d-B	p50f2-AB	p47z	pJA2715	pJA36B	p52d-C	pDP34	p50f2-D	p52d-A	p50f2-CE
Male (M)	46,XY		+	+	+	+	+	+	+	+	+	+	+
Female (F)	46,XX		-	-	-	-	-	-	-	-	-	-	-
2341 (M)	46,XX		+	-	-	-	-	-	-	-	-	-	-
275 (M)	46,XisoYq;	(Lit.33)	-	+	+	+	+	+	+	+	+	+	+
1310 (F)	45,X0/46Xmarker		-	-	-	+	+	+	+	+	-	-	-
618 (M)	46,Xt(Xp22.3,Yp)		+	+	+	+	+	+	+	-	-	-	-
912 (F)	46,Xt(Xp22.3,Yq11)		-	-	-	-	-	-	-	-	+	-	-
2305 (F)	46,XisoYq		-	-	-	-	-	-	-	-	+	+	+
71 (M)	46,XYqdel(Yq11.23)		+	+	+	+	+	+	+	+	+	-	-

Table 1: Hybridization data of patients' DNA with various Y-linked probes.

of snakes and mice (26-29) and transcribed in different tissues (28,30). As studies on mice with the mutation "sex-reversed" (sxr) mapped Bkm related DNA sequences in the neighbouring region of TDF (31, 32), those DNA sequences have been connected to sex-determination and/or differentiation. In addition the simple quadruplets repeats GATA and GACA, the main components of Bkm DNA (27,30), were found on several human Y-derived cosmid clones and mapped to the median part of Yp and the proximal part of Yq (25).

Practically nothing is known about the molecular basis of the expression of sequences involved in male sex determination and differentiation. In a search for Y chromosomal transcribed DNA sequences Northern blots of poly(A)+ RNA of adult human testis tissue were hybridized with different Y chromosome derived probes. Only pJA36B2 was able to detect a poly(A)+ RNA transcript. Quite nothing is known about tissue-specific transcription of this sequence. Preliminary Northern blot experiments of total RNA of human thymus, lung and liver failed to hybridized with pJA36B2, indicating that either the transcription in these tissues is below the limit of detection or that the transcription is limited to certain tissues or developmental stages. Sequence analysis of the transcribed subclone pJA36B2 revealed an

open reading frame of 522 bp (Figure 5). The transcription of the open reading frame sequence was confirmed by positive RNA hybridization signals with subclone J16, which carries the open reading frame sequence, and with two synthetic oligonucleotide probes complementary to the sense strand (data not shown). The data also imply that only part of the transcribed sequence is present in clone pJA36B, as initiation codon and polyadenylation signal are missing, while potential splicing sites can be found. Future experiments have to isolate corresponding cDNA clones to complete the nucleotide sequence of the transcript, characterize the molecular structure of the gene and its transcriptional regulation. The deduced amino acid data are presently used to establish antibodies against a fusion protein and synthetic peptides to characterize any translation products and to search for their biological function.

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Literature

1. Bühler, E.M. (1980) *Hum. Genet.*, 55, 145-175.
2. Burgoyne, P.S. (1982) *Hum. Genet.*, 61, 85-90.
3. Davis, R.M. (1981) *J. Med. Genet.*, 18, 161-195.
4. Cooke, H.J., Brown, W.R.A., Rappold, G.A. (1985) *Nature*, 317, 687-692.
5. Simmler, M.C., Rouyer, F., Vergnaud, G., Nystrom-Lahti, M., Ngo, K.Y., de la Chapelle, A., Weissenbach, J. (1985) *Nature*, 317, 692-697.
6. Rouyer, F., Simmler, M.C., Johnsson, C., Vergnaud, G., Cooke, H.J., Weissenbach, J. (1986) *Nature*, 319, 291-295.
7. Bishop, C., Guellaen, G., Geldwerth, D., Fellous, M., Weissenbach, J. (1984) *J. Mol. Biol.*, 173, 403-417.
8. Koenig, M., Moison, J.P., Heilig, R., Mandel, J.L. (1985) *Nucl. Acid Res.*, 13, 5485-5501.
9. Geldwerth, D., Bishop, C., Guellaen, G., Koenig, M., Vergnaud, G., Mandel, J.L., Weissenbach, J. (1985) *EMBO J.*, 4, 1739-1743.
10. Affara, N.A., Florentin, L., Morrison, N., Kwok,

- K., Mitchell, M., Cook, A., Jamieson, D., Glasgow, L., Boyd, E., Ferguson-Smith, M.A. (1986a) *Nucl. Acid Res.*, 14, 5353-5373.
11. Affara, N.A., Ferguson-Smith, M.A., Tolmie, J., Kwok, K., Mitchell, M., Jamieson, D., Cook, A., Florentin, L. (1986b) *Nucl. Acid Res.*, 14, 5375-5387.
 12. Vergnaud, G., Page, D.C., Simmler, M.C., Brown, L., Rouyer, F., Noel, B., Botstein, D., de la Chapelle, A., Weissenbach, J. (1986) *Am. J. Hum. Genet.*, 38, 109-124.
 13. Müller, U., Donlon, T., Schmid, M., Fitch, N., Richer, C.L., Lalande, M., Latt, S.A. (1986) *Nucl. Acid Res.*, 14, 6489-6505.
 14. Maniatis, T., Fritsch, E.F., Sambrook, J. (1982) *Molecular cloning - a laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
 15. Cooper, D.N., Smith, B.A., Cooke, H.J., Niemann, S., Schmidtko, J. (1985) *Hum. Genet.*, 69, 201-205.
 16. Chirgwin, J.M., Przybyla, A.E., McDonald, R.J., Rutter, W.J. (1979) *Biochem.*, 18, 5294-5299.
 17. Rigby, P.W.J., Dieckmann, M., Rhodes, C., Berg, P. (1977) *J. Mol. Biol.*, 113, 237-251.
 18. Sanger, F., Coulson, A.R., Barrel, B.G., Smith, A.J.H., Roe, B.A. (1980) *J. Mol. Biol.*, 143, 161-178.
 19. Markus, M., Trantravali, R., Der, V.G., Miller, B.A., Miller, D.J. (1976) *Nature*, 262, 65-67.
 20. Goodfellow, P.N., Davis, K.E., Ropers, H.H. (1985b) *Cytogenet. Cell Genet.*, 40, 296-352.
 21. Guellaen, G., Casanova, M., Bishop, C., Geldwerth, D., Andre, G., Fellous, M., Weissenbach, J. (1984) *Nature*, 307, 172-173.
 22. Wolfe, J., Erickson, R.P., Rigby, P.W.J., Goodfellow, P.N. (1984) *EMBO J.* 3, 1997-2003
 23. Page, D.C., Harper, M.E., Love, J., Botstein, D. (1984) *Nature*, 311, 119-123.
 24. Goodfellow, P.N., Darling, S., Wolfe, J. (1985a) *J. Med. Genet.*, 22, 329-344.
 25. Arnemann, J., Jakubiczka, S., Schmidtko, J., Schäfer, R., Epplen, J.T. (1986) *Hum. Genet.*, 73, 301-303.
 26. Singh, L., Purdom, I.F., Jones, K.W. (1980) *Chromosoma*, 79, 137-157.
 27. Epplen, J.T., McCarrey, J.R., Sutou, S., Ohno, S. (1982) *Proc. Natl. Acad. Sci. USA*, 79, 3798-3802.
 28. Schäfer, R., Böltz, E., Becker, A., Bartels, F., Epplen, J.T. (1986a) *Chromosoma*, 93, 496-501.
 29. Schäfer, R., Ali, S., Epplen, J.T. (1986b) *Chromosoma*, 93, 502-510.
 30. Epplen, J.T., Cellini, A., Romero, S., Ohno, S. (1983) *J. Exp. Zool.*, 228, 305-312.
 31. Singh, L., Jones, K.W. (1982) *Cell*, 28, 205-216.
 32. Singh, L., Phillips, C., Jones, K.W. (1984) *Cell*, 36, 111-120.
 33. Schmidtko, J., Arnemann, J., Schmid, M., Baum, F., Mayerova, A., Langenbeck, U., Hansmann, I. (1985) *Hum. Genet.*, 69, 135-137