

Physical and Genetic Maps for Chromosome 10

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A fluorescence *in situ* hybridization (FISH) physical map of 14 polymorphic loci on chromosome 10 covers over 62% of the fractional length of chromosome 10. The positions of three previously mapped loci are confirmed, nine more are refined, and two new loci are cytogenetically mapped. The order of loci determined by FISH agrees with that obtained by genetic linkage studies. When the distance estimates for the physical map are compared to the distance estimates of our existing linkage map, the sex average ratio of the fractional length (FL) per centimorgan (cM) for this portion of chromosome 10 is 0.004 (or 0.4% FL/cM). However, the average ratios for male- and female-specific genetic distances are quite different, in agreement with an overall higher rate of recombination in females (0.008 FL/cM and 0.003 FL/cM, respectively). Moreover, the ratio across the centromere is larger for both the male (0.031 FL/cM) and the female (0.009 FL/cM) than the ratio encompassing the q arm (0.006 FL/cM for males and 0.002 FL/cM for females), suggesting that there is reduced recombination at the centromere in both the male and the female maps when compared to the physical distance generated from FISH on metaphase chromosomes. © 1993 Academic Press, Inc.

INTRODUCTION

One of the broad goals of the human genome initiative is the construction of complete maps for all of the human chromosomes. To this end, several types of maps are being produced. The commonalities in these mapping strategies are the determinations of order and distance of unique DNA sequences along a chromosome. The true order for all types of maps will be the same since all are based on the "identical" strand of DNA constituting each chromosome. Various methods, however, have inherent limitations, associated uncertainties in the data generated, and different metrics. Physical maps are of three types: molecular, cytogenetic, and radi-

ation fragmentation; linkage maps are based on recombination frequency. Each of these four maps has its own metric for measuring distance. The exact number of basepairs between any two points in the genome is the ultimate metric of the physical map.

Currently much molecular mapping is being accomplished by contig assembly and PFGE providing order-of-marker information and estimates of distance; ultimately the distances will become more precise by DNA sequencing. Cytogenetic maps, based on the positions of hybridization along a metaphase chromosome as detected by fluorescence *in situ* hybridization (FISH), use fractional length of the chromosome as their metric. The frequency and location of chromosomal breaks between two points, usually induced by X-ray irradiation of cells in culture, provide the basis for a radiation fragmentation metric, whereas the frequency of meiotic recombination provides the basis for the metric in the linkage map. Confidence in a map is enhanced when the same order is produced by two or more methods. However, the correspondences for the metrics resulting from different methods are not known. A common generalization about the relationship between distances in the molecular and linkage maps is used (i.e., 1 Mb = 1 cM) but is a crude genome-wide average and is assuredly incorrect for most specific places in the genome.

Previously, genetic maps of chromosome 10 have been carefully refined (Wu *et al.*, 1990; Gardner *et al.*, 1991; Lichter *et al.*, 1992a) and physical maps summarized (Smith and Simpson, 1989; Lichter *et al.*, 1991a; Carson and Simpson, 1992; Simpson and Cann, 1992). Here, we present a physical map of much of chromosome 10 as determined by FISH. We chose 14 polymorphic markers that are already in our linkage map to put into our FISH map; this allows us to compare the relationship between the FISH-based physical map and the linkage map.

METHODS

Probes and DNA. Probes and DNA have been previously described (Lichter *et al.*, 1992b; Wu *et al.*, 1990). D10S94 probes are described in Brooks-Wilson *et al.* (1992a,b).

FISH. The FISH was performed according to the procedure of Lichter *et al.* (1990) using C_{ot}1 DNA (BRL) for suppression of highly

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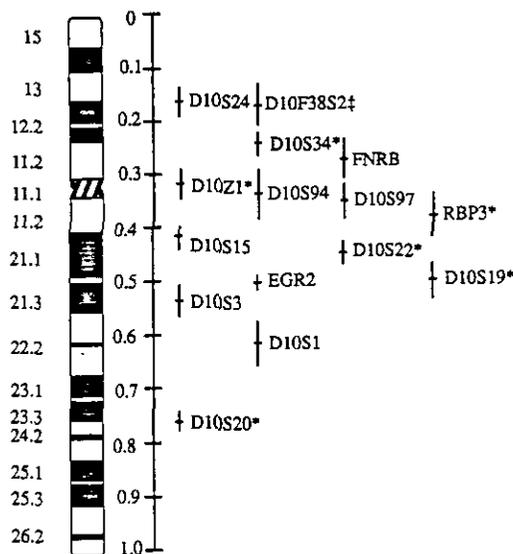


FIG. 1. The fractional length of the chromosome to which each locus maps by FISH. The averages and ranges of the several independent measurements for each probe are given; the number of measurements, mean, and median for each locus are given in Table 1. The starred (*) loci are the framework markers from HGM 11 (Cann and Simpson, 1992); the locus indicated by ‡ is not part of the linkage map and is recognized by the same probe that recognizes D10S97.

repetitive G-C-rich *Alu* sequences in the probes. The chromosomes were counterstained with DAPI to give a G-like banding pattern for easy chromosome identification. All studies (except where noted below) were done with a single probe per hybridization. The fractional length (distance) from the p-terminus was calculated and the range mapped onto a chromosome idiogram to give approximate band designation (Fig. 1). All probes were imaged at least three times (three different chromosomes) with a minimum chromosome length of 8.3 μm to ensure more accurate measurements (Baldini and Ward, 1991). All imaging and measurements were done blindly with respect to presumed location and order of the loci. Two locus pairs (FNRB/D10S34 and RBP3/D10S15) were also studied using double labeling experiments. Both D10S34 and RBP3 were labeled with digoxigenin and FNRB and D10S15 with biotin and all four probes were hybridized simultaneously to the same slide. The digoxigenin probes were detected with anti-digoxigenin-rhodamine and the biotin probes with avidin-FITC, producing two signals per chromosome for each detection filter. Because the mapping positions of all four probes were known (either p or q), it was easy to distinguish between similarly labeled probes (i.e., D10S34 and RBP3 and FNRB and D10S15).

Genetic distance estimates. We transformed our previous estimates of the recombination frequencies between adjacent pairs of loci (Wu *et al.*, 1990; Lichter *et al.*, 1992a,b) into centimorgans (cM) using the Haldane transformation. These distances were then added up over the adjoining intervals, summing outward from the centromere (D10Z1).

Analytical tools. Statistical analyses were performed using JMP (version 4.0.3) for the Macintosh from the SAS Institute to test for linearity and deviations in the slopes. We used D10S15 as the anchor between the long arm and the centromere. D10S15 data were included in the analyses for both data sets (long arm and centromere). The short arm side of the centromere was defined as FNRB; FNRB was used in analyses for both the short arm slope calculations and the centromeric slope calculations.

RESULTS

The results of our FISH experiments and the resultant map of chromosome 10 are summarized in Fig. 1

and Table 1. The 14 loci allow reasonably even coverage of over 62% of chromosome 10, with the exception of the telomeres where no probes have been studied and the short arm where only three loci have been studied in our lab.

Six of these loci are "framework" markers for chromosome 10 (Smith and Simpson, 1989, * in Fig. 1.). The localization of three of these markers greatly refines their position in the physical map: D10S34, D10S19, and D10S20 are now precisely mapped to small subregions of chromosome 10. Data on D10Z1, RBP3, and D10S22 further confirm the positions of these loci along chromosome 10.

The two broad mapping goals are order and distance. The order determined by blind evaluation of the FISH data generally agreed with previously determined and expected results (Wu *et al.*, 1990). However, as discussed below, order was not always clear. We believe that the concordance of the physical and genetic maps further validates the order of markers on chromosome 10 with a few notable ambiguities.

Two probes give hybridization signals at two different loci. The first, pKW6FSacI, produces two distinct signals in the FISH map, one on 10p13 (D10F38S2) and another on 10q11.2 (D10F38S1, also known as D10S97). We used the polymorphic 10q11.2 locus in our analyses (Wu and Kidd, 1990; Lichter *et al.*, 1993). The probe Dry 5-1, which recognizes D10S1, also produces two signals using FISH. Both linkage and somatic cell genetics studies have placed D10S1 on 10q22-q23 (Lichter *et al.*, 1991a; Simpson and Cann, 1992; Wu *et al.*, 1990). This polymorphism is detected as a single variable band among many constant bands, some giving a much more intense signal. The FISH results clearly place the major hybridization signal at 11p11.2-q11 (FLpter on chromosome 11 is 0.357-0.422), an apparently monomorphic locus, with a minor signal at 10q22, the polymorphic locus. Thus, the first polymorphic locus assigned to chromosome 10 (D10S1) is apparently identified by a clone from chromosome 11 and most of the many bands seen on a Southern blot may be chromosome 11 repetitive sequence.

The relative orders of three pairs of loci require comment. The FNRB-D10S34 FISH data imply that FNRB may be proximal to D10S34. Previously, linkage maps have shown that FNRB is distal to D10S34 (Lichter *et al.*, 1992b; Gardner *et al.*, 1991). Three possibilities may explain this apparent discrepancy. First, the observed individual FLpter ranges overlap (Table 1) and both orders were observed in double-labeling experiments on BrdU elongated metaphase spreads. Apparently, the two loci are sufficiently close that loops in the chromatin fiber can obscure true order. The relatively small number of observations precludes any meaningful likelihood analysis for the double-labeling FISH data, although one could model the probability of seeing the wrong order as a decreasing function (from 0.5 to 0.0) of the physical distance between two loci. Second, there may be cross-hybridization of one or more of the probes at these loci to

TABLE 1
FISH Data for Some Polymorphic Chromosome 10 Probes

Locus	Probe	FLpter (range)	Number of observations	Median FLpter	Average FLpter	Corresponding chromosomal bands	Genetic distance from centromere (sex average/M/F)
D10S24	p7A9	0.141–0.178	5	0.160	0.162	10p13–10p12.1	–17.5 cM/–5 cM/–30 cM
FNRB	pB/R2	0.241–0.306	10	0.274	0.274	10p11.2	–4.5 cM/–1 cM/–8 cM
D10S34	cTB14.34	0.220–0.281	19	0.251	0.249	10p11.2	–4 cM/–1 cM/–7 cM
D10Z1	pαRP8	0.294–0.348	18	0.321	0.320	10cen	0 cM
D10S94	c1-6a/pur1-1	0.322–0.374	10	0.346	0.325	10cen–q11.2	0 cM/0 cM/0 cM
D10S97	pKW6ΔSacI	0.330–0.373	8	0.352	0.349	10cen–q11.2	2.4 cM/0 cM/4.8 cM
RBP3	cTBIRBP-9	0.331–0.428	16	0.380	0.382	10q11.2	4.5 cM/1 cM/8 cM
D10S15	pMCK2	0.367–0.461	10	0.414	0.411	10q11.2	6.5 cM/4 cM/9 cM
D10S22	pTB10.163	0.426–0.464	7	0.445	0.446	10q21.1	24 cM/7.5 cM/41 cM
D10S19	pTB10.171	0.462–0.519	4	0.491	0.496	10q21.1–q21.3	41 cM/24 cM/58 cM
EGR2	Zap 32	0.488–0.511	4	0.500	0.500	10q21.1–q21.3	41 cM/24 cM/58 cM
D10S3	Phage 10	0.506–0.528	8	0.517	0.542	10q21.2	53 cM/33 cM/73 cM
D10S1	Dry 5-1	0.572–0.655	3	0.614	0.609	10q22.1–q22.3	80.5 cM/35 cM/126 cM
D10S20	OS-2	0.742–0.779	4	0.761	0.767	10q23.3–q24.1	116 cM/54 cM/178 cM

other similar sequences close by, thereby confusing the interpretation of the FISH observations. (No confusion results when the two sites are far apart—e.g., D10F38S1 and D10F38S2—or on separate chromosomes—e.g., D10S1.) Third, the order determined from linkage data may not be the actual order; it is only the most likely order. Double-labeling FISH experiments were not attempted for the D10S19–EGR2 locus pair but the physical order could not be determined from the separate FISH experiments (Table 1); they were also not resolvable in the linkage map since no crossovers were observed between them. Finally, the RBP3 and D10S15 locus pair is considered separate in linkage maps (Wu *et al.*, 1990). However, these two loci are reported to be on the same 150-kb *Mlu*I fragment (Hyland *et al.*, 1991). Again double-labeling experiments failed to determine the order of these probes because both orders were detected in a small number of observations.

The increasing resolution of the maps of chromosome 10 has led to the possibility of new interpretations of the region—and sex-specific relationships between recombination and physical distances. We have compared these two different mapping strategies by constructing a graph that compares the average position of a probe in the FISH map to the most likely position of the corresponding locus in the linkage map. The sex-average and sex-specific comparisons are graphed in Fig. 2.

DISCUSSION

Comparison of FISH Distances and Genetic Distances

Initial inspection of the sex-average genetic distances and the FISH distances (Fig. 2), shows a slope of 0.003 FL/cM on the q arm, a slightly steeper slope of 0.007 FL/cM on the p arm, and a much steeper slope of 0.014 FL/cM across the centromere. This steeper slope is indicative of a larger physical distance per centimorgan or reduced recombination across the fixed centromeric physical distance. We expected this result because of the

documented reduced male meiotic recombination across the centromere as compared to female meiotic recombination (Wu *et al.*, 1990; Lichter *et al.*, 1992a; Simpson and Cann, 1992; Carson and Simpson, 1992) as seen dramatically in the male-specific graph. Surprisingly, the female graph also shows the same general trend: reduced recombination in the fixed physical FISH distance across the centromere. The male comparison graph has a large slope across the centromere (0.03 FL/cM), because the recombination distance is small (about 2%), whereas the q and p arms have slopes of 0.006 FL/cM and 0.024 FL/cM, respectively. The female comparison graph shows small increases in physical distance per centimorgan on the q and p arms with slopes of 0.002 FL/cM and 0.004 FL/cM, respectively. However, across the centromeric region the slope increases to 0.009 FL/cM, showing apparently greater amounts of physical distance per centimorgan. Although we suspect that the short arm has a slope similar to the long arm, there currently is not enough data to support this conclusion.

In a previous comparison of sex-specific recombination distances to a physical map, the authors used less precise physical mapping data and concluded that very little change occurs around the centromere in females (Carson and Simpson, 1992). Our comparison uses high-resolution FISH mapping that produces greater precision for the physical location of probes and we have mapped more probes in the centromeric region of chromosome 10. Furthermore, recent studies have further refined and characterized the linkage map (Lichter *et al.*, 1992a,b). This comparison not only confirms decreased male recombination across the centromere but also suggests reduced female recombination along the same physical distance.

There is a clear difference in recombination frequency across the centromere between male and female meioses (2 and 20% between FNRB and RBP3 (Lichter *et al.*, 1991a)), and a 5-fold increase of the FL/cM slope between the same two loci in males (relative to the slopes in both arms). The difference that we see in female FL/

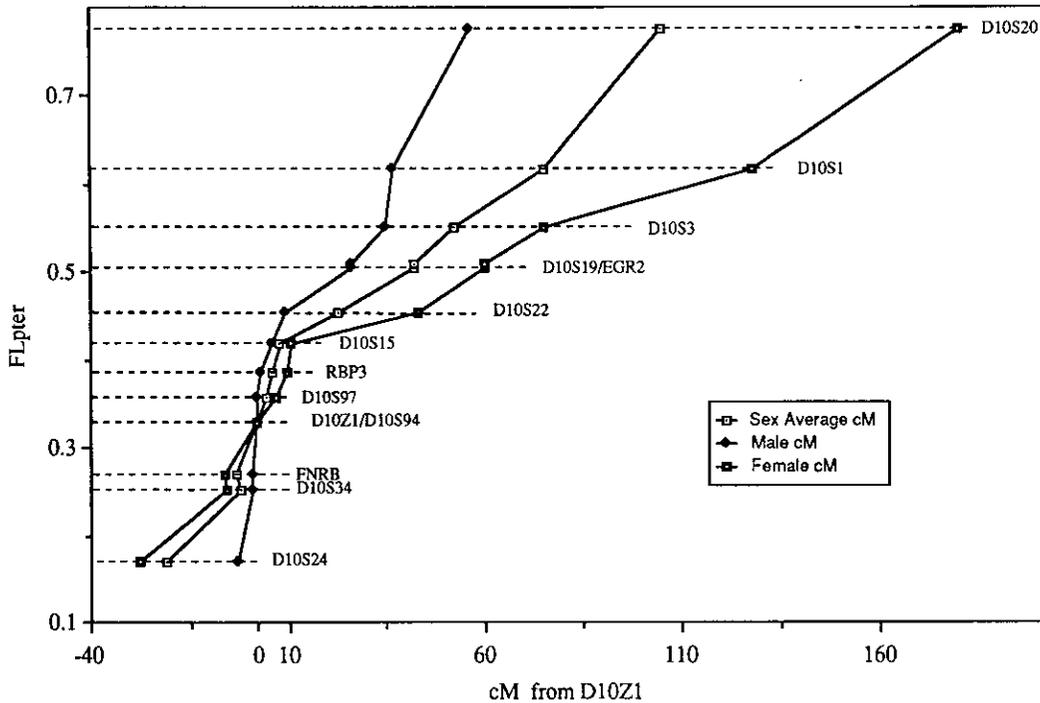


FIG. 2. Sex-average and sex-specific comparison graphs of chromosome 10. Locus positions plotted according to two measurements. The X-axis is the distance from the centromeric locus, D10Z1, of each specific chromosome 10 locus (p arm distances < 0) and the Y-axis of the graph is the mean location of the corresponding probe in the FISH map (with pter at 0.0).

cM across the centromere is nearly as convincing (4.5-fold increase). To test the significance of our initial “visual” interpretation, we proceeded with a more statistically rigorous analysis. There is a significant increase in the slope (FL/cM) derived from female recombination distance across the centromere as compared to that across the long arm. We replotted the FISH data, not using averages of the various measurements (as in Fig. 2) but using the entire dataset (all individual measurements), against the sex-specific recombination distances (Figs. 3A and 3B). Both graphs, derived from ei-

ther the male (Fig. 3A) or the female (Fig. 3B) distances, show a departure from linearity with odds favoring a nonlinear fit of 100,000 to 1 for males and 10,000 to 1 for females. We then plotted data for the centromeric and the long arm subregions separately and determined the slopes and correlation coefficients. The slopes were in agreement with those determined by the averaging method (Fig. 2). All of the correlation coefficients were very large (>0.87), indicating very strong relationships between the measurements of average physical and genetic distances. To test for the significance of the differ-

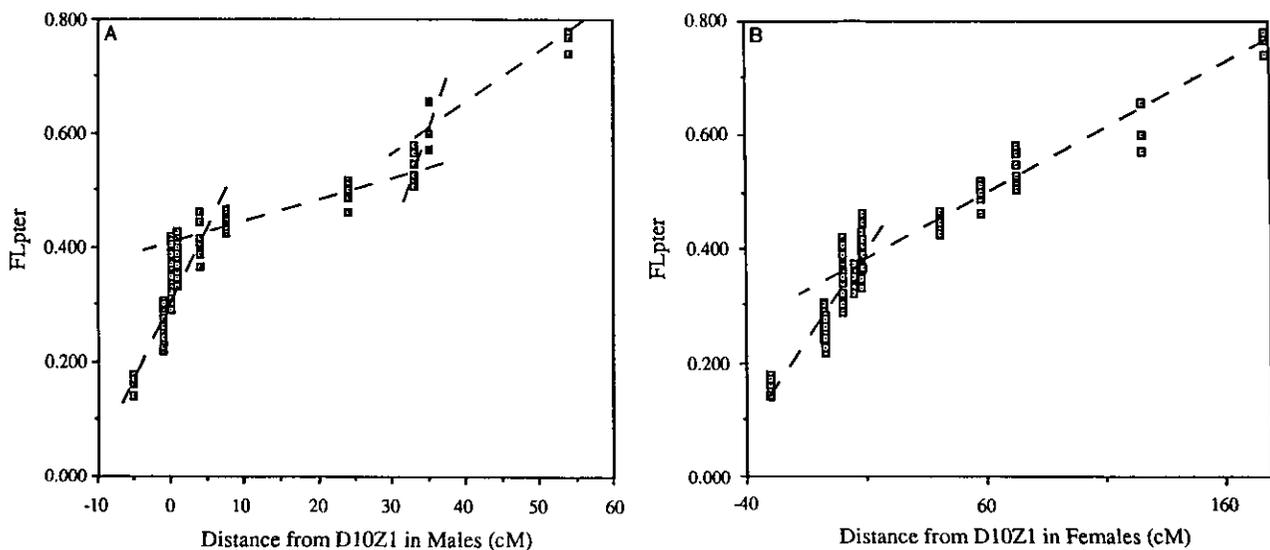


FIG. 3. (A) Male-specific scatter diagram of the comparison data. (B) Female-specific scatter diagram of the comparison data.

ences in the slopes across the centromere and the long arm, we used a modified *t*-test to analyze both the scatter data (Fig. 3) and the averaged data (Fig. 2); both are statistically significant. The probability that the slopes could differ as much as they do by chance is less than 1 in 5000, supporting our conclusion that recombination across the centromere is decreased in females as compared to the long arm. One assumption that we make is little or no uncertainty in the distances in the linkage map. Any errors in genetic distances are likely to be small, as the confidence intervals for most pairwise genetic distances are less than 5 cM (and much less near the centromere). Finally, the ambiguous ordering of the three previously mentioned locus pairs (FNRB-D10S34, RBP3-D10S15, D10S19-DGR2) does not alter our interpretation because the locus pairs are so close together that the distances to the next closest markers on either side are substantial compared to error associated with the distance between members of a pair.

Our conclusion might further be tested by the precise measurement of the number of basepairs/FL and the number of basepairs/cM. Certainly, if the relationship of basepairs to FL is relatively constant (and there is no reason to expect that it is), then this study shows that the axiom of 1 MB = 1 cM is surely incorrect for at least either the pericentromeric region or the long arm of chromosome 10. The inadequacy of this axiom is even more pronounced if the relationship of FL to basepairs is variable in different subregions of chromosomes. Variability in the FL to centimorgan ratios can be caused by differential condensation which may obscure simple relationships between physical distance in basepairs of DNA and the relative lengths of regions of metaphase chromosomes.

We have described two extremes of distance relationships in very small regions that point to the limits of generalizing between different mapping strategies. First, the order of FNRB and D10S34, which are separated by about 2–3% recombination, could not be unambiguously resolved by FISH when each locus was studied separately or in double-labeling experiments in our current study. Second, two loci (RBP3 and D10S15) separated by no more than 150 kb appear to be resolved by linkage data and not by FISH data in this study. Both observations, however, serve to illustrate that any simple relationship between distances measured in basepairs, FL, and cM, derived from any very small region of any chromosome, may not be generalizable to other regions (of any size) in the genome.

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