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Isolation of DNA Markers from a Region between Incontinentia Pigmenti 1 (IP1) X-Chromosomal Translocation Breakpoints by a Comparative PCR Analysis of a Radiation Hybrid Subclone Mapping Panel

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A strategy based on the use of human-specific interspersed repetitive sequence (IRS)–PCR amplification was used to isolate regional DNA markers in the vicinity of the incontinentia pigmenti 1 (IP1) locus. A radiation hybrid (RH) resulting from a fusion of an irradiated X-only somatic cell hybrid (C12D) and a thymidine kinase deficient (TK−) hamster cell line (a23) was identified as containing multiple X chromosome fragments, including DNA markers spanning IP1 X-chromosomal translocation breakpoints within region Xp11.21. From this RH, a panel of subclones was constructed and analyzed by IRS–PCR amplification to (a) identify subclones containing a reduced number of X chromosome fragments spanning the IP1 breakpoints and (b) construct a mapping panel to assist in identifying regional DNA markers in the vicinity of the IP1 locus. By using this strategy, we have isolated three different IRS–PCR amplification products that map to a region between IP1 X chromosome translocation breakpoints. A total of nine DNA sequences have now been mapped to this region; using these DNA markers for PFGE analyses, we obtained a probe order DXS14–DXS422–MTHFDL1–DXS705. These DNA markers provide a starting point for identifying overlapping genomic sequences spanning the IP1 translocation breakpoints; the availability of IP1 translocation breakpoints should assist the molecular analysis of this locus. © 1992 Academic Press, Inc.

INTRODUCTION

The isolation of DNA markers from a targeted chromosomal region has been a required prerequisite for the positional cloning of human genes and the construction of complete regional maps. In addition, the construction and characterization of somatic cell hybrids containing defined human chromosomal fragments has been instrumental in the development of high-resolution physical maps. As part of an effort to clone the incontinentia pigmenti 1 (IP1) locus, we sought a strategy to increase the efficiency of isolating region-specific DNA markers from Xp11.21. The IP1 locus has been mapped to region Xp11.21 by cytologic (Gilgenkrantz *et al.*, 1985; Hodgson *et al.*, 1985; Kajii *et al.*, 1985; Cannizzaro and Hecht, 1987; Crolla *et al.*, 1989) and molecular (Gorski *et al.*, 1991) analyses.

A three-step approach was taken to isolate additional regional DNA markers in the vicinity of the IP1 locus. First, radiation hybrids (RHs) that contained DNA markers spanning IP1 X-chromosomal translocation breakpoints were identified. RHs have been shown to be an effective means of nonselectively retaining small defined human chromosome fragments (Benham *et al.*, 1989; Cox *et al.*, 1989; Zoghbi *et al.*, 1991). Second, to further enrich for DNA markers in the vicinity of the IP1 locus and construct a RH mapping panel, a single RH containing markers spanning the IP1 breakpoints was used to isolate RH subclones. We hypothesized that, for a RH containing multiple human chromosomal fragments, fragments integrated within different hamster chromosomes would segregate independently during passage. By inference this suggested that, by isolating and characterizing a number of RH subclones, it would be possible to (a) identify "enriched" subclones that nonselectively retained DNA markers in the vicinity of the IP1 locus while losing other human fragments present in the parental RH, (b) determine whether DNA markers consistently cosegregated as expected for markers present on a single integrated fragment, and (c) use the RH subclones to construct a regional mapping panel.

Third, RH subclones were analyzed by using interspersed repetitive sequence (IRS)–PCR amplification;

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by using primers directed against human repetitive sequences, it is possible to selectively amplify human-specific DNA sequences (Nelson *et al.*, 1989; Ledbetter *et al.*, 1990). Here, IRS–PCR amplification was used to "fingerprint" RH subclones, estimate the complexity of the human DNA contained within each hybrid, and generate new DNA markers. To facilitate the latter task, a comparative analysis was performed to identify and isolate IRS–PCR products restricted to RH subclones containing DNA markers spanning IP1 breakpoints. IRS–PCR products were mapped to specific intervals within Xp11.21 by using an IP1 somatic cell hybrid mapping panel (Gorski *et al.*, 1991) and were used as probes to extend the physical map of the IP1 locus.

MATERIALS AND METHODS

Somatic cell hybrid and radiation hybrid cell lines. C9-5, B13-3, and C17-1D3 are human–hamster hybrid cell lines and CLX17-A12 is a human–mouse hybrid cell line constructed from Epstein–Barr virus-transformed lymphoblastoid cell lines derived from three unrelated female patients with nonfamilial IP. Hybrids C9-5 and B13-3 contain the translocated X chromosome t(X;9)(p11.21;q34.3) (Gilgenkrantz *et al.*, 1985) and t(X;13)(p11.21;q12.3) (Kajii *et al.*, 1985), respectively; hybrid C17-1D3 contains the derivative X chromosome, and hybrid CLX17-A12 contains the derivative 17 chromosome t(X;17)(p11;p11) (Hodgson *et al.*, 1985; Crolla *et al.*, 1989). The construction and characterization of these hybrids has been described (Gorski *et al.*, 1989, 1991). Hybrids GM06853 and GM06318 contain only a structurally intact human X chromosome (Coriell Medical Institute, Camden, NJ). Hybrid A63-1A contains the translocation chromosome t(X;20)(20pter–q11.1::Xq11.1–xqter); hybrid A48-1Fa contains the translocation chromosome t(X;11)(11pter–11cen::Xcen–Xqter) (Mahtani and Willard, 1988); and hybrid GM10501 contains the translocation chromosome t(X;17)(17qter–17q11.2::Xp11.21–Xqter) (Lafreniere *et al.*, 1991) (Coriell Medical Institute).

A procedure to produce radiation hybrids has been described in detail (Benham *et al.*, 1989). Briefly, RHs containing portions of a human X chromosome were constructed by irradiating a human–hamster hybrid cell line containing a single structurally intact human X chromosome, C12D (Goss and Harris, 1975), with 50,000 rads and performing a polyethylene glycol-mediated fusion with thymidine kinase deficient (TK−) hamster cell line a23 (Westerveld *et al.*, 1971). RHs were screened by hybridization analysis using 32 randomly distributed DNA markers that spanned the length of the X chromosome including markers mapped immediately proximal (DXZ1, Xcen) and distal (DXS146, Xp11.22) to and between (DXS14, Xp11.21) IP1 X-chromosomal translocation breakpoints (Gorski *et al.*, 1991, 1992). RH clone 128 (RH 128) was one of 4 RHs identified which contained these 3 loci and less than 3 other X-chromosomal loci. RH 128 alone was found to survive prolonged passage. RH 128 subclones were obtained by dilutional subcloning; each clone was isolated from a separate plate.

PCR amplification and analysis. Three different primers directed against *Alu* (Nelson *et al.*, 1989) or L1Hs (Ledbetter *et al.*, 1990) human-specific consensus sequences were modified to contain a 5′ *Not*I restriction endonuclease recognition sequence; these included the 5′ *Alu* consensus sequence A-517N, AAGTGCGGCCGCGATCTCGGCTCACTGCAA; the 3′ *Alu* consensus sequence A-TC65N, AAGTCGCGGCCGCTTGCAGTGAGCCGAGAT; and the L1Hs consensus sequence L1HsN, AAGTCGCGGCCGCATGGCACATGTATACATATGTAACT/AAACC. PCR was performed in a 100-μl volume containing 1 μg of hybrid DNA, 50 m*M* KCl, 10 m*M* Tris–HCl, 1.6 m*M* $MgCl_2$, 0.01% gelatin, 250 μM each dNTPs, and either 0.5 μM (A-517N, A-TC65N) or 1 μM (L1HsN) primer. PCR reactions were incubated at 37°C for 1 h, 94°C for 9 min, and, after the addition of 5 U Ampli*Taq* DNA polymerase (Perkin–Elmer/Cetus), 36 cycles of 94°C denaturation (1 min), 55°C annealing (1 min), and 72°C extension (4 min) were performed. Amplification products were electrophoresed in 1.5% agarose and stained with ethidium bromide; single PCR products were excised from a gel, electroluted, and purified by diethylaminoethyl cellulose chromatography (DE52; Whatman, UK) (Schleif and Wensink, 1981). Gel-purified and subcloned amplification products were reamplified by using 1 ng of product and the original primer. To identify unique amplification products, like-sized products were digested with either *Rsa*I or *Alu*I and electrophoresed in 1.5% agarose; products yielding a unique pattern of restriction fragments ("fingerprint") were selected for further characterization. For subcloning, PCR products were sequentially extracted with phenol and chloroform, digested with restriction enzyme *Not*I, reextracted, and ligated to *Not*I-digested plasmid.

DNA probes. DNA markers used in this study have been previously described (Gorski *et al.*, 1991). The localization of these markers relative to IP1 X-chromosomal translocation breakpoints is shown schematically in Fig. 2 and summarized in a companion paper (Gorski *et al.*, 1992). Plasmid DNA was prepared by standard techniques; prior to use as probes, DNA fragments were isolated from agarose gels by electrolution (Maniatis *et al.*, 1982).

Standard and pulsed-field Southern hybridizations. Standard and pulsed-field Southern transfer hybridizations were performed as described (Gorski *et al.*, 1991, 1992). Radioactive probes were prepared by random oligonucleotide priming (Feinberg and Vogelstein, 1983). PCR amplification products to be used as probes were preannealed with human placental DNA to C_0t 100 as described (Sealey *et al.*, 1985). PFGE was performed at 14°C in 0.5× TBE buffer (Maniatis *et al.*, 1982) using a contour-clamped homogeneous electrical field (CHEF) gel system (Chu *et al.*, 1986) (CHEF DRII, Bio-Rad).

RESULTS

Identification and Characterization of Radiation Hybrids

Radiation hybrid 128 (RH 128) was found to contain at least five different loci in the vicinity of the IP1 locus, including loci mapped proximal (DXZ1; Xcen) and distal (DXS146; Xp11.22) to and between (DXS14, DXS343, DXS370; Xp11.21) IP1 X-chromosomal translocation breakpoints (Gorski *et al.*, 1991) (Fig. 1); these results suggested that RH 128 contained an X-chromosomal fragment spanning the IP1 translocation breakpoints. RH 128 did not contain flanking loci DXS7 (Xp11.3) or DXS1 (Xq11) (data not shown). Using an additional 30 probes randomly distributed along the length of the X chromosome, including 22 probes mapped to Xq, hybridization analyses showed that RH 128 contained only two additional loci mapped to Xp22.3 (DXYS20 and STS) (data not shown).

To more fully characterize the X-chromosomal content of RH 128, RH 128 DNA was PCR amplified using *Alu*-directed primer A-TC65N to generate human-specific products (Fig. 1). Ten randomly selected ethidium bromide-stained products were mapped by hybridization to Southern blots containing IP1 somatic cell hybrid-mapping-panel DNA. All 10 products hybridized to all of the mapping panel DNAs, including those containing only Xq (hybrids A48-1Fa and A63-1A) (data not shown). Together, these results indicated that RH 128 contained at least three distinct X chromosome fragments and, although RH 128 contained DNA markers spanning the IP1 X chromosome translocation break-

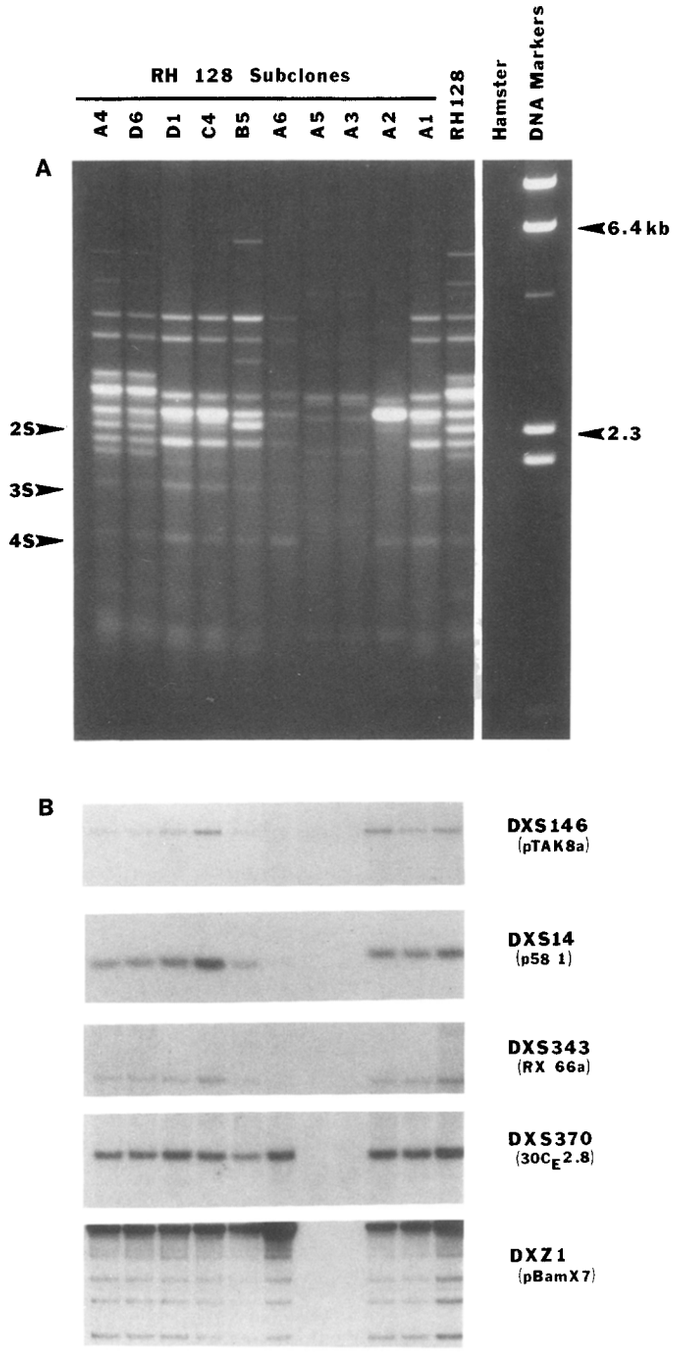

FIG. 1. Comparison of A-TC65N-primed PCR amplification products obtained from DNA isolated from a hamster cell line, RH 128, and 10 independently derived RH 128 subclones (clones A4-A1) electrophoresed in a 1.5% agarose gel (**A**), and autoradiograms of a membrane containing identically ordered *Hind*III-digested RH 128 and RH 128 subclone DNAs sequentially hybridized with DNA markers spanning the IP1 X-chromosomal translocation breakpoints (**B**). Compared to RH 128, subclones A4 and D6 yielded an identical pattern of PCR products; the other subclones yielded a reduced pattern, and subclone A2 yielded the fewest products (**A**). Eight subclones contained all five markers spanning the IP1 breakpoints; subclones A3 and A5 contained none of the markers (**B**). Three PCR products, 2S, 3S, and 4S (arrows) were restricted to those RHs containing the DNA markers (**A**).

points, most RH 128 IRS–PCR products were Xq in origin.

We hypothesized that, for a radiation hybrid containing multiple human chromosome fragments, fragments integrated within different hamster chromosomes would segregate independently during passage. By inference, this suggested that, by isolating and characterizing a number of RH 128 subclones, it would be possible to (a) identify "enriched" subclones that nonselectively retained DNA markers in the vicinity of the IP1 locus while losing other human fragments present in the parental RH, (b) determine whether DNA markers consistently cosegregated as expected for contiguous markers, and (c) construct a RH subclone mapping panel. To facilitate the isolation of additional DNA markers in the vicinity of the IP1 locus, we tested this hypothesis by isolating 24 independent RH 128 subclones; 10 subclones were stable enough to isolate DNA.

Comparative Analysis and Regional Assignment of IRS–PCR Radiation Hybrid Products

Ten RH 128 subclones were characterized by Southern hybridization and IRS–PCR amplification using *Alu* and L1Hs-directed primers. Using primer A-TC65N, RH 128 DNA yielded at least 30 distinct ethidium bromide-stained amplification products ranging from 6.4 to 0.6 kb in size; no products resulted from the amplification of hamster DNA, indicating that the primers were human-specific (Fig. 1). RH 128 and subclones A4 and D6 yielded an identical pattern of products. Eight subclones yielded a reduced but similar pattern of products, which varied by the presence or absence of a small number of specific products. Several subclones (D1, C4, and A1; A5 and A3) yielded apparently identical patterns; subclone A2 yielded the most reduced pattern (Fig. 1). Similar results were obtained using primers A-517N and L1HsN (data not shown). These results demonstrated that the IRS–PCR amplifications were reproducible, distinctive, and specific and suggested that, compared to RH 128, subclones yielding a reduced pattern of amplification products contained fewer X-chromosomal fragments.

To identify RH 128 subclones containing DNA fragments spanning the IP1 breakpoints, probes mapped to regions proximal (pBamX7), distal (pTAK8a), and between (p58-1, RX-66a, 30C$_E$2.8) IP1 X-chromosomal translocation breakpoints were hybridized to hybrid DNA. Eight subclones contained all five markers; subclones A3 and A5 contained none of the markers (Fig. 1). The observed cosegregation of DNA markers suggested that all five markers were contained on a single X chromosome fragment and implied that amplification products derived from this fragment would exhibit the same pattern of distribution within RH 128 subclone DNA.

Eleven distinct ethidium bromide-stained IRS–PCR amplification products were observed to be distributed in a pattern identical to the DNA markers spanning the IP1 breakpoints; these included A-TC65N-primed products 2S, 3S, and 4S (Fig. 1) and 2 A-517N and 6 L1Hs-

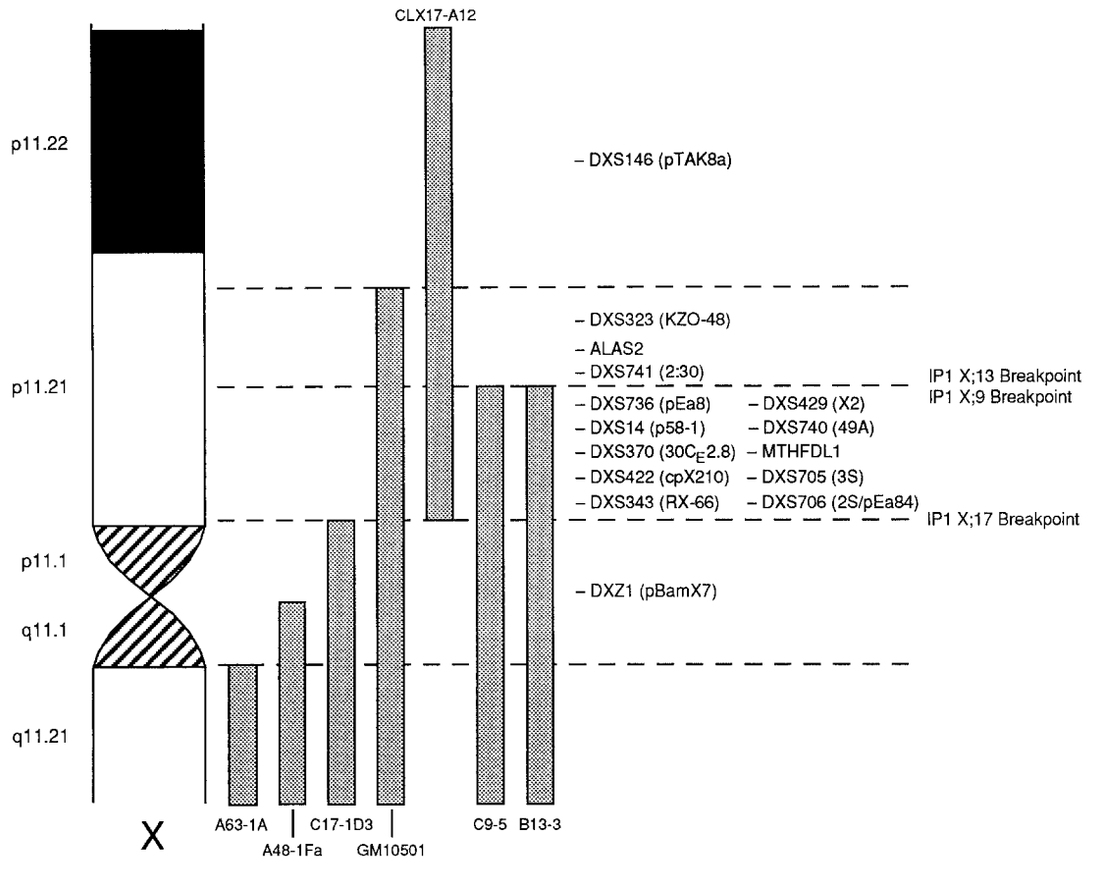

FIG. 2. Idiogram of GTG-banding patterns of the pericentric portion of a prometaphase human X chromosome (ISCN 1985). Bars indicate the X-chromosomal regions present in specific somatic cell hybrids; the exact location of the IP1 X;17 X-chromosomal translocation breakpoint in relation to the Xcen has not been determined (Gorski *et al.*, 1991). X-chromosomal loci found to be in the vicinity of the IP1 locus are shown in their assigned regions: 10 DNA sequences map to a region between IP1 X-chromosomal breakpoints; 3 DNA sequences map to a region distal to the X;13 and X;9 IP1 breakpoints and proximal to the GM10501 breakpoint within Xp11.21; and no loci have been assigned to the potential region between the IP1 X;9 and X;13 breakpoints. The regional assignments of MTHFDL1 (Rozen *et al.*, 1989; Italiano *et al.*, 1991), DXS705, DXS706, and DXS736 are based on mapping data reported in the present work; the regional assignment of DXS741 is based on mapping data reported in a companion paper (Gorski *et al.*, 1992); and the other loci have been previously mapped (Gorski *et al.*, 1991; Lafreniere *et al.*, 1991; Cotter *et al.*, 1992).

primed products (data not shown). To map these products to a specific X-chromosomal region, the 11 amplification products were isolated from gels and reamplified, and low-copy restriction fragments derived from the products were used as probes to hybridize to IP1 somatic cell hybrid-mapping-panel DNA. As shown schematically in Fig. 2, products 3S and 2S mapped to a region between IP1 X chromosome translocation breakpoints. Of the remaining 9 products, 1 mapped to Xq, 2 mapped distal to the GM10501 breakpoint on Xp, and 1 was autosomal; 2 products failed to reamplify and 3 were repetitive (data not shown).

Having isolated PCR products that mapped to a region of interest, we sought to modify this strategy to isolate additional products not identified by ethidium bromide staining. For this purpose, a plasmid library that contained the A-TC65N-primed amplification products of subclone A2 DNA was constructed; 96 recombinant clones were randomly selected, reamplified, and fingerprinted by restriction enzyme digestion. Most products (62 of 95; 65%) were identical and corresponded to the intense 2.4-kb ethidium bromide-stained amplification product seen in the amplified products of subclone A2 DNA (Fig. 1). By fingerprinting, 14 discrete amplification products were identified; 1 product, pEa84, was found to be identical to the previously isolated product 2S (data not shown). To identify products exhibiting a distribution pattern matching that of the DNA markers spanning the IP1 breakpoints, each of the remaining 13 unique products was used as probe and hybridized to membranes containing the PCR products of A-TC65N-primed RH 128 subclone DNA; 8 products showed the desired hybridization pattern and hybridized to an identically sized product present in all of the subclone DNAs except subclone A3 (Fig. 3). By comparison, only 6 of 41 randomly selected A-TC65N-primed RH 128 amplification products yielded the same pattern of distribution (data not shown). Used as a probe and hybridized to IP1 somatic cell hybrid-mapping-panel DNA, product pEa8 mapped to the region between IP1 X chromosome translocation breakpoints (Figs. 2 and 3); 4 products mapped to Xp distal to the GM10501 breakpoint, and 3 were repetitive (data not shown). The localization of the isolated DNA markers, relative to

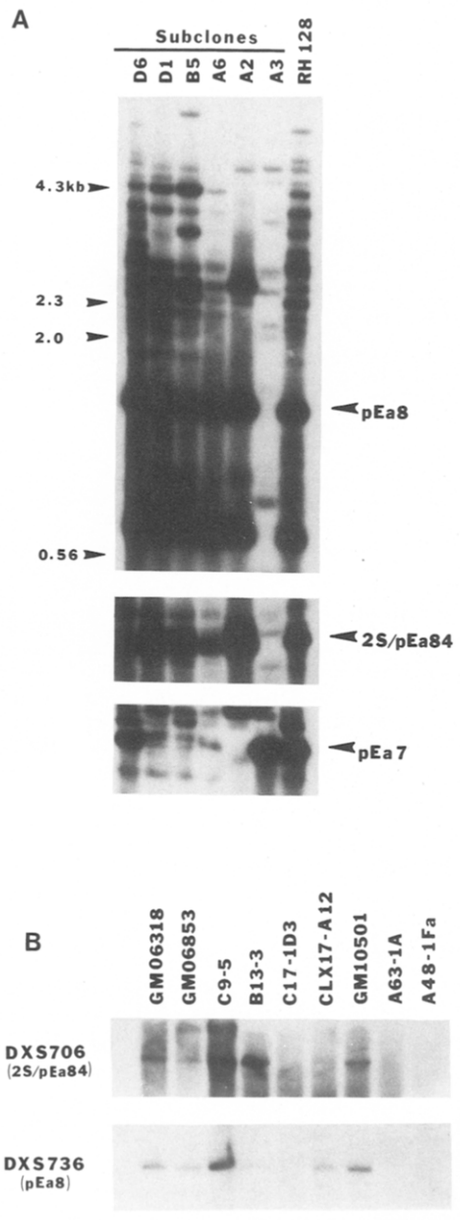

FIG. 3. Amplification products pEa8, 2S/pEa84, and pEa7 sequentially hybridized to 200 ng of A-TC65N-primed RH 128 subclone DNA amplification products (**A**) and *Hin*dIII-digested IP1 somatic cell hybrid mapping panel DNA (**B**). Although each probe hybridized to multiple amplification products, the observed hybridization pattern was probe-specific and each probe preferential hybridized to an identical-sized product (arrows). The hybridization pattern of probes pEa8 and 2S/pEa84 matched that of the DNA markers mapped to the region between IP1 breakpoints; even upon overexposure, as opposed to probe pEa7, neither pEa8 nor 2S/pEa84 detected a like-sized product in subclone A3 DNA (**A**). Probes pEa8 (DXS736) and 2S/pEa84 (DXS706) had identical patterns of hybridization to IP1 somatic cell hybrid mapping panel DNA; both mapped to the same region between IP1 X-chromosomal translocation breakpoints (**B**).

others mapped in the vicinity of the IP1 breakpoints, is summarized schematically in Fig. 2.

Physical Mapping of IRS–PCR Amplification Products

PFGE analyses were performed to better define the region between IP1 X chromosome translocation breakpoints and localize newly isolated DNA markers relative to those previously mapped; three probes could be tentatively overlapped by these analyses (Fig. 4). Probes cpX210 and MTHFDL1 detected a superimposable 660-kb *Sfi*I fragment; probes MTHFDL1 and 3S detected superimposable 1200-kb *Sfi*I fragments, and 1300- to 1600-kb *Sac*II fragments; and probes cpX210 and 3S failed to detect a common restriction fragment. These results tentatively place probes cpX210 and MTHFDL1 within 660 kb and probes MTHFDL1 and 3S within 1200 kb. Probe cpX210 was previously mapped to within 120 kb of probe p58-1 (Gorski *et al.*, 1991); probe p58-1 failed to detect a restriction fragment common to MTHFDL1 and 3S (data not shown). These preliminary data suggest the locus order: DXS14–DXS422–MTHFDL1–DXS705; this order requires confirmation by YAC cloning or additional PFGE studies.

Pulsed-field blots are extremely sensitive to repetitive sequences, more so than Southern blots; even with the use of probe competition conditions (Patel *et al.*, 1991), probes derived from products 2S/pEa84 and pEa8 were found to be too repetitive to be used as probes for PFGE blots (data not shown). Probe 3S was used for a PFGE analysis of somatic cell hybrids containing IP1 derivative chromosomes; hybridized to PFGE blots containing *Eag*I, *Sac*II, and *Sfi*I-digested hybrid C9-5, B13-3, CLX17-A12, and GM06318 DNAs, probe 3S detected restriction fragments of apparently identical size (data not shown). The evident uniformity of restriction fragments detected suggests that these restriction fragments have not been rearranged and implies that the IP1 X-chromosomal translocation breakpoints are located outside the region analyzed.

DISCUSSION

This study demonstrates an approach for rapidly isolating region-specific DNA markers from a RH containing multiple human chromosome fragments. The parental RH used in this study was found to contain at least three human X chromosome fragments, including a set of DNA markers spanning a region of interest within Xp11.21. To facilitate the mapping of DNA markers, we constructed a hybrid mapping panel composed of multiple, discrete subclones derived from a single parental RH. This hybrid panel was used to (a) identify particular subclones which contained the region of interest and yielded a reduced number of amplified products (and presumably contained a reduced number of X chromosome fragments) and (b) construct a mapping panel to assist in identifying regional DNA markers. Human-specific DNA markers were isolated from RH DNA by IRS–PCR amplification; products with the same pattern of distribution as previously mapped IP1 markers were tentatively identified as region-specific DNA markers. Using this approach, we have isolated three different PCR products that map to a region between IP1 X chromosome translocation breakpoints.

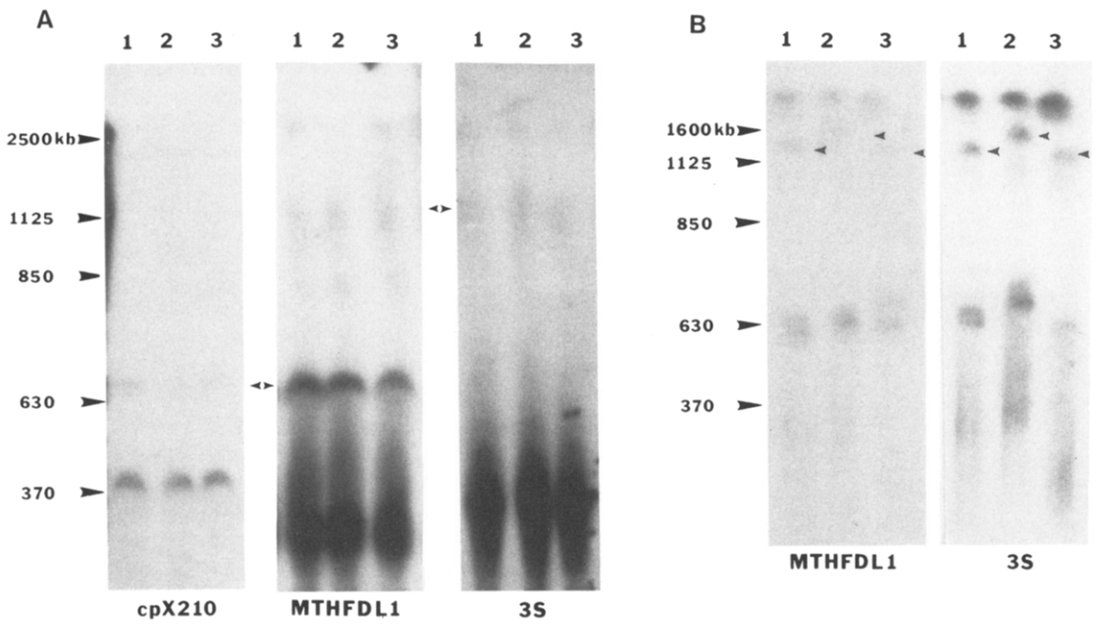

FIG. 4. Southern blot analysis of somatic cell hybrid DNA partially digested with either *Sfi*I (**A**) or *Sac*II (**B**); the same filters were sequentially hybridized with probes cpX210, MTHFDL1, and 3S. DNA was fractionated by CHEF electrophoresis in 1.0% agarose (0.5× TBE) using 200 V at 14°C; switch times were 60 s for 20 h and 120 s for 12 h (**A**) and 60 s for 15 h and 90 s for 9 h (**B**). For *Sfi*I digests, hybrid GM06318 DNA was digested with 30 U (lane **1**), 15 U (lane **2**), and 5 U (lane **3**); for *Sac*II digests, hybrid C9-5 (lane **1**), B13-3 (lane **2**), and CLX17-A12 (lane **3**) DNAs were digested with 30 U. Probe cpX210 detected 400- and 660-kb *Sfi*I fragments; probe MTHFDL1 detected 260-, 660-, and 1200-kb *Sfi*I fragments, and 630- to 660-kb and 1300- to 1600-kb *Sac*II fragments; and probe 3S detected 340- and 1200-kb *Sfi*I fragments, and 660- to 680-kb and 1300- to 1600-kb *Sac*II fragments. Arrows point to restriction fragments corecognized by different probes when autoradiograms are superimposed. The different *Sac*II fragment sizes observed in hybrid C9-5 and B13-3 DNA were not present on other *Sac*II blots and probably represent artifactual differences in quantities of DNA loaded; the partially digested *Sac*II fragments detected by MTHFDL1 and 3S are superimposable.

It can be expected that RHs will have different attributes affecting the ease with which each can be used to construct a hybrid subclone mapping panel; the recovery of region-specific DNA markers from a RH has been hampered by the presence of additional fragments not detected by preliminary hybridization analyses (Benham and Rowe, 1992). The strategy we describe, however, should be generally applicable to other situations in which RHs contain a particular region of interest. RHs have been nonselectively constructed for a relatively large number of human chromosomes including X, 4, 5, 6, 9, 10, 11, 12, 16, and 21 (Benham *et al.,* 1989; Ceccherini *et al.,* 1989; Cox *et al.,* 1989; Brooks-Wilson *et al.,* 1990; Glaser *et al.,* 1990; Florian *et al.,* 1991; Ragoussis *et al.,* 1991; Thomas, 1991; Zoghbi *et al.,* 1991; Sinke *et al.,* 1992); the use of RH subclones to assemble hybrid mapping panels may extend the use of existing RHs and provide additional hybrids for comparative mapping and subcloning.

Our present data do not provide an estimate of the total number of potential IRS–PCR products contained within the region between IP1 X-chromosomal translocation breakpoints; however, our results suggest that a more intensive effort would result in the isolation of additional region-specific DNA markers. Presently, we have isolated approximately one region-specific DNA marker per 800 kb of genomic DNA. This cloning efficiency compares favorably to other, more labor intensive techniques, such as chromosome microdissection (Ludecke *et al.,* 1989), PERT cloning (Nussbaum *et al.,* 1987), and coincidental cloning (Aslanidis and de Jong, 1991). In addition, this achieved density of DNA markers is appropriate for the initiation of additional molecular studies including PFGE mapping, radiation hybrid mapping (Cox *et al.,* 1990), fluorescence *in situ* hybridization analysis (Trask *et al.,* 1991), the isolation of overlapping genomic clones in yeast artificial chromosomes (Schlessinger *et al.,* 1991), and the isolation of sequence tagged sites (Olsen *et al.,* 1989). A total of 10 DNA sequences have now been mapped to a region between IP1 X-chromosomal translocation breakpoints. Together, these sequences provide a starting point for identifying overlapping genomic sequences spanning the IP1 breakpoints; the availability of IP1 translocation breakpoints should assist the molecular analysis of this locus.

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