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A Map of the Distal Region of the Long Arm of Human Chromosome 21 Constructed by Radiation Hybrid Mapping and Pulsed-Field Gel Electrophoresis

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We have used radiation hybrid (RH) mapping and pulsed-field gel electrophoresis (PFGE) to determine the order and positions of 28 DNA markers from the distal region of the long arm of human chromosome 21. The maps generated by these two methods are in good agreement. This study, combined with that of D. R. Cox *et al.* (1990, *Science* 250:245–250), results in an RH map that covers the long arm of chromosome 21 (21q). We have used a subtelomeric probe to show that our map includes the telomere and have identified single-copy genes and markers within 200 kbp of the telomere. Comparison of the physical and RH maps with genetic linkage maps shows "hot spots" of meiotic recombination in the distal region, one of which is close to the telomere, in agreement with previous cytogenetic observations of increased recombination frequency near telomeres.

INTRODUCTION

In the past 10 years, a major effort in molecular genetics has been made in generating maps of genomes. A breakthrough in human gene mapping was the development of DNA markers for linkage analysis, which has resulted in near completion of a genetic map of the human genome at a resolution of about 10–20 cM (e.g., Donis-Keller *et al.*, 1987). The genetic map has been useful for mapping human disease genes to chromosomal regions, but a much higher resolution map is needed for cloning genes and for genome organization studies. High-resolution maps cannot be constructed easily by meiotic linkage methods because their resolution is low at the molecular level, recombination frequency is not linearly correlated with physical distance, and only the subset of markers that recognizes polymorphisms can be mapped.

The development of pulsed-field gel electrophoresis (PFGE, Schwartz and Cantor, 1984) allows the generation of physical maps several megabase pairs in size (for example, Burmeister *et al.*, 1988; Fulton *et al.*, 1989). However, PFGE is not easily applied to the construction of maps of whole chromosomes. With the very large number of probes necessary to create such large maps, DNA probes often recognize fragments of the same size by coincidence. Therefore, an additional method that could be used in conjunction with PFGE to determine the order of markers would be useful.

Somatic cell hybrids containing defined segments of a chromosome of interest can provide information about the order of probes along the chromosome. However, the resolution of maps constructed with such cell lines is usually limited by the small number of available hybrids. In an effort to obtain maps of higher resolution, we have developed a somatic cell hybrid mapping strategy called radiation hybrid (RH) mapping (Cox *et al.*, 1990). In this procedure, a hybrid cell line containing a single human chromosome in a rodent cell background is subjected to a high dose of X-rays, which results in chromosomal fragmentation. The chromosomal fragments are recovered by fusion to a rodent recipient cell line, which nonselectively retains some hamster and human chromosomal pieces from the donor cell line. DNA markers near one another on the chromosome are likely to be re-

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tained or lost together in any particular hybrid cell line. Although many cell lines contain several noncontiguous pieces of DNA from the human chromosome, it is nevertheless possible to use a statistical analysis of cosegregation of markers to construct a map of higher resolution than is possible by classical somatic cell genetic approaches (Cox *et al.*, 1990).

Chromosome 21, the smallest human chromosome, is a paradigm for large-scale human genome mapping efforts. A high-resolution map of chromosome 21 is of particular interest since trisomy 21 results in Down syndrome, the most frequent cause of mental retardation and birth defects in humans. Several meiotic maps of chromosome 21 have been published recently (Tanzi *et al.*, 1988; Warren *et al.*, 1989; Petersen *et al.*, 1989). Gardiner *et al.* (1988, 1990) analyzed chromosome 21 by using somatic cell hybrids and PFGE. In their physical mapping studies, probes close to one another were linked into clusters, and markers were localized into sections along the chromosome. However, the order of markers within sections and the physical distances between many markers could not be determined. Recently, we combined RH mapping with PFGE to determine the order of 14 markers spanning approximately 20 million base pairs on the proximal long arm of chromosome 21 (Cox *et al.*, 1990). Here we use this combined approach to order 28 markers in the distal region of the long arm of chromosome 21 and to construct a continuous physical map of the most telomeric 8000 kbp of the long arm, including the telomere itself. A comparison of the physical map with the genetic linkage map of this segment of the chromosome reveals several hot spots of meiotic recombination. In addition, the physical map helps to define rearrangements that have occurred in this region of the genome during evolution.

MATERIALS AND METHODS

DNA Markers

In this paper we use abbreviations for the locus names determined by the Human Gene Mapping workshops (HGM). For example, S3 represents D21S3, etc. Note, however, that S100B is the gene for the β-subunit of the protein S100 rather than the locus D21S100. Details about the DNA probes used in this study have been described: S3 (pPW231c), S55 (pPW518-1R), and S58 (pPW524-5P) (Watkins *et al.*, 1985); S101 (JG373) (Galt *et al.*, 1989); S15 (pGSE8), S17 (pGSH8), and S19 (pGSB3) (Stewart *et al.*, 1985); S39 (SF13A), S40 (SF14), S41 (SF21), S42 (SF43), S44 (SF50), S49 (SF58), and S51 (SF93) (Korenberg *et al.*, 1987); S123 (B88) and S141 (E73) (Tantravahi *et al.*, 1988); S25 (p10.2) (Millington and Pearson, 1988); pTH2Δ (de Lange *et al.*, 1990); MxA and MxB (Aebi *et al.*, 1989); BCE-I (pS2) (Moisan *et al.*, 1988); PFKL (cPFKL3.0) (Levanon *et al.*, 1987); CD18 (Mac3 and Mac9) (Kishimoto *et al.*, 1987); COL6A1 (cA1) and COL6A2 (cA2) (Weil *et al.*, 1988); SOD1 (pHG-SOD) (Lieman-Hurwitz *et al.*, 1982); S100B (pHS22.4) (Allore *et al.*, 1988). A probe for human α-crystallin-1 (CRYA1) was obtained by amplifying a 3.5-kbp DNA fragment between exons 1 and 3 from the sequences described (McDevitt *et al.*, 1986) by using the polymerase chain reaction (Saiki *et al.*, 1988). After amplification, ends were repaired with Klenow and T4 polymerase, and the product was subcloned into pUC18.

Radiation Hybrid Mapping

RH mapping can be used to determine the order and distance between DNA markers by analyzing the presence or absence of such markers in somatic cell hybrids following exposure to X-rays (Cox *et al.*, 1989, 1990). We used the cell line CHG3, a hybrid cell line containing human chromosome 21 in a hamster cell background (a subclone of 72532X-6, Patterson *et al.*, 1985) to construct the RH map in this study exactly as described (Cox *et al.*, 1990). The distance between two markers as determined by RH mapping is expressed in centirays (cR), analogous to centimorgans (cM) in meiotic linkage mapping. Because RH distance depends on the amount of irradiation used to fragment the chromosomes, it is important to include information about X-ray dose when describing the centiray distance between two markers. A distance of 1 cR_{8000} between two markers corresponds to a 1% frequency of breakage between the markers after exposure to 8000 rads of X-rays (Cox *et al.*, 1990). Four-point analysis is used to calculate the odds favoring one order of four markers over another order (Cox *et al.*, 1990).

Pulsed-Field Gel Electrophoresis

We used DNA from the hybrid cell line CHG3 (see above) and from human blood cells to generate physical maps. To avoid problems in interpreting results due to polymorphisms, the blood cell DNA used throughout this study was prepared from a single individual. The methods for preparation of agarose blocks containing high-molecular-weight DNA, preparation of yeast and bacteriophage λ multimers as size markers, restriction digests, alkaline blotting, and subsequent hybridization of GeneScreen membranes were as described (Herrmann *et al.*, 1987). In general,

TABLE 1

RH Mapping: Analysis of Hybrids and Calculated Distances

Marker A	Marker B	No. of clones observed ++	+−	−+	−−	Total	θ	cR_{8000}	Lod
S40	S49	19	0	1	30	50	0.05	5	12.82
CD18	S25	7	0	1	36	44	0.06	7	7.22
S39	S40	21	0	2	48	71	0.07	7	15.27
S44	COL6A2	15	2	0	33	50	0.09	10	10.08
S40	S141	16	1	1	25	43	0.11	11	9.54
S55	S3	15	2	1	38	56	0.12	13	9.91
S141	CD18	8	0	2	30	40	0.13	13	6.28
COL6A1	COL6A2	14	3	0	31	48	0.14	15	8.39
S44	COL6A1	25	2	3	49	79	0.14	15	14.20
S39	S49	18	1	3	43	65	0.15	16	11.10
S101	S39	16	3	1	41	61	0.16	17	9.84
S49	S141	18	3	1	38	60	0.15	17	10.35
S3	S101	9	3	0	31	43	0.17	18	6.12
S25	S44	13	0	4	37	54	0.18	20	8.15
CD18	S44	16	0	5	47	68	0.18	20	10.19
COL6A1	S100B	21	4	2	46	73	0.19	21	11.40
SOD1	S58	18	4	1	31	54	0.19	22	8.44
S49	CD18	8	2	2	36	48	0.21	23	5.76
S25	COL6A2	4	1	1	17	23	0.22	25	2.61
COL6A2	S100B	10	3	1	28	42	0.23	26	5.38
S141	S25	12	4	1	34	51	0.25	28	6.51
S55	S101	15	4	2	33	54	0.25	29	6.98
S3	S39	14	6	1	45	66	0.26	30	7.40
S101	S40	14	3	3	27	47	0.30	35	5.93
S17	S55	12	5	2	31	50	0.31	37	5.02
S58	S17	13	3	5	31	52	0.34	41	4.95
S17	S3	13	6	3	35	57	0.36	45	4.90
S58	S55	22	9	6	44	81	0.40	52	6.55
SOD1	S17	13	9	6	28	56	0.57	84	2.23

Note. Selected marker pairs, designated Marker A and Marker B, are included. The number of hybrid cell clones analyzed for each marker pair is indicated, with ++ indicating the number of hybrids containing both markers A and B, +− indicating the number of hybrids containing marker A but not marker B, −+ indicating the number of hybrids containing marker B but not marker A, and −− indicating the number of hybrids containing neither marker. θ indicates the estimated frequency of X-ray breakage between Markers A and B, and cR_{8000} indicates the distance between the two markers (5). Marker pairs with a lod score of 3.0 or greater are considered to be significantly linked. Only these marker pairs are used to construct the RH map (5).

PFGE was performed at 15°C in 0.5× TBE buffer. CHEF gel electrophoresis was performed in a CHEF apparatus constructed at the EMBL (Heidelberg, FRG) according to Chu *et al.* (1986). OFAGE was performed in an apparatus obtained from LKB–Pharmacia.

To test whether two probes hybridize to the same fragment, we always used the same filter. Probes were removed from a filter before hybridization with the next probe by placing the filter into a solution containing 2 m*M* Tris/HCl, pH 8, 0.2 m*M* EDTA, 0.1% SDS at 80°C twice for 20 min.

DNA fragments were separated in two ranges of resolution, low resolution (500–3000 kbp) and high resolution (50–900 kbp). Within each range, several sets of filters were prepared. Probes close to each other were compared on identical filters. The prior information from RH mapping and/or genetic mapping therefore helped to determine which probes were likely to be physically linked.

RESULTS

Mapping Strategy

To generate a map of human chromosome 21 distal to the marker SOD1, we first chose 19 markers as landmarks for radiation hybrid mapping. The RH map constructed from these landmarks was then used as an aid in the physical mapping of the most distal region of human chromosome 21, the region including and distal to the marker D21S3. In addition to this initial set of 19 markers, which was analyzed by both RH mapping and PFGE, we analyzed a set of markers by PFGE only (Table 2). These additional markers were included primarily because they were used as

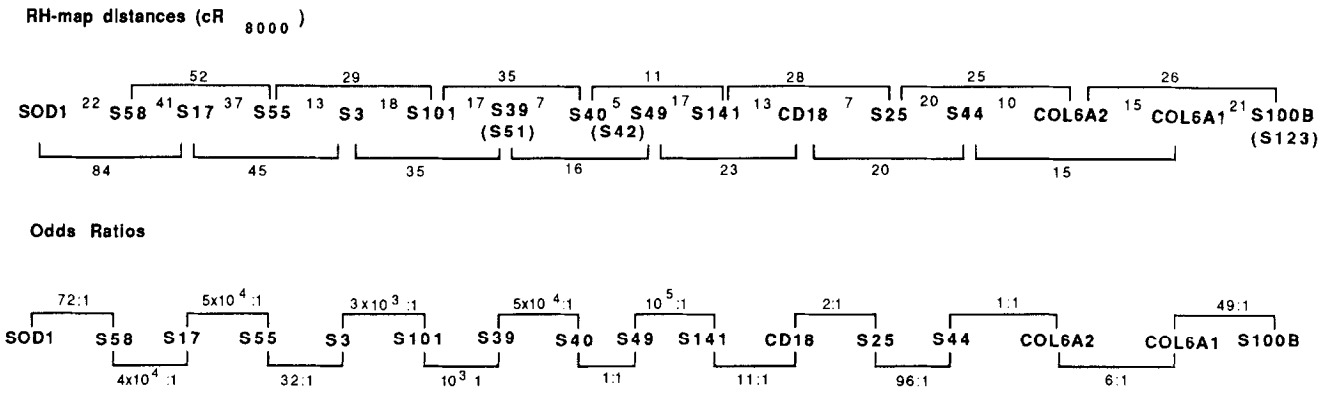

FIG. 1. Radiation hybrid map. The map shows the order of probes, as derived by RH mapping, and the distance between each pair. The telomere is to the right, near S100B. Distances for adjacent markers are shown between the markers, and for nearest neighbor pairs above and below the map. Loci shown in parentheses are those that were not separated by RH mapping from the loci indicated above them, and were not used for the construction of the map. S42 was not separated from either S40 or S49, although these two loci are separated in a cell line for which S42 was not scored. The odds favoring the order indicated compared to the order in which the given marker pair is inverted (5) are shown below the map.

anchor points in recently published genetic maps (Petersen *et al.*, 1989; Tanzi *et al.*, 1988; Warren *et al.*, 1989).

Radiation Hybrid Mapping

Our initial set of 19 markers was analyzed by RH mapping (Cox *et al.*, 1990). Cell hybrids containing chromosome 21 fragments generated by X-ray breakage were scored for the presence or absence of these markers. These primary data were analyzed as pairwise combinations of markers by using a computer program that we developed for RH mapping (Cox *et al.*, 1990). Four of the marker pairs, S100B and S123, S51 and S39, S40 and S42, and S42 and S49, were not separated by X-ray breakage in any of the radiation hybrids that were analyzed. For each of these pairs of markers, only the marker scored for the largest number of hybrids was included in the mapping analysis, which included 16 loci. A sample of the most pertinent marker pairs is listed in Table 1. Pairs of markers with a lod score of 3.0 or greater were considered to be significantly linked, and the two-point distances of only linked marker pairs were used to construct a map. We defined the best order of markers as the one in which the sum of the distances between adjacent markers was minimized. This analysis resulted in the map shown in Fig. 1. Four-point analysis (Cox *et al.*, 1990) was used to calculate the relative likelihood of one order of four markers versus another. Each odds ratio shown below the map in Fig. 1 represents the odds of the illustrated order of four markers compared to the order in which the internal two markers are inverted. Some of these odds ratios are less than 1000:1. In these cases, it is not possible to establish marker order with certainty using the RH data alone. Therefore, pulsed-field gel electrophoresis was used in an effort to obtain a more definitive order.

Long-Range Restriction Mapping of the Most Distal 8000 kbp by PFGE

PFGE mapping requires that markers be physically linked on identical large DNA fragments. To cover large distances, fragments in the size range of 200 to 2500 kbp were separated by CHEF electrophoresis, blotted onto membranes, and hybridized successively with the labeled probes. Enzymes resulting in fragment sizes in this range were *Nru*I, *Mlu*I, *Sal*I, *Not*I, and, in some cases, *Rsr*II and *Cla*I (Figs. 2 and 3). Most of these enzymes were chosen because their sites are less likely to cluster in CpG-rich islands than sites for enzymes such as *Bss*HII, *Sac*II, *Eag*I, etc. (Bird, 1989).

We analyzed DNA isolated from both total blood cells and the hybrid cell line CHG3, which contains human chromosome 21 in a hamster cell background. Due to differential methylation and/or RFLPs between these two sources, the results often complemented each other. For example, the *Cla*I digest of cell line CHG3 shows two fragments linking the loci S51 and S40, which do not obviously share fragments when just blood cell DNA is analyzed (Fig. 2). A *Sal*I fragment present in CHG3 but not in blood DNA also links S40 and S42. Similarly, two large *Nru*I fragments of $\approx$2000 kbp, which are present in blood cell but not in CHG3 DNA, link all loci between S42 and S25/PFKL (Fig. 3). Thus, it was important to have information from both CHG3 and blood cell DNA to construct the map. However, overall the maps from both DNA sources are very similar, indicating that the chromosome 21 region analyzed in hybrid cell line

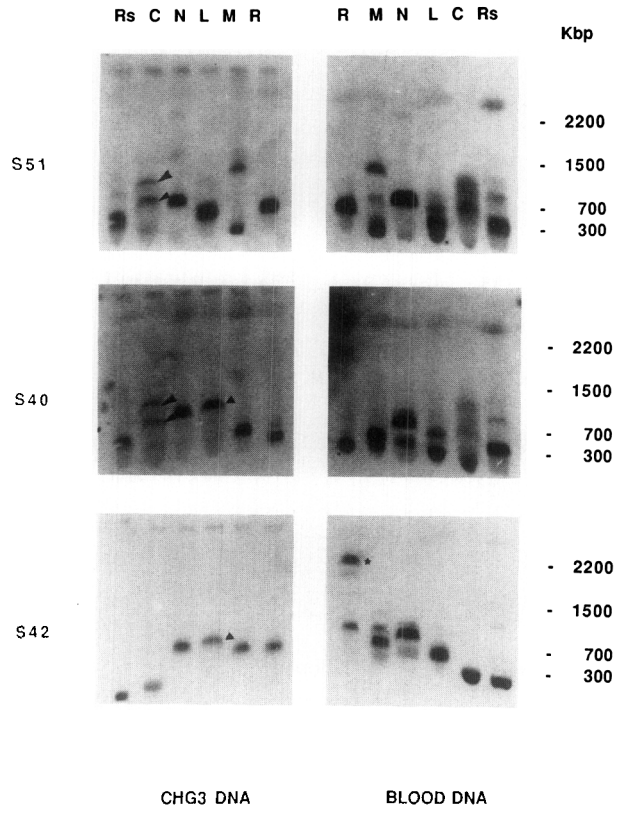

FIG. 2. PFGE comparison of DNA from the cell line CHG3 and human blood cells. One-half of a CHEF gel was loaded with restriction digests of DNA from the cell line CHG3, and the other half was loaded with DNA prepared from human peripheral blood. The migration of the DNA in the gel showed slight curvature, which was taken into account in estimating the sizes of the DNA fragments. Restriction enzymes were Rs, *Rsr*II; C, *Cla*I; N, *Not*I, L, *Sal* I; M, *Mlu*I; and R, *Nru*I. CHEF gel electrophoresis was performed for 7 days at 50 V, with switching times of 20 min. The DNA from the gel was blotted onto a nylon membrane, which was hybridized successively to the probes for the loci indicated. The fragments recognized by the probe for S51 (D21S51) are very similar between the two sources, whereas the other two loci show a number of differences. The arrowheads point to fragments that were particularly useful for linking up the loci and were not present in blood DNA. The *Nru*I fragments (star symbol) of about 2000 kbp are seen only in blood cell DNA and were useful for establishing a continuous map of that region (compare with Fig. 3).

CHG3, on which the RH maps are based, is not grossly rearranged.

Two DNA markers were determined to be physically linked if they recognized PFGE fragments of the same size. Figure 3 shows a total of 10 probes linked to one another on common fragments, spanning a total distance of more than 5000 kbp. In most cases, more than one DNA fragment was shared between probes, making coincidental comigration of shared fragments very unlikely. Although there is only one large (1600 kbp) *Mlu*I fragment in common between S3 and S101, the differential cleavage in CHG3 and blood DNA gives additional evidence that this result is not due to coincidental comigration: for both S3 and S101, the 1600-kbp fragment hybridizes very weakly in blood cell DNA (Fig. 3), but strongly in CHG3 DNA (not shown). In addition, the two complete *Mlu*I-digestion products in blood cell DNA (700 and 1000 kbp) add up to the size of the partial (1600 kbp) fragment within a 10% error margin.

We were unable to obtain unequivocal evidence for physical linkage in the region near CD18. Most rare-cutting restriction enzymes cut at the same place on either side of this gene, suggesting that it is flanked by two very CpG-rich islands. Weak partial *Sal*I, *Rsr*II, and *Cla*I fragments suggest that CD18 is linked to its neighboring loci, S44 and S25/PFKL. Because the RH map (Fig. 1) suggests that the distance between the two flanking markers is not very large, we believe that these comigrating partial restriction fragments are probably identical rather than coincidentally of the same size. The very small fragments recognized by the two CD18 cDNA probes were also recognized by the anonymous probe for S41 (data not shown). Indeed, we found that the cDNA probes for CD18 hybridize to DNA from the phage λ21, from which SF21, which recognizes the locus S41, is derived (Korenberg *et al.*, 1987). We thus established that the phage λ21 contains at least part of the gene for CD18. This phage also contains sites for many rare-cutting restriction enzymes, including *Not*I and *Bss*HII, and thus probably contains one of the CpG-rich islands flanking CD18.

In cases where loci were found to map very close to each other, usually within less than 800 kbp (e.g., in the S40, BCEI, S19, S49 cluster), filters were prepared from DNA fragments separated in the lower resolution range of 50–800 kbp. For this purpose, more frequently cleaving enzymes, such as *Sfi*I, *Bss*HII, and *Eag*I, were used. Some of the data from these filters are included in Table 2. However, the orders of S141 and CRYA1, S25 and PFKL, MxA/B and S39, and BCE-I and S40, each respectively within 300 kbp of the other, could not be established. Similarly, S100B and S123 (see below) and MxA and MxB are each within 100 kbp of the other, and their order is unknown.

In summary, a physical map of the most distal region of chromosome 21 was constructed by PFGE, using a total of 24 probes. This analysis was facilitated by knowledge of the approximate positions of most markers prior to the analysis. Each marker hybridized to at least one large PFGE fragment in common with its neighbor (Table 2). This fact allowed construction of a complete, continuous map spanning about 8000 kbp (Fig. 4). Large DNA fragments of 1100–2200 kbp were most useful for linking many probes into large clusters, whereas the smaller fragments gave the order of markers within these clusters.

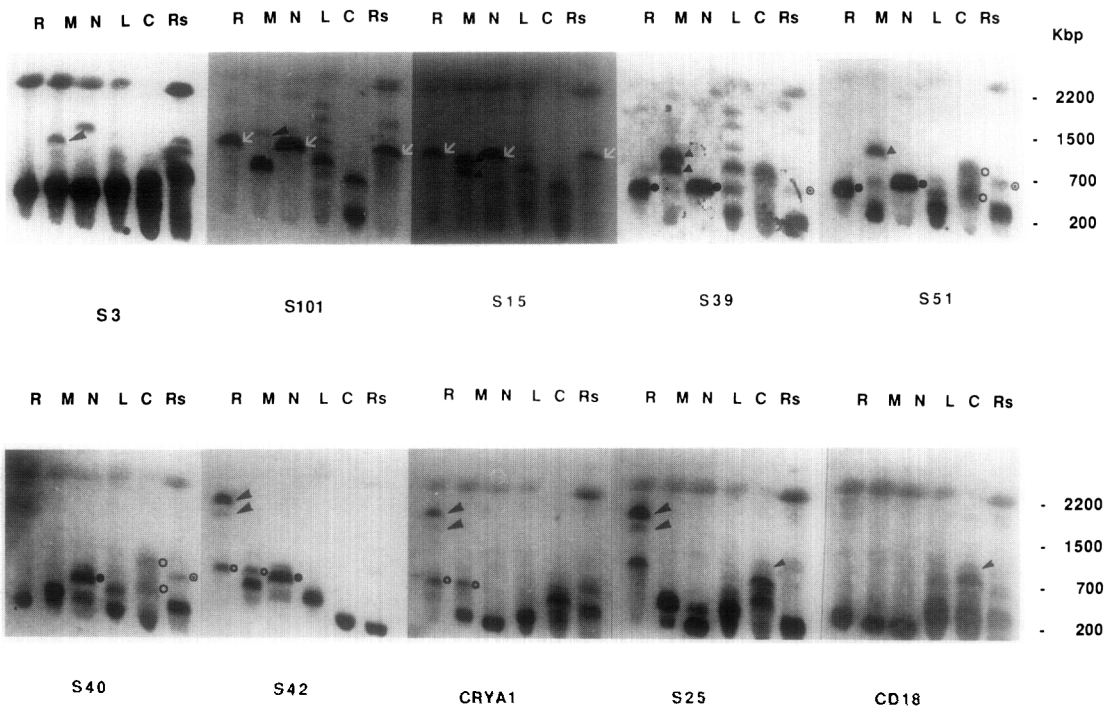

FIG. 3. Physical linkage of 10 loci from 21q22.3 by PFGE. The same CHEF gel described in Fig. 2, showing blood cell DNA digested with the following restriction enzymes: R, *Nru*I; M, *Mlu*I; N, *Not*I; L, *Sal* I; C, *Cla*I; Rs, *Rsr*II. The gel was blotted onto a nylon membrane and was hybridized successively to probes for the loci indicated below each autoradiograph. By overlaying autoradiographs, fragments of identical size recognized by several probes were found. Identical fragments are indicated by the same symbols in successive autoradiographs. The autoradiographs are shown in the order that the loci appear in the genome, from left to right. Approximate sizes, estimated from the sizes of yeast chromosomes, are indicated in kilobase pairs (kbp) on the right.

The most informative large fragments, resulting from *Mlu*I, *Nru*I, and *Not*I digests, are aligned in the map shown in Fig. 4.

Long-Range Map of the 21q Telomere

Recently, de Lange *et al.* (1990) isolated a subtelomeric DNA fragment, pTH2Δ, that recognizes a telomere of chromosome 21, in addition to other human telomeres. Since this probe does not recognize rodent sequences, we used it for PFGE mapping on filters prepared from hybrid cell DNA containing human chromosome 21 on a mouse cell background. Figure 5 shows that several PFGE fragments are shared between this probe and the most distal markers used, S123 and S100B, which codes for the β-subunit of the neuronal calcium-binding protein S100 (Allore *et al.*, 1988). The smallest fragments in common among S100B, S123, and pTH2Δ are about 200 kbp in size (see *Eag*I and *Sna*BI results, Fig. 5). However, a *Nae*I fragment of ≈50 kbp is recognized by pTH2Δ only, placing S100B and S123 at 50 to 200 kbp from the telomere. Thus, single-copy genes and loci can be found at positions within 200 kbp of the telomere. Other genes mapping within 700 kbp of the telomere are collagen VIα1 (COL6A1) and collagen VIα2 (COL6A2). Both genes are within 300 kbp of each other. Their order with respect to the rest of the map was determined by a partial digest with *Cla*I, which resulted in a fragment of about 550 kbp containing both COL6A2 and S100B, but not COL6A1 (Table 2 and Fig. 5).

DISCUSSION

The Map of the Telomeric 8000 kbp of Chromosome 21

We constructed a fine-structure map of the distal region of the long arm of human chromosome 21 by combining RH and PFGE mapping methods. RH mapping was not able to determine the order of loci within the two clusters S40–S49–S42 and S51–S39 due to an insufficient number of hybrids containing breaks between the loci in each cluster, whereas PFGE analysis resolved the order of markers in both cases. Conversely, PFGE did not provide unequivocal evidence for physical linkage between markers S25, CD18, and S44, whereas RH mapping indicated that these markers are closely linked. Although the RH map placed CD18 proximal to S25, the odds favoring this order over the inverted order are only 2:1. Therefore, we believe that the PFGE analysis, which placed

TABLE 2

Fragment Sizes of Markers from 21q22.3 Analyzed by PFGE

Locus (probe)	Enzyme						
	*Not*I	*Mlu*I	*Nru*I	*Sal*I	*Cla*I	*Rsr*II	Others
S3 (pPW231)	700, 1800	700, 1600	600	*300*, 800, *1700*	*250* 600, 800, 1000	1200 1500, 1600	
S101 (JG373)	1400	1000, 1600	1400, *2000*	*300*, 600, 900, 1100, 1500, 2000, 2200	*350* <200, 900	1500 2000	
S15 (E8)	1400	1250, 1400	1400, *2000*	*300*, 600, 900, 1100, 1500, 2000, 2200	*350* <200, 900	100 2000	
S39 (SF13A)	700	<200, 1250, 1400	600, *2000*	*500*, *550*, *1000* 400, 600, 1100, 1500, 2000, 2200	500, 1200	250, 300, 900	
MxA/B	700	<200, 1250, 1400	600, *2000*	*100*, <200, (*1000*)			
S51 (SF93)	700, (*2200*)	<200, 700, 1400	500	*350*, *400*, (*1000*) 450, 600, 750	700, 1200	400, 500, 1000	
S40 (SF14)	(500), 900, 1000	500	250	250, 600, *900*	700, 1200	400, 1000	*Bss*HII: 250
BCE-I (pS2)	nd	nd	nd	nd, *900*	700, 1200		*Bss*HII: 250
S19 (B3)	(500), 900, 1000	500	200	550, *900*	100	400, 1000	*Bss*HII: 250
S49 (SF58)	(500), 900, 1000	500	200	550, *900*	150		*Bss*HII: 250
S42 (SF43)	(500), 900, 1000	600, 850, (1100)	900, 1900, 2000	550, *900*	150	<200, (1200)	*Bss*HII: 100
S141 (E73)	500, *1100*	<200, 850, (1100)	900, 1900, 2000	<200	900, 1100	800	
CRYA1	<200	<200, 850, (1100)	900, 1900, 2000	<200	900	800	
S25 (p10.2)	<200, 400	700	1100, 1900, 2000	300, 600, (800), (1200)	*300*, *600*, 800, 1100, (1500)	*<200*, 500, (1400)	
PFKL	<100	700	ND, 1900, 2000	300, 600, (800), (1200)	*300*, *600*, 800, 1100, (1500)	nd	
CD18 (BLam, LAF-1) S41 (SF21)	<100	<100	<100	300, 600, (800), (1200)	<200, 600, 800, 1400, (1500)	<200, 500, (1200), (1400)	
S44 (SF50)	400, *650*, 1300	400	700	*<200*, 600, 1200	*400*, 600, (1200), (1500)	500, 550, 700, (1200), (1400)	
COL6A1	*700*, 1300	300		300	*600*		*Cla*I partial: No fragment of 550
COL6A2	*700*, 1300	300		300	*250*, *300*		550 (among others)
S100B	*700*, 1300			*<200*		See additional results in Fig. 5	550 (among others)
S123 (B88)	*700*, 1300						
pTH2Δ	*700*, 1300						No fragment of 550

Note. The approximate sizes in kbp are shown for each marker and for seven enzymes. The markers are shown in the order they appear in the genome. Nonitalicized numbers indicate fragments detected in blood cell DNA, whereas italic numbers indicate fragments seen only in DNA from the cell line CHG3. When no sizes are shown, that digest was not analyzed. Numbers indicated in parentheses correspond to faintly hybridizing fragments. Additional digests necessary to determine the order of fragments are listed in the lane marked "others." For the most telomeric region, from COL6A1 to pTH2Δ, additional information is shown in Fig. 5. The sizes were estimated from the known sizes of yeast chromosomes.

S25 proximal to CD18, provides the correct order of these markers. Similarly, although the RH map placed COL6A2 proximal to COL6A1, this order is only six times more likely than the inverted order. Because PFGE analysis provided strong evidence that places COL6A1 proximal to COL6A2, we consider this to be the correct order of these markers. With the exception of these two discrepancies, the order of loci on the RH and PFGE maps is identical. Although RH and PFGE mapping each provided independent evidence for order and linkage of markers in this study, neither method by itself was able to produce an unequivocal map. However, in combination, the two methods were complementary and allowed the correct order of markers to be determined with greater certainty. By carrying out RH mapping prior to PFGE analysis, we were able to determine those probes that were likely to be physically close to one another. This knowledge allowed us to place probes in groups for PFGE analysis, which involves successive

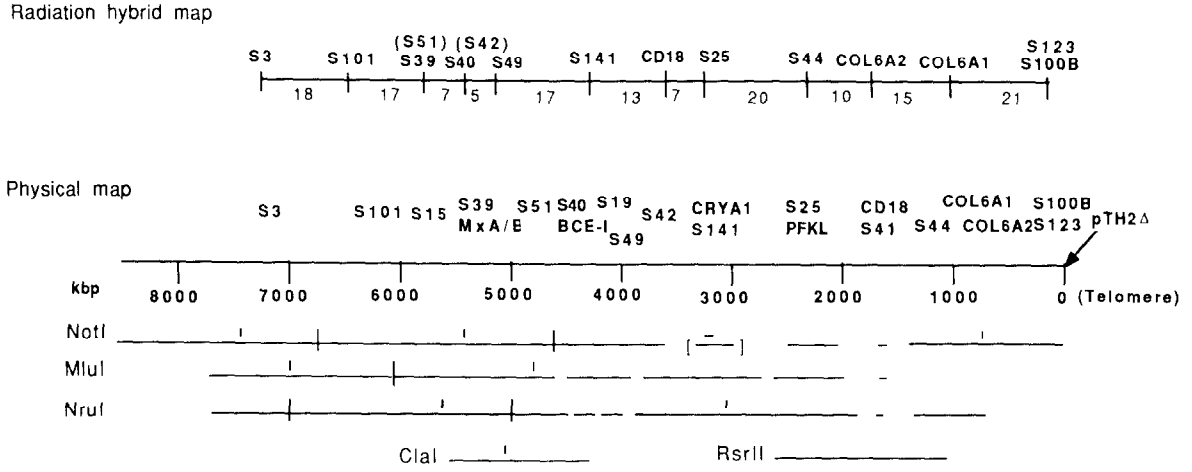

FIG. 4. Comparison of RH- and PFGE-derived maps of the most telomeric 8000 kbp of 21q22.3. **Top:** RH map in which the distances between the DNA markers are drawn proportional to the calculated cR_{8000} value. **Bottom:** The physical map, as determined by pulsed-field gel electrophoresis, is shown. The positions of all markers analyzed are shown above the scale. Position 0 kbp is the telomere (see also Fig. 5). Below the scale, lines indicate the most informative restriction fragments. Completely cleaved sites are indicated by vertical lines, whereas partially cleaved sites are designated by small vertical bars not touching the horizontal line. Empty spaces between horizontal lines are regions where more fragments might exist but could not be detected with the probes available. The order of the probes recognizing the two *Not*I fragments in brackets is not known. The order of probes in the small region around position 4000 is (S40/BCE-I)–S19–S49–S42 and was determined by using additional information not shown in this figure (see Table 2). The exact physical distances around CD18/S41 are not known, but there is tentative evidence for a *Rsr*II fragment of 1400–1600 kbp co-recognized by S25, CD18, and S44. Usually, the margins of the position of a marker are given by the nearest restriction sites flanking the marker (for example, S3 is between the *Not*I site at position 7400 and the *Mlu*I/*Nru*I sites at position 7000).

hybridization of DNA probes to the same filter. Therefore, RH mapping improved both the speed and the efficiency of PFGE mapping.

The PFGE map spans about 8000 kbp of the telomeric region of human chromosome 21, including the telomere itself. This map was constructed with an emphasis on determining continuity and locus order rather than generating a high-resolution map (50–500 kbp) in the vicinity of each probe. In contrast to the order of markers, the sizes determined by PFGE (Table 2) may have considerable error. It is difficult to estimate fragment sizes above 1000 kbp, since there are no good size markers for this range, and pulsed-field gels separating in this size range have lower resolution. Therefore, we do not emphasize exact size determination in this study. In general, size variations of up to 20% between different studies are not uncommon. The mobility of DNA fragments depends on the PFGE system used, the amount of DNA loaded, and other unknown factors. In the Duchenne muscular dystrophy region, fragment sizes were initially overestimated considerably (Burmeister *et al.*, 1988; Den Dunnen *et al.*, 1989), and we believe that current methods still tend to overestimate rather than underestimate fragment sizes.

Gardiner *et al.* (1988, 1990) used PFGE for partial physical mapping of chromosome 21. Probes were localized into sections by using cytogenetic breakpoints, and then linked into clusters defined by comigrating PFGE fragments. A continuous map of the most distal region was not established, and therefore the order of markers within that region cannot be compared with our map. For probes and enzymes used in our study and in the studies of Gardiner *et al.* (1990), DNA fragment sizes agree within a 20% margin of error in the region between S3 and BCE-I (see Fig. 4). In the more distal region, several differences exist, most of which can probably be accounted for by RFLPs at rare restriction sites or by differential methylation. One striking difference, however, is the localization of the marker B88 (D21S123). It was originally reported to lie in distal 21q22.3 (Tantravahi *et al.*, 1988), which is consistent with our localization close to the telomere. Gardiner *et al.* (1990) mapped B88 to 21q22.1. However, recently, Gardiner *et al.* (K. Gardiner, personal communication) found that a different probe was used inadvertently in their studies, and they confirmed the more distal localization when the correct B88 probe was used.

The Complete Map of the Long Arm

This study, in combination with that of Cox *et al.* (1990), provides a continuous map of the long arm of human chromosome 21 with the exception of the region between the centromere and S16. The good agreement between the RH and the PFGE maps shown in this study for the distal region of the long

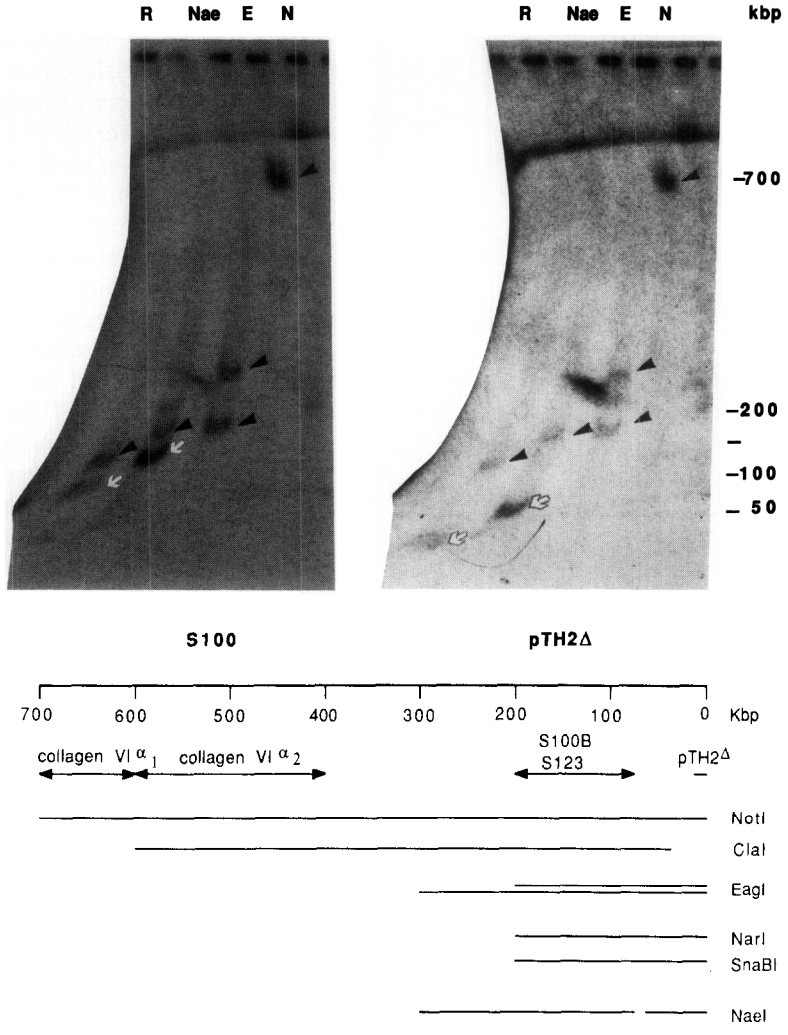

FIG. 5. Physical map of the 21q telomere. **Top:** PFGE analysis of the telomere. An OFAGE gel was prepared with DNA from the cell line SSC16-5, containing human chromosome 21 as the only human chromosome in a mouse background. The DNA was digested with restriction enzymes R, *Nru*I; Nae, *Nae*I; E, *Eag*I; and N, *Not*I and separated in an LKB–Pharmacia OFAGE apparatus at 280 V for 44 h with switching times of 70 s. The DNA was blotted onto nylon membranes and hybridized to the β-subunit of the neuronal gene S100 (S100B) and to pTH2Δ, a probe that recognizes several human telomeres but does not recognize mouse sequences. Black arrowheads point to fragments of identical size recognized by both probes, whereas white arrowheads point to fragments of different sizes. **Bottom:** The map of the telomere as derived from the data shown above and from additional experiments. The relative order of the collagen genes was determined by hybridization of collagen VIα2, S100B, and S123, but not collagen VIα1 and pTH2Δ to a 550-kbp *Cla*I fragment. The two collagen genes are less than 300 kbp apart. The order of S100B and S123 on the chromosome is not known.

arm of chromosome 21 was also observed for the proximal part of the chromosome (Cox *et al.*, 1990). Although, there was no *a priori* reason to expect that RH mapping would provide a good estimate of physical distance, data from both studies indicate that this is the case. The ≈5800 kbp between S3 and S44 are represented by 104 cR_{8000}; thus, 1 cR_{8000} is equivalent to 56 kbp. In the proximal region of the chromosome, 1 cR_{8000} was found to be equivalent to about 52 kbp (Cox *et al.*, 1990). In contrast to maps of other regions of the chromosome, the RH map is expanded about twofold compared to the physical map in the telomeric ≈1500 kbp of the chromosome (see Fig. 4). Additional studies are required to determine whether this telomeric map expansion is a general feature of RH mapping.

Given the direct relationship between RH map units and physical distance, the sizes of regions not yet covered by physical maps can now be estimated. Since 1 cR_{8000} is equivalent to 52–56 kbp, we estimate that the region between S12 and SOD1 (83 cR_{8000}) in the proximal half of human chromosome 21 (Cox *et al.*, 1990) spans about 4500 kbp, whereas the 113 cR_{8000} region between SOD1 and S3 (Fig. 1) is expected to span about 6100 kbp. The total length of the long arm is thus estimated to be 35,000–39,000 kbp (assuming that the distance between S16 and the centromere is 1000–4000 kbp). This figure agrees well

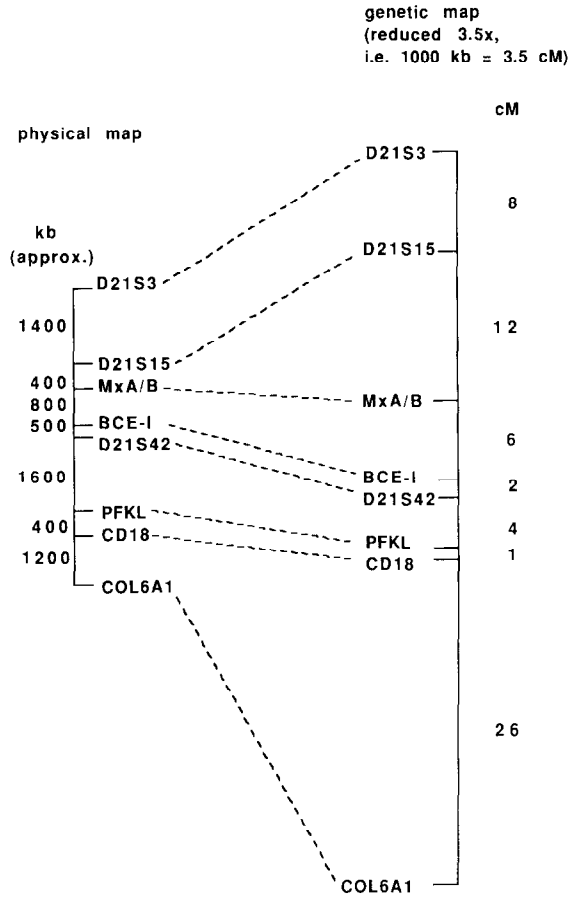

FIG. 6. Comparison of the physical and the genetic maps. Approximate physical distances are shown on the left, and genetic distances [from (28)] are shown on the right. Both distances are drawn to scale, with 3.5 cM equivalent to 1000 kbp, which is the average on human chromosome 21. On the left, markers are positioned according to their physical distances. Since the position of markers on the physical map (Fig. 4) is determined by flanking restriction sites, the physical distances shown here are rough approximations based on the assumption that the locus hybridizing to a probe is halfway between the flanking sites. Dashed lines connecting markers are drawn parallel when the physical and genetic distances correspond to 3.5 cM/1000 kbp, whereas regions of increased recombination rates are shown as nonparallel dashed lines opening to the right, as indicated for the regions between MxA/B and S15, and between CD18 and COL6A1.

with previous estimates based on the cytogenetic length of the chromosome or flow sorting (Van Dyke *et al.*, 1986).

Comparison of Physical and Genetic Linkage Maps of Chromosome 21

Previously, two groups published genetic maps of chromosome 21 (Tanzi *et al.*, 1988; Warren *et al.*, 1989). The position of BCE-I as telomeric to collagen VIα1 (Warren *et al.*, 1989) was recently corrected to a more proximal position of BCE-I (Petersen *et al.*, 1989), which agrees with our results. In all other cases, the order of probes in our study is consistent with the order in the genetic linkage maps. However, there are striking differences between the physical and the genetic maps with respect to the relative distances between markers (Fig. 6). Petersen *et al.* (1989) find a genetic length of about 130 cM for the long arm of human chromosome 21, indicating that, on average, 3.5 cM corresponds to 1000 kbp. Comparing genetic distances to cytogenetic distances, two groups (Tanzi *et al.*, 1988; Warren *et al.*, 1989) conclude that the majority of the genetic distance is contained within the most distal tenth of chromosome 21. The availability of a physical map of the distal region of the chromosome now allows a determination of whether the telomeric expansion of the genetic map is due to local regions of increased recombination ("hot spots") or to a general increase in recombination in this region. Comparison of the physical with the genetic maps points to several hot spots, one of which is near the telomere (Fig. 6): the distance between CD18 and COL6A1 is about 1500 kbp, whereas the genetic distance is 26 cM (Petersen *et al.*, 1989). An additional hot spot may lie between S15 and MxA/B, where 1250 kbp correspond to 12 cM (Fig. 4 and Petersen *et al.*, 1989). Since COL6A1 is within 700 kbp of the telomere (Fig. 5), this is consistent with an increased genetic recombination rate next to telomeres, as was postulated from the analysis of chiasmata frequencies (Laurie and Hulten, 1985).

Relevance to the Study of Down Syndrome

Down syndrome is a complex disorder with variable phenotypes (Jackson *et al.*, 1976) and results from having three copies of chromosome 21. Rare cases of patients who are trisomic for parts of chromosome 21 and who show a subset of the complete phenotype (e.g., McCormick *et al.*, 1989; Rahmani *et al.*, 1989) suggest that Down syndrome is caused by trisomy of many genes, each responsible for a different phenotype of the syndrome. Knowledge of the correct order of chromosome 21 markers and the distances between them will be very helpful in defining the extent of the trisomy in such studies.

Although most of chromosome 21 is syntenic with mouse chromosome 16 (see Reeves *et al.*, 1987), recent studies have shown that some human chromosome 21 genes map to other mouse chromosomes: collagen VIα1, collagen VIα2, S100B, CD18 (MacDonald *et al.*, 1988) and PFKL (M.B. and G. MacDonald, unpublished) have been assigned to mouse chromosome 10, and CryA1 (α-crystallin 1) and the gene for cystathione-β-synthetase (CBS) have been mapped to mouse chromosome 17 (Münke *et al.*, 1988; Skow and Donner, 1985). These studies did not define the extent and location of the homologous regions on human

chromosome 21. Here, we show that the most telomeric probes are localized on mouse chromosome 10 and extend over 2500–3000 kbp (see Fig. 4). The markers homologous to mouse chromosome 17 are more proximal on human chromosome 21. The most distal markers syntenic with mouse chromosome 16 are MxA/B (Reeves *et al.*, 1988), about 5000 kbp from the telomere (Fig. 4). Therefore, phenotypes of Down syndrome caused by genes located in the most distal 5000 kbp of chromosome 21 are not expected to be present in mice trisomic for mouse chromosome 16.

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