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Isolation and Analysis of DNA Markers Specific to Human Chromosome 15

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Summary

Chromosome-specific DNA markers provide a powerful approach for studying complex problems in human genetics and offer an opportunity to begin understanding the human genome at the molecular level. The approach described here for isolating and characterizing DNA markers specific to human chromosome 15 involved construction of a partial chromosome-15 phage library from a human/Chinese hamster cell hybrid with a single human chromosome 15. Restriction fragments that identified unique- and low-copy loci on chromosome 15 were isolated from the phage inserts. These fragments were regionally mapped to the chromosome by three methods, including Southern analysis with a mapping panel of cell hybrids, in situ hybridization to metaphase chromosomes, and quantitative hybridization or dosage analysis. A total of 42 restriction fragments of unique- and low-copy sequences were identified in 14 phage. The majority of the fragments that have been characterized so far exhibited the hybridization pattern of a unique locus on chromosome 15. Regional mapping assigned these markers to specific locations on chromosome 15, including q24-25, q21-23, q13-14, q11-12, and q11. RFLP analysis revealed that several markers displayed polymorphisms at frequencies useful for genetic linkage analysis. The markers mapped to the proximal long arm of chromosome 15 are particularly valuable for the molecular analysis of Prader-Willi syndrome, which maps to this region. Polymorphic markers in this region may also be useful for definitively establishing linkage with one form of dyslexia. DNA probes in this chromosomal region should facilitate molecular structural analysis for elucidation of the nature of instability in this region, which is frequently associated with chromosomal aberrations.

Introduction

A single human chromosome 15 consists of ~1 × 10^8 bp composing nearly 3% of the total human haploid genome (Mendelsohn et al. 1973; Southern 1982). Relatively few genes or DNA markers have been assigned to this chromosome. Genetic diseases that have been mapped to this chromosome include the Prader-Willi syndrome (PWS), one form of dyslexia, Tay-Sachs disease, and congenital lipoid adrenal hyperplasia (McKusick 1986).

Chromosome 15 is an acrocentric chromosome with satellite-rich heterochromatic centromere and stalk regions (Miklos and John 1979; Mattei et al. 1984). The role of satellite-rich heterochromatin is not clear, but it has been shown to interfere with meiotic crossover and to preserve linkage groups in regions near the centromere (Miklos and John 1979). Variations within the constitutive heterochromatin (heteromorphisms) appear to be a general feature of chromosome 15 (Kurnit 1979; Butler et al. 1986). Interspersed satellite DNAs composed of short repeated sequences could possibly mediate unequal

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crossover, resulting in quantitative variability of constitutive heterochromatin present in cytogenetic heteromorphisms (Kurnit 1979).

The proximal long arm of chromosome 15 appears to be particularly labile, as it is frequently associated with chromosomal translocations, small bisatellited additional chromosomes (SBACs), and deletions. These chromosomal rearrangements, especially interstitial deletions in the 15q11-q13 region, have been found to occur in patients with PWS (Ledbetter et al. 1981; Mattei et al. 1984; Bray and Wilson 1986; Butler et al. 1986).

Cytogenetic and molecular evidence suggest that the proximal long arm region of chromosome 15 is rich in palindromic or inverted repeat sequences, a situation that may lead to the instability of this region (Cavalier-Smith 1974; Lejeune et al. 1979; Donlon et al. 1986). Cytogenetic evidence favoring the presence of such palindromic sequences is based on observations that the proximal long arm region of chromosome 15 is very often translocated onto other autosomes' telomeres, which are themselves palindromic structures (Cavalier-Smith 1974; Lejeune et al. 1979). Molecular evidence comes from analysis of a phage library constructed from this region, in which several recombinant phage would grow only in the recombination-deficient bacterial strain *E. coli* DB1257 (Donlon et al. 1986). It has been shown that this bacterial strain permits the propagation of palindromic inserts that do not grow in recombination-proficient strains of *E. coli,* presumably owing to the destabilizing effect of such inserts (Wyman et al. 1985). Furthermore, electron micrographs of heteroduplex formation of these phage inserts show many stem-loop structures characteristic of inverted repeat sequences (Donlon et al. 1986). A variety of mechanisms have been suggested for the role that these inverted repeat sequences might play in chromosomal rearrangements occurring during DNA replication (Kurnit 1979). Furthermore, a role for replication in the lability of this region has also been suggested by evidence for the paternal inheritance of de novo deletions in PWS patients (Butler and Palmer 1983; Butler et al. 1986).

To facilitate molecular analysis of human chromosome 15 and its associated genetic diseases, we initiated a study aimed at isolation and characterization of DNA markers specific to this chromosome. The approach involved construction of a partial genomic phage library from a human/Chinese hamster cell hybrid containing a single human chromosome 15. Restriction fragments that identified unique- and low-copy loci on chromosome 15 were isolated and regionally mapped to the chromosome by three different methods. Several markers that exhibited RFLPs should be useful for genetic linkage analysis, particularly with regard to dyslexia. The markers that were mapped to the proximal long arm of chromosome 15 should be important not only for the molecular analysis of PWS but also for the elucidation of both the nature of instability of this chromosomal region and the frequent association of this region with chromosomal abnormalities.

Material and Methods

Cell Lines

The Chinese hamster ovary cell line CHO-K1 (K1) was used as a source of hamster DNA (Kao and Puck 1968). The human fibrosarcoma cell line HT1080 (HT) was used as a source of human DNA (Croce 1976). Human/Chinese hamster (CHO-K1) somatic cell hybrids were developed and characterized as described elsewhere (Kao et al. 1969, 1976). A human/CHO-K1 cell hybrid 15A retaining a single, intact human chromosome 15 as the only human chromosome was used for constructing a partial human chromosome 15–specific library. Human/CHO-K1 cell hybrids possessing various deletions and translocations of chromosome 15 were used for regional mapping. These included the hybrid cell line CP22, which retains a human chromosome 21 and a partial chromosome 15 from 15pter-q14. Also included were the hybrids 8a (XVII-10A-8a) and 14a (XVII-10A-14a), provided by Dr. U. Francke (Oliver et al. 1978). The hybrid 8a carries the der(15),t(15,22) including the short arm and the proximal long arm from 15pter-q14. The hybrid 14a carries the der(22),t(15,22) including the distal long arm of chromosome 15 from 15q14-qter. All of these cell lines and hybrids were cultured in F12 medium supplemented with 10% FCS.

Lymphoblastoid cell lines from PWS patients with cytogenetically defined deletions were used for quantitative hybridization analysis. The lymphoblastoid cell lines DON5 and DON10, provided by Dr. S. Latt, were derived from patient donors having a small deletion in the 15q11.2 region (Donlon et al. 1986). The lymphoblastoid cell lines GM9024, GM9133, and GM9189, provided by Dr. M. Butler and the National Institute of General Medical Sciences' Human Genetic Mutant Cell Repository in

Camden, NJ, were derived from PWS patients carrying a large deletion from 15q11-q13 (Butler et al. 1986). Lymphoblastoid cell lines were cultured in RPMI 1640 medium supplemented with 2 mM glutamine and 10% or 15% FCS.

Lambda Phage and Bacterial Strains

The lambda phage used for library construction was the EMBL4 replacement vector designed by Frischauf et al. (1983). The nonselective bacterial host used for growing this phage was the *E. coli* strain LE392 (Young and Davis 1983). The lambda lysogens BHB2688 and BHB2690 were used for preparation of in vitro packaging extracts (Scalenghe et al. 1981; Maniatis et al. 1982). The phage clone 7-72 contains a low-copy insert isolated previously by screening a Charon-4A human genomic library for unique- and low-copy sequences (Kao et al. 1982).

DNA Isolation, Restriction Enzyme Digestion, Gel Electrophoresis, Filter Hybridization, and Autoradiography

The methods used for isolating DNA from mammalian cell lines were those described elsewhere (Gusella et al. 1979). Similar methods were used for isolating DNA from blood lymphocytes following separation from erythrocytes by centrifugation. The methods used for growing phage for isolation of vector DNA were essentially as described by Maniatis et al. (1982). The rapid method for isolating small quantities of phage DNA for restriction-enzyme digestion and Southern analysis has been described elsewhere (Kao et al. 1982). Restriction-enzyme digestions were carried out by procedures provided by the suppliers (Boehringer Mannheim, New England Biolabs, or International Biotechnologies, Inc.). DNA fragments were separated by agarose-gel electrophoresis. The concentration of the agarose gel used was determined on the basis of the desired resolution of DNA fragment size. Following electrophoresis, gels were stained with ethidium bromide (1.0 μg/ml), and DNA fragments were visualized and photographed under UV light. The DNA was transferred from gels to nitrocellulose or Gene Screen Plus® (New England Nuclear) filters by the method of Southern (1975). The same procedure was followed for hybridization with Gene Screen Plus® filters, except that the solutions were those described by the manufacturer (New England Nuclear). For whole-phage and genomic DNA, 0.2–0.5 μg was labeled with [^{32}P]-dCTP to a high specific activity of $\geq 1 \times 10^8$ cpm/μg DNA by nick-translation (Rigby et al. 1977) utilizing a commercial nick-translation kit (Bethesda Research Laboratory). Electroeluted fragments were labeled with [^{32}P]-dCTP to specific activities $>5 \times 10^8$ cpm/μg DNA by the random-primer oligonucleotide-labeling method of Feinberg and Vogelstein (1983).

Construction of a Partial Human Chromosome-15 Genomic Library

The procedure for library construction with DNA from the hybrid cell line 15A and the vector EMBL4 was similar to that described by Frischauf et al. (1983). In brief, high-molecular-weight DNA isolated from 15A containing a single human chromosome 15 was digested with the restriction enzyme *Mbo*I under conditions defined empirically to give randomly cut 20–25-kb restriction fragments. These fragments were ligated into vector arms prepared from the phage EMBL4, and the recombinant DNA molecules were packaged into phage particles in vitro as described elsewhere (Maniatis et al. 1982). An in situ plaque-hybridization method (Benton and Davis 1977) using [^{32}P]-labeled total human DNA as the probe was used to screen for recombinant phage containing human (but not Chinese hamster) DNA. Recombinants containing human DNA were distinguished by the presence of human-specific moderately repetitive sequences (Gusella et al. 1980).

Identification, Isolation, and Characterization of Chromosome 15–Specific Unique- and Low-Copy DNA Fragments

DNA isolated from recombinant phage containing human inserts was digested with *Eco*RI, *Hin*dIII, and *Ava*I. The DNA fragments were separated by electrophoresis as described, transferred to nitrocellulose, and hybridized with [^{32}P]-labeled total human DNA. Under these conditions, DNA fragments that were visible on the ethidium bromide–stained gel but did not exhibit a hybridization signal on the autoradiogram and were not part of the lambda genome represented unique- and low-copy sequences (Gusella et al. 1980). These fragments were isolated by electroelution from preparative agarose gels in which ~200 μg of digested recombinant-phage DNA was separated. To confirm the origin and to determine the complexity of the isolated restriction fragments, they were [^{32}P]-labeled and used as hybridization probes

on nitrocellulose filters containing restriction digests of DNA from the cell lines K1, HT, and the hybrid 15A.

In Situ Hybridization

In situ hybridization was similar to that of Harper and Saunders (1981) and has been used routinely in our laboratory (Law et al. 1986; Kao et al. 1987). In brief, human chromosomes were prepared from peripheral blood lymphocyte cultures synchronized with BrdU and thymidine and incubated with colcemid (0.1 μg/ml) for 10 min prior to harvest. Slides were treated with RNase (100 μg/ml) for 1 h at 37 C, rinsed in 2 × SSC, and dehydrated in ethanol. The chromosomes were denatured in 70% formamide/2 × SSC at 70 C for 2 min and dehydrated in ethanol. The probes were oligolabeled with [^{3}H]-dCTP, [^{3}H]-dATP, and [^{3}H]-TPP (New England Nuclear) (Feinberg and Vogelstein 1983). Hybridization was carried out at 37 C for 18 h in 50% formamide/2 × SSC at 41 C, then in 2 × SSC at 41 C, and then at room temperature, followed by dehydration in ethanol and air drying. Slides were coated with Kodak NTB2 nuclear-track emulsion, stored at 4 C for 14 days, and developed in Kodak Dektol. The slides were stained with Hoechst H33258 (1 μg/ml in 2 × SSC), rinsed and exposed under UV light, and then stained with 7% Giemsa (Fisher) in phosphate buffer at pH 6.8.

Screening for RFLPs

DNAs isolated from peripheral blood samples of eight unrelated individuals and from the hybrid cell line 15A were used in Southern blot analysis with each DNA marker as the probe. Eight restriction enzymes—including *Eco*RI, *Hin*dIII, *Bam*HI, *Pvu*II, *Hae*III, *Rsa*I, *Msp*I, and *Taq*I—were routinely used for all probes in the screening procedure.

Results

Library Construction and Identification of Chromosome 15–Specific Restriction Fragments

Twenty-four recombinant phage containing human DNA—and thus chromosome-15 material—were isolated after screening a partial phage library constructed from the cell hybrid 15A. Forty-two restriction fragments composed of unique- or low-copy sequences were identified in 14 phage by using the enzymes described. It was possible to identify multiple unique-sequence fragments in several phage and, in some cases, to identify unique-sequence fragments with more than one enzyme. Twenty-five of these fragments were isolated by electroelution from preparative agarose gels and analyzed for their chromosome-15 origin and complexity. The majority of the fragments characterized in this manner showed the hybridization pattern of a unique locus on chromosome 15. Several fragments showed hybridization patterns of low-copy loci in the genome, and at least one or all of these loci were specific to chromosome 15, including 23(1.5), 23(1.7), 33(1.7), 46(1.0), and 7-72. Two fragments, 33(1.7) and 46(1.0), cross-hybridized to hamster DNA, indicating possible sequence conservation in these species. (We designate the fragments as 23[1.5], etc., implying phage clone 23 [1.5-kb subfragment], etc.)

Regional Localization of Restriction Fragments to Chromosome 15 by Southern Blot Analysis Using a Hybrid Mapping Panel

DNA fragments were physically mapped to two specific regions of chromosome 15 by their hybridization pattern to a hybrid mapping panel of DNA. Given the chromosome-15 composition of this mapping panel, any marker detected in HT, 15A, CP22, and 8a but not in 14a was mapped to the 15pter-q14 region. Conversely, any marker detected in HT, 15A, and 14a but not in CP22 and 8a was mapped to the 15q14-qter region. This was particularly helpful for identifying phage with inserts from the proximal long arm region, as it has been reported that ribosomal DNA sequences, which compose the majority of the short arm of chromosome 15, are difficult to clone (Ray et al. 1985). The results of these mapping experiments are summarized in table 1.

In addition to the isolation of DNA restriction fragments to be used as probes for regional mapping to a hybrid mapping panel, entire phage were [^{32}P]-labeled and used as probes in hybridization at high stringency. High-stringency hybridization in the presence of 50% formamide at 54 C has been shown to prevent hybridization of Alu-type moderately repetitive sequences, which are present throughout the genome (Fisher et al. 1984). Under these conditions it was possible to rapidly map entire phage as described above, without the potentially laborious task of isolating a unique sequence from the phage insert (table 1). However, there are some limitations to this mapping procedure, probably reflecting the DNA sequence composition of the insert. In one case, loss of

Table 1

Regional Mapping with a Hybrid Mapping Panel

Phage Clone	Probe[a]	Enzymes Used to Produce Subfragments	Mapping Panel[b]						Map Position
			K1	HT	15A	CP22	8a	14a	
23	Whole phage		−	+	+	+	+	−	15pter-15q14
	1.7 kb	*Eco*RI	−	+	+	+	+	−	
	1.5 kb	*Eco*RI	−	+	+	+	+	−	
24	Whole phage		−	+	+	+	?[c]	?[c]	
28	Whole phage		−	+	+	+	+	−	
	1.0 kb	*Eco*RI	−	+	+	+	+	−	
33	Whole phage		+	+	+	+	+	−	
	1.7 kb	*Ava*I	+	+	+	+	+	−	
40	1.3 kb	*Eco*RI	−	+	+	+	+	−	
46	1.0 kb	*Ava*I	+	+	+	+	+	−	
54	2.5 kb	*Ava*I	−	+	?[d]	+	?[d]	−	
74	Whole phage		−	+	+	+	+	−	
85	2.4 kb	*Hin*dIII	−	+	+	+	+	−	
7-72	Whole phage		−	+	+	+	+	−	
18	Whole phage		−	+	+	−	−	+	15q14-15qter
20	3.7 kb	*Hin*dIII	−	+	+	−	−	+	
21	Whole phage		−	+	+	−	−	+	
22	Whole phage		−	+	+	−	−	+	
24	1.0 kb	*Hin*dIII	−	+	+	−	−	+	
42	2.2 kb	*Hin*dIII	−	+	+	−	−	+	
83	4.1 kb	*Hin*dIII	−	+	+	−	−	+	
84	Whole phage		−	+	+	−	−	+	
	1.0 kb	*Eco*RI	−	+	+	−	−	+	

[a] Either whole phage or restriction fragments isolated from phage inserts were used as hybridization probes.

[b] Cell lines used in the mapping panel were as described in Material and Methods.

[c] Low-copy loci identified by the recombinant phage 24 may map to both regions of chromosome 15.

[d] Owing to the complexity of the insert, it was not possible, with certainty, to regionally map this phage or restriction fragment to either of these chromosomal regions.

hybridization signal altogether was observed at the high-stringency hybridization of 54 C, suggesting that the human insert was composed entirely of repetitive elements with low homology in the genome. In another case, it was not possible to effectively reduce the signal from repetitive elements by hybridization at 54 C, suggesting the presence in the genome of repetitive elements with considerable homology but distinct from the Alu family, which was shown not to hybridize under these conditions (Fisher et al. 1984). The limitation inherent in the regional mapping of whole phage with such homologous repetitive elements may be overcome by using a mapping panel of hybrids with few human chromosomes.

Regional Mapping by In Situ Hybridization

To determine more specifically the regional map position of markers on chromosome 15, DNA fragments were hybridized to metaphase chromosomes in situ. The DNA markers used as probes for in situ hybridization included several that previously had been shown, by Southern blot analysis with the hybrid mapping panel, to map to the proximal long arm region from 15pter-q14; these included the markers 85(2.4), 7-72, 28(1.0), and 23(1.7). The markers 20(3.7) and 83(4.1), which mapped to the more distal long arm region from 15q14-qter, were also included, as these markers were shown to reveal RFLPs (see below). The results of the in situ hybridization with each of the six DNA markers described above are presented in figure 1. These results were consistent with the regional mapping using the hybrid panel (table 1) and further localized the markers to more refined regions on chromosome 15.

Regional Mapping by Dosage Analysis

An approach that relies on the availability of cells from PWS patients with cytogenetically defined dele-

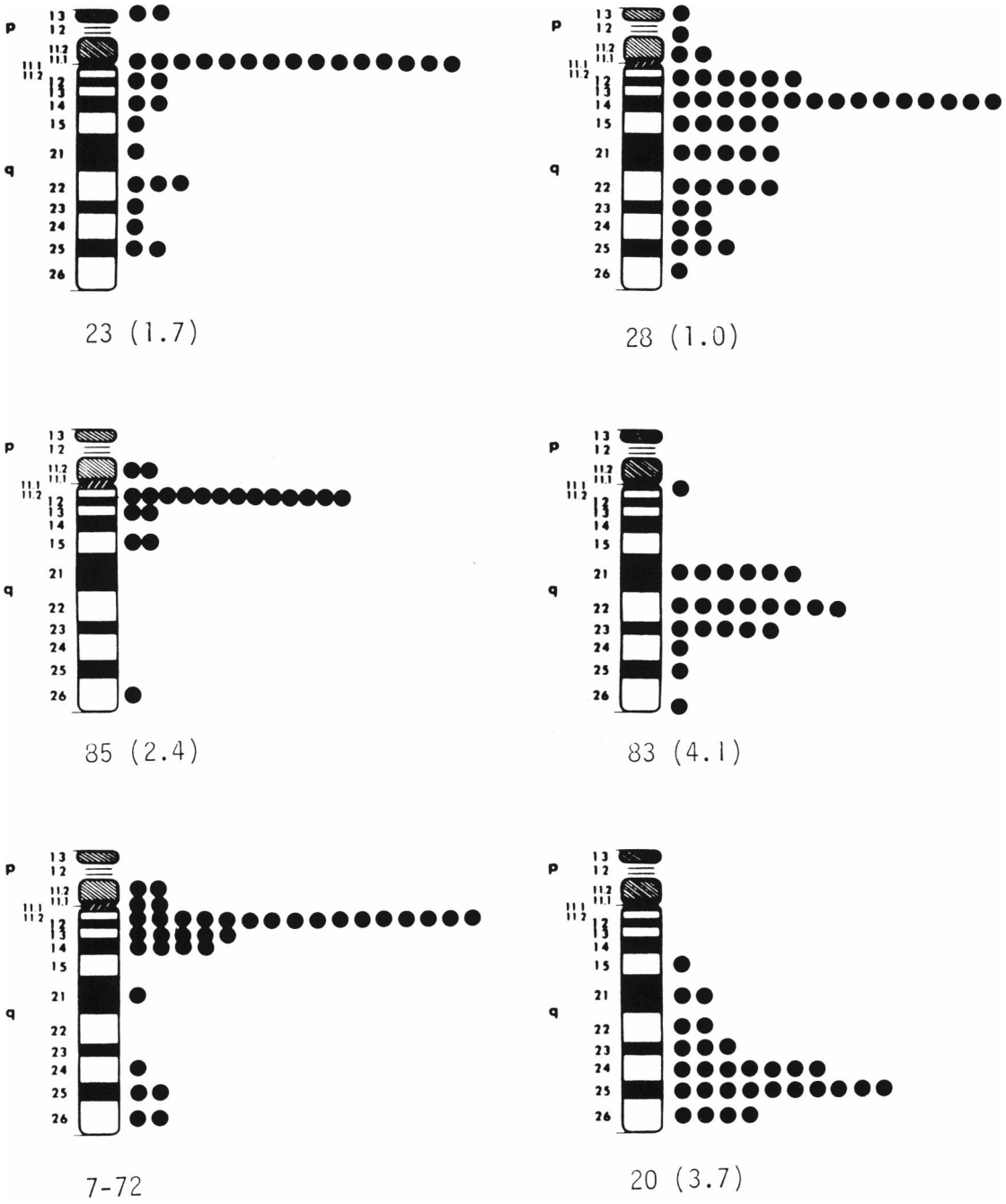

Figure 1 [^{3}H]-labeled probes were hybridized to metaphase chromosomes from normal human lymphocytes, and the distribution of grains on chromosome 15 was established, which led to a peak of hybridization indicating the location of the probe on the chromosome. The background of grains in these experiments ranged between three and seven grains per spread, and the specific labeling of chromosome 15 among all cells examined was 15%–20%. For regional localization of chromosome 15–specific probes, only spreads with grains on chromosome 15 were analyzed in detail. Twenty-five cells were analyzed for probe 23(1.7), 15 cells for 85(2.4), 32 cells for 7-72, 45 cells for 28(1.0), 18 cells for 83(4.1), and 25 cells for 20(3.7). The mapping positions for these probes are as follows: 15cen for 23(1.7), 15q11-q12 for 85(2.4) and 7.72, 15q11-q14 for 28(1.0), 15q21-q23 for 83(4.1), and 15q24-q26 for 20(3.7).

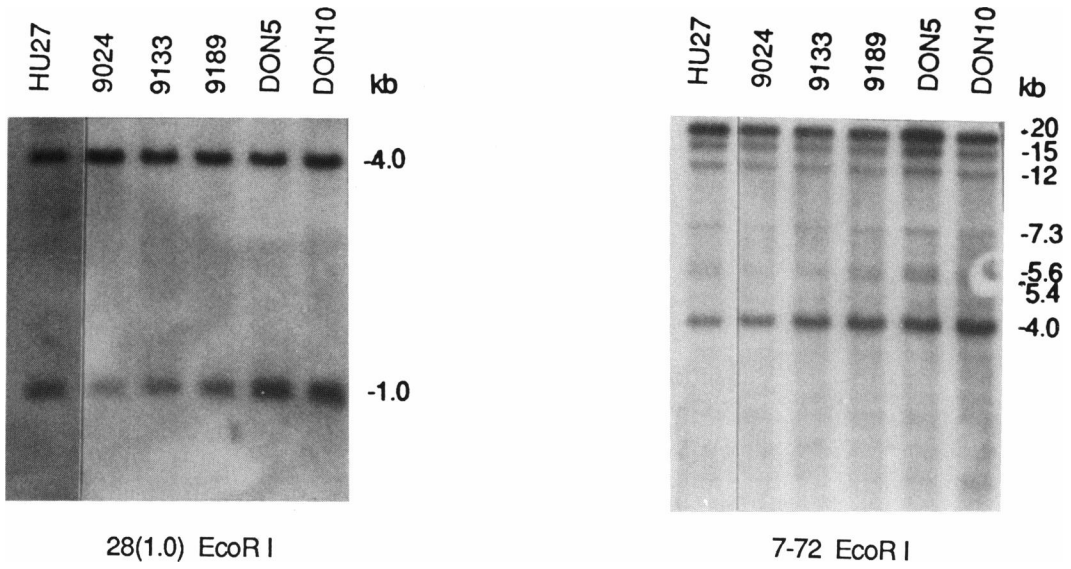

Figure 2 Dosage analysis with DNA markers in the proximal long arm region of chromosome 15. *Left,* The DNA marker 28(1.0) was used as a probe in quantitative Southern hybridization analysis with *Eco*RI-digested DNA from a normal individual (HU27), PWS patients with a large 15q11-q13 deletion (9024, 9133, 9189), and PWS patients with a small 15q11.2 deletion (DON5, DON10). *Right,* A similar analysis was performed with the marker 7-72 as probe. At least four hybridization experiments with each probe were performed to confirm the results. The 4.0-kb band was an internal control by hybridization to the marker 84(1.0) mapped to the distal long arm of chromosome 15, region q14-qter.

tions within the 15q11-q13 region of one chromosome-15 homologue was used to confirm and refine the regional map position of markers in this region. DNA from normal individuals, which have two homologues of chromosome 15, has two copies of any given locus on chromosome 15, while DNA from PWS patients with a deletion in one chromosome-15 homologue would have only one copy of the locus in the deletion region of chromosome 15. For Southern analysis with a probe in the deletion region, the hybridization signal with PWS DNA should be half the intensity of that for DNA from a normal individual. Also included in each hybridization was the chromosome-15 marker 84(1.0), shown to map to the more distal long arm region of 15q14-qter. This probe was used as an internal diploid standard for comparison.

The results of these experiments (fig. 2) confirm the in situ map position of markers 28(1.0) and 7-72. The marker 28(1.0) showed reduced dosage in PWS patients GM9024, GM9133, and GM9189, who had a large deletion in the 15q11-q13 region, but not in PWS patients DON5 and DON10, who had a small deletion in the 15q11.2 region. Similar results were seen for the three *Eco*RI loci of 7.3, 5.6, and 5.4 kb that were identified by the marker 7-72 on chromosome 15. These loci also showed reduced dosage in patients with the large 15q11-q13 deletion—but not in those patients with the smaller 15q11.2 deletion. Thus, these dosage results further localized probes 28(1.0) and 7-72 to 15q11.2-q13.

The results for the other markers were not conclusive and need to be studied further. The probes 85(2.4) and 23(1.7) did not appear to show reduced dosage in PWS patients with any sized deletions (data not shown), suggesting a map position very close to the centromere and possibly in 15q11.1. It should be mentioned that these types of experiments can be very ambiguous, depending on the uniformity of DNA transfer and hybridization, and should be repeated several times before reaching a conclusion. The alternative to using dosage analysis for regional mapping with naturally occurring deletions involves isolation of the deleted chromosome 15 from its cytogenetically normal homologue by fusion of the patients' cells with rodent cells. While this procedure can be time consuming and might not be adopted as a general practice, it is superior in the interpretation of results, as only the absence of a hybridization signal must be ascertained, rather than differences in hybridization intensity of 2:1.

Table 2

RFLP Analysis

Marker, Physical Map Position,[a] and RFLP Enzyme	Chromosomes Analyzed	Allele Frequencies
20(3.7), 15q24-q25:		
*Msp*I	17	.65/35
*Hin*dIII	17	.765/.235
*Bam*HI	17	.765/.235
*Hae*III	17	.765/.235
*Pvu*II	17	.765/.235
23(1.7), 15cen:		
*Eco*RI	17	.70/.30
83(4.1), 15q22:		
*Hin*dIII	20[b]	.85/.15
*Bam*HI	17	.941/.059
85(2.4), 15q11-q12:		
*Hae*III	17	.59/.41

[a] As determined by hybrid mapping panel analysis and in situ hybridization.

[b] Additional chromosomes were included because polymorphic alleles were found in the regional mapping study with *Hin*dIII-digested clone-panel DNA.

Identification of Markers Revealing RFLPs

Unique- and low-copy DNA markers specific to chromosome 15 were used as probes to detect RFLPs in Southern blot analysis with DNA from eight unrelated individuals and the hybrid 15A. The markers and the restriction enzymes that revealed useful polymorphisms are listed in table 2; allele frequencies and physical locations are also indicated. The hybridization patterns of these loci are shown in figure 3.

In figure 3*a* it was apparent that the polymorphic alleles identified by 20(3.7) with enzymes *Hin*dIII, *Bam*HI, *Hae*III, and *Pvu*II were each characterized by a 200-bp size difference between alleles, suggesting that the RFLP revealed by 20(3.7) with these enzymes was an insertion/deletion polymorphism. This interpretation was supported by the observation that the genotypes of individuals 3 and 9 and of the hybrid 15A were the same with all four restriction enzymes. The polymorphism identified with the enzyme *Msp*I was of the more common type, owing to a change in the restriction-enzyme site.

The DNA marker 23(1.7) identified two polymorphic *Eco*RI alleles of 2.9 and 3.1 kb (fig. 3*c*). These alleles also appeared to show polymorphism in the hybridization intensity among different individuals. The variation in hybridization intensity of these alleles, as well as the broad hybridization signal seen in some individuals, may be interpreted as being due to the presence of a minisatellite, composed of short tandemly repeated core sequences, in these fragments (Jeffreys et al. 1985).

It was difficult to determine the frequency of the polymorphic alleles identified by the marker 85(2.4) for *Hae*III-digested DNA (fig. 3*d*). This probe detected a nonpolymorphic 3.2-kb *Hae*III allele and an apparently polymorphic 0.4-kb allele (or more than one 0.4-kb allele, depending on hybridization intensity). In individuals 2, 3, 5, 7, and 9 and in the hybrid 15A it was also possible to detect a 0.6-kb allele with a hybridization intensity less than that of the 0.4-kb alleles. This hybridization pattern suggested that in some individuals one of the 0.4-kb fragments became a 0.6-kb fragment owing to an *Hae*III restriction site polymorphism, resulting in a heterozygous individual with three or more copies of the 0.4-kb allele and one copy of the 0.6-kb allele. The presence of the 0.6-kb allele was confirmed by stringent washing of the original filter as this fragment became even more distinct. However, owing to the inability to resolve the 0.4-kb *Hae*III alleles, the frequencies of the 0.4- and 0.6-kb alleles as shown in table 2 were an approximation.

Discussion

The combined approaches of somatic cell genetics and molecular cloning techniques were used to isolate and map DNA markers specific to human chromosome 15. A partial library constructed from the

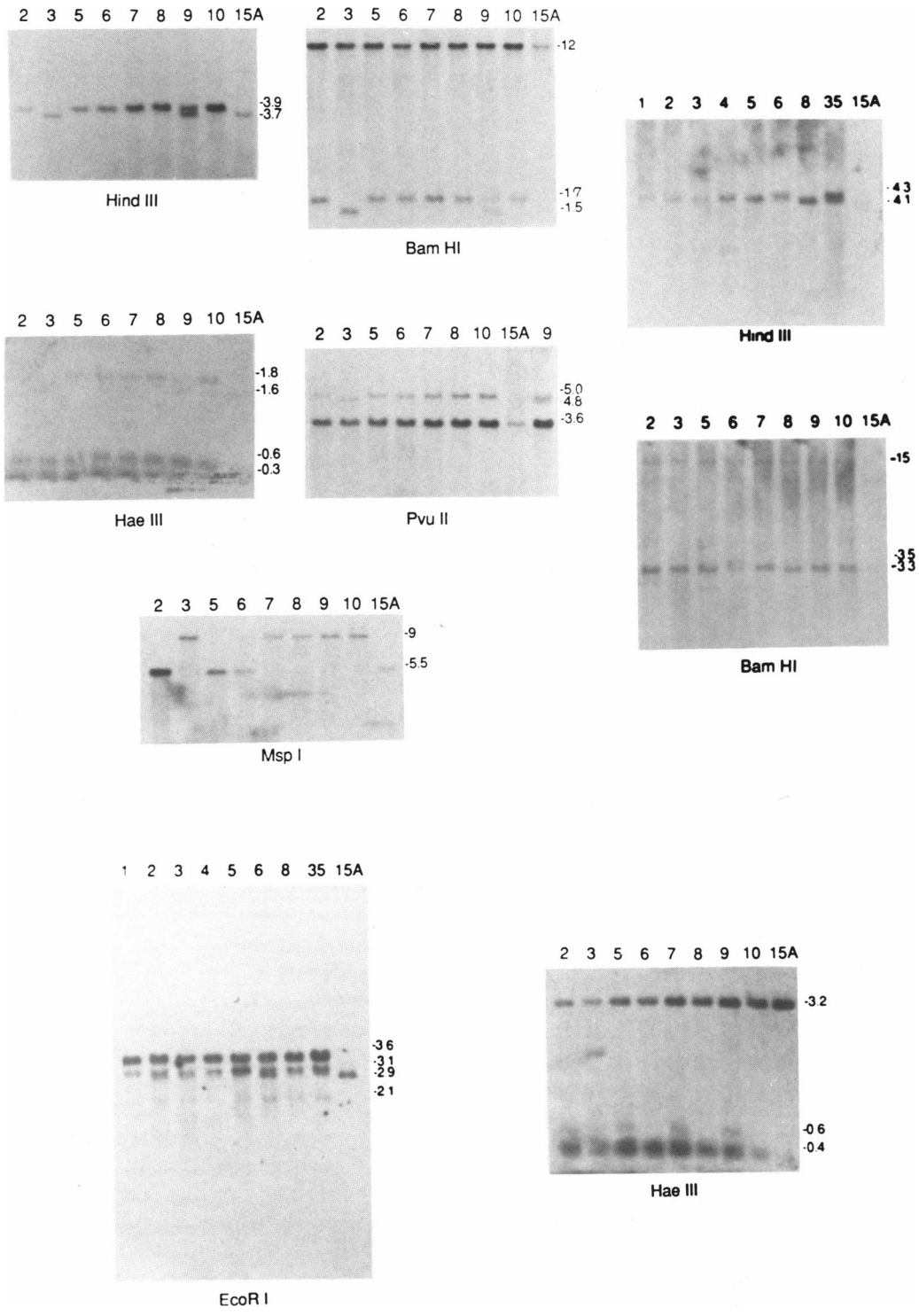

Figure 3 Screening for RFLPs with chromosome 15–specific markers by Southern blot analysis. In panel *a*, the *Hin*dIII marker 20(3.7) identified polymorphic alleles of 3.9 and 3.7 kb with *Hin*dIII, of 1.7 and 1.5 kb with *Bam*HI, of 1.8 and 1.6 kb with *Hae*III, of 5.0 and 4.8 kb with *Pvu*II, and of 9.0 and 5.5 kb with *Msp*I. In panel *b*, the *Hin*dIII marker 83(4.1) identified polymorphic alleles of 4.1 and 4.3 kb with *Hin*dIII and of 3.3 and 3.5 kb with *Bam*HI. In panel *c*, the *Eco*RI marker 23(1.7) identified polymorphic alleles of 3.1 and 2.9 kb with *Eco*RI. In panel *d*, the *Hin*dIII marker 85(2.4) identified polymorphic alleles of 0.4 and 0.5 kb with *Hae*III. Note that the most frequent polymorphic allele (table 2) is listed first for each marker.

hybrid 15A, which contained an intact human chromosome 15, made it theoretically possible to isolate markers representing the entire chromosome. Such markers should be useful in linkage analysis because of their ability to identify unique, polymorphic loci covering the entire chromosome. For chromosome 15, comprising ~100 cM, a minimum of three evenly spaced, highly polymorphic markers would be required to construct a complete linkage map of the chromosome, as described elsewhere (Botstein et al. 1980; Drayna et al. 1984; White et al. 1985). This would require screening many chromosome 15–specific markers for their ability to detect RFLPs and for their physical map position. Each of the four markers 20(3.7), 83(4.1), 23(1.7), and 85(2.4) reveals polymorphic loci on chromosome 15. Each marker identifies alleles that are anticipated to be informative in family linkage analysis.

The *Hae*III polymorphism detected by the DNA marker 85(2.4), which maps to the 15q11-q12 region, and the *Eco*RI polymorphism detected by the DNA marker 23(1.7), which maps to the centromeric region, will be useful for linkage analysis in the proximal long arm of chromosome 15. For example, a tentative assignment for one form of dyslexia, a very common reading disorder (Vellutino 1987), was made to the proximal long arm region of chromosome 15 (Smith et al. 1983). This assignment was obtained on the basis of family analysis, which showed linkage between centromeric heteromorphisms of chromosome 15 and dyslexia, with LOD scores >3 (Smith et al. 1983). Expansion of this study to include more families resulted in LOD scores <3, suggesting either heterogeneity in the inheritance of dyslexia or a nongenetic component. Further testing with two polymorphic DNA markers, D15S1 and D15S2, which map to the q14-q21 and q15-q22 regions of chromosome 15, respectively, did not confirm the linkage, presumably because the loci were too distal from the putative dyslexia locus, which is thought to lie proximal to the centromere (Smith et al. 1986). The markers 85(2.4) and 23(1.7) might be very useful for establishing a linkage with chromosome 15 and one form of dyslexia. Furthermore, DNA markers near the centromeric region may be particularly suitable for linkage analysis, as the heterochromatic constitution of this region precludes meiotic crossover (Miklos and John 1979).

The polymorphic markers 85(2.4) and 23(1.7) could also be useful in determining the parental origin of chromosome-15 abnormalities associated with PWS. Linkage analyses, using heteromorphisms of both the centromere and the short arm of chromosome 15 such as the heteromorphism described above, were carried out to determine a paternal origin of the de novo deletion of chromosome 15 in PWS patients (Butler and Palmer 1983; Butler et al. 1986). One of the shortcomings of using cytogenetic heteromorphisms for linkage analysis is that not all individuals are informative for these heteromorphisms, thus limiting the analysis. The use of informative polymorphic DNA markers in this region of chromosome 15 should overcome this restriction. The markers 20(3.7) and 83(4.1), which map to 15q24-q26 and 15q21-q23, respectively, identify compound polymorphic loci with more than one restriction enzyme (table 2) and should be useful for establishing linkage for genes in the distal long arm region of chromosome 15.

To understand the molecular and biochemical basis of the phenotype associated with PWS, it is necessary to identify the functional gene(s) associated with the disorder. The development of DNA markers specific to this region of chromosome 15 represents a first step in an attempt to understand the complex molecular and genetic basis of this disorder. Several DNA markers with loci in the proximal long arm region of chromosome 15 have been isolated and characterized in the present study; these include the markers 85(2.4), 28(1.0), 7-72, 23(1.5), 23(1.7), 46(1.0), 33(1.7), 40(1.3), and 54(2.5). Three markers—85(2.4), 28(1.0), and 7-72—were shown by clone-panel analysis and by in situ hybridization to map to the 15q11-q13 region. Dosage analysis confirmed the map position of 28(1.0) within this region and also confirmed the map position of several other chromosome-15 loci identified by 7-72. Dosage analysis suggested that the polymorphic marker 85(2.4) may actually flank the deletion region and lie proximal to the centromere. These markers may themselves be valuable for molecular analysis and diagnosis of PWS or be useful in the development of additional markers for such purposes.

It has been suggested that the proximal long arm of chromosome 15 may contain inverted repeats and/or palindromic sequences that are involved in the cytogenetic behavior of this region (Cavalier-Smith 1974; Lejeune et al. 1979; Donlon et al. 1986). Although our studies have shown no direct evidence for the presence of inverted repeats, some indication of a

chromosomal region rich in repetitive sequences has been evident (Donlon et al. 1986). In the present study, DNA fragments isolated from the proximal long arm region appeared to have more complex genomic structures and in some cases showed unusual patterns of hybridization (data not shown). Contrary to these results, markers that were characterized and mapped to the distal long arm of chromosome 15 were all of the unique-copy class. Perhaps repeated elements, unique to the palindromic sequences and inverted-repeat class, are related to the cytogenetic activity of this chromosome. The multiplicity of chromosome-15 rearrangements associated with PWS—including deletions, balanced and unbalanced translocations, extra chromosome 15–derived material (such as SBACs), and apparently normal chromosomes—indeed suggests a more fundamental cause, such as transposition, in the etiology of PWS (Riccardi 1981). Recently it has been suggested that one role for the genome's moderately repetitive sequences—especially for the SINES (including Alu) and the LINES (including L1)—is that of a retroposon or transposable element in the genome (Finnegan et al. 1986; Weiner et al. 1986).

The potential to study human chromosome 15 at a molecular level is very exciting. Analysis of the PWS through studies on the proximal long arm of human chromosome 15 may provide important insights into the organization of the genome in this region, especially with respect to the role of repetitive elements. The technology is now available to identify genetic loci and gene products associated with human disease (Cavanee et al. 1984; Monaco et al. 1985, 1986; Friend et al. 1986; Hoffman et al. 1987; Lee et al. 1987). Once these genes are isolated, it will be possible to analyze their genomic structure and regulation and to identify the mutations that lead to disease.

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