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# Regional Assignments of Three Polymorphic DNA Segments on Human Chromosome 15

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Hybridization of probe pDP151 (locus *D15S2*) to genomic human DNAs digested with EcoRI revealed allelic restriction fragments 9 and 11 kilobase-pairs (kb) in length. Hybridization of pDP151 to EcoRI-digested DNAs from 21 Chinese hamster × human hybrid cell clones containing different subsets of human chromosomes demonstrated cosegregation of the 9 and 11 kb EcoRI fragments with human chromosome 15. *D15S2* and two other polymorphic loci previously mapped to chromosome 15—*D15S1* and *D15S6*—were localized to specific regions on human chromosome 15. Eight Chinese hamster × human somatic cell hybrid clones derived from a human donor heterozygous for a balanced translocation between chromosomes 15 and 22 [t(15;22)(q14;q13.3); Oliver et al, Cytogenet Cell Genet 22:503–510, 1978] were studied. After digestion of human and hybrid DNAs with HindIII and Southern blotting, pDP151 (*D15S2*) and pMS1-14 (*D15S1*) hybridized to fragments of 4 and 4.5 kb, respectively. Further, pMS1-14 (*D15S1*) and p9-1a (*D15S6*) hybridized to EcoRI fragments of 3.5 and 3.2 kb. All fragments cosegregated with the der(22) derivative chromosome containing region 15q14→15qter. In situ hybridization of these probes to normal human chromosomes mapped the corresponding loci with greater precision: *D15S1* to 15q15→15q21, *D15S2* to 15q15→15q22, and *D15S6* to 15q22→15q24.

**Key words: DNA markers, somatic cell hybrids, in situ hybridization**

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## INTRODUCTION

Unique DNA sequences mapped to specific chromosomal regions, especially if polymorphic (restriction fragment length polymorphisms; RFLP) [Botstein et al, 1980] can provide useful markers for genetic disorders and tools for chromosome analysis. RFLPs can result from sequence variation in human DNA caused by insertion or deletion of DNA sequences of variable length, as has been seen at the *D14S1* locus [Wyman and White, 1980]. RFLPs more commonly arise from single base pair changes that alter the recognition sites of restriction endonucleases [Barker et al, 1984]. Linkage relationships between RFLP markers and a specific genetic disorder can then be established. If a genetic disorder is not associated with a distinct biochemical or chromosomal abnormality, but is closely linked to the polymorphic DNA sequence, the DNA segment itself can become a marker for the disease in preclinical diagnosis, carrier detection, and prenatal genotype prediction. Furthermore, mapped arbitrary DNA sequences can be used as markers for the identification of chromosomal regions involved in complex rearrangements and in unidentified "marker" chromosomes.

We report here the detection of an EcoRI RFLP by probe pDP151 and the assignment of the homologous locus (*D15S2*) to human chromosome 15 using Southern blot analysis of DNA from Chinese hamster × human somatic cell hybrid clones containing different subsets of human chromosomes. We have carried out regional assignments of DNA sequences recognized by probes pDP151, pMS1-14 (locus *D15S1*) [de Martinville et al, 1983], and p9-1a(12) (locus *D15S6*; Sheer et al, unpublished results), by analysis of hybrids with rearranged human chromosomes 15, and by in situ hybridization to normal metaphase chromosomes. The results suggest a linear order of these DNA loci on the chromosome that can now be tested by family linkage studies.

## MATERIALS AND METHODS

### DNA Probes

The derivation of pMS1-14 has been described elsewhere [Barker et al, 1984]. The probe pMS1-14 is a 2.9 kb random human sequence subcloned in pBR322. The corresponding locus has been mapped to chromosome 15 by Southern blot analysis of somatic cell hybrids [de Martinville et al, 1983]. The probe detects a two-allele MspI polymorphism with a PIC of 0.37 [Barker et al, 1984]. Plasmid pDP151 consists of a 2.6 kb random genomic EcoRI-HindIII restriction fragment isolated from a human library [Lawn et al, 1978] and subcloned into pBR322. Locus *D15S2*, identified by probe pDP151, was included in Table IV, Polymorphic DNA Clones, in the Report of the Committee on Human Gene Mapping by Recombinant DNA Techniques in Human Gene Mapping 7 [Skolnick et al, 1984] but has not otherwise been reported. p9-1a(12) was subcloned from cosmid 9-1a as a random PstI fragment ~ 1 kb in size in pAT153. The corresponding locus, called *D15S6*, has been mapped to 15pter→15q22 (Sheer et al, unpublished results). RFLPs near this locus are presently being looked for.

### Hybrid Cell Lines

DNA was prepared from 21 different Chinese hamster × human hybrid cell clones containing different subsets of human chromosomes and derived from six

series of somatic cell hybrids. Two different Chinese hamster cell lines (V79/380-6 and Don/a23) were fused with diploid human cells (either fibroblasts or leukocytes) from six human donors who carried defined chromosome rearrangements [Francke et al, 1976; Francke and Pellegrino, 1977; Oliver et al, 1978; Francke and Francke, 1981; Francke, 1984]. The human chromosome contents of the hybrids were defined by chromosome analysis and enzyme marker and DNA marker studies. Hybrid series XVII used for regional mapping of genes on chromosome 15 was derived from a human donor with a balanced reciprocal translocation between chromosomes 15 and 22, t(15;22)(q14;q13.3) [Oliver et al, 1978]. When these clones were expanded in culture, their chromosome content was reconfirmed by karyotype analysis.

### Southern Blot Analysis

High-molecular-weight DNAs were purified from human leukocytes and fibroblasts and from human × Chinese hamster hybrid cell lines as previously described [de Martinville et al, 1982]. DNA samples were digested to completion with restriction endonucleases EcoRI and/or HindIII (New England Biolabs) and analyzed by Southern filter hybridization [Southern, 1975]. The DNA probes were nick-translated with $^{32}$P-labeled nucleotides [Rigby et al, 1977]. Hybridization was performed in 5X SSC for 16 hr at 65°C, followed by washing twice for 10 min each in 2X SSC + 0.1% SDS and 0.1X SSC + 0.1% SDS at the same temperature (1X SSC = 0.15 M NaCl, 0.015 M sodium citrate; SDS = sodium dodecyl sulphate).

### In Situ Hybridization

For in situ hybridization, normal human metaphase spreads were prepared from methotrexate synchronized peripheral lymphocyte cultures. The three chromosome 15-specific probes were nick-translated with three tritium-labeled nucleotides ([$^3$H]dATP, [$^3$H]dCTP, [$^3$H]dTTP) to specific activities of $2 \times 10^7$ cpm/$\mu$g as described [Münke et al, 1984]. The procedures of hybridization and staining with quinacrine and Wright stain were as described elsewhere [Harper and Saunders, 1981; Kirsch et al, 1982]. Between 61 and 105 metaphase cells from two individuals were examined after hybridization with each of the probes.

## RESULTS

A two-allele RFLP was detected by hybridizing probe pDP151 to EcoRI-digested human genomic DNAs. Among 66 unrelated U.S. white individuals examined, 32 exhibited a single homologous EcoRI fragment of 9 kb (eg, Fig. 1A, lane 1), 30 had homologous EcoRI fragments of 9 and 11 kb (eg, Fig. 1A, lane 13), and four had a single homologous EcoRI fragment of 11 kb. These results suggested that the 9 and 11 kb EcoRI fragments are allelic and have frequencies of 0.7 and 0.3, respectively. Codominant Mendelian inheritance of the 9 and 11 kb EcoRI fragments was observed in families (data not shown). Since no RFLP was observed following hybridization of pDP151 to human genomic DNAs digested with other enzymes, such as MspI, TaqI, HindIII, and BamHI, we assume that the two-allele EcoRI RFLP is due to base pair substitution.

Probe pDP151 when hybridized to EcoRI cleaved Chinese hamster × human hybrid DNA revealed a much smaller cross-reacting Chinese hamster-specific fragment (not shown) in addition to the 9 and 11 kb human fragments. The human-

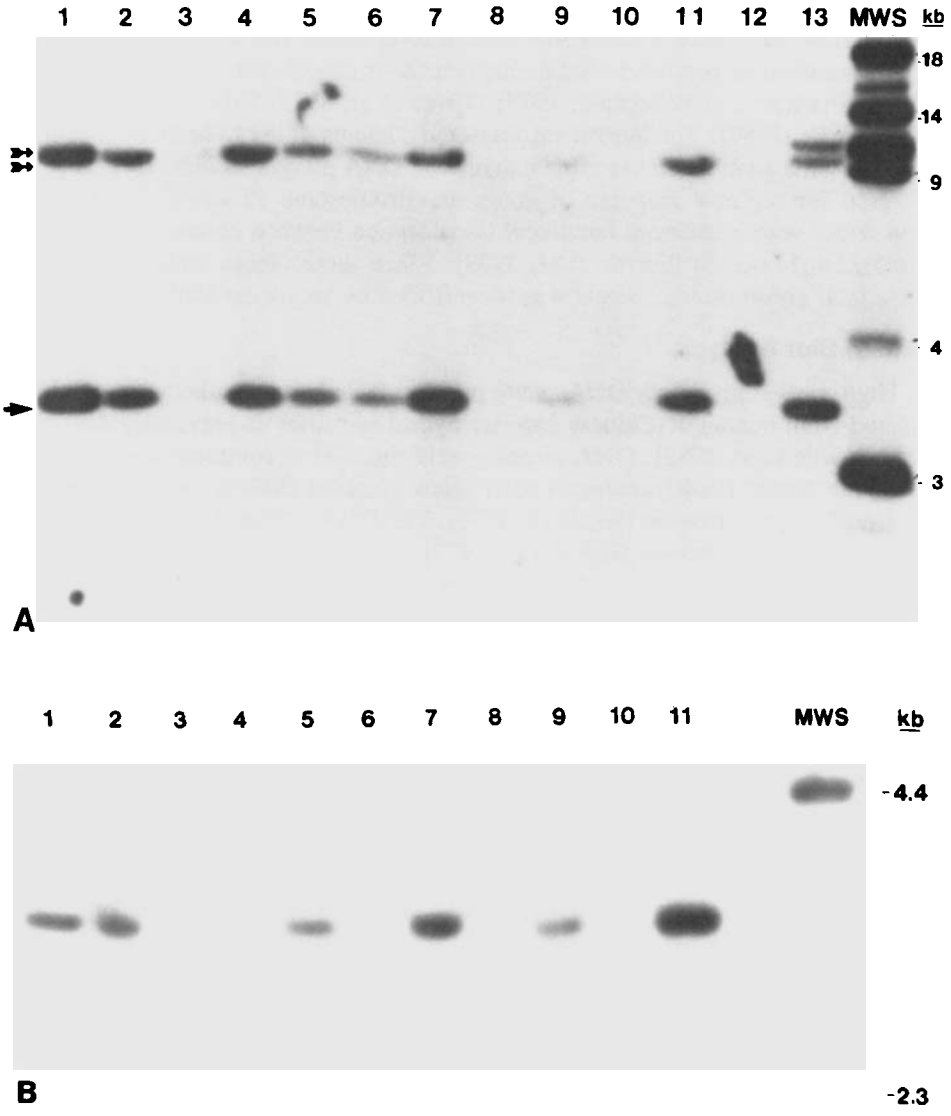


Fig. 1. A, mapping of *D15S1* and *D15S2* to human chromosome 15. Probes pMS1-14 and pDP151 were hybridized together to human, rodent, and hybrid DNA cleaved with EcoRI. In human DNA samples (lanes 1 and 13), a single fragment of ~3.5 kb hybridized with pMS1-14 (single arrow). pDP151 revealed fragments of 9 kb (lane 1) and 9 plus 11 kb (lane 13), demonstrating the polymorphism revealed by this probe (double arrow). The human t(15;22) donor of hybrid series XVII was homozygous for the 9 kb fragment (lane 7). Hybrid clones in lanes 2, 4, 5, 6, 9, and 11 were positive with both probes. Hybrids in lanes 4 and 9 had region 15q14→qter as the only chromosome 15 material. Hybrids in lanes 3 and 10 lacked the 15q14→qter derivative but carried the 15pter→q14 derivative chromosome and were negative for the human fragment. The hybrid in lane 8 had no chromosome 15 material and did not show either human fragment. Lane 12 contained Chinese hamster DNA. All lanes were loaded with 10 μg DNA. B, regional mapping of *D15S6*. Hybridization of p9-la(12) to human, rodent, and hybrid DNA cleaved with EcoRI. Probe p9-la(12) hybridized to single human-specific fragment of 3.2 kb (lanes 1 and 11). Hybrid samples in lanes 2, 5, 7 and 9 were positive for the human fragment; those in lanes 3, 4, 6, and 10 were negative. Positive lanes contained the 15q14→15qter region.

specific fragments cosegregated with human chromosome 15 in all 20 hybrid clones informative for chromosome 15 (Table I). The pDP151 fragment segregated discordantly with all other human chromosomes in at least 21% of the hybrids. These results assign *D15S2* to human chromosome 15.

The DNA segments homologous to pDP151, pMS1-14 and p9-1a, were regionally localized on chromosome 15 using hybrids that contained defined parts of chromosome 15 [Oliver et al, 1978] (Table II). DNA was cleaved to completion with EcoRI. The human donor was homozygous for the 9 kb fragment revealed by pDP151 (Fig. 1A). EcoRI fragments of 3.5 kb for pMS1-14 (Fig. 1A), and 3.2 kb for p9-1a (Fig. 1B) were revealed. When cleaved with HindIII, fragments of 4 kb for pDP151 (Fig. 2A) and 4.5 kb for pMS1-14 (Fig. 2B) were observed. A weak Chinese hamster fragment was observed only with probe pDP151 (Fig. 2A). The human fragments detected by all three probes cosegregated with the human derivative chromosome containing region 15q14→qter (Table II).

In situ hybridization of each of the three probes was carried out in two experiments using chromosomes from different donors (Fig. 3). In situ hybridization of $^{3}$H-

**TABLE I. Correlation of Human pDP151 Sequences With Human Chromosomes in 21 Chinese Hamster × Human Somatic Cell Hybrids**

| Hybridization/ chromosome | Human chromosomes[a] | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | X | Y |
| +/+ | 3 | 5 | 8 | 6 | 6 | 3 | 4 | 9 | 4 | 3 | 5 | 8 | 6 | 8 | 10 | 7 | 4 | 8 | 7 | 8 | 7 | 11 | 4 | 5 |
| −/− | 6 | 7 | 5 | 9 | 7 | 6 | 10 | 6 | 9 | 8 | 5 | 7 | 5 | 1 | 10 | 3 | 10 | 6 | 7 | 5 | 6 | 2 | 1 | 8 |
| +/− | 6 | 6 | 2 | 3 | 5 | 4 | 6 | 1 | 5 | 7 | 5 | 3 | 5 | 2 | 0 | 4 | 7 | 3 | 4 | 3 | 3 | 0 | 3 | 6 |
| −/+ | 3 | 3 | 4 | 1 | 3 | 4 | 0 | 4 | 1 | 2 | 4 | 2 | 5 | 4 | 0 | 7 | 0 | 4 | 2 | 5 | 4 | 7 | 2 | 2 |
| Discordant hybrids | 9 | 9 | 6 | 4 | 8 | 8 | 6 | 5 | 6 | 9 | 9 | 5 | 10 | 6 | 0 | 11 | 7 | 7 | 6 | 8 | 7 | 7 | 5 | 8 |
| Informative hybrids | 18 | 21 | 19 | 19 | 21 | 17 | 20 | 20 | 19 | 20 | 19 | 20 | 21 | 15 | 20 | 21 | 21 | 21 | 20 | 21 | 20 | 20 | 10 | 21 |
| Percent discordant | 50 | 43 | 32 | 21 | 38 | 47 | 30 | 25 | 32 | 45 | 47 | 25 | 48 | 40 | 0 | 53 | 33 | 33 | 30 | 38 | 35 | 35 | 50 | 38 |

[a]Only intact human chromosomes present in at least 10% of cells were included. Data on rearranged chromosomes or chromosomes present in less than 10% were excluded.

**TABLE II. Regional Mapping of *D15S1, D15S2,* and *D15S6* on Human Chromosome 15**

| Series XVII hybrid subclone | Human chromosome 15 regions present (average copy No./cell) | | | Hybridization signal | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | HindIII | | EcoRI | | |
| | Normal 15 | 15q14→ qter | 15pter→ q14 | D15S1 pMS | D15S2 pDP | D15S1 pMS | D15S2 pDP | D15S6 9-1a |
| XVII-10A | 0.6 | 0.7 | 0.4 | + | + | + | + | + |
| XVII-10A-11a | 0.6 | 0.2 | 0 | + | + | + | + | + |
| XVII-10A-13a | 0 | 0.8 | 0.2 | + | + | + | + | + |
| XVII-10A-14a | 0 | 0.7 | 0 | + | + | + | + | + |
| XVII-18B-2a | 0 | 0.3 | 0 | (+)[a] | (+) | (+) | (+) | (+) |
| XVII-10A-1a | 0 | 0 | 0.5 | − | − | − | − | − |
| XVII-10A-8a | 0 | 0 | 0.6 | − | − | − | − | − |
| XVII-18B-10a | 0 | 0 | 0 | − | − | − | − | − |

[a](+), weak signal.

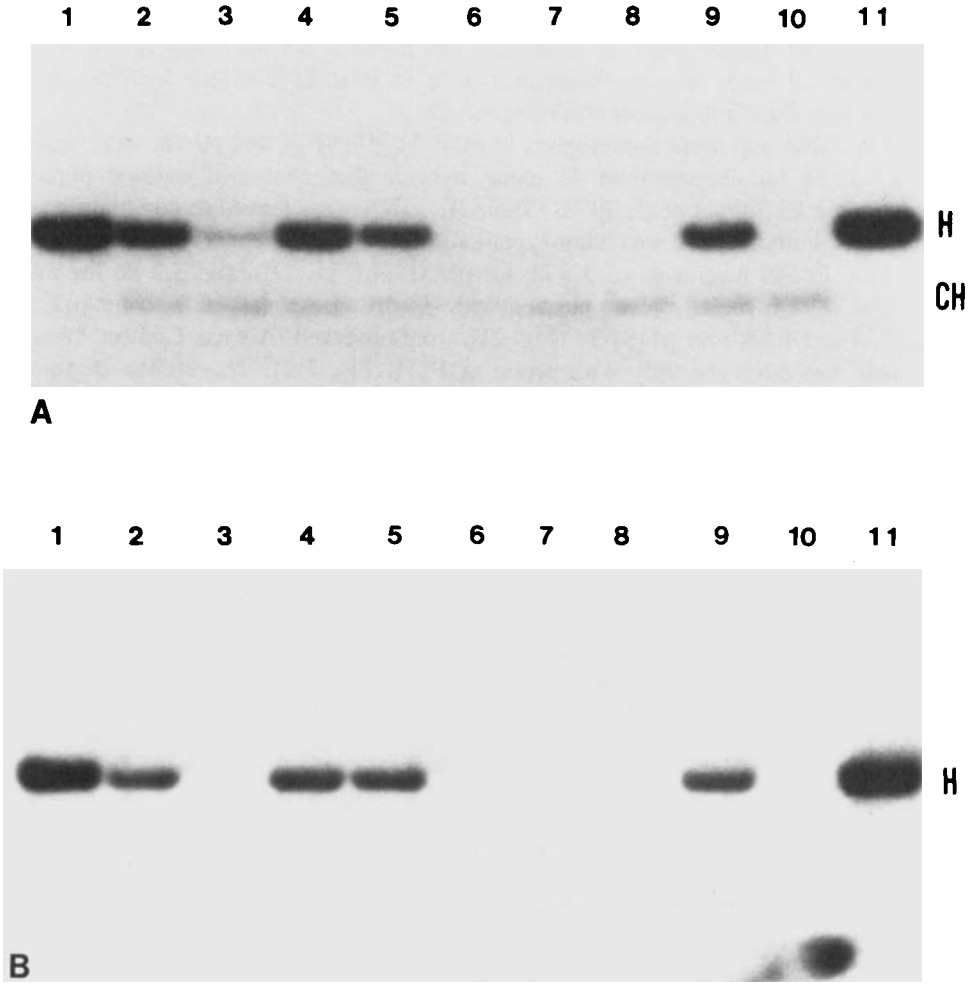


Fig. 2. Regional mapping of *D15S1* and *D15S2* on chromosome 15. A, hybridization of pDP151 to human, rodent, and hybrid DNA cleaved with HindIII. Human DNA (lanes 1 and 11) produced a single HindIII band ~4 kb in size (labeled H). A smaller cross-hybridizing fragment (labeled CH) was present in Chinese hamster DNA (lane 10) and in all Chinese hamster × human hybrid samples. Hybrids in lanes 2, 3, 4, 5, and 9 were positive for the human fragment, and hybrids in lanes 6, 7, and 8 were negative. The hybrids whose DNA was in the lanes positive for the human fragment all contained the human derivative chromosome carrying the region 15q14→15qter (those in lanes 3 and 4 had this region as their only chromosome 15 material), whereas hybrid DNA in lanes negative for the human fragment either had no chromosome 15 material (lane 6) or only the derivative 15 carrying region 15pter→15q14 (lanes 7 and 8). B, hybridization of pMS1-14 to human, rodent, and hybrid DNA. Human DNA (lanes 1 and 11) produced a single HindIII band of ~4.5 kb (labeled H), whereas Chinese hamster DNA (lane 10) did not cross-hybridize with pMS1-14. The same pattern of hybridization to human-specific fragments was seen on this filter loaded with the same samples as in A, indicating that pMS1-14 also maps to region 15q14→15qter. All lanes were loaded with 5 μg DNA. The weak human band in lanes 3 correlated with low frequency of the derivative 22 chromosome.

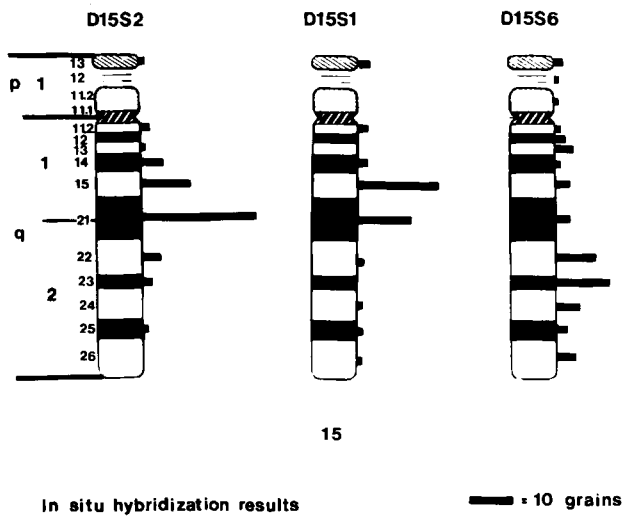


Fig. 3. Distribution of silver grains over chromosome 15 after in situ hybridization with tritiated DNA probes.

labeled pDP151 to normal human mitotic chromosomes has revealed 326 grains over all chromosomes in 105 cells. Twenty-three percent of cells were labeled over 15q15→15q22, and 41 grains (13% of total) were at this site.

With the pMS1-14 probe, 33 of 327 grains (10%) in 88 cells were located at region 15q15→15q21. Thirty-eight percent of cells were labeled over 15q15→15q21.

For 9-1a(12), 172 grains were scored over 61 cells. Nine grains were seen over region 15q15→15q25, with 18% of cells labeled over this site. In a second experiment (Fig. 3), 37 cells with grains over chromosome 15 were analyzed. There was a distinct peak (26 of 50 grains total) over region 15q22→15q24.

## DISCUSSION

We have assigned three polymorphic DNA segments to regions of human chromosome 15. Analysis of somatic cell hybrids with a chromosome 15 rearrangement has defined the smallest region of overlap (SRO) for *D15S1* and *D15S2* as 15q14→15qter. For *D15S6*, the SRO is 15q14→15q22, since this locus has previously been assigned to 15pter→15q22 (J. Trowsdale, personal communication) using the somatic cell hybrids of Sheer et al [1983]. Furthermore, in situ hybridization of the probes to normal human chromosomes has placed *D15S1* in bands 15q15→15q21, *D15S2* in bands 15q15→15q22, and *D15S6* in bands 15q22→15q24. Although the autoradiographic silver grain distributions overlap to a large extent, a tentative linear order can be proposed based on sites of major grain accumulation: cen—*D15S1*—*D15S2*—*D15S6*—tel (Fig. 4). From the physical localization, it seems possible that these loci are within measurable genetic distance. Establishment of the precise distances and linear order by family linkage studies could form the beginning of a genetic map of chromosome 15 [White et al, 1985]. In fact, such studies using pDP151 and pMS1-14 were reported at HGM8 [Kimberling et al, 1985] suggesting close linkage ($\Theta = 0.07$) and placement of *D15S1* and *D15S2* in the "distal half of the long arm." Their tentative gene order, however, was cen—*D15S2*—*D15S1*.

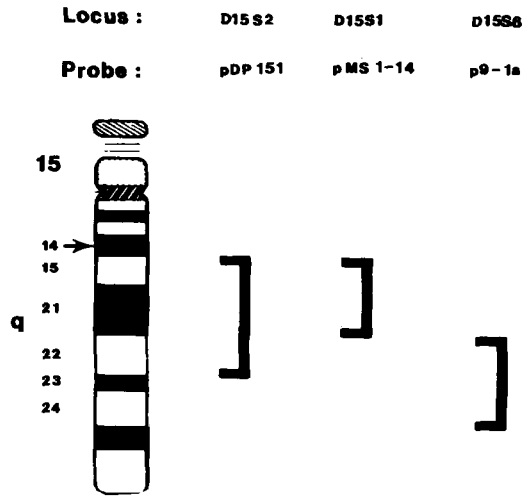


Fig. 4. Summary of regional assignments based on somatic cell hybrid and in situ hybridization results. Arrow indicates the t(15;22) translocation breakpoint.

Chromosome 15 carries the locus for hexosaminidase A (*HEXA*), the enzyme deficient in Tay-Sachs disease (15q22→15q25.1) [Ferguson-Smith and Westerveld, 1979]; $\beta_2$-microglobulin (*β2M*) has been mapped to 15q14→qter [Oliver et al, 1978] and further refined to 15q14→15q22 by Sheer et al [1983]. The Prader Willi syndrome (PWS)-associated chromosomal rearrangements occur in bands 15q11→15q12 [Ledbetter et al, 1982; Charrow et al, 1983]. Our regional assignments demonstrate that all three loci, *D15S1*, *D15S2*, *D15S6*, are too far away from the region altered in Prader Willi syndrome to be useful in linkage studies of PWS or in studies of the copy number or genomic organization of these sequences in PWS patients. They might, however, be useful in linkage studies with *HEXA*, and *D15S1* and *D15S2* have been useful in linkage analysis with the proposed chromosome 15-specific reading disability [Smith et al, 1983]. These linkage data are now considered tentative [Fain et al, 1985].

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## REFERENCES

Barker D, Schäfer M, White R (1984): Restriction sites containing CpG show a higher frequency of polymorphism in human DNA. Cell 36:131–138.

Botstein D, White RL, Skolnick M, Davis RW (1980): Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32:314–331.

Charrow J, Balkin N, Cohen MM (1983): Translocations in Prader-Willi syndrome. Clin Genet 23:304–307.

de Martinville B, Schäfer M, White R, Francke U (1983): Chromosomal assignments of three random RFLP loci defined by base-pair changes in *MspI* sites. Mol Biol Med 1:415–424.

de Martinville B, Wyman AR, White R, Francke U (1982): Assignment of the first random restriction fragment length polymorphism (RFLP) locus *D14S1* to a region of human chromosome 14. Am J Hum Genet 34:216–226.

Fain PR, Kimberling WJ, Ing PS, Smith SD, Pennington BF (1985): Linkage analysis of reading disability with chromosome 15. Cytogenet Cell Genet 40:625.

Ferguson-Smith MA, Westerveld A (1979): Report of the committee on the genetic constitution of chromosomes 13–22. Cytogenet Cell Genet 25:59–73.

Francke U (1984): Random X inactivation resulting in mosaic nullisomy of region Xp21.1→p21.3 associated with heterozygosity for ornithine transcarbamylase deficiency and for chronic granulomatous disease. Cytogenet Cell Genet 38:298–307.

Francke U, Busby N, Shaw D, Hansen S, Brown MG (1976); Intrachromosomal gene mapping in man: Assignment of nucleoside phosphorylase to region 14cen→14q21 by interspecific hybridization of cells with a t(X;14)(p22;q21) translocation. Somatic Cell Genet 2:27–40.

Francke U, Francke B (1981): Requirement of the human chromosome 11 long arm for replication of herpes simplex virus type 1 in nonpermissive Chinese hamster × human diploid fibroblast hybrids. Somatic Cell Genet 7:171–191.

Francke U, Pellegrino M (1977): Assignment of the major histocompatibility complex to a region of the short arm of human chromosome 6. Proc Natl Acad Sci USA 74:1147–1151, 5776.

Harper ME, Saunders GF (1981): Localization of single copy DNA sequences on G-banded human chromosomes by in situ hybridization. Chromosoma 83:431–439.

Kimberling WJ, Ing PS, Smith SD, Fain PR (1985): Linkage relationships of two DNA probes on chromosome 15 with each other and with the centromere. Cytogenet Cell Genet 40:668.

Kirsch IR, Morton CC, Nakahara K, Leder P (1982): Human immunoglobulin heavy chain genes map to a region of translocations in malignant B lymphocytes. Science 211:301–303.

Lawn RM, Fritsch EF, Parker RC, Blake G, Maniatis T (1978): The isolation and characterization of linked λ- and β-globin genes from a cloned library of human DNA. Cell 15:1157-1174.

Ledbetter DN, Mascarello JT, Riccardi UM, Harper UD, Airhart SD, Strobel RJ (1982): Chromosome 15 abnormalities and the Prader-Willi syndrome: A follow-up report of 40 cases. Am J Hum Genet 34:278–285.

Münke M, Lindgren V, de Martinville B, Francke U (1984): Comparative analysis of mouse x human hybrids with rearranged chromosome 1 by in situ hybridization and Southern blotting: High resolution mapping of *NRAS, NGFB,* and *AMY* on human chromosome 1. Somatic Cell Mol Genet 22:506–510.

Oliver N, Francke U, Pellegrino MA (1978): Regional assignment of genes for mannosephosphate isomerase, pyruvate kinase-3 and $\beta_2$ microglobulin expression on human chromosome 15 by hybridization of cells from a t(15;22)(q14;q13.3) translocation carrier. Cytogenet Cell Genet 22:503–510.

Rigby PWJ, Dieckmann M, Rhodes C, Berg P (1977): Labelling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J Mol Biol 113:237–251.

Sheer D, Hiorns LR, Stanley KF, Goodfellow PN, Swallow DM, Povey S, Heisterkamp H, Griffen J, Stephenson JR, Solomon E (1983): Genetic analysis of the 15;17 chromosome translocation associated with acute promyelocytic leukemia. Proc Natl Acad Sci USA 80:5007–5011.

Skolnick MH, Willard HF, Menlove LA (1984): Report of the committee on human gene mapping by recombinant DNA techniques. Cytogenet Cell Genet 37:210–273.

Smith SD, Kimberling WJ, Pennington BF, Lubs HA (1983): Specific reading disability: Identification of an inherited form through linkage analysis. Science 219:1345–1347.

Southern E (1975): Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–517.

White R, Leppert M, Bishop DT, Barker D, Berkowitz J, Brown C, Callahan P, Holm T, Jerominski L (1985): Construction of linkage maps with DNA markers for human chromosomes. Nature 313:101–105.

Wyman AR, White R (1980): A highly polymorphic locus in human DNA. Proc Natl Acad Sci USA 77:6754–6758.

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