A long range restriction map of the distal human X chromosome short arm around the steroid sulfatase locus

Xiao-Miao Li, Pauline Yen, T.Mohandas and Larry J.Shapiro

Howard Hughes Medical Institute Laboratories, Departments of Pediatrics and Biological Chemistry, UCLA School of Medicine and the Division of Medical Genetics, Harbor/UCLA Medical Center, Torrance, CA 90509, USA

Received October 16, 1989; Revised and Accepted March 23, 1990

ABSTRACT

The distal short arm of the human X chromosome is of interest because it contains genes which escape X chromosome inactivation and because it is subject to frequent deletions in human patients. The steroid sulfatase gene has been particularly well studied as an example of a gene which escapes X inactivation and which is included in a number of these deletion events. For these reasons a physical map of the region around the STS gene would be of interest. We have constructed a rare cutting enzyme map of this area and have determined the position of several nearby markers with respect to STS. We have also oriented the 5' and 3' ends of the STS gene on this map and have determined the centromeric and telomeric portions of the region. Finally, we have shown that this map can be used to locate deletion breakpoints in STS deficient patients.

INTRODUCTION

The human X chromosome comprises 5% of the human haploid genome or approximately 150 megabases of DNA. The X is the most extensively mapped and studied portion of the human chromosome complement due to the large number of inherited conditions resulting from mutations of X-linked genes and due to the ease of linkage mapping and somatic cell genetic manipulations of the X. The majority of genes on the X chromosome are regulated by X inactivation in female somatic cells, however, several loci on the distal X short arm have been identified which escape this process. Presumably those genes, such as *MIC2*, which are located in the pseudoautosomal portion of the X and thus have identical homologues on the Y, do not require X inactivation to produce dosage compensation between males and females (1). However, several other loci unique to the X such as the steroid sulfatase gene (*STS*) also escape X inactivation, perhaps because of their evolutionarily recent derivation from a pseudoautosomal organization (2).

In the mouse, functional copies of *STS* are located on both the X and Y chromosomes and are capable of undergoing meiotic recombination during spermatogenesis. The X encoded murine gene escapes inactivation (3,4). In humans, there is no functional *STS* locus on the Y chromosome but there is a nonprocessed pseudogene on the long arm of the Y (5,6). This may reflect

the occurrence of a pericentric inversion of the Y prior to the divergence of New World monkeys and higher primates from prosimians. Steroid sulfatase deficiency is a common human inborn error of metabolism. It affects one in every 4,000–5,000 males in many social and ethnic groups spread over a diversity of geographic locations (2). Recent work suggests that about 90% of such individuals have sustained deletions of at least the entire 146 kb *STS* gene (7–10). Most of these patients have a skin condition known as ichthyosis. A few subjects have more complex phenotypes which appear to be due to more extensive deletions of this region, some of which are associated with visible cytogenetic abnormalities. Based on these observations, it is likely that de novo deletion events are more common in this region of the genome. This may be true for other regions of the X chromosome as well, such as the dystrophin locus which is often partially deleted in patients with Duchenne muscular dystrophy (11). In an effort to elucidate the basis for these deletions, the characterization of their breakpoint may be helpful. We wish to determine whether the deletions are of a uniform size or have breakpoints clustered in a given region and the nature of the sequences at newly created junctions. To date we have cloned and characterized one such deletion junction (10). Further work will require mapping of deletion breakpoints at some distance from the *STS* locus. To this end we have constructed a rare cutting enzyme restriction map of this region using pulsed field gel electrophoresis (12,13) which should assist the study of several aspects of the genetics of the X short arm.

MATERIALS AND METHODS

Cell line and DNA probes

Cell line: IMR 90 is a fibroblast cell line derived from normal female fetal lung tissue. The long range restriction map was generated with rare cutting enzymes. Most of these enzymes are methylation sensitive, and the methylation state of genomic DNA varies from individual to individual and from tissue to tissue. To avoid such variables, IMR 90 was the principal DNA source used in generating the maps presented here, although many of the restriction sites identified were confirmed in other cell lines. High molecular weight DNA from patients with typical clinical features of X-linked ichthyosis and *STS* gene deletions was also prepared. All of these subjects also lacked sequences which hybridized with GMGX9. Cell lines CF85, CF94, CF108, and

CF126 are mouse-human hybrid cell lines in which the relevant human X chromosome as well as a few autosomes have been retained on a murine background. Their derivation has been described previously (14). CF85 and CF126 contain an X chromosome with a terminal deletion including STS, MIC2 and other pseudoautosomal markers. The X in CF108 is derived from a patient with an X/Y translocation previously shown to have an X chromosome breakpoint distal to STS but proximal to MIC2. The X/Y translocation in CF 94 has an breakpoint proximal to STS.

Probes: pSTS331 contains the entire human STS cDNA (14). The STS 3' probe is a 1 kb genomic fragment from the 3' flanking region of the STS gene (5). MIC2 is a cell surface antigen gene located in the pseudoautosomal region of the X and Y chromosomes (15). A genomic clone of MIC2, p19B, was used as the probe. 38j (DXS283)(16), GMGX9 (DXS237)(17), dic 56 (DXS143)(18) and 18-55 (DXS70)(19) are X-specific anonymous markers. S232 (20) (DXS278) is a probe which detects a highly polymorphic locus on Xp and a monomorphic locus on Yq. The genetic linkage of these probes is shown in Figure 1. S232 actually hybridizes to a series of restriction fragments spread over more than 1 megabase. As a part of a study of the organization of this repeating element, we have isolated cosmids containing S232 cross hybridizing sequences (21). From two of these, single copy probes, CS21I and CS17C have been obtained.

Preparation of DNA in agarose plugs

Agarose plugs containing DNA were prepared by a modification of a protocol of Gardiner et al. (22). Briefly, fibroblasts at a concentration of 3×10^7 /ml in $1 \times$ PBS (phosphate buffered saline) were mixed 1:2 v/v with 1% InCert agarose (FMC) in 0.125 M EDTA pH 8.0 at 45°C. The mixture was pipetted into a 1 ml glass pipet, 1/8 inch in diameter. After the entire pipet was chilled to 4°C, the contents were removed from the back of the pipet into a petri dish and cut into 6 mm plugs. The plugs were added to 2.5 volumes of ESP buffer containing 1% N-lauroylsarcosine (Sigma), 0.5 M EDTA, pH 8.0 and 1 mg/ml proteinase K (Sigma). After 24 hours incubation at 50°C, the plugs were washed extensively with excess TE (10 mM Tris-HCl 1 mM EDTA pH 8.0) 4 times at room temperature (about 1 hour per wash) and stored at 4°C.

Molecular weight markers were λ concatamers and yeast *S. cerevisiae* strain AB972 chromosomes, prepared by the method of Schwartz and Cantor (12) and Carle and Olson (13). Yeast *S. pombe* strain 972H⁻ DNA was prepared by the method of Smith et al. (23).

Restriction enzyme digestion

Prior to digestion, plugs were equilibrated with restriction enzyme buffer suggested by the manufacturer (New England Bio Labs). The digestions were routinely carried out for 5–6 hours in 100 μ l containing 30–40 units of enzyme. In the digestions with NaCl concentration above 20 mM, 4 mM spermidine was added. The reactions were stopped by adding 100 μ l of 0.5 M EDTA. For double digestions, the first digestion buffer was removed. 100 μ l ESP buffer was added to the plugs and incubation was carried out at 50°C over night. Proteinase K was inactivated by 2 mM PMSF (phenylmethylsulfonyl fluoride) in TE for 30 minutes at room temperature. The plugs were washed extensively with two changes of TE, and the second digestion was carried out as described above. After digestion, the plugs were loaded directly onto a 1% agarose gel (BRL).

Figure 1. Genetic linkage map of the distal short arm of the human X chromosome. The closed bar at the left end represents the pseudoautosomal region. The genetic distances (in centimorgans) between the markers were determined by A: Yates et al (29) and B: Knowlton et al (20).

PFGE, blotting and hybridization

A Beckman GeneLine Transverse Alternating Field Electrophoresis System was used for pulsed field gel electrophoresis. DNA fragments were routinely separated on a 1% agarose gel at 20°C for 24 hours at 170 mA with a pulse frequency of 60 seconds. To separate DNA fragments over 1 mb long, the gel was run at 44 V with a pulse frequency of 75 seconds for 12 days at 18°C. After electrophoresis, the gel was stained with ethidium bromide and exposed to short wave length UV light for 1–5 minutes to break DNA into smaller fragments suitable for transfer. DNA was then blotted onto Bio-Rad zeta-probe nylon membrane by alkaline transfer (Bio-Rad Bulletin 1233). The hybridization conditions were as described previously except (14) that 1% SDS (instead of 0.1%) was used in the hybridization and less stringent washing condition ($2 \times$ SSC, 0.1% SDS at 65°C) was applied to probes 38j, dic 56, 18-55 and the STS 3' probe. The filters were air dried and exposed to Kodak XAR-5 film at -70°C with double DuPont intensifying screens. The filters were reprobbed after the signal was stripped by washing with boiling $0.1 \times$ SSC, 0.1% SDS twice, one minute each. Filters were stored in $2 \times$ SSC at 4°C.

RESULTS

A restriction map of the region around the STS locus

DNA was digested with Not I, Mlu I, Nru I, Sfi I, Nae I, Sal I, and Sac II singly and in various combinations. Additional partial digestions were carried out. Following electrophoresis and transfer, filters were hybridized with CS17C STS cDNA, STS 3' genomic, GMGX9, and CS21I probes as shown in Fig. 2. Fragment lengths were assigned as indicated in Table I, the map in Fig. 2 was derived from these data and results of digestions with two enzymes. Of note is the establishment of the orientation of the 5' and 3' ends of the STS gene with regard to the restriction map and the X chromosome centromere and telomere. Although the STS cDNA probe and the STS 3' probe hybridize with the same 890 kb Nae I fragment, in Mlu I digests the STS cDNA probe recognizes bands of 1400 and 1300 kb while the 3' flanking genomic probe which is about 6 kb 3' of STS gene hybridizes with a new band of 990 kb. The separation of the STS gene and the 3' flanking sequence by a Mlu I site was confirmed by double digestions with Mlu I and Nae I which showed that the 3' probe and the cDNA probe hybridized with a 620 kb fragment and a 280 kb fragment, respectively. Thus the Mlu I site identified by the * in Fig. 2 must be at the very 3' end of the STS gene. Additional experiments have shown that this Mlu I site (*) is either polymorphic or subject to variable methylation such that its presence cannot always be established. However, when detected it orients the 5' and 3' ends of the STS gene with respect to this map. In addition, we have recently obtained yeast artificial chromosome (YAC) clones spanning the entire STS gene as well

Figure 2. Long range restriction map around the STS locus. IMR90 DNA was digested with various restriction enzymes and the fragments separated by PFGE and blotted with various probes around the STS locus. The same blot was used in panels A–E, with the probe stripped between each hybridization. *S. cerevisiae* chromosomes were used as the size standard as indicated at the left side of panel A. The *Mlu* I site marked with a * at the middle of the map is positioned very close to the 3' end of the STS gene and its presence orients the direction of transcription of the STS gene. The deletion breakpoint of the X chromosome in the hybrid cell lines CF85 and CF126, as indicated, determines the telomere-centromere orientation of the map.

Table 1. Approximate Sizes of Restriction Fragments Detected with Xp probes

Probes	Restriction Enzymes						
	Not I	Mlu I	Sfi I	Nru I	Nae I	Sal I	Sac II
Mic 2	1400	1400	250	N	100	450	200
38J	1380	1500	400	1280	350	380	N
CS17C	N	1400 1300(w)	750	700	400 200 100	600 400	N
STS cDNA	1700	1400 1300(w)	890(w) 800	1450 950(w) 870(w)	890	700 300	890
STS 3'	1700	990	890(w) 800	N	890	700	N
GMGX9	1700	990	890(w) 800	N	N	15,25 100 300	N
CS21I	1700	990	460	1280	N	N	N
Dic56	1300	1500	200	870	N	470 400 100	N
18-55	500	1300	280	1200	N	370 280	N

The restriction digestions were carried out on IMR90 DNA. *S. cerevisiae* strain AB972 chromosomes were used as size standards; lengths were determined by comparison to yeast chromosomes provided by Beckman Instruments, Inc. Weak hybridizing fragments are indicated by (w). When the digestions were not done, 'N' is indicated.

Figure 3. Partial digestion and PFGE link markers on the distal short arm of the human X chromosome. IMR90 DNA was partially digested with Not I or Mlu I and blotted with Xp probes. Yeast *S. pombe* chromosomes were used as size standard. The same filter was used for the various probes so the hybridizing fragments can be compared. Arrows between the blots point to the common fragments that hybridize with different probes.

as 3' and 5' flanking sequences. The Not I site located distal (telomeric) to the STS gene is contained within one of these YAC clones and is situated close to the 5' end of the gene as shown in Figure 2 (unpublished). These data establish with reasonable certainty the orientation of the STS gene with respect to the centromere of the X chromosome.

GMGX9 must be physically close to STS as it is deleted in most but not all STS deficient subjects (9,17). However, the orientation and distance between the two loci was not established. By hybridization of GMGX9 to the same filters as shown in Figure 2, panels B & C, the GMGX9 locus was mapped to a position about 500 kb 3' to STS. GMGX9 hybridizes with the same Sfi I and Mlu I bands as does the STS 3' probe, but recognizes different Sal I bands.

S232 is a probe which recognizes a highly polymorphic set of restriction fragments on the X chromosome as well as a monomorphic series of DNA fragments on the Y chromosome (20). The 7 kb insert of this clone appears to recognize a series of sequences that are repeated at least four times in the Xp22.3 region. Each of these S232 elements is extensively polymorphic such that essentially every female subject studied is heterozygous for alleles characterized by variable length restriction fragments with several enzymes. We have isolated human cosmids containing S232-like sequences from the X chromosome and characterized them (21). CS17C and CS21I, two single copy sequences adjacent to these S232 like elements, have been isolated and their organization on the X chromosome has been studied by hybridizing these sequences to conventional Southern blots of genomic DNA from hybrid cell lines containing X chromosomes with a variety of terminal deletions. CF108 has a breakpoint distal to STS but proximal to 38j and it contains CS17C, STS, GMGX9, CS21I and dic 56 sequences. CF94 has a breakpoint proximal to STS and it lacks CS17C, STS, GMGX9,

CS21I sequences but hybridizes with dic 56. CF85 and CF126 have breakpoints between GMGX9 and CS21I since these cell lines are positive for 21I and dic56 sequences but negative for GMGX9, STS, CS17C and 38j. Thus CS21I must be proximal (centromeric) to STS and distal to dic 56. Linkage analysis supports the notion that some polymorphic loci detected by S232 are proximal to STS (20). However S232 like sequences are probably spread over a sizeable distance as occasional recombinants within a polymorphic S232 haplotype are observed. Studies of CS17C and adjacent sequences in STS⁻ patients showed that CS17C maps distal to STS (21).

When CS21I was hybridized to the same PFGE blots as in Fig.2 panels B, C, and D, different sized fragments were detected for all enzymes as compared with STS cDNA, STS 3' probe and GMGX9 except for Mlu I (Fig.2E). With this digestion, CS21I gives a strong signal for the same bands as does the STS 3' probe and GMGX9 suggesting that it is located 3' to most of the restriction sites but within the same Mlu I fragment. This established the orientation of STS gene, with its 3' end toward the centromere. CS17C hybridized to the same Mlu I fragments as the STS cDNA probe, suggesting that CS17C is within 1.3 mb of the STS gene (Fig. 2, panel A).

Mapping by partial digestion

Not I and Mlu I give the largest fragments around the STS locus. By partial digestion, we obtained even larger bands which might be useful in locating markers even farther from STS. However with these very large fragments, care was taken to compare intensity and band shape as well as absolute position before indicating that two probes were likely to hybridize to the same partial digestion fragment.

38j is a probe which detects a locus telomeric to STS near, but not within the pseudoautosomal region (16) while MIC2 is

Figure 4. Detection of the breakpoints in STS deletion patients. DNAs isolated from six STS deletion patients (G,DB,RF,A,W,V), a normal male (M) and a normal female (F) were digested with Sfi I, the fragments separated with PFGE and hybridized with CS17C (panel A) or CS21I (panel B). In normal individuals, CS17C detects a 750 kb (band a) X fragment and a 380 kb (band c) Y fragment, while CS21I detects a 460 kb (band d) X fragment. All the patients gave altered X fragments.

a pseudoautosomal locus. Hybridization of MIC2, 38j and CS17C to the same blot containing genomic DNA partially digested with Not I showed that all these three probes hybridized with a 5 mb fragment and 38 j and CS17C share a 3.5 mb fragment in addition (Figure 3A). It thus appears that MIC2 and 38j are within 5 mb and 3.5 mb of CS17C, respectively. In a similar manner, the hybridization of various probes to these same filters was undertaken. As shown in Figure 3B and 3C, STS cDNAs and dic 56 appear to hybridize with the same 2.8 mb Not I fragment and the same 3 mb Mlu fragment, and dic 56 and 18-55 share the same 5 mb fragment. A maximum distance physical map is therefore generated which link 6 markers over 14 mb on the distal short arm of the human X chromosome.

Studies of STS deficient patients

Since one of the goals of this work is to establish the physical location of breakpoints in STS deficient patients, we have undertaken a preliminary evaluation of six patients using probes CS17C and CS21I. All these patients have deleted the STS and GMGX9 loci. DNA from the patients was digested with Sfi I and blotted with CS17C and CS21I. As shown in Figure 4A, CS17C detects a 750 kb fragment (band a) on the X chromosome and a 380 kb fragment (band c) on the Y chromosome in a normal male while in a normal female, only the 750 kb fragment is present. All the STS deficient patients give an altered X fragment of 480 kb (band b) in addition to the Y fragment. When the same blot was hybridized with CS21I, the same 480 kb fragment was detected in the patients while the normal controls gave a 460 kb fragment (band d in Figure 4B). These results suggest that the deletion breakpoints in these patients occur somewhere within the 480 kb Sfi fragment recognized by these two probes. These observations should permit the evaluation of breakpoint heterogeneity and location in additional patients.

DISCUSSION

In this communication, we describe and present a rare-cutting enzyme restriction map of the distal X chromosome short arm

around the steroid sulfatase locus. The purpose of this study was to provide the physical landmarks required to further characterize this genetically interesting region. Location of additional genes which escape X inactivation and the identification of breakpoints in chromosome rearrangements, particularly X/Y translocations should be facilitated by this data. The organization of the interesting S232 polymorphic complex should be amenable to elucidation given this map. Finally, it should be possible to identify the breakpoints in the many patients who have STS deletions.

At present, there are five expressed genes on the human X chromosome which have been shown to escape X inactivation. These include *MIC2*(1), *Xg^a*(2), *STS*, *STS* (2), *ZFX* (24) and *AIS9T* (25,26). All are situated on the X short arm. *ZFX* is considerably more proximal than *MIC2*, *Xg^a* or *STS*, while *AIS9T* is mapped to proximal Xp (27,28). The presence of clusters of rare cutting enzyme sites may indicate the presence of CpG islands associated with expressed sequences and thus provide landmarks for identifying other genes. While it has been shown that *STS* and *MIC2* escape X inactivation in a position independent manner even when relocated by chromosome rearrangements, it will be of interest to see whether escape from inactivation is a general property of genes in this region.

We have already been able to make use of this map and several single copy sequences isolated from the S232 cross hybridizing regions to locate the breakpoints of at least one X/Y translocation chromosome and to obtain information on the location of several STS deletion boundaries. Of STS deficient patients studied to date, 10% have either point mutations or small deletions or insertions. Two individuals with partial deletions of 40 kb (10) and 100 kb (9, Li et al, unpublished) are known to us. The remainder of subjects lack at least the entire 146 kb STS gene. Data from several groups show that while some STS deficient subjects retain GMGX9 sequences, most are deleted for this locus as well. From our mapping data, this would indicate that the centromeric extent of these deletions is at least 500 kb 3' of the STS gene. On the telomeric side of STS, all patients with interstitial deletions are positive for 38j sequences but are negative for sequences which hybridize with a clone approximately 100 kb obtained by chromosome jumping from the 5' end of STS (Yen et al, unpublished). Thus the deletions extend at least 100 kb toward the telomere from STS and are usually at least 750 kb in size. Quite recently we have utilized S232 related probes to locate the 3' boundaries of these deletions more precisely and find that many of the patients' breakpoints cluster 900–1000 kb proximal to the 3' end of the STS gene. It is likely that further such mapping studies will enable us to determine the extent of heterogeneity in deletion breakpoints and to facilitate their cloning and sequencing, as we have done for one of the intragenic partial deletions (10) so that some understanding of the mechanism which produces these very frequent deletions may be obtained.

ACKNOWLEDGEMENTS

The authors thank Jean Weissenback for donating 38j, Malcolm Ferguson-Smith for GMGX9, Lou Kunkel for pdic56 and 18-55, and Peter Goodfellow for MIC2. S232 was obtained from Collaborative Research. Nelva J. Hitt assisted in the preparation of the manuscript and Siao Ping Tsai and Hsiao-Fen Huang provided skillful technical assistance. This work was supported in part by grants from the NIH HD12178 and HD19445 and the March of Dimes (1-639) and by the Howard Hughes Medical Institute.

REFERENCES

1. Goodfellow, P., Pym, B., Mohandas, T., and Shapiro, L.J. (1984) *Am. J. Hum. Genet.* 36, 777–782.
2. Shapiro, L.J. (1985) In: *Advances in Human Genetics*, H. Harris and K. Hirschhorn, (Eds.), Plenum Publishing Co., 331–381.
3. Keitges, E., Rivest, M., Siniscalco, M., and Gartler, S.M. (1985) *Nature* 315, 226–227.
4. Keitges, E.A., Schorderet, D.F., and Gartler, S.M. (1985) *Genetics* 116, 465–469.
5. Yen, P.H., Marsh, B., Allen, E., Tsai, S.P., Ellison, J., Connolly, L., Neiswanger, K., and Shapiro, L.J. (1988) *Cell* 55, 1123–1135.
6. Fraser, N., Ballabio, A., Zollo, M., Persico, G., and Craig, I. (1987) *Development* 101, 127–132.
7. Conary, J.T., Lorkowski, G., Schmidt, B., Pohlmann, R., Nagel, G., Meyer, H.E., Krentler, C., Cully, J., Hasilik, A., and von Figura, K. (1987) *Biochem. Biophys. Res. Commun.* 144, 1010–1017.
8. Bonifas, J.M., Morley, B.J., Oakey, R.D., Kan, Y.W., and Epstein, Jr., E.J. (1987) *Proc. Natl. Acad. Sci. USA* 84, 9248–9251.
9. Ballabio, A., Carozzo, R., Parenti, G., Gil, A., Zollo, M., Persico, M.G., Gillard, E., Affara, N., Yates, J., Ferguson-Smith, M.A., Frants, R.R., Eriksson, A.W., and Andria, G. (1989) *Genomics* 4, 36–40.
10. Shapiro, L.J., Yen, P., Pomerantz, D., Martin, E., Rolewic, L. and Mohandas, T. (1989) *Proc. Natl. Acad. Sci. USA* 86, 8477–8481.
11. Lindlof, M., Kiura, A., Kaariainen, H., Kalimo, H., Lang, H., Pihko, H., Rapola, J., Somer, H., Somer, M., Savontaus, M.-L., and de la Chapelle, A. (1989) *Am. J. Hum. Genet.* 44, 496–503.
12. Schwartz, D.C. and Cantor, C.R. (1984) *Cell* 37, 67–75.
13. Carle, G.R., Frank, M. and Olson, M.V. (1986) *Science* 232, 65–68.
14. Yen, P.H., Allen, E., Marsh, B., Mohandas, T., Wang, N., Taggart, R.T. and Shapiro, L.J. (1987) *Cell* 49, 443–454.
15. Goodfellow, P.J., Darling, S.M., Thomas, N.S., and Goodfellow, P.N. (1986) *Science* 234, 740–743.
16. Levilliers, J., Quack, B., Weissenbach, J., and Petit, C. (1989) *Proc. Natl. Acad. Sci. USA* 86, 2296–2300.
17. Gillard, E.F., Affara, N.A., Yates, J.R.W., Goudie, D.R., Lambert, J., Aitken, D.A., and Ferguson-Smith, M.A. (1987) *Nucleic Acids Res.* 15, 3977–3985.
18. Middlesworth, W., Bertelson, C., Kunkel, L.M. (1985) *Nucleic Acids Res.* 13, 5723.
19. Kunkel, L.M., Lalande, M., Monaco, H.D., Flint, A., Middlesworth, N. and Latt, S.A. (1985) *Gene* 33, 251–258.
20. Knowlton, R.G., Nelson, C.A., Brown, V.A., Page, D.C., and Donis-Keller, H. (1989) *Nucleic Acids Res.* 17, 423–436.
21. Yen, P.H., Li, X.-M., Tsai, S.-P., Johnson, C., Mohandas, T. and Shapiro, L.J. (1990) *Cell* (in press).
22. Gardiner, K., Laas, W., and Patterson, D. (1986) *Som. Cell Mol. Genet.* 12, 185–195.
23. Smith, C.L., Malsumoto, T., Niwa, O., Klco, S., Fan, J.-B., Yanagida, M. and Cantor, C.R. (1987) *Nucleic Acids Res.* 15, 4481–4489.
24. Schneider-Gadicke, A., Beer-Romero, P., Brown, L.G., Nussbam, R. and Page, D.C. (1989) *Cell* 57, 1247–1258.
25. Brown, C.J. Poveis, V.E., Munroe, D.L., Sheinin, R., and Willard, H.F. (1989) *Som. Cell & Molec. Genet.* 15, 173–178.
26. Brown, C.J. and Willard, H.F. (1989) *Am. J. Hum. Genet.* 45, 592–598.
27. Zacksenhaus, E., Sheinin, R. and Wang, H. (1990) *Cytogenet. Cell Genet.* (in press).
28. Brown, C.J. and Willard, H.F. (1990) *Am. J. Hum. Genet.* 46, 273–279.
29. Yates, J.R.W., Goudie, D.R., Gillard, E.F., Aitken, D.A., Affara, N.A., Clayton, J.F., Tippet, P.A. and Ferguson-Smith, M.A. (1987) *Genomics* 1, 52–59.