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Nucleotide Sequence and Chromosomal Mapping of the Human c-*yes*-2 Gene

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We molecularly characterized the second gene, c-*yes*-2, of two copies of *yes*-related genes which we previously found to contain in the human genome. First, nucleotide sequence analysis revealed that the c-*yes*-2 gene is a pseudogene of the c-*yes*-1 gene. Second, by using two independent methods, hybridization of both DNAs from sorted chromosomes and metaphase spreads with c-*yes*-2 DNA, we assigned the c-*yes*-2 gene to chromosome 22q11.2. This chromosomal localization is inconsistent with that given in our previous report. The failure of proper mapping in our experiment might have been caused by instability of hybrid cell clones.

Key word: c-*yes*-2 — Pseudogene — Chromosome 22

Protein tyrosine kinase activity is the crucial function of proteins encoded by a number of both retroviral oncogenes and activated proto-oncogenes isolated by DNA transfection techniques.[1, 2] This activity is also associated with a number of receptors for growth factors. All of these protein tyrosine kinases share a homologous amino acid sequence, called the kinase domain, that exhibits the tyrosine kinase activity.[1] Previous findings showed overlap between the retroviral transforming proteins of the tyrosine kinase family and the growth factor receptors. The c-*erbB*-1 and the c-*fms* genes encode the epidermal growth factor receptor and mononuclear phagocyte growth factor receptor, respectively.[3, 4] Nucleotide sequence analysis of v-*ros* and v-*kit* showed that they could encode the receptors for growth factors.[5, 6] Thus, at least several proto-oncogenes, from which retroviral oncogenes are generated, are involved in the regulation of normal cell growth, and quantitative and/or qualitative alterations of the proto-oncogene products mediate abnormal cell growth leading to neoplastic transformation. In contrast, the c-*src*,[7] the c-*yes*[8] and the c-*fgr*[9, 10] gene products are not typical growth factor receptors, since they are lacking in both the ligand binding domain and the transmembrane domain. Recently, we and other investigators molecularly cloned a number of *src*-related genes, *fyn* (*syn/slk*),[11, 12] *lyn*,[13] *lck*[14, 15] and *hck*,[16, 17] all of which could also encode nonreceptor-type proteins similar to the *src* gene product. Interestingly, each of the six *src*-related genes, c-*yes* (M. Nishizawa, unpublished result), c-*fgr*,[9] *fyn*,[11] *lyn*,[13] *lck*[17] and *hck*,[17] shares the identical exon-intron organization to that of the *src* gene at all exon-intron boundaries so far examined, suggesting that they were generated by the gene duplication of an ancestral gene that had already acquired the present exon-intron organization. Our previous analysis using human-mouse hybrid cells showed that the human genome contained at least two *yes*-related genes, c-*yes*-1 and c-*yes*-2, and that the c-*yes*-1 is located on chromosome 18 while the c-*yes*-2 is on chromosome 6.[18] Subsequent analysis of the human c-*yes*-1 genome clones revealed that the c-*yes*-1 gene is localized on chromosome 18 at band q21.3[19]

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and is actively transcribed.[8] The human c-*yes*-1 gene encodes a polypeptide of 543 amino acids that is structurally similar to the c-*src* gene product, pp60$^{c\text{-}src}$.[8] The c-*yes*-2 gene is clearly distinct from the known *src*-related genes because of the differences of chromosomal location and of gene structure. However, the c-*yes*-2 gene has not been analyzed further. Here we molecularly cloned most of the c-*yes*-2 gene and determined the nucleotide sequence of the cloned DNA. In addition, we corrected our previous mapping of c-*yes*-2 gene on the human chromosome by using two independent methods.

Materials and Methods

Cloning of the Human c-*yes*-2 Gene Human placental DNA digested with *Bam*HI was fractionated by electrophoresis on 0.7% agarose gel. The *Bam*HI fragments (approximately 9–20 kbp) were recovered from the gel and molecularly cloned using λL47.1 DNA as a vector. This phage library was screened by plaque hybridization to the 1.5 kbp *Sin*I fragment of the v-*yes* gene labeled with ^{32}P by nick translation. We used relatively relaxed conditions for hybridization, as previously described,[20] because of the divergence between the v-*yes* and the human c-*yes*-2 sequence. From about 5×10^5 plaques, 13 positive clones were isolated. DNAs from two of these clones reacted with all probes of v-*yes*, subA, subB and subC (Fig. 1).

Nucleotide Sequence Analysis The nucleotide sequence was determined by the dideoxy chain-termination method[21] using M13mp18, M13mp19 and pUC19 as cloning-sequencing vectors.[22]

Chromosome Mapping Chromosomal location of the c-*yes*-2 gene was determined by two independent methods, Southern blot hybridization of DNAs from sorted chromosomes and *in situ* hybridization of metaphase spreads with c-*yes*-2 specific DNA. Metaphase chromosomes were prepared from three cell lines, GM0131, whose karyotype is normal, GM6321 that carries reciprocal translocation t(9;22)(q13;q13.3), and GM1137 that carries reciprocal translocation t(6;10)(p25;p11), which were provided by the Human Genetic Mutant Cell Repository (Institute for Medical Research, Camden, N.Y.) and then sorted into eight fractions using a fluorescence-activated cell sorter as described previously.[23,24] DNA samples were prepared from each fraction of the sorted chromosomes as described[25] and analyzed by Southern blot hybridization.

In situ hybridization to metaphase chromosomes was performed according to our previous report.[19]

Results and Discussion

Molecular Cloning of the Human c-*yes*-2 Gene Southern blot analysis of DNAs from human-mouse hybrid cells showed that the human genome contains at least two copies of the v-*yes*-related sequence.[18] One of them (c-*yes*-2) represented by the 1.9-kbp *Eco*RI fragment, was localized on chromosome 6, while the other (c-*yes*-1) was on chromosome 18 (ref. 18 and see below for correction). The 1.9-kbp *Eco*RI fragment reacted with each of the three probes that includes the 5′-terminal (subA), middle (subB), or the 3′-terminal (subC) portion of the 1.8-kbp v-*yes* sequence, suggesting that c-*yes*-2 is a processed *yes*-related gene (Fig. 1). A 14-kbp *Bam*HI fragment also reacted with all three probes, subA, subB and subC (Fig. 1). We did not detect this *Bam*HI fragment in the human-mouse cell hybrids, H/B 3-2 and H/B 5-1, that were proved to contain c-*yes*-1 sequence but not c-*yes*-2 sequence, represented by the 1.9-kbp

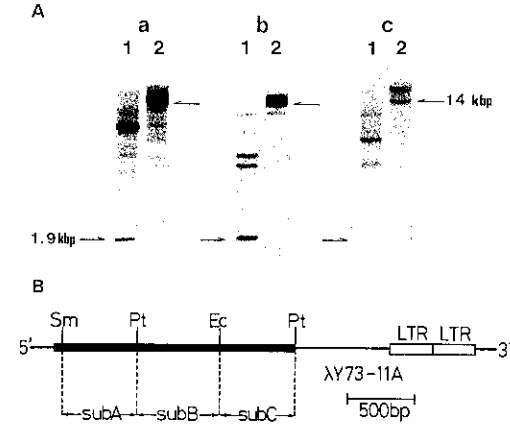

Fig. 1. Identification of the human c-*yes*-2 gene. A. Southern blot analysis of the human DNA. Human embryo fibroblast DNA (10 μg) was digested with either *Eco*RI (lane 1) or *Bam*HI (lane 2). The digested DNA was fractionated by electrophoresis on 0.9% agarose gel and then subjected to Southern blot analysis using the ^{32}P-labeled probe, subA (panel a), subB (panel b) or subC (panel c). B. Schematic illustration of the DNA fragments used as probes in hybridization analyses. λY73-11A is a DNA clone of the Y73 genome. The heavy line indicates the v-*yes* sequence. The restriction sites are abbreviated as follows: Ec, *Eco*RI; Pt, *Pst*I; Sm, *Sma*I.

*Eco*RI fragment (data not shown). Thus, we molecularly cloned the 14-kbp *Bam*HI fragment at the *Bam*HI site of the λL47.1 *Bam*HI arm. In this 14-kbp *Bam*HI fragment, all three probes (subA, subB, subC) hybridized only to the 1.5-kbp *Bam*HI-*Eco*RI fragment (Fig. 2). We determined the nucleotide sequence of this region and found an about 1.8-kbp sequence that is highly homologous (95%) with the corresponding sequence of the human c-*yes*-1 cDNA (nucleotides 352-1812) (Fig. 3). However, it was concluded for the following reasons that the c-*yes*-2 is not a functional gene but is a processed pseudogene of c-*yes*-1. First, the c-*yes*-2 gene is an intronless gene. Second, five point mutations generate five termination codons in the *yes*-coding frame, insertion of two G residues causes a frame shift, and also, insertion of an *Alu* family flanked by direct repeats interrupts the *yes*-coding frame. All these mutations disrupt the putative coding frame which could otherwise encode the *yes*-related protein (Fig. 3). Figure 4 shows homology as high as 86% of the insertion sequence of c-*yes*-2 with the consensus *Alu* sequence.[26] Third, the 3′-terminal 200-bp outside of the c-*yes*-2 sequence that is homologous to c-*yes*-1 coding region has 90% homology to the corresponding noncoding region of the c-*yes*-1 cDNA (data not shown). Since the 3′ noncoding sequence of the c-*yes*-1 cDNA is considerably different from those of the other *yes*-related genes, *fyn*[11] and *lyn*,[13] the c-*yes*-2 gene appears to be a pseudogene of the c-*yes*-1 gene. At this moment, however, we do not know whether other vertebrate genomes contain the *yes*-related pseudogene(s).

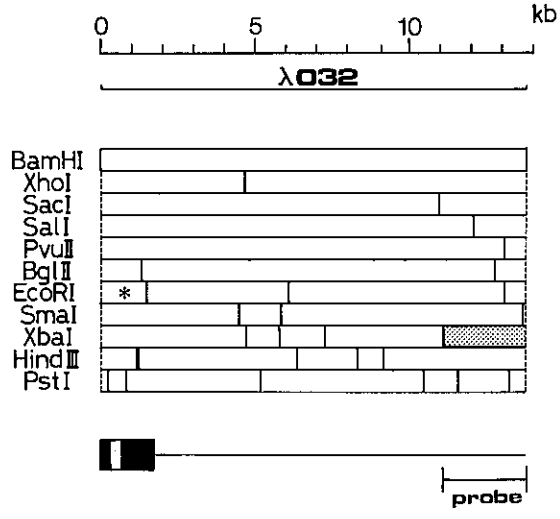

Fig. 2. Restriction endonuclease map of the human c-*yes*-2 gene locus. The *Bam*HI-*Eco*RI fragment that reacts with all three probes (subA, subB, subC) is marked by an asterisk. The v-*yes* related sequence is indicated by two black boxes, and an *Alu* family repeat by an open box. The DNA probe used in chromosome mapping is indicated by a dotted box.

Chromosome Mapping of the c-*yes*-2 Gene To determine the chromosome location of the c-*yes*-2 gene more precisely, we performed hybridization of both sorted chromosomes and metaphase spreads with the c-*yes*-2 locus-specific probe, the 2.7-kbp *Xba*I-*Bam*HI fragment prepared from the human c-*yes*-2 DNA clone (Fig. 2). Metaphase chromosomes were prepared from two human cell lines, GM0131 and GM6321, and separated into eight fractions with a fluorescence-activated cell sorter. DNAs were prepared from each fraction of the sorted chromosomes and analyzed by Southern blot hybridization using the c-*yes*-2 specific probe (Fig. 5). The karyotypically normal cell, GM0131, produced one positive signal in fraction "H" that contained chromosomes 21 and 22. The other cell, GM6321, carries reciprocal translocation t(9;22)(q13; q13.3) that produces derivative chromosomes, der(9) and der(22). The chromosome der(9) contains q13.3-qter of chromosome 22, while the chromosome der(22) contains pter-q13.3 of chromosome 22. These derivative chromosomes can be sorted at different fractions from those of normal homologs. Southern blot analysis of DNAs from the sorted chromosomes of GM6321 cells revealed two positive signals, one in a fraction "e" that contains the chromosome der(22) and the other in fraction "h" that contains the normal chromosome 22. These results indicate that the human c-*yes*-2 gene is located on chromosome 22 pter-q13.3. To localize the human c-*yes*-2 gene more precisely, we performed *in situ* hybridization experiments according to our reported method. The probe used for this experiment is the 2.7-kbp *Xba*I-*Bam*HI fragment of the c-*yes*-2 clone outside of *yes*-related sequence (Fig. 2), flanked by a 172-bp pUC19-derived sequence.

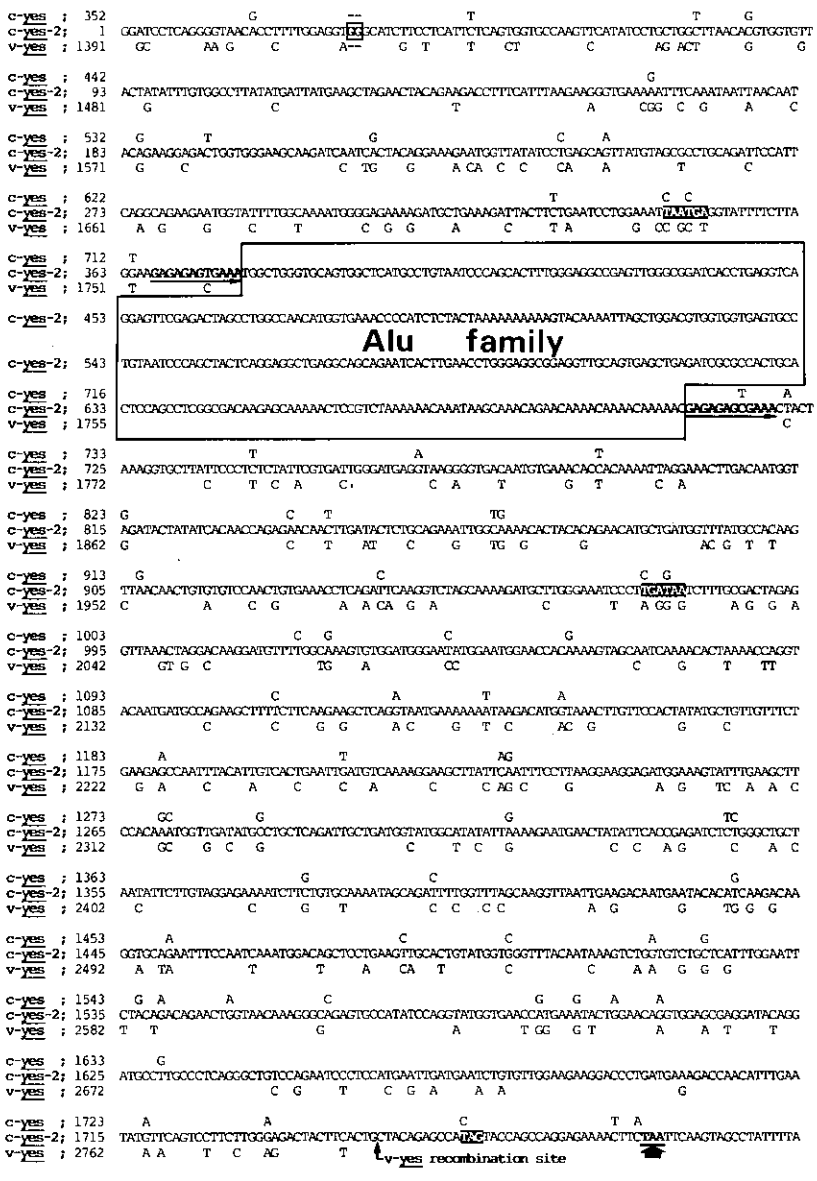

Fig. 3. Nucleotide sequence of the human c-*yes*-2 gene. The nucleotide sequences of human c-*yes*, human c-*yes*-2 and v-*yes* genes are compared. Nucleotides of c-*yes* and v-*yes* are shown only where they are different from those of c-*yes*-2. Nucleotides of the c-*yes*,[8] v-*yes*[28] and c-*yes*-2 are numbered on the left. Two inserted nucleotides are boxed. An *Alu* family repeat is also boxed. The two direct repeats are shown by arrows. Termination codons found in the *yes*-coding frame are written in white letters. The termination codon of c-*yes* is indicated by an arrow.

Analysis of metaphase chromosomes of 168 cells revealed that 26% (44 of 168) had silver grains at the proximal long arm of chromosome 22, 22q11.1-q12 (Fig. 6). Of 46 grains on chromosome 22, 24 (52.2%) were over q11.2. This result is consistent with the chro-

mosome localization determined by Southern blot analysis of DNAs from the sorted chromosomes.

Our previous analysis of the human-mouse cell hybrids using the v-*yes* probe showed that the c-*yes*-2 gene was localized on chromosome 6, which is inconsistent with the results described above. One possibility is that the molecularly cloned genetic locus of "c-*yes*-2" is different from the "c-*yes*-2" locus represented by the 1.9-kbp *Eco*RI fragment. However, this is unlikely because no other *Eco*RI or *Bam*HI fragments except the 1.9-kbp *Eco*RI and the 14-kbp *Bam*HI fragments

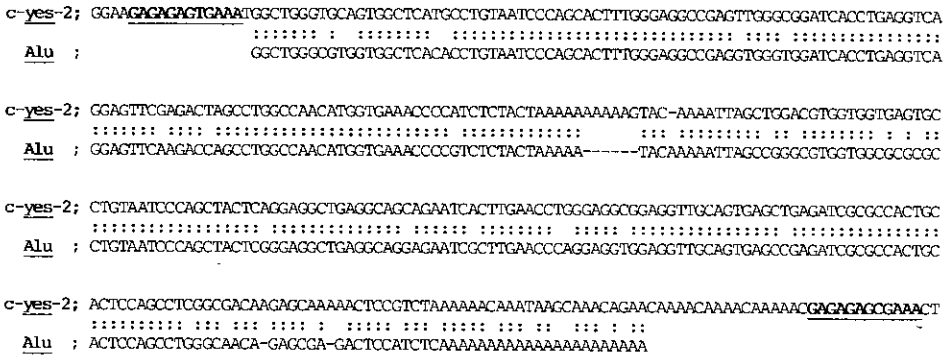

Fig. 4. Comparison of the c-*yes*-2 *Alu* sequence with the consensus. The *Alu* sequence from the c-*yes*-2 locus is compared with a consensus human *Alu* sequence.

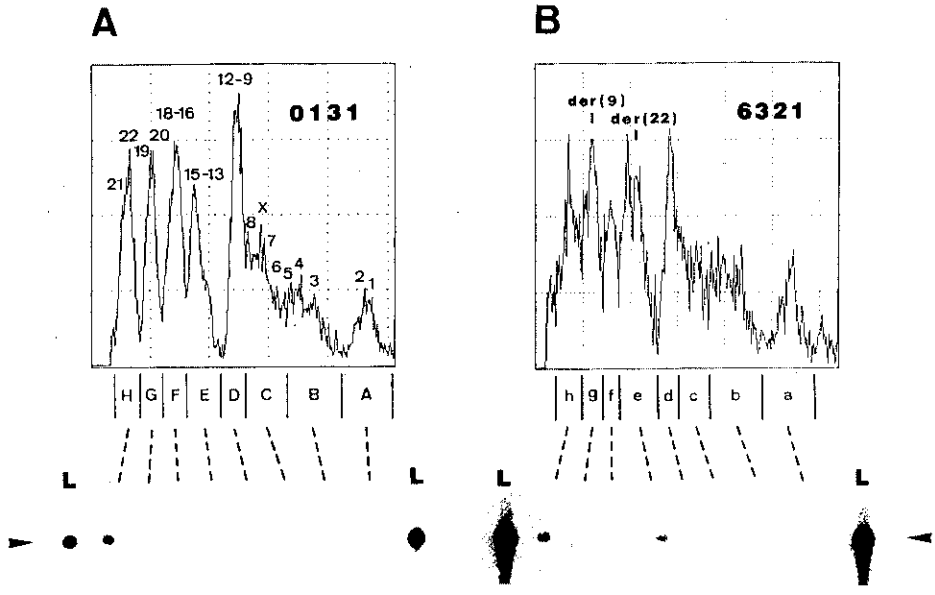

Fig. 5. Assignment of the c-*yes*-2 gene to chromosome 22 pter-q13.3. A. Sorting chromosomes from GM0131 cells and Southern blot analysis of DNAs from sorted fractions (A to H). Each DNA sample was digested with *Hin*dIII and subjected to Southern blot analysis using the c-*yes*-2 specific probe (Fig. 2). Total DNA digested with *Hin*dIII was used as the standard (lanes L). B. Sorting chromosomes from GM6321 cells containing the reciprocal translocation t(9;22)(q13; q13.3), and Southern blot analysis of DNAs from the sorted fractions (a to h).

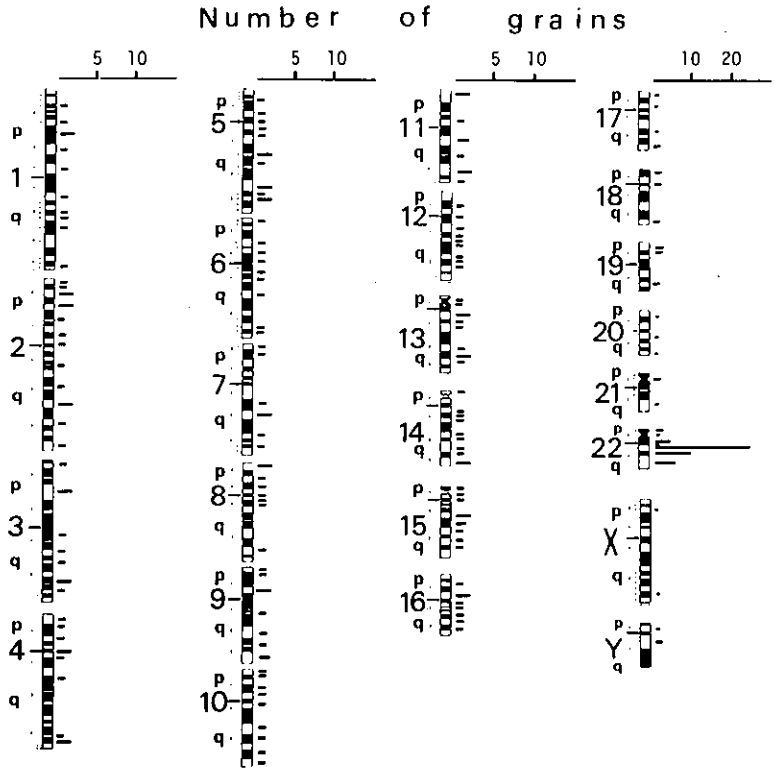

Fig. 6. Assignment of the c-*yes*-2 gene by using an *in situ* hybridization technique. The histogram shows the localization of grains on the human chromosomes. Significant clustering of grains is shown on chromosome 22 at band q11.2.

react with all three probes, subA, subB and subC (Fig. 1) and because Southern blot analysis using the 14-kbp *Bam*HI specific probe revealed that DNAs from ten different hybrid cells which retained the 14-kbp *Bam*HI fragment also contained 1.9-kbp *Eco*RI fragment and *vice versa* (data not shown). Another possibility is that the inconsistent result may represent a difference in sensitivity between DNA hybridization analysis and the detection of the human chromosomes in the hybrid cells. Although chromosome analysis was done on a minimum of 30 metaphases per hybrid clone, it sometimes failed to give a representative result because of the instability of the hybrid cell clones. In contrast, DNAs were extracted from approximately 10^7 cells and could provide representative samples. In addition to c-*yes*-2, we assigned one *yes*-related gene, *fyn*, to chromosome 6 by using the same human-mouse hybrid cells.[11] This raised the possibility that the *fyn* gene might also be located on chromosome 22. Although the chromosomal location of *fyn* had been confirmed by *in situ* hybridization experiments,[27] we performed Southern blot hybridization of DNAs from sorted chromosomes using the *fyn* probe (Fig. 7), in order to re-confirm our previous data. With the karyotypically normal GM0131 cells, a positive signal was observed in fraction "C" that contained chromosomes 6, 7, 8 and X. The other cell, GM1137, carries reciprocal translocation t(6; 10)(p25; p11) that produces two derivative chromosomes, der(6) and der(10). The chromosome der(6) contains p25-qter of chromosome 6 and the chromosome der(10) contains pter-p25 of chromosome 6. Analysis of the GM1137 cell revealed two positive signals, one in fraction "b" that contains chromosome der(6) and the

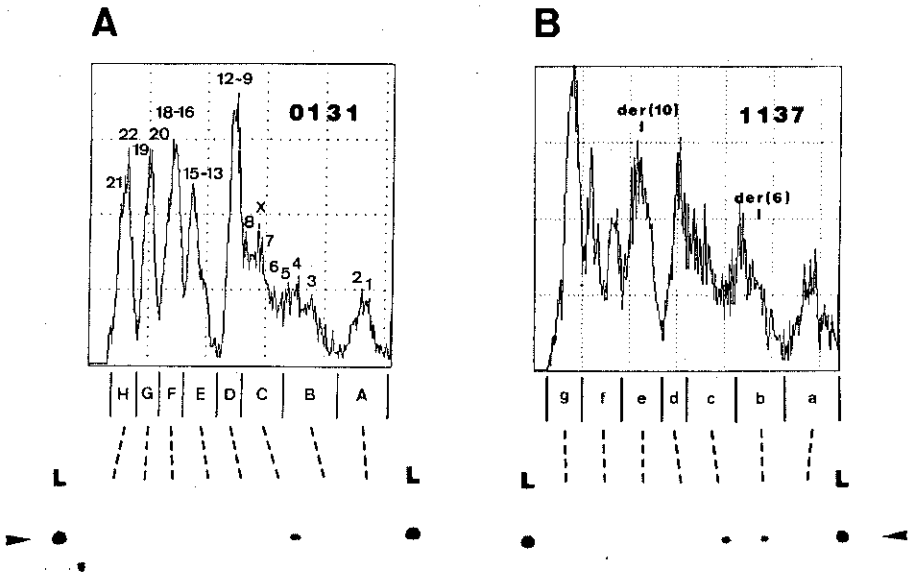

Fig. 7. Assignment of the *fyn* gene to chromosome 6 p25-qter. A. Sorting chromosomes from GM0131 cells and Southern blot analysis of DNAs from the sorted fractions (A to H). Each DNA sample was digested with *Pvu*II and subjected to Southern blot analysis with the ^{32}P-labeled *fyn* probe. B. Sorting chromosomes from GM1137 cells containing the reciprocal translocation t(6;10)(p25;p11) and analysis of DNAs from the sorted fractions (a to g).

other in fraction "c" that contains chromosome 6. These data led to the conclusion that the human *fyn* gene is located on 6p25-qter, and confirmed our previous result, chromosome 6 at band q21.[27] Chromosome mapping of the *fyn* gene by using the sorted fractions of chromosomes revealed that the set of human-mouse hybrid cells we used could not discriminate between chromosome 6 and chromosome 22.

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