Am. J. Hum. Genet. 44:695–703, 1989

Isolation of Additional Polymorphic Clones from the Cystic Fibrosis Region, Using Chromosome Jumping from D7S8

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Summary

The cystic fibrosis (CF) locus has been located, by both linkage analysis and physical mapping, to a 900-kb region of 7q22–31 flanked by D7S8 (J3.11) and D7S23 (XV-2c). Using a 100-kb general jumping library, we isolated two sequential jump clones, J31 and J29, to one side of the D7S8 region and one jump clone, J32, to the other side of D7S8, so that the total region covered is about 300 kb. Three new RFLPs were detected by J29 and J32. Using PFGE mapping and the three jump clones, we found it possible to orient D7S8 on the chromosome and, by linkage analysis, to further narrow the CF region by 100 kb. The orientation of D7S8 will be useful for directing the isolation of other jump clones toward the CF locus. Though the newly described RFLPs are in considerable linkage disequilibrium with D7S8 polymorphisms, they increase the informativeness of genetic markers in the D7S8 region and should be useful in prenatal diagnosis.

Introduction

Cystic fibrosis (CF) is the most common lethal genetic disease in the white population, with a carrier frequency of 4%–5%. CF is a generalized disorder of exocrine glands that is characterized by elevated sweat chloride levels, intestinal obstruction, pancreatic insufficiency, sterility in males, and chronic lung disease leading to respiratory failure and death (Lloyd-Still 1983). An abnormal regulation of chloride ion channel activity in secretory epithelial cells has been proposed to be directly involved in the pathogenesis of CF (Knowles et al. 1983; Quinton and Bijman 1983), and recent studies suggest that the CF defect probably lies in a cyclic AMP-dependent pathway regulating the channel (Frizzell et al. 1986; Welsh and Liedtke 1986; Schoumacher et al. 1987). The precise metabolic defect, however, is not known.

Received October 24, 1988; revision received January 6, 1989.

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0002-9297/89/4405-0010$02.00

Since the molecular defect in CF has not been identified by biochemical or protein studies, the CF problem lends itself to the reverse-genetics approach. With this approach the disease gene is captured by virtue of its chromosomal map position, without direct knowledge of its function. The encoded protein, the genetic mutation, and the metabolic defect can then be determined. Recent successes of this approach include cloning the genes responsible for retinoblastoma (Friend et al. 1986), chronic granulomatous disease, (Royer-Pokora et al. 1986) and Duchenne muscular dystrophy (Monaco et al. 1986).

The first step in the reverse-genetics approach is to map the disease gene to a particular chromosome by RFLP linkage analysis. The CF gene has been mapped to the long arm of chromosome 7 (7q22–7q31.1) in a region flanked by the *met* proto-oncogene and the anonymous DNA segment J3.11 (D7S8) (Tsui et al. 1985; (Dean et al. 1985; Wainwright et al. 1985; White et al. 1985*b;* Beaudet et al. 1986; Lathrop et al. 1988). This region has been estimated to be about 2 cM by recombinational analysis and 1.5 million base pairs (Mb) by physical mapping with pulsed-field gel electrophoresis (PFGE) (Drumm et al. 1988; Poustka et al.

1988). On average, 1 cM of genetic distance corresponds to about 1,000 kb of physical distance.

Because the size of the region between the flanking markers *met* and D7S8 is very large, attempts to isolate the CF gene by the usual approach of chromosome walking with cosmid or lambda vectors require a very large number of steps and could be halted by the presence of any DNA segment that could not be efficiently cloned in such vectors. While the use of yeast artificial chromosomes (YACs) (Burke et al. 1987) would result in the cloning of all intervening sequences without the problems associated with many small steps, there have been several technical difficulties in making and screening YAC libraries that have thus far hindered their wide application. Thus, an approach that does not require cloning all the intervening DNA between the nearest DNA marker segment and the gene itself but yet relies on conventional vectors would be advantageous. Estivill et al. (1987*a*) isolated clones XV-2c and CS.7 from the CF region by selection for hypomethylated HTF islands. Linkage analysis revealed that these two markers are in strong linkage disequilibrium with CF. Subsequent identification of recombination events suggests that CF lies to the D7S8 side rather than to the *met* side of XV-2c (Berger et al. 1987; Estivill et al. 1987*b*; Farrall et al. 1988). Recently, by screening a flow-sorted chromosome 7–specific library and using the single-copy DNA segments in hybridization analysis with a panel of somatic cell hybrids, Rommens et al. (1988) isolated two DNA markers, D7S122 and D7S340, which map between *met* and D7S8 and are in close linkage disequilibrium with CF.

We have applied the technique of chromosome jumping to further delimit the CF region and to identify the CF gene. Chromosome jumping, as described by Collins and Weissman (1984) and independently by Poustka and Lehrach (1986), depends on the circularization of very large DNA fragments, followed by cloning of the junction fragments of these circles which bring together DNA sequences that were originally located a considerable distance apart in the genome. Using the technique of chromosome jumping, we previously obtained a clone called CF63 which is about 100 kb 3′ to the *met* oncogene in the direction of CF (Collins et al. 1987). We now report the isolation of jumps to both sides of D7S8, followed in one direction by a second jump, so that the total region covered around D7S8 is about 300 kb. Using PFGE mapping and these three jump clones, we have found it possible to orient D7S8 on the chromosome. With these clones, three new RFLPs have been defined, which, though in partial disequilibrium with D7S8, will add to the informativeness of genetic markers in this region. Family analysis with these clones further narrows the candidate region for the CF gene.

Material and Methods

Cell Lines and DNA Preparation

1EF2/3, a hamster-human somatic cell hybrid, carries as its only human component a portion of the long arm of human chromosome 7 and is positive for *met* and D7S8 (Arfin et al. 1983; Rommens et al. 1988). 1EF 2/3 was a gift from John Wasmuth. Cell lines 3.1.0 and HD-1 are two Epstein-Barr virus (EBV)–transformed human lymphocyte cell lines. DNA was prepared from human lymphocytes and from cell lines following solubilization in 1% Triton X-100 and centrifugation (Bell et al. 1981). The nuclear pellets were resuspended in 0.25 mM EDTA, 1% SDS and were incubated with proteinase K (10 μg/ml) for 16–20 h at 50°C. After extraction with phenol and chloroform: isoamyl alcohol (24:1), DNA was precipitated with ethanol (2 vol) and was redissolved in TE (10 mM Tris HCl [pH 7.5], 1 mM EDTA).

Library Preparation, Screening, and Subcloning

The construction of the 100-kb general chromosome-jumping library was as described elsewhere (Collins et al. 1987); this same library was amplified prior to screening by overlaying the plate stock with 12 ml SM buffer (50 mM Tris HCl [pH 7.5], 10 mM $MgSO_4$, 100 mM NaCl, 0.1% gelatin/plate. The plates were stored at 4°C overnight, and the bacteriophage suspension was recovered and titered. Human genomic phage libraries were prepared in lambda FIX, lambda DASH (Stratagene), and EMBL-3 (Frischauf et al. 1983). For libraries prepared in lambda FIX, human DNA was partially digested with *Mbo*I and was inserted into the *Xho*I site after both vector and insert DNA were filled in with the first two nucleotides (Zabarovsky and Allikmets 1986). For libraries prepared in lambda DASH and EMBL-3, human DNA was partially digested with *Sau*3A and was ligated into the *Bam*HI cloning sites. The jumping library and the genomic phage libraries were plated out to 1×10^6 clones for each screening. All libraries were plated on bacterial host LE392 or TAP 90 (Patterson and Dean 1987) at a density of 40,000 plaques/150-mm plate and were screened by standard techniques. Positive plaques were picked and placed in 500 μl SM buffer. Small amounts of DNA were prepared from positive phage clones and were sub-

cloned by ligating *Eco*RI-digested phage DNA to phosphatase-treated *Eco*RI-cut pBRΔAva (Richards et al. 1988) or pUC18.

Restriction-Mapping Jump Subclones

The insert in either pBRΔAva or pUC18 was mapped by digestion with *Eco*RI plus *Ava*I, followed by Southern blotting and sequential probing with supF, human DNA, and the probe used to screen the jump library. Because the supF gene contains an *Ava*I site, the start piece and the jump piece in each jump subclone can be identified (Collins 1988).

DNA Preparation and Restriction-Endonuclease Digestion for PFGE

Cells were grown in Dulbecco's modified Eagle's media (Hazleton Biologics, Inc., Lenexa, KS). Lymphoblastoid cells were grown in 75-cm^2 tissue culture flasks (Corning, Corning Glass Works, Corning, NY) to a density of 10^7 cells/50 ml. IEF2/3 cells were grown on 150-mm culture dishes (Falcon, Cockeysville, MD) to confluency. Cells were harvested by centrifugation at 120 *g* for 5 min, were washed once with PBS, and then were resuspended to a concentration of 2×10^7 cells/ml. DNA in cell blocks was prepared, and restriction-endonuclease digestion was carried out as previously described by Drumm et al. (1988). Enzymes were from New England Biolabs (Beverly, MA).

Probes

DNA was isolated from the cloning vectors by endonuclease digestion and low-melting agarose-gel electrophoresis and was labeled to 10^9 cpm/μg with ^{32}P-dCTP (Amersham) by oligonucleotide-primed labeling (Feinberg and Vogelstein 1983).

PFGE Mapping

Single, double, and partial digests of high-molecular-weight DNA embedded in LMT agarose were carried out as described elsewhere (Drumm et al. 1988). DNA was separated by contour-clamped homogenous electric field (CHEF) gel electrophoresis (Chu et al. 1986) or by field inversion gel electrophoresis (FIGE) (Carle and Olson 1986). Gels were 1% agarose in running buffer. CHEF gels were run for 96 h at 90 V, 14°C on the LKB pulsaphor with a hexagonal array electrode insert in 0.5 × Tris acetate buffer (20 mM Tris-acetate, 1 mM EDTA [pH 8.0]). Switching times were ramped from 30 s to 5 min. FIGE gels were run by means of a programmable time ramp (DNAstar, Madison, WI) using a forward ramp from 3 to 180 s and a reverse ramp from 1 to 60 s operating over 48–72 h at 180 V and a temperature of 14°C. The running buffer was 0.0445 M Tris borate/0.0445 M boric acid/0.001 M EDTA. After electrophoresis and ethidium bromide staining, DNA was nicked by exposure to 254 nM UV light for 1 min and then was denatured and transferred to Gene Screen (Dupont). Transfer and hybridizations were as described by Drumm et al. (1988).

RFLP Identification and Linkage Analysis

DNA from 6–18 unrelated Caucasians was digested, run on 1% agarose gels, blotted to nylon membranes, and hybridized as described elsewhere (Dean et al. 1987). For clones containing repetitive sequences, blots were prehybridized with 500 μg human placental DNA/ml for 24 h. Putative RFLPs were confirmed by hybridization to DNA from 3-generation pedigrees (White et al. 1985*a*) and were analyzed in a collection of CF families having two or more affected offspring. Data from recombinant families were repeated. Standard linkage disequilibrium (Δ) was calculated as follows:

$$\Delta = \frac{(n_{ii}n_{jj}) - (n_{ij}n_{ji})}{\sqrt{(n_{ii} + n_{ij})(n_{ji} + n_{jj})(n_{ij} + n_{ji})(n_{ij} + n_{jj})}},$$

where n_{ij} is the number of observed haplotypes with allele i at the first locus and allele j at the second locus (Beaudet et al. 1986).

Results

The general 100-kb jumping library was screened with J3.11, a 0.7-kb *Eco*RI-*Hin*dIII plasmid subclone isolated from the phage clone 3.11.2 (D7S8) (Bartels et al. 1986). J3.11 sits at one end of a 6.2-kb *Eco*RI fragment and therefore biases the selection of clones that jump in the direction of the *Eco*RI site in the 0.7-kb fragment rather than toward the *Eco*RI site at the other end of the 6.2-kb fragment (Collins 1988) (fig. 1). One positive clone, called J31, was isolated and subcloned into pBRΔAva. J31 consists of a 3.0-kb start piece (J3.11 positive by hybridization) connected to a 600-bp jump piece by the 200-bp supF gene. The restriction maps of the phage clone 3.11.2 and the start piece in J31 were compared; when the topology of circle formation (Collins et al. 1987; Collins 1988; Richards et al. 1988) was considered, the direction of the jump clone could be unequivocally determined. Specifically, the 3-kb start piece in J31 contains the 0.7-

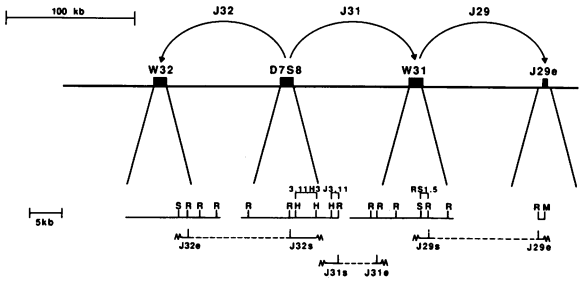

Figure 1 Isolation of jump clones by using the 100-kb general jumping library. The four cloned regions of DNA and the three approximately 100-kb jumps connecting them are shown in the upper half of the figure. Jumping clones are designated with a "J" followed by a number (e.g., J31); walks from these clones are designated with a "W" followed by the same number (e.g., W31). At the bottom of the fig. is an expanded view of the cloned regions, with the limits of the jumping clones (s = the start of a jump; e = the end of a jump). The sawtooth indicates the location of the supF gene. Restriction sites are as follows: R = *Eco*RI; H = *Hin*dIII; S = *Sal*I; M = *Mbo*I.

kb *Eco*RI-*Hin*dIII fragment; therefore, J31 jumped in the direction of the *Eco*RI site in J3.11, as shown in figure 1. A 3-kb start piece in a clone that jumped in the opposite direction would contain a 0.2-kb *Eco*RI-*Hin*dIII fragment from the opposite end of the 6.2-kb *Eco*RI fragment.

The 600-bp jump piece in J31 was subcloned into pUC18 and was used to screen a human phage genomic library which yielded W31, a phage clone with a 13-kb insert. W31 was oriented in relation to J31 after restriction-enzyme digestion, Southern transfer, and probing with the jump piece of J31. The 1.5-kb *Eco*RI-*Sal*I fragment of W31 (RS1.5) was then used to screen the jump library to continue jumping in the same direction (fig. 1). RS1.5 was chosen because it sits at one end of the largest *Eco*RI fragment (5 kb) in W31. The jump clone J29 was isolated. To jump in the opposite direction as J31 and J29, 3.11H3 (also referred to as p3H-3 [Dean et al. 1987]), a 2.5-kb *Hin*dIII fragment located at the opposite end of the 6.2-kb fragment as J3.11, was used as probe to screen the jump library. J32 was obtained and the 400-bp jump piece of J32 (J32e in fig. 1) was used as probe to screen a total human genomic library. This yielded phage W32 containing a 10.1-kb insert. Comparison of the restriction maps of the start piece of J32 with 3.11.2 indicates that J32 jumped in the opposite direction as J31 and J29.

A detailed physical map of the *met*-D7S8 region has been reported (Drumm et al. 1988; Poustka et al. 1988) and can be used to order additional cloned DNA segments in this region. Probes near the HTF island lying between *met* and CF (Estivill et al. 1987*a*; Rommens et al. 1988) are particularly useful for the detailed PFGE mapping of these newly isolated clones. To further aid in the mapping of this region, we used JG2X, a clone obtained by successive chromosome jumping and walking from pH131 (D7S122), a probe isolated by Rommens et al. (1988) and known to be approximately 1,000 kb to the *met* side of D7S8.

3.11H3, J31, and J29 were found to hybridize to a *Bss*HII fragment of more than 1,400 kb in cell lines 3.1.0 and 1EF2/3 and to a 650-kb *Bss*HII fragment in HD-1. J32 and JG2X both hybridize to a 1,315-kb *Bss*HII fragment in 3.1.0 and 1EF2/3 (fig. 2). In HD-1, JG2X hybridizes to a 110-kb *Bss*HII fragment, and J32 hybridizes to a 350-kb *Bss*HII fragment (fig 2). This PFGE data therefore shows that J32 has jumped from D7S8 toward the CF locus, because J32 and JG2X are on the same *Bss*HII fragment in 3.1.0 and 1EF2/3 that there is a substantial degree of disequilibrium between these RFLPs. To determine the extent of linkage

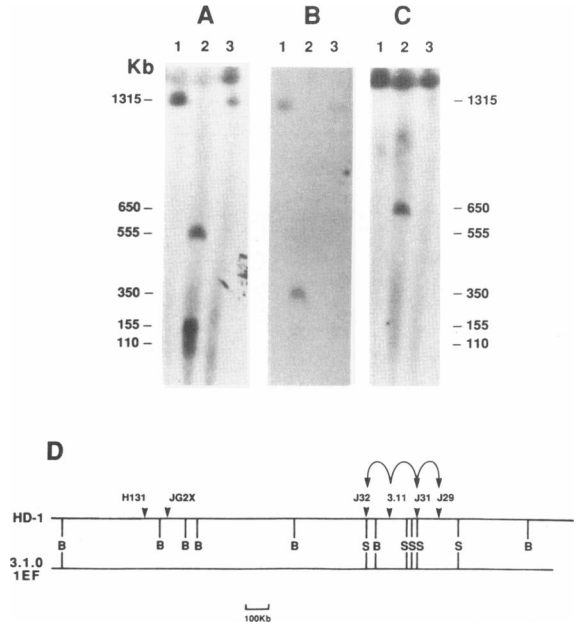

Figure 2 The direction of the jumps in relation to the CF locus was determined by PFGE mapping using CHEF gel electrophoresis. Panels *A*–*C* show the results of probing a *Bss*HII blot with JG2X, J32e, and 3.11H3, respectively. In each panel, lane 1 is 3.1.0 DNA (EBV-transformed lymphoblast), lane 2 is HD-1 (EBV-transformed lymphoblast), and lane 3 is 1EF2/3 (hamster-human somatic cell hybrid carrying a portion of 7q). Panel *D* shows the resulting *Bss*HII (B) map. *Sal*I sites (S) around J3.11 that were used to determine the distance of jumps are also indicated.

whereas D7S8, J31, and J29 are on a different fragment. J31 and J29 therefore must represent two sequential jumps away from the CF locus.

PFGE mapping was also used to determine the size of the jumps. While the jumping library was originally prepared by circularizing molecules of approximately 100 kb from a preparative pulsed-field gel (Collins et al. 1987), previous analysis in well-characterized regions indicate that a range of jump sizes, from 35 kb to 120 kb, exist in this library (Collins et al. 1987; Kenwrick et al. 1988; M. C. Iannuzzi, M. L. Drumm, N. Hidaka, L.-C. Tsui, and F. S. Collins, unpublished data). For the jumps around D7S8, we were aided by the presence of *Sal*I sites in the W31 and W32 regions (figs. 1, 2). Thus, for example, the *Sal*I site in W32 was shown to represent one end of a 160-kb *Sal*I fragment which contains J3.11 (fig. 2). In a *Sal*I-*Bss*HII double digest, J32e hybridizes to a 30-kb fragment, and J3.11 hybridizes to a 130-kb fragment (data not shown). Two small (10–15-kb) *Sal*I fragments occur adjacent to this fragment, with the *Sal*I site in W31 representing the far end of the second fragment. Thus, although the precise position of J3.11 cannot be determined, the combined span of the J32 and J31 jumps is approximately 185 kb. J29 resides on a 210-kb *Sal*I fragment bounded at one end by the *Sal*I site in W31. The distance between J29 and J31 cannot be precisely determined from these data.

Whole phage clones and single-copy probes were used to search for RFLPs. While the W31 locus failed to reveal any polymorphisms after sampling of 20 enzymes, both the J29 and W32 clones detected frequent RFLPs (fig. 3). Probe J29 detects a two-allele *Pvu*II RFLP with fragments of 9 and 6 kb (table 1). The J29 clone also detects an *Eco*RV polymorphism. The J32 clone detects a *Sac*I RFLP with 15- and 6-kb alleles.

When these newly described RFLPs were analyzed in a group of 36 unrelated Caucasians, along with the *Msp*I polymorphism of D7S8, few additional heterozygotes were detected. In fact, 60% of the individuals had the same genotype with all four RFLPs, suggesting

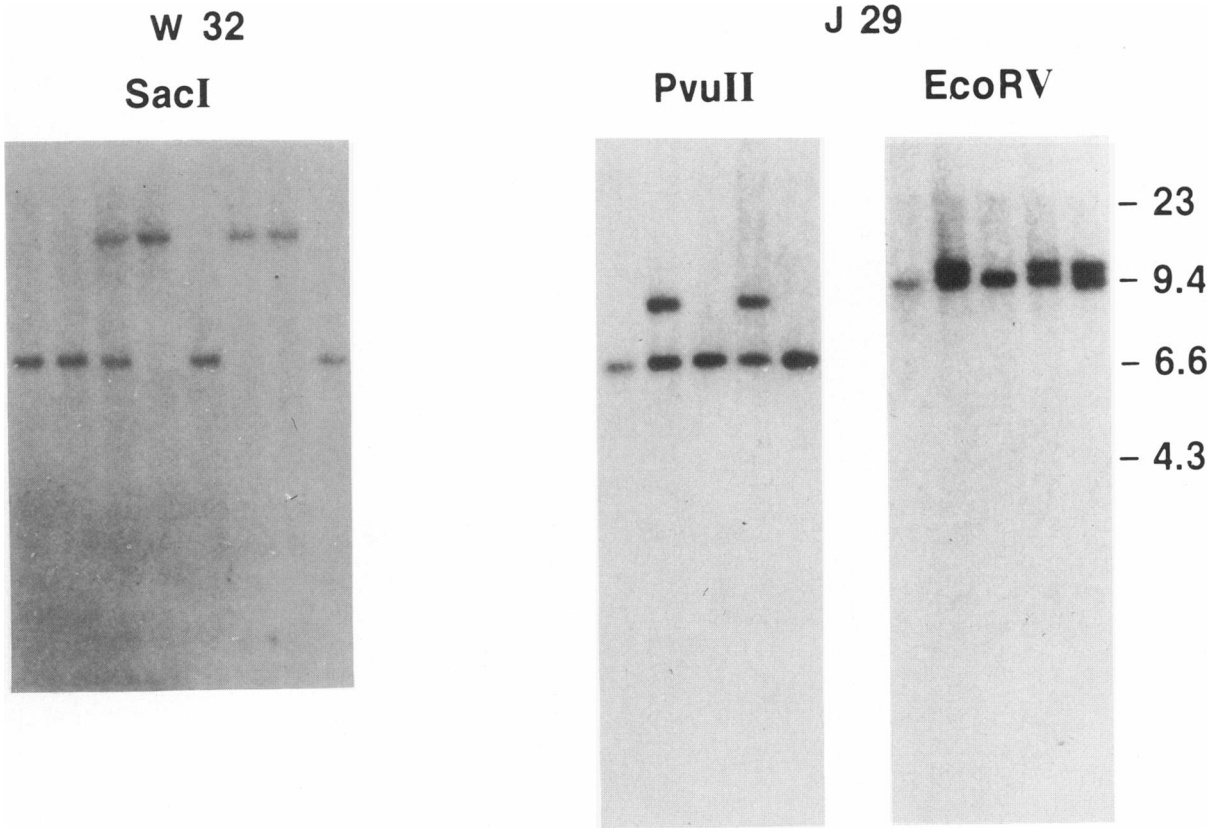

Figure 3 Newly identified RFLPs in the CF region. Southern blots show the alleles of newly described RFLPs detected by jumping probes derived from D7S8.

Table 1

Characteristics of New Polymorphisms

A. Description of RFLPs

Probe, Location	Enzyme	Alleles	Size	Frequency	*n*
J29, 7q31	*Eco*RV	1	12	.31	62
		2	9.5	.69	
	*Pvu*II	1	9.0	.40	196
		2	6.0	.60	
W32, 7q31	*Sac*I	1	15	.36	187
		2	6.0	.64	

B. Disequilibrium Data

	n	Δ	χ^2
W32 *Sac*I vs. CF	104	.10	.9
J29 *Pvu*II vs. CF	94	−.07	.4
J29 *Eco*RV vs. CF	21	−.09	.2
W32 *Sac*I vs. J29 *Eco*RV	24	.70	12*
W32 *Sac*I vs J29 *Pvu*II	91	.83	63*
J29 *Pvu*II vs. J29 *Eco*RV	25	.77	15*
W32 *Sac*I vs. D7S8 *Msp*I	88	.75	49*
D7S8 *Msp*I vs. J29 *Pvu*II	91	.74	50*
D7S8 *Msp*I vs. J29 *Eco*RV	25	.83	17*

NOTE.—Disequilibrium values were calculated in a group of 33 3-generation pedigrees. Comparisons were made between the *Sac*I polymorphism of W32 and the *Eco*RV and *Pvu*II polymorphisms of J29 and CF. In addition, pairwise comparisons were made between the markers themselves by using all unrelated chromosomes for which haplotypes could be generated by including the *Msp*I RFLP of D7S8.

* $P < .005$.

disequilibrium and confirm the linkage of these polymorphisms to CF, the probes were hybridized to DNA from pedigrees of CF families described elsewhere (White et al. 1985*b*, 1986). Table 1B displays the extent of linkage disequilibrium (Δ) between each marker and CF and between the markers themselves. None of the RFLPs show a significant association with CF in this population. This is consistent with the results previously obtained in these families with D7S8 (White et al. 1985*b*). However, each marker pair in the D7S8 region displays significant disequilibrium, with Δ values ranging between .70 and .83.

Despite the high degree of disequilibrium between the markers, several of the parents previously uninformative for D7S8 were informative for one or more of the RFLPs detected by the jumping clones. A recombinant between D7S8 and CF (individual 8920 in family CF-1380 [White et al. 1986]) is also recombinant with W32 (fig. 4). These data exclude the CF mutation from the interval between D7S8 and W32 and suggest that further jumps are required to reach the CF gene.

Discussion

A large number of disease genes have been mapped, by RFLP linkage analysis, to relatively small chromosomal regions. In physical terms, however, the RFLP markers most often lie megabase distances away from the genes of interest. Such distances obviate applying

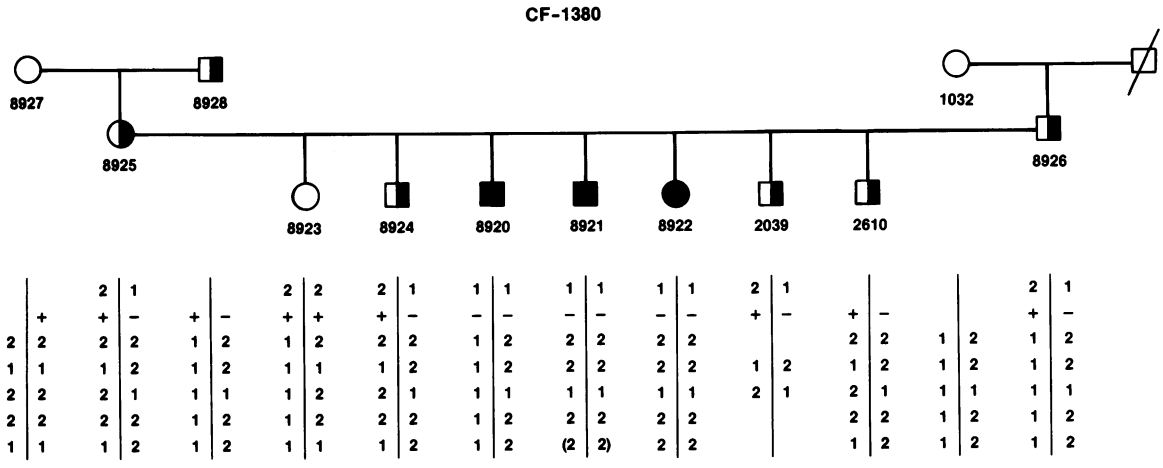

Figure 4 CF family 1380 displaying recombination between CF and W32. Displayed below the pedigree are the haplotyped alleles for *met*, the CF gene, and the D7S8 region probes. RFLPs are as follows: W 32 S = W32 *Sac*I; 311 M = J3.11 *Msp*I; T = J3.11 *Taq*I; J 29P = J29 *Pvu*II; EV = J29 *Eco*RV. Individual 8920 is a paternal recombinant between CF and W32S.

the usual approach of chromosome walking for cloning. This is the case for the CF gene, which by linkage analysis and PFGE mapping has been located to a 915-kb region flanked by the chromosome 7 markers XV-2c and D7S8 (Drumm et al. 1988; Poustka et al. 1988). Molecular cloning techniques that may be applied to cloning a gene at this great distance include chromosome jumping (Collins and Weissman 1984; Poustka and Lehrach 1986), YACs (Burke et al. 1987), and cloning from preparative pulsed-field gels (Michiels et al. 1987).

The use of YACs allows cloning large (100–1,000-kb) DNA is specially designed plasmid vectors containing the necessary yeast components. However, technical difficulties in both making and screening YAC libraries have so far precluded their wide availability and general application. Cloning from preparative pulsed-field gels depends on identifying a specific band on the gel by hybridization with the marker DNA segment and on using the DNA from the band to generate a library that is "enriched" for closely linked sequences. It is often difficult to map and orient the randomly selected clones, however, and extraneous DNA comigrating to the same region on the gel as the DNA of interest results in a high background of unrelated clones.

The use of chromosome jumping as a general approach for cloning genes at a distance from a starting RFLP marker has several advantages. First, chromosome jumping together with PFGE mapping can be used to physically orient any DNA segment on the chromosome, which can then direct subsequent isolation of clones. The *met* region was previously oriented in this way (Collins et al. 1987), and now the orientation of D7S8 on the chromosome has been determined. By means of chromosome jumping, three jump clones—including two sequential jumps in one direction and one jump in the opposite direction—have been isolated. These three jump clones, J32, J31, and J29, allow orientation by physical mapping. To have oriented this region by genetic mapping would have required a recombination within this region, which has so far not been detected. The physical location of D7S8 is also resolved; since J32 crosses a *Bss*HII site (fig. 2), D7S8 must be within about 100 kb of this site.

A second advantage of chromosome jumping is that it is possible to bias the selection of clones in a particular direction away from the starting screening probe. By choosing a segment which sits close to the *Eco*RI site at one end, clones that jump in the direction of the *Eco*RI site will be selected (fig. 1) (Collins 1988). By choosing a segment at the opposite end of the *Eco*RI fragment, the jump will be biased in the opposite direction. We have found that bias increases as the size of the *Eco*RI fragment increases above 4 kb and that little bias occurs when one uses a probe derived from a *Eco*RI fragment less than about 3 kb. Another advantage is that chromosome jumping, unlike chromosome walking, is not impeded by either repetitive DNA sequences or recombinogenic regions and can be used to skip over such troublesome regions.

Chromosome jumping is also a convenient way to generate new RFLP markers at a distance from one another. The jump clones may be expanded, by chromosome walking, to search for informative RFLPs and to aid the genetic analysis of large regions. These polymorphisms should increase the informativeness of the locus, allowing an increase in the evidence for linkage, better evidence for gene order, and a greater applicability and accuracy of genetic diagnosis. In addition, any recombination observed between the jumps will provide additional data on both the orientation of the clone and the localization of the gene. While the jump pieces in the jump clones tend to be small, overlapping phage, cosmids, or both have been obtained, without difficulty, for all jump clones isolated to date. Using a moderately rigorous search for RFLPs (six to nine individuals and 10–30 enzymes), we were able to locate polymorphisms with two of the three jump clones reported here. These RFLPs increase the percentage of individuals heterozygous at this region; in the families surveyed, four of 21 grandparents uninformative for D7S8 became informative with the new polymorphisms. By demonstrating that the W32 probe is still recombinant in family 1380, we have been able to narrow the location of the CF gene by approximately 100 kb.

The examination of linkage disequilibrium by using the W32 and J29 RFLPs has provided several interesting results. Despite the fact that these clones span about 280 kb, there is a high degree of disequilibrium between each of these markers and D7S8. This suggests that in the history of Caucasians very little recombination has occurred in this interval. We are unaware of any other human markers this far apart that show such substantial disequilibrium. By contrast, none of the markers is in significant disequilibrium with the CF mutation. Even when the markers are combined in haplotypes, the common haplotypes appear to be in equilibrium with CF (data not shown). This is consistent with the lack of disequilibrium with CF previously noted in these families (White et al. 1986). It will be of interest to see the level of disequilibrium of these markers in populations that do show disequilibrium

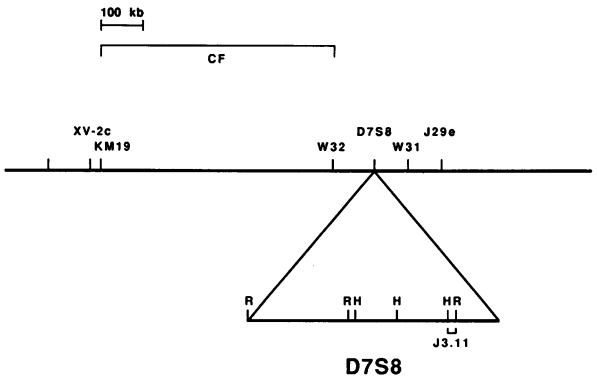

Figure 5 Summary map of the CF region

between D7S8 and CF (Beaudet et al. 1986, Schmidtke et al. 1987).

One disadvantage of chromosome jumping is that PFGE mapping is required to independently verify the direction and the size of the jumps. The three jump clones likely span about 220–320 kb, and, while the distance between J32 and J31 was determined to be 185 kb by PFGE, the distance from J31 and J29 has not yet been defined because of the absence of rare-cutter restriction sites in this region.

The use of chromosome jumping and PFGE mapping has achieved several goals relative to the CF problem. The CF region has been further narrowed by 100 kb (fig. 5). D7S8 has been oriented so that new jump clones in the direction of CF can be isolated and used to further delimit the CF region. J32 and J29 detect three new RFLPs and increase the informativeness of the D7S8 region for application to prenatal diagnosis.

Acknowledgments

M.C.I. and F.S.C. acknowledge support from the Cystic Fibrosis Foundation and NIH grant DK39690-01. We thank Mark Leppert and Ray White for providing DNA and data from the Utah CF families. We also thank M. Oskarsson for the EMBL-3 and λ-DASH genomic libraries, and Lap-Chee Tsui and members of his laboratory for numerous discussions. This project has been funded at least in part with federal funds from the Department of Health and Human Services under contract N01-CO-74102 with Program Resources, Inc. F.S.C. is an Associate Investigator of the Howard Hughes Medical Institute.

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