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Physical Mapping of the Cystic Fibrosis Region by Pulsed-Field Gel Electrophoresis

MITCHELL L. DRUMM,* CASSANDRA L. SMITH,† MICHAEL DEAN,‡ JEFFERY L. COLE,* MICHAEL C. IANNUZZI,* AND FRANCIS S. COLLINS*

*Departments of Internal Medicine and Human Genetics and the Howard Hughes Medical Institute, University of Michigan, Ann Arbor, Michigan 48109; †Departments of Microbiology and Psychiatry, Columbia University College of Physicians and Surgeons, New York, New York 10032; and ‡Biological Carcinogenesis Development Program, Program Resource Incorporated, Frederick Cancer Research Facility, Frederick, Maryland 21701

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The gene for cystic fibrosis (CF) is known to be flanked by the closely linked DNA markers *met* and J3.11 on chromosome 7. Using the technique of pulsed-field gel electrophoresis, we have constructed a complete overlapping restriction map of approximately 3000 kb of DNA in this region. The *met* and J3.11 probes are found to be between 1300 and 1800 kb apart, which compares well with their genetic distance of 1–2 cM. The CF gene must be located within this interval, and the availability of this physical map should be of considerable utility in mapping additional clones as the search for the gene proceeds.

INTRODUCTION

Cystic fibrosis (CF) is the most common autosomal recessive disease in the Caucasian population, affecting approximately 1 in 2000 newborns. CF is characterized by chronic pulmonary disease, pancreatic insufficiency, elevated sweat chloride levels, and sterility in males (Lloyd-Still, 1983). Decades of research at the biochemical and physiologic levels have thus far failed to reveal the nature of the metabolic defect, though recent evidence indicates that the abnormality may lie in a failure of chloride channel responsiveness to adrenergic stimuli (Frizzell *et al.*, 1986; Welsh and Liedtke, 1986).

Because of the difficulty in defining the metabolic abnormality by a direct approach, considerable excitement has been generated recently by the application of "reverse genetics" to the CF problem. In this approach the gene is sought not by knowledge of its function, but by its map position (Ruddle, 1984; Orkin, 1986). Such an approach has recently been successful in identifying the genes for chronic granulomatous disease (Royer-Pokora *et al.*, 1986), Duchenne muscular dystrophy (Monaco *et al.*, 1986), and retinoblastoma (Friend *et al.*, 1986).

The first step in this procedure is the localization of the gene to a particular chromosome by linkage analysis; this was accomplished for CF 2 years ago by identification of linkage to an anonymous marker DOCR-917 on chromosome 7q (Tsui *et al.*, 1985). Analysis of CF families with other markers on chromosome 7 subsequently demonstrated that two markers lie within 1 cM of the gene: the *met* protooncogene (White *et al.*, 1985) and the random probe J3.11 (Wainwright *et al.*, 1985). Recent multipoint analysis has shown that the odds in favor of CF lying in between these two markers are very high (Beaudet *et al.*, 1986; Lathrop *et al.*, 1987). This delimits the location of the CF gene to a region of approximately 2 cM; on the average, this should represent about 2000 kb of physical distance (Smith *et al.*, 1987b). Two factors make this only a rough estimate, however: the width of the 95% confidence limits on the *met*–J3.11 linkage distance, and the variability in the relationship between genetic distance and physical distance.

Other markers in this interval have subsequently been cloned. Using the technique of chromosome jumping (Collins and Weissman, 1984; Poustka and Lehrach, 1986), we obtained a clone called CF63 which is approximately 100 kb 3′ to the *met* gene (Collins *et al.*, 1987a), in the direction of CF (see below for evidence of directionality of the *met* gene in this region). By selection for clones rich in hypomethylated CpG sequences, Estivill *et al.* (1987) obtained DNA fragments XV-2c and CS.7, which lie between *met* and J3.11 by linkage analysis and which detect restriction fragment length polymorphisms (RFLPs) in strong linkage disequilibrium with the CF mutation.

Linkage disequilibrium indicates that most CF is likely due to a mutant allele with a single origin. It is

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difficult, however, to use this information to precisely delimit the location of the CF gene, since disequilibrium may not be a linear function of proximity to the gene. In this situation, it is essential to develop a physical map of the region so that the limits of the region of candidate genes can be fully defined and in order to develop signposts in the area which can be used to unequivocally order probes on the chromosome.

The recent development of pulsed-field gel electrophoresis (Schwartz *et al.*, 1983; Schwartz and Cantor, 1984; Carle and Olson, 1984; Carle *et al.*, 1986; Cantor *et al.*, 1986; Chu *et al.*, 1986) has made possible the construction of long-range physical maps by providing the means to separate DNA fragments as large as 10,000 kb. When combined with restriction enzymes which cut only rarely in the genome and Southern blotting techniques to identify single-copy fragments in complete and partial digests, this approach provides a means of constructing a map of a region of several megabases (Smith *et al.*, 1986, 1987b; Smith and Cantor, 1987). This has been recently accomplished in man, for example, for the Duchenne muscular dystrophy region (van Ommen *et al.*, 1986; Burmeister and Lehrach, 1986; Kenwrick *et al.*, 1987), the major histocompatibility complex (Lawrance *et al.*, 1987), the human β-globin region (Collins *et al.*, 1987b), and regions of chromosome 4p near the Huntington disease locus (Poustka *et al.*, 1987). We have now applied this approach to the CF region, using *met*, CF63, and J3.11 as molecular probes. A detailed restriction map has been constructed with a variety of enzymes which cut rarely in genomic DNA, and the CF gene has been localized to a region of approximately 1800 kb.

MATERIALS AND METHODS

DNA Preparation

DNA was prepared essentially as described by Smith *et al.* (1986). Briefly, human lymphoblastoid cells from cell line 3.1.0 were harvested by centrifugation at 120*g* for 5 min, washed once with phosphate-buffered saline (PBS), and then resuspended to a concentration of 2×10^7 cells/ml. The cells were warmed to 50°C and mixed with an equal volume of molten Sea Plaque (FMC) low-melting-temperature agarose, which was 2% in 125 m*M* EDTA. The mixture was then cast into 0.1-ml blocks, which were cooled and placed into lysis solution at a concentration of 1.5 blocks/ml of solution. Lysis solution was 1% *N*-lauroylsarcosine (Sigma), 0.5 *M* EDTA, pH 8, and 0.5 mg/ml proteinase K. Blocks were incubated for 6 h at 50°C, and incubation was repeated twice with fresh lysis solution. After lysis, the blocks were rinsed several times with TE (10 m*M* Tris, pH 7.5, 1 m*M* EDTA) and then incubated at room temperature with gentle rocking in two changes of TE with 1 m*M* phenylmethylsulfonyl fluoride (PMSF), each for 2 h. Blocks were then rinsed several times with TE and stored at 4°C.

Restriction Digests

One-third of a block, or approximately 3 μg of DNA, was digested by adding the block to 25 μl of 10× restriction enzyme buffer, 190 μl of water, and 15 to 20 U of enzyme. Digests were carried out for 2 h at 37°C. Enzymes were from New England Biolabs (Beverly, MA).

Electrophoresis, Blotting, and Hybridization

All gels were 1% agarose in 0.5X Tris-borate-EDTA (Maniatis *et al.*, 1982) as electrophoresis buffer. Field-inversion gels (Carle *et al.*, 1986) were run using ramping programmed by a DNAStar Pulse unit (Madison, WI) for 65 h at 165 V; forward times ranged linearly from 6 to 240 s, and the ratio of forward to reverse times was always 3:1. Orthogonal field (OFAGE) gels were run on an apparatus constructed according to Carle and Olson (1984) for 20 h and a pulse time of 40 s. Separation of fragments above 1000 kb was carried out using the LKB Pulsaphor (LKB-Pharmacia, Houston) for 5 to 7 days at 95 V, with a pulse time of 30 to 70 min.

After electrophoresis, gels were exposed to 1 min of 254-nm uv light on a transilluminator box and then soaked in 0.5 *M* NaOH, 1.5 *M* NaCl for 20 min. Capillary blotting to GeneScreen (DuPont) was carried out for 30 h using 0.5 *M* NaOH, 1.5 *M* NaCl as the transfer solution. The blots were neutralized in 1 *M* ammonium acetate, 20 m*M* NaOH for 5 min, air-dried for 6 h, and baked 20 min under vacuum at 80°C, and then the DNA was uv-crosslinked to the filter by a 1.5-min exposure to a 330-nm light source. This protocol is similar to one described by Herrmann *et al.* (1986).

Probes were labeled by the random priming method of Feinberg and Vogelstein (1984), boiled, and then preannealed for 90 min with a 5000-fold excess (w/w) of sonicated human genomic DNA (Litt and White, 1985).

Hybridizations were carried out using a modification of the Church and Gilbert (1984) protocol for genomic sequencing. Blots were prehybridized at 65°C for 1 h in 15 ml of 0.5 *M* sodium phosphate (pH 7.2), 7% SDS, and 1 m*M* EDTA (1 *M* sodium phosphate is 134 g $NaH_2PO_4 \cdot 7H_2O$ and 4 ml phosphoric acid per liter). Probes were added to sealed bags and hybridization was carried out for 24 to 36 h at 65°C. Washes were 3× 15 min with 40 m*M* sodium phosphate, 0.5% SDS, 1 m*M* EDTA, at 65°C with shaking.

Blots were routinely stripped by preheating 1 m*M* Tris, pH 8, 0.1 m*M* EDTA, 1% SDS to 80°C, adding this to filters, and incubating at 65°C for 20 min with shaking. Filters were rinsed with water and placed against X-ray film for 20–24 h to show that stripping was complete.

RESULTS

The following probes were used to construct the maps: metH, CF63, and 3.11H3. metH is a subclone from the 3′ end of *met* (White *et al.*, 1985); CF63 is a clone derived by chromosome jumping (Collins *et al.*, 1987a), mapping approximately 100 kb further 3′ to metH; and 3.11H3, also referred to as p3H-3 (Dean *et al.*, 1987), was isolated from a λ clone hybridizing to J3.11 and is a 2.5-kb *Hin*dIII fragment mapping 2.7 kb from J3.11 (D7S8, Wainwright *et al.*, 1985). These probes were used to screen genomic digest blots, and the resultant fragment sizes for single enzyme digests, including partial cleavage products, are listed in Table 1; fragment sizes for double digests are listed in Table 2.

Separate maps of the *met* and D7S8 regions were first constructed and these two maps were then linked together in order to construct a map spanning the region between metH and J3.11.

metH/CF63 map

Using metH as a probe and a combination of *Not*I complete and *Sfi*I partial digests, a 140-kb *Sfi*I fragment is generated (Fig. 1). In *Sfi*I partial digests, the most prominent partial bands are at 165 and 560 kb. This suggests that the 140-kb *Sfi*I fragment is flanked by a 25-kb *Sfi*I fragment on one side and a 420-kb fragment on the other side. With an *Sfi*I + *Not*I double digest, the 560-kb band disappears and a doublet of 320 and 345 kb appears, indicating that a *Not*I site is present in the 420-kb *Sfi*I fragment. With this information, the map shown in Fig. 1 can be readily constructed. With this map as a foundation, other restriction sites were added using the data in Tables 1 and 2. Representative lanes of a single FIGE blot probed with metH, CF63, and 3.11H3 are shown in Fig. 2.

CF63 detects a 35-kb *Sfi*I primary fragment and a 60-kb *Sfi*I partial fragment (Fig. 2B). Because both CF63 and metH detect *Nru*I fragments of 115 and 135 kb, we therefore conclude that the probes are no more than 115 kb apart and are most likely separated by a single 25-kb *Sfi*I fragment. The 115-kb primary and 135-kb partial *Nru*I fragments detected by both metH and CF63 are cleaved by *Sfi*I to 65- and 85-kb fragments detected by metH and a 30-kb fragment detected by CF63, placing the *Sfi*I sites relative to the *Nru*I sites as shown in Fig. 3A. These same *Nru*I fragments are cleaved by *Mlu*I to give 60- and 80-kb fragments for metH and 55 kb for CF63. The placement of *Mlu*I sites with respect to *Nru*I sites is shown in Fig. 3B. The 560-kb *Sfi*I partial band detected by metH is cut by *Mlu*I, reducing it to 300 kb, the same size fragment as generated by *Mlu*I alone. When CF63 is used as the probe, the pattern of bands is identical to *Sfi*I alone, in which a 35- and a 60-kb band are detected. This would suggest that the *Mlu*I site separating metH and CF63 is extremely close to an *Sfi*I site and gives the map shown in Fig. 3C, the same map which is predicted by superimposing Fig. 3A on Fig. 3B.

No difference between the digestion products of *Bss*HII, *Sac*II, and *Nae*I has been observed on blots

TABLE 1

Sizes (in kb) of Restriction Fragments Generated by the Enzymes Listed and Detected by the Probes Given

	Enzyme								
Probe	*Bss*HII	*Mlu*I	*Nae*I	*Not*I	*Nru*I	*Sac*II	*Sal*I	*Sfi*I	*Xho*I
metH	240	300	240	850	115	240	630	140	550
	400	460	400		135	400		165	
		1250			1815			560	
		2250						585	
CF63	240	950	240	850	115	240	ND	35	550
	400	1250	400		135	400		60	
		2250			1815			140	
3.11H3	1200	1000	ND	5000	1700	1000	160	1050	120
		1950			1815				
		2250							

Note. For each digest, the smallest fragment is assumed to be a complete digestion product, while those listed below it are partial cleavage products. All digests were considered complete for this cell line, since neither increased enzyme amount nor incubation time altered hybridization patterns of probes from those listed here. Sizes of the largest partial cleavage products, which are difficult to size accurately, are estimated on the basis of addition of the sizes of primary fragments.

TABLE 2

Cleavage Products (in kb) from Double Enzyme Digestions

Probe	Enzymes							
	*Bss*HII *Sfi*I	*Nru*I *Sfi*I	*Mlu*I *Sfi*I	*Mlu*I *Bss*HII	*Mlu*I *Sac*II	*Sfi*I *Sac*II	*Mlu*I *Nru*I	*Not*I *Sfi*I
metH	140	65	140	140	140	140	60	140
	165	85	165	240	240		100	165
	300		300	300	300		105	320
							130	345
CF63	35	30	35	100	100	35	55	35
		55	60					60
								140
3.11H3	980	550	1000	920	920	980	720	ND
		720						

Note. As in Table 1, the smallest fragments represent complete digests with both enzymes, the larger fragments presumably represent partial digest products with one or both enzymes.

screened with metH or CF63. This implies that the sites for these enzymes are clustered and may represent HTF (*Hpa*II Tiny Fragment) islands (Bird, 1986). There appear to be three such islands in the vicinity of *met* and CF63, as shown by double digests of any of these three enzymes with *Mlu*I. These double digests cleave the primary 240-kb *Bss*HII, *Sac*II, and *Nae*I bands to 140-kb bands detected by metH and 100-kb bands detected by CF63. The 560-kb *Sfi*I partial digestion fragment is cleaved to 300 kb by *Bss*HII, *Sac*II, and *Nae*I in the same manner as that described for *Mlu*I and *Sfi*I above. This is explained by one of the *Mlu*I sites being in the same cluster as a *Sac*II, *Bss*HII, and *Nae*I site, as shown in Fig. 3D. Combining Figs. 3C and 3D, the map shown in 3E is obtained. The *Not*I site discussed in Fig. 1 can be added to this map by lining up the 140- and 25-kb *Sfi*I fragments with those in Fig. 3E. This places the 420-kb *Sfi*I fragment which contains the *Not*I site to the left of metH. The other end of the *Not*I fragment and the *Mlu*I fragment containing CF63 are shown on the map in Fig. 3F.

Since CF63 represents a defined jump in the 3′ direction from *met,* the orientation of the *met* map relative to CF and J3.11 can also be determined. We (Collins *et al.,* 1987a) and others (White *et al.,* 1986) have previously noted the presence of a polymorphic *Not*I site 3′ to *met* which is present (Fig. 3F) in about 10% of chromosomes. When this site is present, *met* appears on a 470-kb *Not*I fragment rather than on the more usual 850 kb. The sequence XV-2c cloned by Estivill *et al.* (1987), which by linkage analysis must be closer to CF than is *met,* lies on a 350-kb *Not*I fragment in chromosomes with the polymorphic *Not*I site and on an 820-kb fragment (which we size at 850 kb) in chromosomes lacking this site. This indicates that

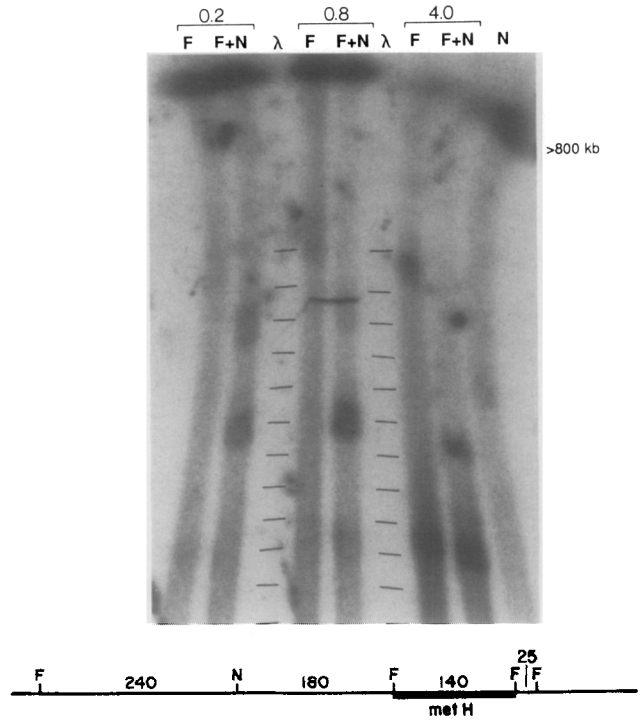

FIG. 1. Genomic digests of lymphoblastoid DNA with *Sfi*I (F), *Sfi*I + *Not*I (F + N), or *Not*I (N), run on an OFAGE gel with 30-s pulses for 25 h. All *Not*I digests were carried out using 4 U/μg DNA to ensure complete digestion, while *Sfi*I concentration was varied to give different degrees of partial digestions. From **left** to **right,** the *Sfi*I concentrations are 0.2 U/μg DNA, 0.8 U/μg DNA, and 4.0 U/μg DNA. Markers are drawn in (λ) and consist of concatenated λcI857, which are multiples of 48.5 kb. Notice the 560-kb *Sfi*I fragment which is cleaved to 320 kb by *Not*I (compare F to F + N in the 4.0 set). Below is the *Sfi*I/*Not*I map constructed from the data generated from the blot, illustrating the positions of the 420- and 25-kb *Sfi*I fragments which flank metH, and the *Not*I site located in the 420-kb fragment. There is probably an additional *Not*I site about 140 kb to the left of this, because of the 460/485 kb doublet in the 0.2 F + N lane, but this has not yet been confirmed on other blots.

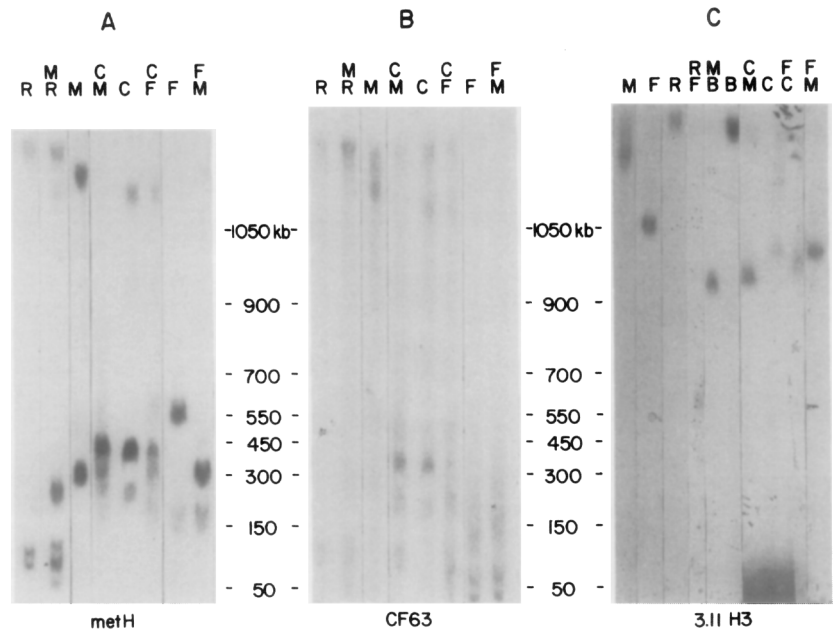

FIG. 2. Lanes from a FIGE Southern blot of lymphoblastoid DNA with combinations of the enzymes *Bss*HII (B), *Sac*II (C), *Sfi*I (F), *Mlu*I (M), and *Nru*I (R). (**A** and **B**) Probes were metH and CF63, respectively, and both probes are shown hybridizing to the same *Nru*I and *Sac*II fragments, but different *Mlu*I and *Sfi*I fragments. (**C**) Lanes from the same blot are shown after probing with 3.11H3. As is seen here, there are no fragments on this blot which hybridize to both 3.11H3 and either of the other two probes.

XV-2c must lie on the 3′ side of *met*. Since linkage analysis places these markers in the order *met*–[XV-2c–CF]–J3.11 (Beaudet *et al.*, 1986; Estivill *et al.*, 1987), this proves that CF and J3.11 also lie on the 3′ side of *met*. Such a conclusion is consistent with other data based on a chromosomal rearrangement within the *met* gene in the MNNG-HOS cell line (Scambler *et al.*, 1986).

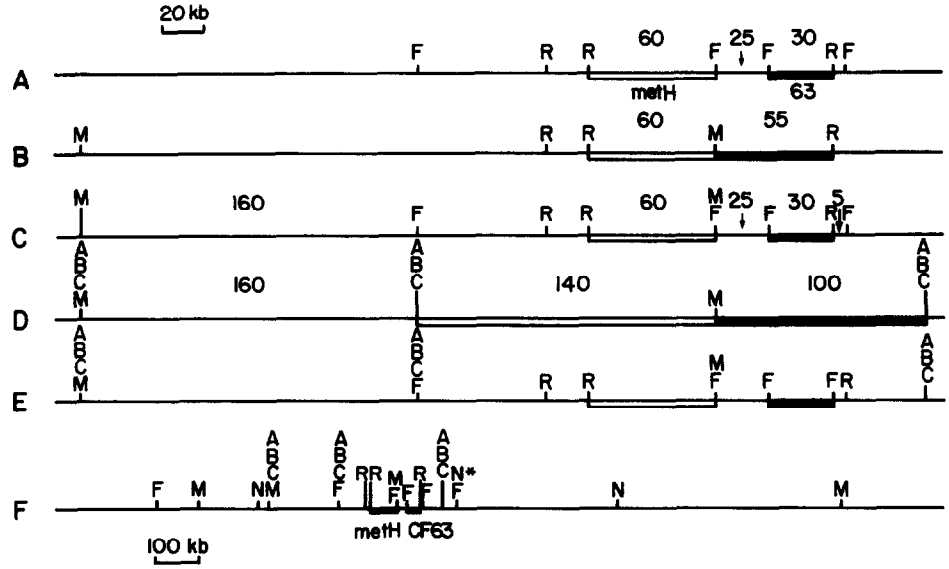

FIG. 3. Construction of metH/CF63 regional map. (**A**) The map generated by digests with *Nru*I (R), *Sfi*I (F), and *Nru*I + *Sfi*I; (**B**) generated from *Nru*I and *Mlu*I (M); (**C**) from *Mlu*I and *Sfi*I; (**D**) from *Mlu*I combined with any of the three enzymes *Nae*I (A), *Bss*HII (B), or *Sac*II (C). Superimposing maps **A**, **B**, **C**, and **D** on each other gives the map shown in **E**. A composite map of the region, shown in **F**, includes *Not*I sites indicated in Fig. 1, as well as the *Sfi*I sites determined by partial digests. N* is a polymorphic *Not*I site. Numbers above maps are sizes in kilobases of fragments.

D7S8 Regional Map

The ground work of this map is based on the double digestion product of *Sfi*I and *Nru*I (Fig. 2C). These two enzymes yield a fragment of 550 kb from an *Sfi*I fragment of 1050 kb and an *Nru*I fragment of 1700 kb. *Nru*I combined with *Mlu*I cuts the 1150-kb *Mlu*I fragment into a 720-kb fragment, *Sac*II plus *Mlu*I shortens the 1000-kb *Sac*II fragment to 920 kb, and *Sac*II and *Sfi*I result in a 970-kb fragment. (These sizes were deduced primarily from OFAGE or Pulsaphor blots, which can separate fragments in this range better than can the FIGE blot shown in Fig. 2C). The map consistent with these data is shown in Fig. 4. This also includes other data from Tables 1 and 2, including restriction sites for partial cleavage products, as for *Nru*I and *Sfi*I.

Joining of the Maps

To link the two maps, *Mlu*I partial digests were analyzed using a long pulse time to resolve fragments as large as 3000 kb. To construct these blots we found it necessary to run partial digests over a wide range of enzyme concentrations, and often to correlate the results of more than one experiment. Two examples of partial *Mlu*I blots are shown in Fig. 5. When the blots were probed with metH, a complete digest band of 300 kb was seen, as well as partial bands of 460, 1250, and 2250 kb. Reprobing with 3.11H3 gives partial bands at 1950 and 2250 kb in addition to the 1000-kb primary band. These patterns are consistent with a map in which a 300-kb metH and a 1000-kb 3.11H3 *Mlu*I fragment are separated by a 950-kb *Mlu*I fragment containing CF63, as shown in Fig. 5. In confirmation of this conclusion, CF63 does reside on a 950-kb *Mlu*I fragment (not well shown in Fig. 2B, but visible in other OFAGE and FIGE blots which are not shown). Partial *Nru*I digests also demonstrate that metH and 3.11H3 detect the same 1815-kb *Nru*I fragment. When this information is combined with the partial *Mlu*I data in Fig. 5, the final map in Fig. 6 is generated. The orientation of the D7S8 regional map relative to the *met*–CF63 map must be as shown; the alternative orientation could not explain the *Mlu*I and *Nru*I results.

DISCUSSION

Locating of a disease gene by "reverse genetics" requires one to move from a primary genetic map, generated by linkage analysis, to a physical map which can be used to delimit the DNA region within which the gene must reside. While the genetic map suggests the size of the physical region by the relationship of 1 cM ≅ 1000 kb, there can be marked local deviations from this correspondence due to nonlinearity of recombination rates along a chromosome. In this study we have used the two markers *met* and J3.11, which are known to be tightly linked to CF, as well as a jumping clone derived from *met* (CF63), to construct a long-range restriction map of the CF region covering approximately 3000 kb on chromosome 7. The summary map is shown in Fig. 6.

A few caveats about physical mapping of large chromosomal regions should be kept in mind. The recognition sites for most of the enzymes used (except *Sfi*I) contain CpG sequences, and methylation of the cytosine residues can potentially prevent restriction (Smith *et al.*, 1987b). Any variation in the methylation of these sites from cell line to cell line or tissue to tissue could result in a difference in restriction fragment lengths and thus in the map. Strictly speaking, the map presented is that for B-cell line 3.1.0 and may differ for other cell lines. Partial methylation may result in incomplete digestion, even with large quantities of restriction enzyme. Examples are the two *Mlu*I sites between metH and 3.11H3. Incomplete cutting at these *Mlu*I sites leads to the strong signal for the 2250-kb fragment in Fig. 5.

A related issue is that in diploid cell lines, the possibility of restriction site polymorphism must be considered when two bands are detected by a single probe in a complete digest, especially if the intensities of the two bands are similar. An example is the polymorphic *Not*I site shown in Fig. 6. Without studies of familial inheritance, it may be impossible to distinguish this

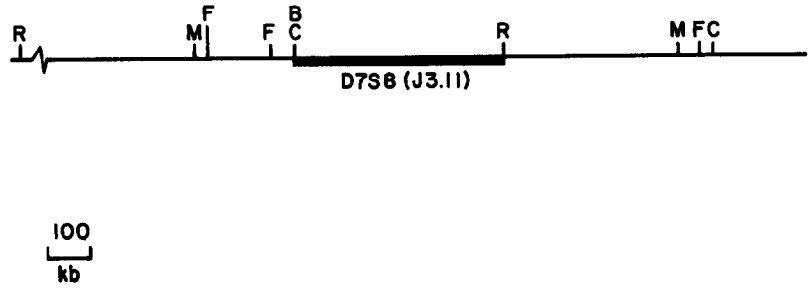

FIG. 4. D7S8 regional map constructed from single and double digests with *Bss*HII (B), *Sac*II (C), *Sfi*I (F), *Mlu*I (M), and *Nru*I (R). Sizes of fragments from these digests are listed in Table 1 and 2.

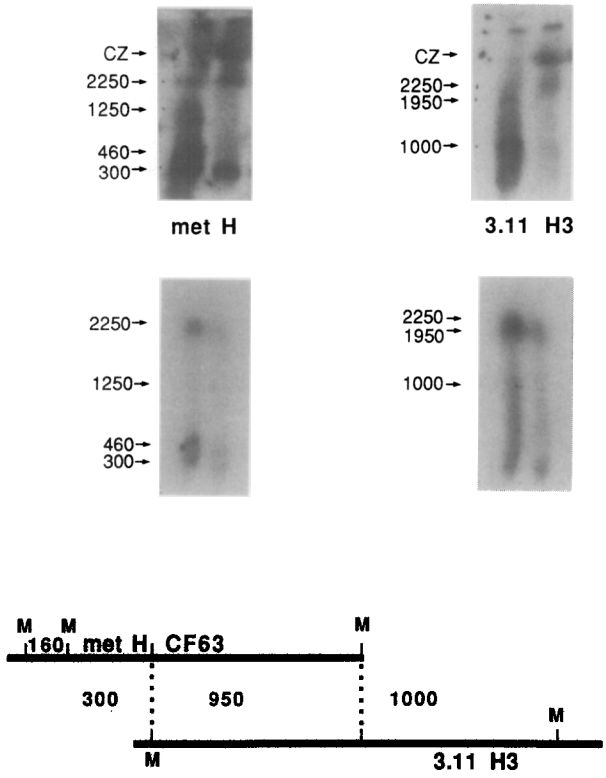

FIG. 5. *Mlu*I partial digest Southern blots probed sequentially with metH and 3.11H3. The **upper** panel, which is from a gel run on an LKB Pulsaphor as described by Smith *et al.* (1987a), resolves intermediate partial fragments, including bands at 460 and 1250 kb for metH and at 1000 and 1950 kb for 3.11H3. The **lower** panel, also from a Pulsaphor, but run as described under Materials and Methods, does not show some of these intermediate fragments but clearly shows both probes hybridizing to the same 2250-kb partial *Mlu*I fragment. In both blots the lane on the left is a somewhat more complete digest than the lane on the right, though both are intentional partials. The apparent signal at the lower end of the size range with probe 3.11H3 in the lower panel is an artifact due to nonspecific hybridization to small amounts of degraded DNA in these samples. **Below** is a map consistent with the data from the two blots in which metH and 3.11H3 can be linked together.

result from partial digestion due to methylation. The overall map will not, however be affected so long as the same cell line is used throughout.

A final caveat is that the sizes of the fragments reported in Tables 1 and 2 must be considered to have a potential error of at least 10%. Sizing of PFGE bands is quite sensitive to the amount of DNA per lane (Collins *et al.*, 1987b); while we attempted to keep the DNA per lane under 3 μg, some variation in the size of a fragment from gel to gel was often seen. In our experience, one must be particularly careful in sizing DNA fragments larger than 800 kb on FIGE gels; as also noted by others (Ellis *et al.*, 1987), above this range the relationship of fragment size to mobility breaks down.

The map in Fig. 6 indicates that *met* and J3.11 are between 1300 and 1800 kb apart, which is in reasonable agreement with their genetic distance of about 2 cM. The CF gene must reside within this region. Two clusters of sites, so-called HTF islands (Bird, 1986), appear in this interval, one just 3′ to *met*, and the other about 400 kb closer to J3.11. This latter island has recently been cloned by Estivill *et al.* (1987) and contains sequences in strong disequilibrium with the CF gene. The suggestion that the CF transcript itself is encoded in this segment has not, however, been confirmed. There is, of course, no reason why the CF gene, which is expressed in a highly tissue-specific manner, need be associated with an HTF island. The physical map in Fig. 6 can be of considerable utility in ordering additional cloned DNA segments from this region. Coupled with fine linkage analysis in CF families in which recombinations between *met* and CF or CF and J3.11 have occurred, physical mapping should allow the narrowing of the actual gene location to a region of 200–300 kb. A region of this size would be small enough to clone in overlapping cosmids, which

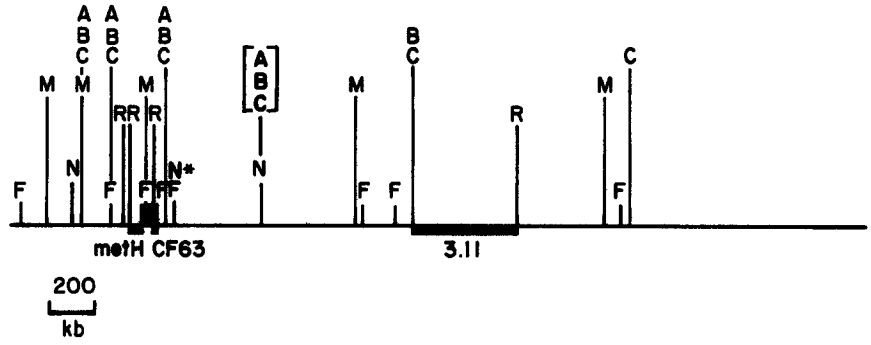

FIG. 6. Composite map linking regional maps of Figs. 3F and 4 together. The distance between metH and 3.11H3 is estimated from this map to be between 1300 and 1800 kb. A, *Nae*I; B, *Bss*HII; C, *Sac*II; F, *Sfi*I; M, *Mlu*I; N, *Not*I; and R, *Nru*I. Additional *Sfi*I, *Nae*I, *Bss*HII, and *Sac*II sites may be located in the region near the center of the map, but cannot be detected by the probes used. In brackets are the *Nae*I, *Bss*HII, and *Sac*II sites reported by Estivill *et al.* (1987) to be present close to the *Not*I site in the center of the map, which is the location of the XV-2c and CS-7 probes they have derived. The sites in brackets do not cut in the B-lymphoblast cell line used for our mapping data.

could be screened exhaustively for candidate transcripts. With this strategy there is good reason to be optimistic that in the near future the cloned CF gene will be in hand.

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