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High-Resolution Mapping of Human Chromosome 11 by in Situ Hybridization with Cosmid Clones

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Cosmid clones containing human DNA inserts have been mapped on chromosome 11 by fluorescence in situ hybridization under conditions that suppress signal from repetitive DNA sequences. Thirteen known genes, one chromosome 11–specific DNA repeat, and 36 random clones were analyzed. High-resolution mapping was facilitated by using digital imaging microscopy and by analyzing extended (prometaphase) chromosomes. The map coordinates established by in situ hybridization showed a one to one correspondence with those determined by Southern (DNA) blot analysis of hybrid cell lines containing fragments of chromosome 11. Furthermore, by hybridizing three or more cosmids simultaneously, gene order on the chromosome could be established unequivocally. These results demonstrate the feasibility of rapidly producing high-resolution maps of human chromosomes by in situ hybridization.

EFFORTS TO CONSTRUCT A HIGH-RESolution map of the human genome have intensified significantly during the past few years. Analysis of the inheritance of restriction fragment length polymorphisms in family pedigrees has permitted the construction of linkage maps for most regions of the human genome (*1*) as well as the identification of the genetic loci for many diseases (*2*). Physical mapping techniques provide a complement to genetic linkage. Standard agarose gel electrophoresis gives resolution in the 1- to 10-kb range, and pulsed field techniques extend this range to the megabase level. However, gel electrophoresis methods are not useful for producing an initial localization for a DNA sequence that has not been mapped previously. Furthermore, they do not directly provide the ability to order DNA sequences more than a few hundred kilobases apart or the ability to assign DNA sequences to specific chromosomal regions. For initial chromosome assignment and precise gene localization, somatic cell genetics, fluorescence-activated cell sorting of metaphase chromosomes, and in situ hybridization have been widely used.

The most direct method for identifying the chromosomal locus of a segment of human DNA is by in situ hybridization. Although unique sequences less than 1 kb long can be localized by using isotopically labeled probes, autoradiographic development times are long (often weeks or months), extensive statistical analysis is required, and the mapping precision is limited by the necessity of having to capture the emitted isotopic signal by an emulsion overlay. In contrast, nonisotopically labeled probes offer markedly improved speed and spatial resolution, but in general they have suffered from a lack of sensitivity. However, several groups have reported the detection of unique sequence targets of 6 kb or less by nonisotopic in situ hybridization (*3–5*).

Previous studies have demonstrated that it is possible to suppress the hybridization signal from ubiquitous repeated sequences, such as the Alu and Kpn elements, by adding appropriate competitor DNAs to the probe mix. Such suppression strategies, which exploit the rapid reassociation kinetics of repetitive sequences, have been used to facilitate the selective hybridization of unique sequence subsets from probes for Southern blotting (*6*) and for in situ hybridization (*5, 7–10*). In this report, we describe the use of chromosomal in situ suppression (CISS) hybridization (*11*) in conjunction with fluorescent detection of hybridized probes for the rapid and precise localization of large numbers of cloned genomic DNA segments and for the development of a physically based map of human chromosome 11. Furthermore, a detailed comparison between results obtained by CISS hybridization and map order derived by somatic cell genetics reveals a one to one correspondence between the two methods.

To determine the chromosome to which a probe has hybridized, a technique for chromosome identification compatible with CISS hybridization is required. Conventional Giemsa banding of chromosomes before hybridization lowers hybridization efficiency, whereas posthybridization staining quenches fluorescence. Although it is possible to do Giemsa banding after hybridization (*4*), this process requires the relocalization of specific metaphase spreads and two separate photographic steps. However, alternative banding or labeling methods for direct chromosome identification are available. For example, quinacrine banding, bromodeoxyuridine banding, and diamidinophenylindole banding are compatible with fluorescent detection of probes by in situ hybridization (*12*). As an alternative strategy we have used cohybridization with a differently labeled probe (or probe set), such as an Alu DNA BLUR clone (*13*), which gives a banding pattern resembling R banding (*14*), or with probes tagging a particular chromosome such as a previously mapped cosmid clone or a chromosome-specific DNA repeat (*12, 15*). A DNA library from a sorted human chromosome that decorates the cor-

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responding chromosome from the terminus on the p arm (pter) to qter (*8–10*) has also been used. Figure 1A shows the assignment of cosmid XB2, labeled with digoxigenin, to chromosome 11 by cohybridization with a pool of insert DNA from a chromosome 11 recombinant library (*11, 16*) labeled with biotin. The cosmid was detected by fluorescein isothiocyanate (FITC), whereas the chromosome decoration was achieved by using rhodamine (red). When mapping probes of known chromosomal origin, we routinely counterstained the spreads with propidium iodide (PI) (Fig. 1, B and C), as this fluorochrome can be readily excited by the laser-scanning microscope used in this study. For simultaneous hybridization strategies, it is important to have different probe-labeling procedures that result in comparable fluorescent signals. Therefore, a variety of probe modification techniques were tested for their use in cosmid hybridization protocols. Conventional biotinylation (Fig. 1C), dinitrophenol (DNP) modification (Fig. 1B), and digoxigenin modification of probe (Fig. 1A) lead to similar probe detection sensitivities and thus are suitable for mapping and cohybridization of cosmid probes.

Several factors influenced the intensity and specificity of the hybridization signal; in particular, the purity of the DNA probe and the size of the probe fragments used in hybridization reactions (*17*). By carefully controlling these parameters, we routinely obtained strong and specific fluorescent hybridization signals in the absence of appreciable background fluorescence. Furthermore, a highly specific signal was generated from each of the four chromatids in more than 80% of the metaphase spreads examined when cosmid DNA probes were used, thus minimizing the statistical analysis required to establish map coordinates; similar hybridization efficiencies were reported by Landegent and co-workers (*7*).

Although the mapping of cosmid clones can be done with conventional fluorescence microscopy, digital imaging microscopy was used to facilitate the collection and analysis of the hybridization data. To produce digitized images we used a laser-scanning confocal microscope in a photon-counting mode. Thus the fluorescence signals could be quantitated directly, the signal-to-noise ratio could be enhanced by Kalman optical filtering, and the image could be stored in digital form. Sections of these images could be expanded to focus in on a particular chromosome of interest, the length and width of this chromosome could be measured, and the epicenter of the hybridization signal could be calculated, all electronically. A typical example of such an analysis, with biotinylated cosmid 4.13 DNA as the probe, is shown in Fig. 1D. The length and width of the chromosome 11 depicted was established by using a cursor to delineate the boundary parameters, and a line was drawn through the long axis of the chromosome so that it passed through the fluorescent signal on one chromatid. The relative photon density in each pixel along this line, in both the FITC and PI channels, was displayed against chromosome length on the *X*-axis (Fig. 1D, inset). The map position of the probe is expressed as the fractional length (FL) of the total chromosome relative to a fixed reference point, which we have here arbitrarily chosen as pter and designated FLpter. When chromosomes with highly variable polymorphic heterochromatin regions are analyzed (for example, chromosomes 1, 9, 16, and Y), reference points should be carefully chosen, so that the size variation of the heterochromatin does not affect the precision of the mapping procedure.

The map position of each cosmid could be roughly assigned by visual inspection; however, multiple chromosomes from each hybridization reaction were analyzed as outlined above to define the locus more precisely. These studies indicated that the precision of the chromosomal map coordinate was dependent on the degree of chromosome condensation (Table 1). Much less variation of the FLpter values is observed when more extended chromosomes are examined. The map position of clone 4-4B (based on elongated prometaphase chromosomes of 9 to

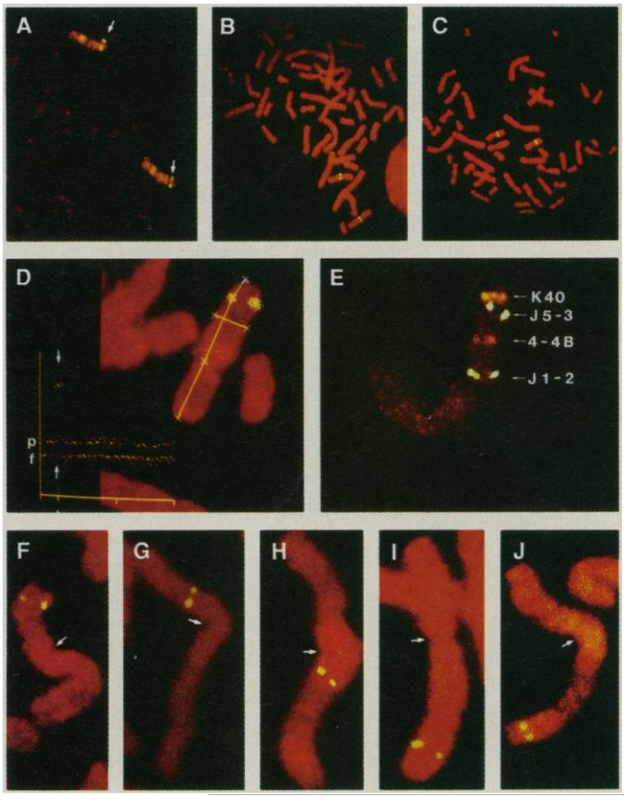

Fig. 1. CISS hybridization with cosmid DNA probes (*11*). (**A**) Chromosomal assignment of cosmid XB2 by simultaneous decoration of chromosome 11. Digoxigenin-labeled probe was cohybridized with biotinylated DNA from human chromosome 11 and detected by FITC (yellow) and rhodamine (red). Chromosome counterstain with DAPI is not shown and therefore only chromosome 11 is seen clearly. Arrows denote cosmid signal. (**B**) Mapping of DNP-labeled cosmid CL15, containing the gene for muscle glycogen phosphorylase. Signal is detected indirectly with FITC. (**C**) Biotinylated cosmid XB11 detected directly with avidin-FITC after CISS hybridization. In (B) and (C) chromosomes were counterstained with PI. Each picture is a digitized image taken as described (*11*). The preparation of metaphase chromosome spreads and the labeling of DNA probes were carried out as described (*25*). (**D**) Precise mapping of cosmid sequences. Biotinylated cosmid 4.13 was detected with FITC, and chromosomes were counterstained with PI. FITC and PI fluorescence was quantitated by laser scanning in the photon-counting mode. Analysis of the digitized image is described in the text. The X's denote chromosome boundary parameters. Arrows in the insert denote the position and the extent of changes in FITC signal intensity; this also provides a rapid assessment of the signal-to-noise ratio and the ability to compute the epicenter of the hybridization signal; the latter is especially useful when signal sizes are large (as shown here). Abbreviations: f, FITC; and p, PI. (**E**) Simultaneous visualization of a set of four cosmids on the short arm of chromosome 11. DNP-labeled cosmids K40 (β-globin) and 4-4B and biotin-labeled cosmids J5-3 and J1-2 were combined in a CISS hybridization experiment and detected by rhodamine and FITC, respectively. DAPI counterstain is not shown, but enhancement of rhodamine and FITC images reveals the outline of the whole chromosome by background fluorescence. The hybridization signals can be split (for example, J1-2 on the left chromatid); this phenomenon was observed occasionally during our study and seems to be dependent on chromosome preparation and denaturation. (**F** to **J**) Localization of labeled cosmid DNA clones on extended (prometaphase) human chromosomes 11. Hybridization signals (FITC) and PI counterstain were recorded by digital imaging and analyzed as described in the text. Each chromosome 11 is oriented such that pter is at the top of each panel, and the panels are arranged in the order of the cosmid localization in relation to pter. Arrows indicate centromere position. DAPI-banding was used to evaluate chromosome morphology when needed. The cosmids are (F) J5-3, (G) J13-4, (H) 1.1, (I) XB1, and (J) 8.5.

Table 1. Map coordinates of the cosmid 4-4B on chromosome 11 as a function of the degree of chromosome condensation.

Chromosome length* (μm)	Ratio of chromosome width to length*	FLpter*
5 to 6	0.28 to 0.41	0.16 to 0.24
6 to 7	0.29 to 0.41	0.16 to 0.23
7 to 8	0.18 to 0.25	0.17 to 0.22
8 to 9	0.12 to 0.24	0.18 to 0.22
9 to 14	0.08 to 0.17	0.20 to 0.22

*Range of values from seven to eight chromosomes per size class.

14 μm) is therefore given as 0.20 to 0.22, indicating the whole range of FLpter values observed on elongated chromosomes. We determined the FLpter values of the clones described below by analyzing 10 to 20 long chromosomes 11. Five or more chromosomes longer than 9.0 μm were used to define the narrowest FLpter range. An additional important criterion for selecting chromosomes for detailed mapping was that both chromatids of each chromosome exhibit a discrete signal and that both signals be perpendicular to the long axis of the chromosome.

To test the feasibility of physical mapping by in situ hybridization, 50 clones containing chromosome 11 DNA inserts (*18*) were analyzed as outlined above. In contrast to clones containing known genes, all random cosmids were done as a blind study. Typical hybridization signals observed with five of these cosmid clones on elongated prometaphase chromosomes are shown in Fig. 1, F to J. Each panel shows a single chromosome 11 oriented such that pter is at the top. The ability to obtain a rough regional localization of the hybridization signal by visual inspection should be readily apparent from these examples. The chromosomal loci of all clones, expressed as FLpter values, are given in Fig. 2. Many of the clones can be localized to within 2% of the total chromosome length. Although the conventional chromosome 11 ideogram is included in Fig. 2 for reference, the mapping data must be considered only in terms of FLpter values because no cytological banding was done. Expressing map location of these clones in terms of chromosomal bands would decrease overall mapping precision because many are clearly defined with subband resolution.

Thirteen known genes were included in the clones analyzed. An initial assessment of how well in situ linkage maps would correlate with cytological map positions established by other genetic methods was made by a comparative study of these clones (Table 2). In all cases but one, the FLpter value fell within the designated cytological boundaries defined by prior studies. Furthermore, the FLpter values gave a more precise localization of the genes within these intervals. The lone exception noted was with the ETS1A oncogene homolog that was reported to lie in band 11q23 (*19*). The observed range of FLpter values (0.94 to 0.98) would place this gene further toward the telomere, and extrapolation onto the chromosome 11 ideogram would place the ETS1 locus in band 11q24 or 11q25. The analysis of chromosomes expressing the fragile site fra(11)(q23) suggests that the ETS1 locus is located distal to the FRA11B locus, placing it in 11q24 or 11q25 (*20*). This result also distinguishes the ETS locus from the Ewings sarcoma 11;22 translocation located in 11q23. This observation is consistent with the result presented here.

A more detailed comparison between chromosomal map position determined by CISS hybridization and map order defined by somatic cell genetic techniques was carried out on the short arm of chromosome 11. Figure 3 summarizes a series of hybridization results obtained by using a set of cosmids previously unmapped by either technique. The hybrid mapping panel was derived from a monochromosomal hybrid line, J1, containing chromosome 11 as the only human chromosome (*21*). This mapping panel has been characterized extensively and has proved consistent in previous studies with mapping of chromosome 11 loci by genetic linkage, translocation, and deletion mapping (*22*). The 12 clones analyzed in this study fall within eight intervals in the somatic cell hybrid mapping panel; CISS hybridization provides map order for a minimum of 10 of the 12 clones. The map order given by the two techniques is in full agreement. Eleven of the 12 clones gave a single discrete signal by CISS hybridization, whereas clone J2-2 hybridized to two sites in close proximity, suggesting the possibility of a duplication of genomic sequences corresponding to this clone. Both signals for this clone were consistent with the map position derived by the somatic cell genetic mapping panel. In addition, in three cases where two cosmid clones fell within one hybrid deletion interval, their order relative to pter was discernible by CISS hybridization (J5-3 and J2-2; J10-3 and J4-17; J10-17 and 4-4B).

Gene order established by CISS hybrid-

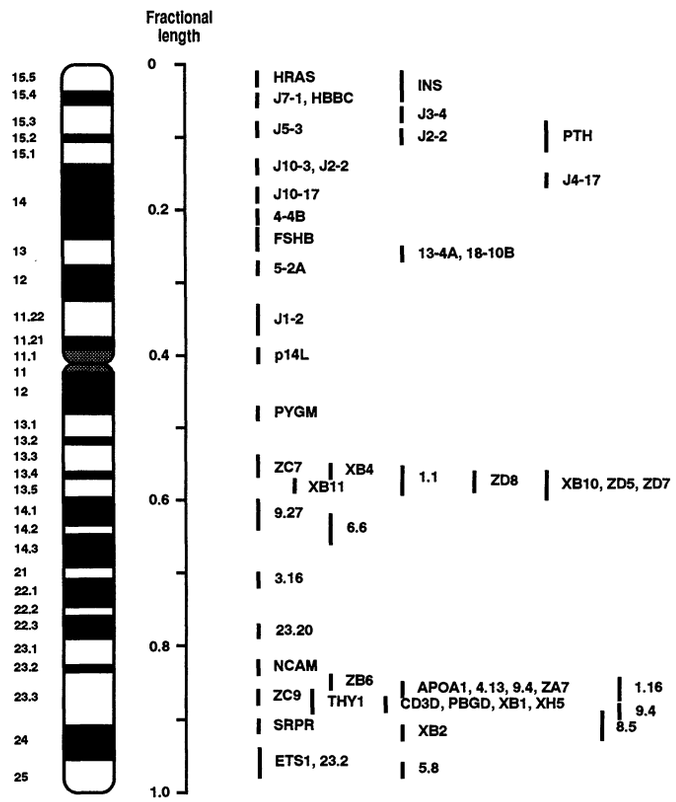

Fig. 2. Diagrammatic summary of the mapping data for all 50 probes used in this study. Vertical bars indicate the range of FLpter values obtained for each probe on elongated chromosomes 11. Two cosmid probes, J2-2 and 9.4, delineate two adjacent signals, apparently reflecting sequence homology of two loci; in both cases the signal nearer to pter was weaker. Similarly, cosmid probes 8.5 and T3δ1.7 (CD3D) showed two close signals that, however, did not differ in their range of map coordinates. The fine mapping was not carried out with chromosomal banding; therefore, assignment to chromosomal bands on the chromosome 11 ideogram would be by coincidence of FLpter values and band position only. Two of these clones, λ15B (FSHB) and HTHY1 (THY1), each gave additional hybridization signals on an autosome other than chromosome 11.

ization can be confirmed by the use of two fluorophores simultaneously (Fig. 1E). Here two pairs of cosmids are detected, one with rhodamine and one with FITC. The map order derived from Fig. 2 predicts that these four clones should give an alternating pattern of fluorescein and rhodamine hybridization signals. This is indeed the observed result. As many as 12 clones have been successfully hybridized simultaneously (*12*). The use of combinatorial analysis allowed us to confirm map order for all of these clones by the two-fluorophore technique. Again, the map order was consistent with the somatic cell genetic mapping panel.

The strategies described in this report have the potential to substantially increase the rate at which high-resolution physical maps of human chromosomes can be created. Each step in the mapping process—probe production, probe labeling, preannealing to suppress signal from interspersed repeat DNA, and in situ hybridization itself—can be carried out on many clones in parallel with ease. Large amounts of DNA are not required for probe production, so cultures of 10 ml or less are adequate for DNA preparation. Probes labeled with biotin, DNP, or digoxigenin give a discrete signal on both chromatids with high efficiency, thus leading to a dramatically reduced analysis time in comparison to similar experiments with radioisotopic labeling and detection. Furthermore, eliminating the need to identify and isolate single copy segments for each genomic clone substantially decreases the effort required to map a cloned genomic DNA segment.

A wide spectrum of cloning techniques are compatible with CISS. Genomic DNA sequences cloned in phage, cosmid, or yeast artificial chromosome vectors have been used successfully. An initial appraisal of chromosome localization of a series of cloned DNA segments can be obtained within 1 to 2 days. High-resolution mapping requires additional time for detailed analysis.

This study also provides a confirmation of the accuracy with which genomic DNA sequences can be localized by CISS hybridization. Map positions, for both known and previously unmapped clones, derived by CISS hybridization and somatic cell genetics agreed to the limits of resolution of the latter technique. These results give confidence in the likelihood that accurate map positions will be given by CISS hybridization for regions of the genome less clearly defined than chromosome 11. In the context of linkage mapping, we report here the localization of 50 probes along chromosome 11, which is estimated to contain roughly 140 to 150 megabase pairs (~140 to 150 centimorgans). Therefore, CISS hybridization will be a powerful tool to supplement current efforts in generating a 1- to 3-cM map of the human genome.

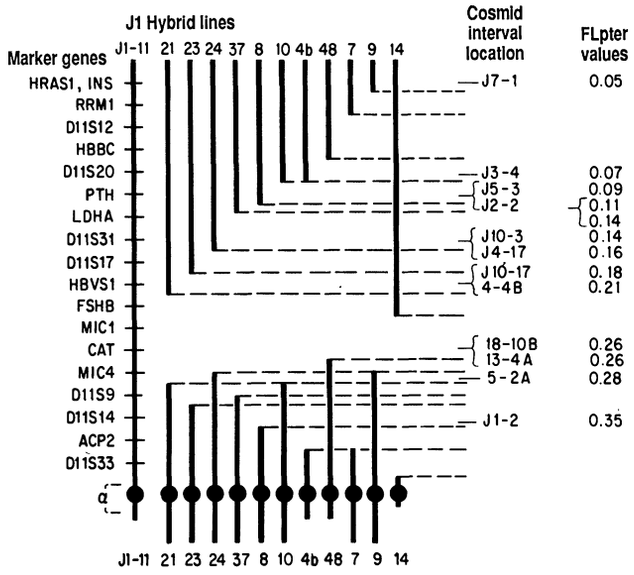

Fig. 3. Comparison of cosmid mapping by CISS hybridization with mapping by Southern blot analysis of somatic cell hybrid DNA. The J1 hybrid cell lines with different deletions in 11p (delineated by the gap in the vertical bold lines) are listed across the top of the figure. These cell lines as well as control human and hamster lines were analyzed by Southern blotting with 12 cosmid probes. The marker genes were previously typed (*22*) in the J1 hybrids (and in additional hybrids from five patients with 11p translocations). Their spacing is arbitrarily chosen and not to scale. The intervals defined by these marker genes are given by the horizontal dashed lines. After evaluation of all Southern blot data with one cosmid probe, the interval in which each probe is located was assigned as indicated. In the right column the median of the range of FLpter values is given for each of these cosmids to indicate the in situ hybridization mapping data.

The use of deletions, duplications, and translocations will be of value in mapping chromosomal regions of high interest with greater definition. Current strategies for using such reagents for mapping require Southern blotting or segregation of aberrant chromosomes in somatic cell hybrids, techniques that are both technically demanding and time consuming. CISS hybridization permits direct visualization of the presence or absence of a genomic DNA sequence in a given deletion or duplication or its position relative to a translocation breakpoint in a rapid and straightforward manner. For example, we have visualized separation of two cosmid clones from 11p13 that closely span the breakpoint of an 11p13:2p11 translocation in a Potter's syndrome patient (*12*). The combination of CISS hybridization and somatic cell hybrids, particularly radiation-reduced hybrids (*22, 23*), should be especially useful in developing a detailed map of a limited chromosomal region.

The direct ordering of genomic clones by CISS hybridization through the use of two fluorophores demonstrates the value of a

Table 2. Correlation of FLpter values and cytological map location for known genes. Abbreviations: HHMI, Howard Hughes Medical Institute at New Haven, Connecticut, Human Gene Mapping Library; HRAS1, Harvey rat sarcoma virus 1/oncogene homolog; INS, insulin; HBBC, β-hemoglobin complex; PTH; parathyroid hormone; FSHB, follicle-stimulating hormone/β-polypeptide; PYGM, muscle glycogen phosphorylase; NCAM, neural cell adhesion molecule; APOA1, apolipoprotein A-1; PBGD, porphobilinogen deaminase; THY1; Thy-1 cell surface antigen; CD3D, antigen CD3D, delta peptide; SRPR, signal recognition particle receptor (docking protein); and ETS1, avian erythroblastosis virus/E-26 oncogene homolog.

HHMI symbol	DNA clone	Cytological map location	FLpter value* (*n*)
HRAS1	pT24-Hras	11p15.5	0.01 to 0.03 (10)
INS	phins321	11p15.5	0.01 to 0.05 (9)
HBBC	K40	11p15.5	0.04 to 0.06 (10)
PTH	pPTHg108	11pter-p15.4	0.08 to 0.12 (12)
FSHB	λ15B	11p13	0.22 to 0.26 (17)
PYGM	CL15	11q12-q13	0.47 to 0.49 (10)
NCAM	HNCAM 2.2	11q23	0.82 to 0.84 (22)
APOA1	13.27	11q23-11qter	0.85 to 0.87 (17)
PBGD	11.25	11q22.3-qter	0.87 to 0.89 (10)
THY1	HThy1	11q22.3-q23	0.86 to 0.90 (13)
CD3D	T3δ1.7	11q23	0.87 to 0.89 (12)
SRPR	7.24	11q24-q25	0.90 to 0.92 (18)
ETS1	ETS1A	11q23.3 (11q24)	0.94 to 0.98 (12)

*Range of values, *n* = number of chromosomes measured.

simultaneous multiparameter analytical approach. The development of additional fluorophore-reporter combinations will further simplify determination of gene order and reduce the total number of analyses required. Additional fluorochromes also will permit the development of banding patterns that precisely fit the needs of an experimental situation through the choice of an ordered set of clones for a given chromosome or chromosomal region.

In the context of these applications, the limits of resolution of CISS hybridization are an important question. At least two parameters appear to affect the resolution of CISS hybridization: position on the chromosome and the degree of chromosome condensation. Elongated, prometaphase chromosomes give higher resolution and show less variability of FLpter values than more compact metaphase chromosomes. Regions of the chromosome that include the centromere and telomeres often show somewhat higher variability in map position than probes in other chromosomal locations, suggesting the possibility that local chromatin structure plays a significant role in determining the limits of resolution. In related studies, it has been shown that the resolution of CISS hybridization is on the order of 1 Mbp (*24*). Thus, interconnection between CISS hybridization and pulsed-field gel analysis should be possible. Methods in which the chromosomal DNA can be analyzed in a more extended state, such as after premature chromosome condensation or chromosome shattering, or by using DNA in interphase nuclei, and refinements in optical imaging techniques provide routes to improving spatial or lateral resolution.

Challenges presented in mapping the human genome occur at many levels. Mapping a large number of clones rapidly, efficiently, and accurately to develop a regularly spaced array of cloned genomic sequences covering the genome is an important initial goal. A second challenge is to develop more detailed maps of specific chromosome regions, particularly in situations where deletions, translocations, or duplications define genes of medical genetic significance. A third goal is to develop strategies for ordering a large number of independently isolated clones or previously ordered overlapping clone sets that span a discrete region several megabases in size. The data presented here indicate that CISS hybridization has the potential to make a major contribution to each of these endeavors.

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11. The conditions for CISS hybridization were carried out as described (*8*). Briefly, 20 to 50 ng of labeled probe DNA was combined with 1.5 to 3 μg of human placental DNA and sufficient salmon sperm DNA to obtain a total of 10 μg of DNA in 10 μl of hybridization cocktail. After denaturation of the probe mixture (75°C for 5 min), preannealing of repetitive DNA sequences was allowed for 5 to 15 min (37°C) before application to separately denatured chromosome specimens. Alternatively, in cases (for example, p14L) where no suppression and therefore no competitor DNA is needed, probe mixtures were denatured and then cooled on ice. When cosmid signals were obtained in parallel with a specific decoration of chromosome 11, 300 ng of pooled, labeled inserts from a chromosome 11 library (*16*) was combined with the differentially labeled cosmid DNA probe. To obtain Alu banding simultaneously with the probe signal, the competitor DNA was substituted by 300 ng of differentially labeled pBS-Alu4, and preannealing was reduced to a few seconds. Alternatively, 100 ng of labeled pBS-Alu4 was denatured in hybridization cocktail, cooled on ice, and combined with a preannealed probe just before application to slides. After overnight incubation and posthybridization washes (*8*), the specimens were incubated with blocking solution [3% bovine serum albumin (BSA), 4× SSC (saline sodium citrate) or, when BSA cross-reacting DNP antibodies (anti-DNP) were used, 5% nonfat dry milk, 4× SSC] for 30 to 60 min at 37°C. For detection, all protein reagents were made up in 1% BSA, 4× SSC, and 0.1% Tween 20 (BSA cross-reacting antibodies were preincubated in this solution for 30 min at 37°C) and then incubated with the specimen (37°C, 30 min) and followed by washes (4× SSC, and 0.1% Tween 20, three times for 3 min at 42°C). Biotin-labeled probes were detected by incubation with FITC-conjugated avidin DCS (5 μg/ml) (Vector Laboratories, Burlingame, CA) or TRITC-conjugated ExtrAvidin (5 μg/ml) (Sigma). The signal of some short DNA probes (for example, pT24-Hras) was amplified as described [D. Pinkel, T. Straume, J. W. Gray, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 2934 (1986)]. DNP-labeled probes were detected by incubation with rabbit–anti-DNP (7 μg/ml) (Sigma) and a second incubation with FITC- or rhodamine-conjugated goat–anti-rabbit antibodies (8 μg/ml) (Boehringer Mannheim). Digoxigenin-labeled probes were incubated first with sheep–anti-digoxigenin Fab fragments (2.5 μg/ml) (Boehringer Mannheim) and then with FITC-conjugated donkey–anti-sheep antibodies (7 μg/ml) (Sigma). For single probe hybridizations, labeled DNA was detected by FITC-conjugates, and chromosomal DNA was counterstained by PI (200 ng/ml PI in 2× SSC, 5 min at room temperature). For hybridizations with multiple differentially labeled probes, chromosomal DNA was counterstained (*8*) or banded [D. Schweizer, *Hum. Genet.* **57**, 1 (1981)] with diamidinophenylindole (DAPI). After mounting in antifading solution (*8*), the slides were evaluated on a Nikon Optiphot microscope equipped for conventional epifluorescence microscopy. For fine mapping, a modified version of the Bio-Rad laser scanning confocal microscope (Lasersharp MRC 500) was used in the photon counting mode (integration period of 0.1 to 0.3 ms per pixel) to produce digital images. The 488-nm line from an argon ion laser was used for excitation. In dual label experiments narrow band pass filters were used to obtain separate images of each fluorochrome (550-nm filter for FITC; 610-nm filter for PI or rhodamine). In some cases, the 532-nm line from an Amoco Microlaser [frequency-doubled diode-pumped Nd:YAG (yttrium-aluminum-garnet)] was used to excite rhodamine. The two separate images of one object were stored and then overlayed electronically. For image optimization, digital filtering was applied. Photographs were taken from the video screen.
12. P. Luchter *et al.*, unpublished data.
13. A human Alu DNA probe was generated by subcloning a genomic DNA fragment derived from the recombinant lambda phage λHA15 [fragment D, compare: B. Calabretta, D. L. Robberson, H. A. Barrera-Saldana, T. P. Lambrou, G. F. Saunders, *Nature* **296**, 219 (1982)] into plasmid vector pBSM13+ (Stratagene). The resulting DNA clone, pBS-Alu4, contains four Alu elements homologous to BLUR 2 and BLUR 8.
14. L. Manuelidis and D. C. Ward, *Chromosoma* **91**, 28 (1984); J. R. Korenberg and M. C. Rykowski, *Cell* **53**, 391 (1988); R. K. Moyzis *et al.*, *Genomics* **4**, 273 (1989).
15. T. Cremer, D. Tesin, A. H. N. Hopman, L. Manuelidis, *Exp. Cell Res.* **176**, 199 (1988).
16. For delineation of human chromosome 11 the total DNA inserts of the library LA11NS02 derived from sorted chromosome 11 [M. A. Van Dilla *et al.*, *Biotechnology* **4**, 537 (1986)] were prepared as described (*8*).
17. Small-scale preparation of probe DNA was as described [T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982)] and was followed by ribonuclease treatment, phenol and chloroform extraction, and ethanol precipitation. Alternatively, some probes were prepared at medium scale and purified by CsCl density equilibrium centrifugation. RNA-contaminated probes give higher levels of nonspecific fluorescent background. Nick translation was preferred to the random primer extension labeling procedure because the size of the generated probe molecules is easier to control and labeling can be carried out at higher DNA concentrations. The critical size range of probe molecules (smaller than 500 bp, preferably 150 to 250 bp long) was achieved by empirically varying the amount of deoxyribonuclease in the nick translation reaction. The size distribution of the probe DNA was verified by analyzing a portion of DNA (denatured) on an agarose gel run under nondenaturing conditions.
18. Cosmid clones from the short arm of chromosome 11 (4-4B, 5-2A, 13-4A, 18-10B, J1-2, J2-2, J3-4, J4-17, J5-3, J7-1, J10-3, and J10-17) were isolated from a DNA library derived from hybrid cell line J1-11 (*21*). Replica colony filters were screened [Maniatis *et al.*, in (*17*)] for human positives by hybridization with radiolabeled [random primer method, A. P. Feinberg and B. Vogelstein, *Anal. Biochem.* **137**, 266 (1984)] total human DNA, a cloned human repeat probe (BLUR 11), or the C_0t-1 fraction of human repetitive DNA. C_0t-1 DNA was isolated as described [C. Shih and R. A. Weinberg, *Cell* **29**, 161 (1982)]. Human cosmids were mapped by preannealing radiolabeled DNA with total sheared human DNA (*6*) and hybridizing to Southern blots of Eco RI–digested J1 cell hybrid DNA as outlined by T. Glaser *et al.* [*Nature* **321**, 882 (1986)]. The map position of each cosmid was based on scoring hybridization signals for 19 J1 hybrids and assigning

position based on a J1 hybrid map of 11p (*22*). DNA probes for known genes on chromosome 11p were provided as follows: cosmid K40 [F. S. Collins and S. M. Weissman, *Prog. Nucleic Acid Res. Mol. Biol.* **31**, 315 (1984)] containing the β-globin gene cluster (HBBC) by N. Guttmann-Bass (Jerusalem, Israel) plasmids phins321 and pPTHg108 [G. I. Bell, D. S. Gerhard, N. M. Fong, R. Sanchez-Pescador, L. B. Rall, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 6450 (1985)] containing the insulin gene (INS) and the parathyroid hormone gene (PTH), respectively, by G. Bell (University of Chicago) and H. Kronenberg (Massachusetts General Hospital); plasmid pT24-Hras (Shih and Weinberg, above) containing the Harvey-*ras* proto-oncogene 1 (HRAS1) by H. E. Ruley (Cold Spring Harbor Laboratory); phage λ15B [P. C. Watkins *et al.*, *DNA* **6**, 205 (1987)] containing the follicle-stimulating hormone β polypeptide (FSHB) by P. Watkins (Bethesda Research Laboratories); and plasmid p14L (*22*) containing a centromeric nonalphoid repeat element by T. Glaser (Oregon Health Sciences University). Cosmid clones mapping to the long arm of chromosome 11 were isolated from a genomic library constructed in cosmid vectors sCos-1 (G. A. Evans, K. A. Lewis, B. E. Rothenberg, *Gene*, in press) by using DNA from a somatic cell hybrid TG5D1-1 carrying 11q13-11qter as the only human material in a mouse erythroleukemia (MEL) cell background [C. L. Maslen *et al.*, *Genomics* **2**, 66 (1988)]. Cosmid clones were isolated by cross-hybridization with total human DNA and archived in 96-well microtiter plates; many of the clones were organized into "contigs" of overlapping clones by using a pooled hybridization multiplex procedure (G. A. Evans and K. A. Lewis, *Proc. Natl. Acad. Sci. U.S.A.*, in press). Cosmid clones are designated by archive grid coordinates: 1.1, 1.16, 2.23 (XB1), 3.16, 3.17 (XB2), 4.13, 4.16 (XB4), 5.8, 6.6, 7.21 (XB10), 7.24 (SRPR), 8.5, 8.15 (XB11), 9.4, 9.27, 11.25 (PBGD), 11.34 (XH5), 13.27 (AP0A1), 17.26 (ZD7), 18.4 (ZA7), 18.29 (ZD8), 19.18 (ZC7), 19.21 (ZC9), 22.7 (ZB6), 23.2, 23.20, and 23.23 (ZD5). [The nomenclature of these clones according to *Nomenclature for Physical Mapping of Complex Genomes* (Document AO2, available from National Technical Information Service, Department of Commerce, Springfield, VA) is SALKc-3-1.1, SALKc-3-4.16, and so forth, but only the grid coordinate numbers are used here.] Cosmids containing known genetic loci were located by using cDNA or oligonucleotide probes (for example, PBGD), and additional anonymous loci (for example, XB1 or ZB6) containing as yet uncharacterized genes were identified by the presence of putative HTF islands (G. Hermanson and G. A. Evans, unpublished data). Some cosmid clones carrying genes known to be located on 11q, CL15 (PYGM), ETS1a (ETS1), HNCAM2.2 (NCAM), HTHY1 (THY1), and T3δ1.7 (CD3D) were isolated from a human genomic cosmid library constructed in cosmid vector pWE15 [G. M. Wahl *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 2160 (1987)]. Where appropriate, the clone designation is followed by the genetic loci in parentheses.

19. C. De Taisne *et al.*, *Nature* **310**, 581 (1984); J. J. Yunis *et al.*, *Genomics* **4**, 84 (1989); M. Budarf, B. Sellinger, C. Griffin, B. S. Emanuel, *Am. J. Hum. Genet.* **45**, 128 (1989).
20. R. N. Simmers and G. R. Sutherland, *Hum. Genet.* **78**, 144 (1988).
21. The J1 hybrid cell line, possessing a standard complement of hamster chromosomes, but retaining only human chromosome 11, was isolated originally by C. P. Jones, P. Wuthier, T. T. Puck [*Somatic Cell Genet.* **1**, 235 (1975)]. Cell surface antigen loci on the short arm of human chromosome 11 were used as selective markers to isolate a panel of deletion derivatives of the J1 cell line after mutagen treatment [F. T. Kao, C. Jones, T. T. Puck, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 193 (1976)]; these lines have been used extensively to map a variety of DNA clones on chromosome 11 [J. F. Gusella *et al.*, *ibid.* **77**, 2829 (1980); (*22*)]. The panel of hybrids used here possesses segments of 11p with varying internal deletions spanning the WAGR region.
22. T. Glaser, D. Housman, W. H. Lewis, D. Gerhard, C. Jones, *Somatic Cell Mol. Genet.*, in press.
23. S. J. Goss and H. Harris, *Nature* **255**, 680 (1975); D. R. Cox *et al.*, *Genomics* **4**, 397 (1989).
24. P. Lichter *et al.*, *Cytogenet. Cell Genet.*, in press.
25. Metaphase spreads were prepared from human lymphocyte cultures with normal karyotypes (46, XX and 46, XY). In order to obtain elongated chromosomes, cultured cells were treated with Colcemid (0.1 μg/ml) for 10 to 30 min, and hypotonic treatment (0.075*M* KCl) was carried out for 12 to 18 min, followed by standard methanol-acetic acid fixation. An optional synchronization of the lymphocyte cultures was carried out as described [J. J. Yunis, *Science* **191**, 1268 (1976)]. Probe labeling was generally carried out by nick translation, substituting dTTP with (i) bio-11-dUTP [D. J. Brigati *et al.*, *Virology* **126**, 32 (1983); (ii) DNP-11-dUTP [dinitrophenol-*N*-hydroxysuccinimide ester was used to convert 5-(3-amino)allyl-dUTP to DNP-11-dUTP as described by P. R. Langer, A. A. Waldrop, D. C. Ward, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6633 (1981)], or (iii) with dTTP and digoxigenin-11-dUTP (Dig-11-dUTP) (Boehringer Mannheim) in a ratio of 3:1. Unincorporated nucleotides were separated from the probe DNA by centrifugation through 1 ml of Sephadex G-50 (Pharmacia) columns in the presence of 0.1% SDS.

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U1-Specific Protein C Needed for Efficient Complex Formation of U1 snRNP with a 5′ Splice Site

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One of the functions of U1 small nuclear ribonucleoprotein (snRNP) in the splicing reaction of pre-mRNA molecules is the recognition of the 5′ splice site. U1 snRNP proteins as well as base-pair interactions between U1 snRNA and the 5′ splice site are important for the formation of the snRNP–pre-mRNA complex. To determine which proteins are needed for complex formation, the ability of U1 snRNPs gradually depleted of the U1-specific proteins C, A, and 70k to bind to an RNA molecule containing a 5′ splice site sequence was studied in a nitrocellulose filter binding assay. The most significant effect was always observed when protein C was removed, either alone or together with other U1-specific proteins; the binding was reduced by 50 to 60%. Complementation of protein C–deficient U1 snRNPs with purified C protein restored their 5′ splice site binding activity. These data suggest that protein C may potentiate the base-pair interaction between U1 RNA and the 5′ splice site.

THE U1 SMALL NUCLEAR RIBONUcleoprotein (snRNP) particle is the most abundant member of the class of major snRNPs (U1, U2, U4+U6, and U5) that are essential cofactors in mRNA splicing (*1–3*). The 165-nucleotide (nt) U1 RNA is complexed with at least ten proteins, which can be divided into two classes, U1-specific proteins (70k, A, and C) and common U snRNP proteins (B′, B, D, D′, E, F, and G) (*4*). One of the functions of U1 snRNP in pre-mRNA splicing is the recognition of the 5′ splice site (*5, 6*). Although base-pairing between the 5′ end of U1 snRNP and the 5′ splice site is crucial for this recognition step (*7*), a role for U1 snRNP proteins has also been demonstrated (*5, 8*). The identity of the essential snRNP proteins and their exact functions are not known.

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We used U1 snRNPs that were gradually depleted of the U1-specific proteins (A, C, and 70k), to investigate whether one or more of these proteins are involved in 5′ splice site binding (Fig. 1A). U1 snRNP particles, deficient in protein C (ΔC), protein A (ΔA), or proteins A and C [Δ(A,C)] were obtained by chromatography of snRNPs U1 to U6 (which had been affinity purified by antibody to m_3G) on Mono Q ion-exchange resin at elevated temperatures (25° to 37°C) (*9*). A U1 snRNP particle lacking 70k, A, and C [Δ(70k,A,C)] was obtained by Mono Q chromatography at 37°C. Depletion of the respective U1-specific proteins by this procedure was, however, not always 100% effective. The ΔA and Δ(A,C) U1 snRNPs used for these studies, for example, contained residual amounts of A protein [less than 5% of the amount of A present in wild-type (wt) U1 snRNPs]. The fraction of U5 snRNPs contaminating the wt and ΔC U1 snRNPs was about 10 and 5%, respectively, as estimated by RNA gel