Radiation Hybrid Mapping: A Somatic Cell Genetic Method for Constructing High-**Resolution Maps of Mammalian Chromosomes**

DAVID R. COX,* MARGIT BURMEISTER, E. ROYDON PRICE,† SUWON KIM, RICHARD M. MYERS

Radiation hybrid (RH) mapping, a somatic cell genetic technique, was developed as a general approach for constructing long-range maps of mammalian chromosomes. This statistical method depends on x-ray breakage of chromosomes to determine the distances between DNA markers, as well as their order on the chromosome. In addition, the method allows the relative likelihoods of alternative marker orders to be determined. The RH procedure was used to map 14 DNA probes from a region of human chromosome 21 spanning 20 megabase pairs. The map was confirmed by pulsed-field gel electrophoretic analysis. The results demonstrate the effectiveness of RH mapping for constructing high-resolution, contiguous maps of mammalian chromosomes.

ONSTRUCTION OF A HIGH-RESOLUTION MAP OF THE HUman genome has been of interest to geneticists for the past 50 years, but only recently, with the advent of significant technical advances in molecular and somatic cell genetics, has the possibility of obtaining such a map become a reality. The use of restriction fragment length polymorphisms (RFLP) in conjunction with genetic linkage analysis has allowed the construction of meiotic linkage maps for each of the 23 human chromosomes with an average resolution of 10 to 15 centiMorgans (cM) (1). These maps have proved valuable for localizing human disease genes in the genome, and, in a few instances, they have provided the basis for isolating disease genes (2). The ability to separate human chromosomes from one another, either in rodent-human somatic cell hybrids or by physical chromosome sorting, has also led to significant advances in defining a map of the human genome. Hundreds of human loci have been assigned to specific human chromosomes with these techniques (3). Furthermore, in situ hybridization now provides a means of localizing molecular probes to specific positions on human chromosomes (4).

12 OCTOBER 1990

Despite these technical advances, present-day maps of human chromosomes are very crude in molecular terms. On average, 1 percent meiotic recombination between two markers on a human chromosome corresponds to 1 megabase pairs (Mb) of DNA. In situ hybridization can localize markers to within 2 percent of total chromosome length, but in molecular terms, this again represents several million base pairs. Pulsed-field gel electrophoresis (PFGE), which can separate DNA fragments of several million base pairs in agarose gels, provides a potentially powerful means for constructing long-range physical maps of human chromosomes when used in conjunction with restriction enzymes that cut infrequently in human DNA (5). However, in practice, the paucity of useful rare-cutter enzymes and the nonrandom distribution of rare-cutter sites in human genomic DNA make it difficult to order DNA sequences more than a few hundred kilobase pairs (kb) apart with this technique alone. Thus, obtaining long stretches of contiguous order information at the 100- to 500-kilobase level of resolution remains a difficult task. In an attempt to overcome some of the problems in the construction of high-resolution, contiguous maps of human chromosomes, we have developed a somatic cell genetic mapping approach, radiation hybrid (RH) mapping, which provides a general method for ordering DNA markers spanning millions of base pairs of DNA at the 500-kb level of resolution. We now describe the use of RH mapping, in conjunction with PFGE, to construct a highresolution map of the proximal 20 Mb of the long arm of human chromosome 21.

Theory and practice of radiation hybrid mapping. In this method, which is based on earlier studies by Goss and Harris (6), a high dose of x-rays is used to break the human chromosome of interest into several fragments. These broken chromosomal fragments are recovered in rodent cells, and approximately a hundred such rodent-human hybrid clones are analyzed for the presence or absence of specific human DNA markers. The further apart two markers are on the chromosome, the more likely a given dose of xrays will break the chromosome between them, placing the markers on two separate chromosomal fragments. By estimating the frequency of breakage, and thus the distance, between markers, it is possible to determine their order in a manner analogous to meiotic mapping.

We began with a Chinese hamster-human somatic cell hybrid (CHG3) containing a single copy of human chromosome 21 and very little other human chromosomal material (7). This cell line was exposed to 8000 rad of x-rays, which fragmented the chromosomes and resulted in an average of five human chromosome 21 pieces per cell (8). Because broken chromosomal ends are rapidly healed after

D. R. Cox is in the Department of Psychiatry and the Department of Biochemistry and Biophysics, M. Burmeister is in the Department of Psychiatry and the Department of Psychiatry, and R. M. Myers is. R. Price and S. Kim are in the Department of Psychiatry, and R. M. Myers is in the Department of Physiology and the Department of Biochemistry and Biophysics, University of California at San Francisco, San Francisco, CA 94143.

^{*}To whom correspondence should be addressed. †Present address: Department of Genetics, Harvard University School of Medicine, Boston, MA 02115



x-irradiation, resulting in the fusion of human and hamster fragments, the human chromosomal fragments are usually present as translocations or insertions into hamster chromosomes. However, some cells contain a fragment consisting entirely of human chromosomal material with a human centromere (9). A dose of 8000 rad of x-rays results in cell death, and therefore we rescued the irradiated donor cells by fusing them with nonirradiated hamster recipient cells (GM459) deficient in hypoxanthine phosphoribosyl transferase (HPRT). The fused cells were allowed to grow in HAT medium (100 µM hypoxanthine, 1 µM aminopterin, 12 µM thymidine), which kills the recipient cells, and selects for donor-recipient hybrids that retain the hamster HPRT gene from the irradiated donor cell (10, 11). We isolated 103 independent somatic cell hybrid clones, each representing a fusion event between an irradiated donor cell and a recipient hamster cell, and assayed for the retention of 14 DNA markers of human chromosome 21 by Southern (DNA) hybridization analysis (Fig. 1 and Table 1) (12) although not all hybrids were analyzed for every marker. Even though this fusion scheme did not select for the retention of human chromosomal sequences, each of the 14 chromosome 21 markers was nonselectively retained in 30 to 60 percent of the radiation hybrids (Table 1).

Fig. 1. Southern hybridization analysis of human chromosome 21 DNA markers in selected radiation hybrids. Genomic DNA from human cells, CHG3 cells, the Chinese hamster cell line GM459, and 13 radiation hybrids (Hybrid clones) was digested with Eco RI. The resulting DNA fragments were subjected to electrophoresis in an agarose gel, and then transferred to an MSI membrane (Micron Separations, Inc., Westboro, Massachusetts) (11). The membrane was hybridized with a mixture of five ³²P-labeled human DNA fragments, which recognize the five chromosome 21 loci indicated on the left. The position of Hind III-digested bacteriophage λ DNA fragments, used as a size standard, is indicated on the right. Because each of the five human probes recognizes a different sized Eco RI fragment in human DNA and none of the probes hybridize with hamster DNA, it is possible to analyze each hybrid for all five loci simultaneously. All five loci are present in human and CHG3 genomic DNA, whereas only subsets of the loci are present in most of the radiation hybrid clones. In this figure, a blank lane separates the lane containing GM459 DNA and the lane containing hybrid clone 9 DNA.

Nonselective retention of human chromosomal fragments seems to be a general phenomenon under these fusion conditions, although in some cases, the frequency of retention may be lower than 30 percent (11-14).

Each radiation hybrid often retains more than one human chromosomal fragment, which complicates estimates of the frequency of breakage between any two markers based on observed marker segregation. For instance, a hybrid that retains two markers, A and B, could have resulted from a break between A and B, with retention of the markers on two separate fragments, or from no break between A and B, with both markers retained on a single fragment. Similarly, a hybrid that has lost both markers A and B could have resulted from breakage between A and B, with a loss of two chromosomal fragments, or from no breakage between A and B, with loss of a single fragment containing both A and B. Thus, it is not possible to determine the frequency of breakage between two markers directly from the observed marker segregation in the hybrids. However, if we assume that breakage between two markers is independent of marker retention, and that the retention of one fragment is independent of the retention of any other, we can estimate the frequency of breakage, θ , by the following equation

$$\theta = [(A^+B^-) + (A^-B^+)]/[T(R_A + R_B - 2R_A R_B)]$$

where (A^+B^-) is the observed number of hybrid clones retaining marker A but not marker B, (A^-B^+) is the observed number of hybrid clones retaining marker B but not marker A, T is the total number of hybrids analyzed for both marker A and B, R_A is the fraction of all hybrids analyzed for marker A that retain marker A, and R_B is the fraction of all hybrids analyzed for marker B that retain marker B (15); θ is analogous to a recombination frequency in meiotic mapping. However, unlike a meiotic recombination fre-



Fig. 2. An RH map of 14 DNA markers from the proximal region of the long arm of human chromosome 21. (**A**) Distances between linked markers, expressed in cR_{8000} (Table 1), were used to construct a map that includes the entire set of markers in an order such that the sum of distances between adjacent markers is minimized. This procedure does not determine the order of S8 and APP, as inversion of these markers results in an identical minimum distance. (**B**) The odds against permutation of adjacent loci on the RH map.

These odds compare the likelihood of the given order of four adjacent markers versus the likelihood of the order in which the two internal markers are inverted. For example, the order S16-S48-S46-S4 is 10^6 times more likely than the order S16-S48-S48. The odds ratios at the left end of the map represent a comparison of the likelihood of S16-S48-S46-S4 versus S48-S16-S46-S4 (4 × 10^3 :1), whereas those at the right end compare the likelihood of APP-S12-S47-SOD1 versus APP-S12-SOD1-S47 (400:1).

Fig. 3. PFGE analysis of the human chromosome 21 loci S16 and S48. DNA from human blood cells was digested with the restriction enzymes shown above each lane, and fragments were separated by CHEF gel electrophoresis, with switching times of 30 seconds. Multimers of the phage λ ZAP (Stratagene, La Jolla, California), used as size markers, are present in the left lane of each panel. "Lim." denotes the region of limiting mobility in the gel. DNA was transferred to a membrane (MSI) and hybridized (20) successively to probes E9, which recognizes locus S16, and SF105, which recognizes locus S48. Because Sma I, Xho I, and Cla I do not cleave DNA that is methylated at their respective sites, partial methylation in genomic blood cell DNA results in partial digestion with these enzymes and leads to multiple fragments that hybridize to each probe. The two probes hybridize to the same size bands in genomic DNA digested with Sma I and Xho I. The smallest fragment recognized by both probes is a Sma I fragment of about 150 kb, defining the maximum distance between the two loci. The size estimates take into account a significant curvature of the gel.

quency, which can vary from 0 to 0.5, θ varies from 0 to 1.0. A θ value of 0 indicates that two markers are never broken apart, whereas a θ value of 1.0 indicates that two markers are always broken apart and are therefore unlinked. A lod score (logarithm of the likelihood ratio for linkage) identifies those marker pairs that are significantly linked, as in the case of meiotic linkage analysis. For our chromosome 21 data set, a lod score of 3.0 or more is taken as evidence for significant linkage (16).

Although θ is a good estimate of the distance between markers that are close together, it can underestimate the distance between more distant markers. The mapping function, $D = -\ln(1 - \theta)$, which assumes no interference and is analogous to the Haldane mapping function in meiotic linkage analysis (17), can be used to make a more accurate estimate, D, of distance between two markers; D is expressed in centiRays (cR), analogous to centiMorgans. Because the frequency of breakage between two markers, and thus

Fig. 4. Physical linkage of five human chromosome 21 loci and comparison to the RH map. Genomic DNA from CHG3 cells was cleaved with the enzymes Not I (N) and Sal I (L), and the fragments were separated by CHEF-gel electrophoresis. The lanes between the Not I and Sal I digests correspond to Sna BI digests, which were uninformative. The DNA was transferred to Nylon membranes and hybridized successively to probes specific for the loci S16, S13, S46, S4, and \$52 (20). Approximate sizes, derived from yeast chromosomes as size standards (5, 20), are indicated to the right. Open diamonds, a Not I fragment shared by S16 and S13; closed diamonds, a second Not I fragment shared between S13 and S46; arrows, a third Not I fragment shared between S4 and S52; open triangles, three Sal I fragments shared between S46 and S4, the largest of which is also recognized by S52. A restriction map derived from these data and data obtained with the additional rare-cutter restriction enzymes Eag I and BssH II (21) is shown below the five autoradiogram panels. Horizontal arrows below each locus indicate the relative position of that locus on the map, except for \$13, the position of which is shown by a vertical arrow. Vertical bars designate cleavage sites for the indicated restriction enzymes. Dotted lines indicate partial Sal I digestion products. The scale indicates distance in kilobase pairs (kb). The RH map of these five loci is shown at the bottom of the figure, with the distances between adjacent loci expressed in centiRays for an x-ray dose of 8000 rad (cR_{8000}). Comparison of the physical map with the RH map indicates that the order of loci and relative distances between loci are similar.



D, depends on the amount of irradiation, 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 percent frequency of breakage between the markers after exposure to 8000 rad of x-rays.

Although it is easy to calculate a lod score, θ , and D for any single pair of markers, such determinations are tedious for a large number of pairwise marker combinations. As a result, we developed a computer program in which a Lotus spread sheet was used to determine marker segregation from raw data. This program calculates the lod score, θ , and D for each pairwise combination of markers (18). The output generated by this program for selected pairwise combinations of the 14 chromosome 21 DNA markers is



RESEARCH ARTICLE 247

Loci	Clones (no.)	Reten- tion		
S16	81	0.59		
S48	96	0.58		
S46	71	0.54		
S4	96	0.50		
S52	67	0.46		
S11	94	0.56		
S1	91	0.47		
S18	95	0.37		
S8	71	0.41		
APP	71	0.34		
S12	94	0.36		
S111	68	0.32		
S47	85	0.42		
SOD1	64	0.41		

Table 1. Retention of human chromosome 21 DNA in radiation hybrids. The 14 chromosome 21 loci, together with the number of clones assayed for each locus and the fraction of analyzed clones retaining the locus is shown.

shown in Table 2. To construct an RH map of this set of 14 markers, we first identified those pairs of markers that are significantly linked. We then used only this set of linked marker pairs to determine the "best" map, defined as that which included the entire set of markers in an order such that the sum of the distances between adjacent markers is minimized. This process of identifying the best map was carried out by trial and error, resulting in a map of 14 markers spanning a distance of 341 cR_{8000} (Fig. 2A). We obtained the same map whether we used a lod score of 3.0 or greater or the more stringent criterion of 4.0 or greater as evidence of significant linkage.

Because RH mapping is a statistical procedure, the RH map defined as the best map does not necessarily represent the actual order of markers on the chromosome. Therefore, some measure of the relative likelihood of one order versus another is required. The likelihood of any particular order of four markers can be calculated by extending the method used to determine the likelihood of the order of any pair of markers. However, it is not practical to use this approach to calculate the likelihood of an order for more than four markers. We consider that one order is significantly more likely than another when the ratio of their likelihoods is greater than 1000:1. To facilitate the comparison of various marker orders, we have developed a second computer program that uses a Lotus spread sheet to calculate the likelihood of each of the 12 possible orders of a set of four markers and to list these orders from the most likely to the least likely (19). With this method, it is possible to distinguish regions of the RH map where confidence in the marker order is strong as opposed to weak. For example, the order \$16-\$48-\$46-\$4 is more than 1 million times more likely than the order \$16-\$46-S48-S4 (Fig. 2B), providing strong evidence in favor of the first order. In contrast, the order S52-S11-S1-S18 is only 56 times more likely than the order S52-S1-S11-S18. In this case, the data are not strong enough to determine an unambiguous order of \$1 and \$11. Overall, this type of analysis indicates that the order determined for the majority of markers on the RH map is significantly more likely

Fig. 5. PFGE analysis of the human chromosome 21 loci APP, S12, and S111. Genomic DNA from the cell line CHG3 was cleaved with the enzymes Not I, Eag I, Sal I, BssH II, and Mlu I, and separated by FIGE with Program 7 of a P100 FIGE apparatus (MJ Research, Cambridge, Massachusetts). DNA was transferred to GeneScreen Nylon membranes and successively hybridized to probes that recognize the loci APP, S12, and S111. Probes for all three loci recognize a common BssH II fragment. Probes for APP and S12, but not S111, recognize a common Sal I fragment, whereas probes for S12 and S111, but not APP, recognize a common Eag I fragment. These data establish the order of these three loci as APP-S12-S111. The map shown below the figure is an approximation since double digests to position the Eag I and Sal I sites relative to each other were not performed.

248

than any alternative order (Fig. 2B).

Confirmation of the RH map by PFGE. In PFGE mapping, large DNA fragments are separated in agarose gels subjected to alternating electric fields. The DNA fragments are transferred to membranes, which are then hybridized to the markers in question (20). Markers are determined to be physically linked when they recognize identical large DNA fragments on such a membrane.

The optimal resolution range of PFGE usually requires that markers are spaced every 500 kb on average. Therefore, we expected that only those marker pairs that were close to each other by RH mapping would be shown to be physically linked by PFGE analysis. Indeed, S16 and S48, which were determined to be 8 cR₈₀₀₀ apart by RH mapping, were found to be within 150 kb of each other by PFGE analysis (Fig. 3). Similarly, the two loci S1 and S11, which had an RH map distance of 11 cR₈₀₀₀, were found to lie within 150 kb of each other by PFGE analysis (21). Additional PFGE mapping data further confirmed the order of DNA markers generated by RH mapping. We reasoned that enough markers were available in the region between \$16 and \$52 that it should be possible to establish a continuous physical map by PFGE. To facilitate this analysis, we used an additional locus, S13, recognized by the probe M21 (Fig. 4). Because this probe contains sites for the rare-cutter restriction enzymes Not I and BssH II, it is a useful "linking clone" that recognizes different large Not I and BssH II fragments extending in either direction from the locus. The order (S16/S48)-S13-S46-S4-S52 is given unambiguously by shared restriction fragments with two enzymes, Sal I and Not I (Fig. 4). A continuous restriction map spanning 4500 kb was constructed from these results. A comparison of the PFGE and RH maps from this region (Fig. 4)



SCIENCE, VOL. 250

demonstrates that the order is identical and distances between markers are similar.

One region where RH mapping could not determine the order of markers unambiguously was between S8 and S12. The odds for the order S18-S8-APP-S12 compared to S18-APP-S8-S12 were only 43:1. Therefore, in this region, additional mapping information was necessary to determine a definitive order. PFGE analysis showed that S8 and S18, but not APP and S12, recognize a Not I fragment of about 4.5 Mb, whereas APP, S111, and S12 recognize a common Not I fragment of about 3000 kb (21). PFGE analysis indicates that these three loci are within less than 1300 kb of each other and that their order is APP-S12-S111 (Fig. 5). Thus, these DNA markers can be grouped into the mutually exclusive clusters S18-S8 and APP-S12-S111. These PFGE results are consistent with the order S18-S8-APP-S12 and they exclude the order S18-APP-S8-S12. In addition, this analysis provides order information for the markers S12 and S111, which was not obtained by RH mapping because of the lack of x-ray breakage between these two markers. Thus, it is possible to clarify ambiguous marker orders by combining data from PFGE and RH mapping.

Several regions of chromosome 21 have been analyzed by both RH mapping and PFGE, and therefore it is possible to determine the relation between RH map units and physical distance. The region between \$16 and \$52 covers about 3500 kb as determined by PFGE (Fig. 4). This same region spans 66 cR₈₀₀₀ as determined by RH mapping. Therefore, in this case, where we know that there are no gaps in the physical map, 1 cR₈₀₀₀ corresponds to 53 kb. A similar analysis of the region between S52 and S111, which is estimated to span 10,800 kb, showed that 1 cR₈₀₀₀ corresponds to an average of 51 kb (22). Finally, in another study of the distal region of the long arm of human chromosome 21, it was shown that 1 cR₈₀₀₀ corresponds to 56 kb (23). Thus, we found that distance estimated by RH mapping is directly proportional to physical distance. This was surprising, since there is no a priori reason why hot spots of x-ray breakage should not occur in some regions of the chromosome, distorting the relation between RH map units and physical distance. Although we found no evidence for hot spots of xray breakage on chromosome 21, such regions may exist in other parts of the genome.

Applications of RH mapping. Our RH and PFGE mapping studies have allowed us to construct a high-resolution map of the proximal half of human chromosome 21q. This map together with a map generated similarly on the distal region of 21q (23), provides a complete, continuous map of the long arm of chromosome 21. Our maps are in good agreement with both physical (24) and meiotic (25) maps previously described. The RH map is, in general, confirmed by our PFGE analyses.

During the construction of these maps, it became clear that, in many instances, RH and PFGE mapping are complementary. Our PFGE mapping studies grouped the DNA markers into four clusters: (S16/S48)-S46-S4-S52, S1-S11, S18-S8, and APP-S12-S111. Although the order of markers within each cluster could be determined by PFGE, the orders and distances between the clusters could not be established by this technique alone. In contrast, RH mapping allowed the construction of a continuous map, but was not able to resolve the orders of some markers that could be determined by PFGE; for example, S12 and S111. In other cases—for example, S16 and S48–RH mapping was able to determine the order of markers, whereas PFGE was not. Therefore, even though RH mapping is a statistical rather than a physical mapping method, when combined with PFGE, it is an efficient means of establishing physical maps of human chromosomes.

Because RH mapping does not depend on the availability of a selectable marker for the chromosome of interest, this method can

Table 2. Distances between DNA markers determined by RH mapping. Selected pairwise combinations of the 14 chromosome 21 DNA markers used to construct the RH map are listed under markers A and B. For each marker pair, the number of radiation hybrids that retain both marker A and marker B (++), marker A and not marker B (+-), marker B and not marker A (-+), neither marker A nor marker B (--), and the total number of hybrids analyzed for both markers (sum) are listed, followed by the estimated frequency of breakage between the two markers (θ) , and the estimated distance between the markers (RH map units). Each estimated distance is followed by the standard deviation of that estimate. The lod score for each pair of markers, is a measure of the likelihood that the two markers are linked.

Marker		Clones observed (no.)				θ	RH map	Lod	
Α	В	++	+	-+		Sum	v	units (cR ₈₀₀₀)	score
S16	S48	46	2	1	32	81	0.08	8 ± 5	18.31
S48	S46	37	2	1	31	71	0.09	9±5	15.97
S8	APP	24	4	0	42	70	0.12	13 ± 7	13.60
S11	S1	43	7	0	41	91	0.15	17 ± 7	16.96
S16	S46	36	4	2	29	71	0.17	19 ± 8	12.42
S46	S4	32	6	1	32	71	0.20	22 ± 9	11.51
S47	SOD1	20	2	5	35	62	0.23	26 ± 11	8.63
S4	S52	27	4	4	32	67	0.24	27 ± 10	9.53
S46	S52	29	6	2	29	66	0.24	28 ± 11	9.40
APP	S12	18	6	2	45	71	0.25	28 ± 11	8.77
S48	S4	46	10	2	38	96	0.25	29 ± 9	13.29
S8	S12	20	9	0	42	71	0.27	31 ± 11	8.63
S18	APP	20	6	4	40	70	0.31	37 ± 13	7.59
S12	S47	27	4	9	45	85	0.32	38 ± 12	9.22
S18	S8	22	4	7	37	70	0.33	40 ± 14	7.32
APP	S47	17	6	5	40	68	0.34	42 ± 14	6.48
S1	S18	30	12	5	43	90	0.38	48 ± 14	8.12
S52	S1	24	6	7	28	65	0.40	51 ± 17	5.40
S52	S11	27	4	12	24	67	0.47	64 ± 20	4.32
S1	S8	22	9	7	29	67	0.48	66 ± 20	4.13
S11	S18	30	22	5	36	93	0.56	83 ± 21	4.29
<u>\$4</u>	S11	36	11	17	30	94	0.60	91 ± 23	3.45
S12	SOD1	13	6	13	31	63	0.64	101 ± 33	1.70

be used to map any mammalian chromosome present as a single copy in a Chinese hamster cell. In theory, it should also be possible to use a Chinese hamster cell containing single copies of several heterologous chromosomes as a donor cell line. Although radiation hybrids generated from such a donor would be useful for constructing maps of these heterologous chromosomes, these hybrids would be less useful as a source of DNA markers from a specific chromosomal region. Occasionally, we have observed that a particular combination of donor and recipient cell lines does not yield viable hybrids after irradiation and cell fusion. In such cases, we have been able to obtain hybrids by using a different recipient Chinese hamster cell line.

One consequence of the high frequency of retention of human DNA fragments in radiation hybrids is that many hybrid cells retain more than one human chromosomal fragment. Fortunately, because of its statistical nature, RH mapping does not require knowledge of the number of human chromosomal fragments in a particular hybrid in order to construct a map. However, it is not advisable to use an individual radiation hybrid as a reagent to map probes or to isolate probes from a specific chromosomal region without cytogenetic characterization to determine whether or not that radiation hybrid contains a single contiguous human chromosomal fragment. Extensively characterized cell lines that are demonstrated to contain a single chromosomal fragment can be valuable reagents for both regional mapping and isolation of new DNA markers (14, 26).

We have found that many, but not all, human chromosomal fragments in radiation hybrids are retained in a stable fashion (11). Fragment instability does not adversely affect RH mapping if a

single batch of DNA from each hybrid is used to score all markers. However, fragment instability, combined with the large amount of hamster DNA relative to human DNA in hybrid cells, significantly reduces the hybridization signals obtained with some radiation hybrids.

RH mapping involves the analysis of a single copy of the human chromosome of interest, unlike meiotic mapping, in which two copies of a human chromosome must be distinguished from one another by DNA polymorphisms. Therefore, even nonpolymorphic DNA markers, which cannot be used for meiotic mapping, can be used for RH mapping. This ability to use a wider spectrum of DNA markers and the fact that all probes are informative in every cell line are major strengths of RH mapping. Another advantage is that the range of resolution of RH mapping can be varied by altering the xray dose used to fragment the chromosomes. We have found that 8000 rad is a useful dose for RH mapping, as it produces maps in a range of resolution not easily obtained by other mapping methods.

REFERENCES AND NOTES

- 1. H. Donis-Keller et al., Cell 51, 319 (1987); P. O'Connell et al., Genomics 5, 738 (1989)
- 2. B. Royer-Pokora et al., Nature 322, 32 (1986); A. P. Monaco et al., ibid. 323, 646 (1986); J. M. Rommens et al., Science 245, 1059 (1989); J. R. Riordan et al., ibid., p. 1066.
- 3. P. J. McAlpine et al., Cytogenet. Cell Genet. 51, 13 (1989).
- 4. P. Lichter et al., Science 247, 64 (1990).
- D. C. Schwartz and C. R. Cantor, Cell 37, 67 (1984); D. P. Barlow and H. Lehrach, Trends Genet. 3, 157 (1987).
- S. J. Goss and H. Harris, Nature 255, 680 (1975); J. Cell Sci. 25, 17 (1977)
- The CHG3 cell line is a subclone of the hamster-human hybrid cell line 72532X-6 [D. Patterson et al., Ann. N.Y. Acad. Sci. 450, 109 (1985)].
- 8. In an effort to determine the extent to which human chromosome 21 was fragmented by 8000 rad of x-rays, we used in situ hybridization with biotinylated human genomic DNA as a probe to analyze the chromosomes of donor cells at the first mitotic division after x-irradiation (11). Although 8000 rad of x-rays results in the death of donor cells, such irradiated cells are able to undergo one cell division. We found that the number of human chromosomal segments per cell followed a Poisson distribution, with an average of five segments per cell. Based on an estimated size of human chromosome 21 of 40 Mb, these results indicate that 8000 rad resulted in a chromosome break approximately every 8 Mb.
- 9. D. R. Cox and R. M. Myers, unpublished observations
- 10. The recipient hamster cell line, GM459, was obtained from the NIGMS Human Mutant Cell Repository, Camden, NJ.
- 11. Irradiation and cell fusion were carried out exactly as described [D. R. Cox et al., Genomics 4, 397 (1989)].
- The origins of probes used in our study are as follows: PW228C (D21S1), PW233F (D21S4), PW245D (D21S8), PW236B (D21S11), PW267D (D21S12), and PW511-1H (D21S52) [P. Watkins et al., Nucleic Acids Res. 13, 6075 (1985); M. Van Keuren et al., Am. J. Hum. Genet. 38, 793 (1986)]. SF85 (D21S46), SF103 (D21S47), and SF105 (D21S48) [J. R. Korenberg, M. L. (D21346), SF105 (D21347), and SF105 (D21348) [J. K. Korenberg, M. L. Croyle, D. R. Cox, Am. J. Hum. Genet. **41**, 963 (1987)]. GSM21 (D21S13), GSE9 (D21S16), and GSB1 (D21S18) [G. Stewart, P. Harris, J. Gatt, M. A. Ferguson-Smith, *Nucleic Acids Res.* **13**, 4125 (1985)]. (Because probe GSB1 contains repetitive sequences, we used only a subset of the entire 6-kb Eco RI fragment of this probe to obtain unambiguous results in both RH mapping and PFGE analysis. The 6-kb probe was digested with Hae III, and the largest resulting fragment, which is about 3 kb in length, was used as a probe.) FB68L (APP) [R. Tanzi et al., Science 235, 880 (1987)], SOD-1 (SOD1) [Y. Groner et al., N.Y. Acad. Sci. 450, 122 (1987)]. 133 (1985)]. (A 4.1-kb Bgl II fragment, which contains a portion of intron 4 and all of exon 5 of the SOD1 gene, was excised from SOD-1 and used as a probe.) MBpcq (D21S111), a plasmid carrying a 694-bp insert comprising nucleotides 249 to 943 of λpq [C. Wong et al., Proc. Natl. Acad. Sci. U.S.A. 86, 1914 (1989)] The insert was generated by PCR amplification of human genomic DNA and cloned into pUC18. We have abbreviated the locus names; for example, S16 indicates the locus D21S16. Insert isolation, DNA (Southern) blot preparation, and hybridization have been described (11).
- F. Benham et al., Genomics 4, 509 (1989).
 P. J. Goodfellow, S. Povey, H. A. Nevanlinna, P. N. Goodfellow, Somat. Cell Mol. Genet. 16, 163 (1990).
- 15. Although it is not possible to determine the frequency of breakage between two markers directly from the observed marker segregation in the hybrids, it is possible to estimate the frequency of breakage between two markers with an algorithm. We can define the observed marker segregation in terms of four unknowns: θ , P_A , the probability of retention of a fragment containing A and not B; P_B , the probability of retaining a fragment containing B and not A; and P_{AB} , the probability of retaining a fragment that contains A and B without a break between them. In this model, the fraction of hybrids retaining marker A but not marker B, $(A^+B^-)/T$, can be represented as $(A^+B^-)/T = \theta P_A(1 - P_B)$. Similarly, $(A^-B^+)/T =$

 $\theta P_B(1 - P_A), (A^+B^+)/T = (1 - \theta)P_{AB} + \theta P_A P_B, \text{ and } (A^-B^-)/T = (1 - \theta)(1 - P_{AB}) + \theta(1 - P_A)(1 - P_B).$ Solving for θ in terms of P_A and P_B gives $\theta = [(A^+B^-) + (A^-B^+)]/T(P_A + P_B - 2P_A P_B)$. It is not possible to determine P_A and P_B directly from the observed data. However, if we use R_A as an estimate of P_A , and R_B as an estimate of P_{B_1} it is possible to use the above equation to estimate θ . The fact that these estimates of P_A and P_B , when used in the above equations, define values of $(A^+B^-)/(A^-B^+)$ that are not significantly different from the observed values of $(A^+B^-)/(A^-B^+)$ for each marker pair, indicates that the model, as well as values of (A B)/(A B') for each marker pair, indicates that the model, as well as the estimates of P_A and P_B, are appropriate. Simpler models assuming P_A = P_B, which require that (A⁺B⁻) = (A⁻B⁺), do not fit the observed data. R_{AB}, which is an estimate of P_{AB}, can be described in terms of R_A, R_B, and θ as follows: R_{AB} = {[(A⁺B⁺)/T] - θR_AR_B}/(1 - θ).
16. The likelihood of obtaining the observed data, L_θ, for a given pair of markers is defined as L_θ = [(1 - θ)R_{AB} + θR_AR_B]^{(A+B+})[θR_A(1 - R_B)]^{(A+B-})[θ(1 - R_A)-R_B]^{(A-B+})[(1 - θ)(1 - R_{AB}) + θ(1 - R_A)(1 - R_B)]^{(A-B-}). The lod score for the marker pair is defined as lod = log [L₁/(L₀ = 1)] where L₀ = 1 is the likelihood

- marker pair is defined as $\log = \log [L_{\theta}/(L_{\theta} = 1)]$ where $L_{\theta} = 1$ is the likelihood assuming that the two markers are not linked; that is, $\theta = 1$. This lod score can be used to identify marker pairs that are significantly linked, in a manner analogous to meiotic mapping. We arbitrarily set a lod score of 3.0 as evidence of significant linkage between two markers. Because the prior probability of linkage between two markers is greater for RH mapping than for standard meiotic mapping (8), a lod score of 3.0 or greater is a more stringent criterion of significance than is typically used in meiotic linkage analysis.
- 17. J. Haldane, J. Genet. 8, 299 (1919).
- 18. This computer program is available upon request.
- 19. The likelihood for a particular order of four markers, A, B, C, and D, can be calculated by an equation analogous to that already described (16). In the case of four markers, however, there are 16 possible observed classes of hybrids $(A^+B^+C^+D^+, A^-B^+C^+D^+, A^+B^-C^+D^+, and so on)$ in the likelihood calculation. In addition, calculation of the likelihood requires estimates of the frequency of breakage between markers A and B, markers B and C, and markers C and D, as well as estimates of ten retention frequencies (PA, PB, PC, PD, PAB, PBC, PCD, PABC, P_{BCD} , and P_{ABCD}). We use the values of θ for markers A and B, B and C, and C and D, calculated as described in (15), as estimates of frequency of breakage between these markers. P_A , P_B , P_C , P_D , P_{AB} , P_{BC} , and P_{CD} are also estimated as described in (15). R_{AC} , calculated as described in (15), is used to estimate P_{ABC} . Because P_{AC} is the probability of retaining both markers A and C on a fragment with no breakage between them, $P_{AC} = P_{ABC}$, given the marker order ABCD. Similarly, R_{BD} is used to estimate P_{BCD} , and R_{AD} is used to estimate P_{ABCD} . Because the equation used to calculate the likelihood of a particular order of four markers consists of over 50 terms, it is not included here. However, this equation is the basis of the computer program we have developed to compare the likelihoods of different marker orders. This program is available on request.
- For PFGE analysis, DNA from the cell line CHG3 (7) or human peripheral blood cells was cleaved with restriction endonucleases that cut rarely in mammalian genomes, and the resulting fragments were separated by field inversion gel electrophoresis (FIGE) [G. F. Carle, M. Frank, M. V. Olson, *Science* 232, 65 (1986)] or contour-clamped homogeneous electric field gel electrophoresis (CHEF) [G. Chu, D. Vollrath, R. W. Davis, *ibid.* 234, 1582 (1986)]. DNA was blotted onto GeneScreen membranes (New England Nuclear, Boston), which were treated and hybridized to DNA inserts as described [B. G. Herrmann, D. P. Barlow, H. Lehrach, Cell 48, 813 (1987)]. Chromosomes of Saccharomyces cerevisiae and Schizosaccharomyces pombe were used as size markers. Estimates of fragment length may have an error as great as 20 percent.
- 21 M. Burmeister, unpublished observations
- 22. S1 and S11 recognize a 3300-kb Not I fragment, S18 and S8 recognize a 4500-kb Not I fragment, and APP, S12, and S111 recognize a 3000-kb Not I fragment [M. Burmeister, unpublished observations]. These three fragments together comprise about 10,800 kb of DNA, which can be used as an estimate of the distance between S52 and S111 if we assume that any remaining gaps in this region of the chromosome are small. Since the RH map distance from S52 to S111 is 211 cR8000, 1 cR8000 corresponds to an average of 51 kb in this region of the chromosome.
- M. Burmeister et al., Genomics, in press.
 K. Gardiner et al., Somat. Cell Mol. Genet. 14, 623 (1988); K. Gardiner et al., EMBO J. 9, 25 (1990); M. J. Owen, L. A. James, J. A. Hardy, R. Williamson, A. M. Goate, Am. J. Hum. Genet. 46, 316 (1990). In some instances, there are differences in the sizes or number (or both) of fragments observed in these various studies as compared to our data. These differences most likely arise from polymorphisms in the blood or hybrid cell line DNA used in each study. Nevertheless, the order of markers and the distances between them are generally consistent with our results
- R. E. Tanzi et al., Genomics 3, 129 (1988); A. C. Warren, S. A. Slaugenhaupt, J. G. Lewis, A. Chakravarti, S. E. Antonarakis, *ibid.* 4, 579 (1989); M. B. Peterson et al., Am. J. Hum. Genet. 45 (suppl. 1), abstr. 157 (1989)
- C. A. Pritchard, D. Casher, E. Uglum, D. R. Cox, R. M. Myers, Genomics 4, 408 27.
- (1989); T. Glaser, E. Rose, H. Morse, D. Housman, C. Jones, *ibid.* 6, 48 (1990). We thank D. Patterson for hybrid cell line 72532X-6; Y. Groner, J. Gusella, G. Stewart, R. Tanzi, and P. Watkins for providing DNA probes; A. Chakravarti, J. Ott, D. T. Bishop, C. Falk, and M. Boehnke for stimulating discussions concerning the mathematical treatment of RH data; C. Murray for help in the initial scoring of hybrid cells; and S. Rider and A. Porter for in situ hybridization analysis. Supported by grants from the NIH and the Wills Foundation (D. R. C. and R. M. M.) and a postdoctoral fellowship from the Gene Technology Program of the Deutscher Akademischer Austauschdienst (M. B.).

5 June 1990; accepted 30 August 1990