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In situ hybridization of two cloned chromosome 7 sequences tightly linked to the cystic fibrosis locus

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Abstract. Two DNA sequences closely linked to the cystic fibrosis locus have been sublocalized to 7q31.3 → q32 by in situ hybridization. These findings are consistent with previously pub-

lished maps of that region of human chromosome 7. The cystic fibrosis locus therefore maps to the 7q31.3 → q32 region, a more distal location than had been inferred from previous data.

The genetic locus for cystic fibrosis, CF, has been mapped to the long arm of human chromosome 7 on the basis of extensive genetic linkage information (reviewed by Tsui et al., 1986). The regional localization of the CF locus has been inferred from studies of the closely linked, flanking DNA markers MET (White et al., 1985) and D7S8 (Wainwright et al., 1985). It has been established by linkage studies that both MET and D7S8 are less than 1 centimorgan from CF, and that MET maps proximal to the centromere and D7S8 maps distal (Beaudet et al., 1986; Lathrop et al., 1988); long range restriction maps containing MET and D7S8 have also been constructed (Drumm et al., 1988; Poutska et al., 1988; Rommens et al., in preparation). However, the regional localization of MET and D7S8 has not been straightforward; MET was originally localized to a broad region, 7q21 → q31, by in situ hybridization (Dean et al., 1985) and D7S8 was placed in bands 7cen → q22 (Bartels et al., 1986) by error which led to the first assignment of CF to 7cen → q22 (Wainwright et al., 1985).

Most of the recent subregional localization studies (Tsui et al., 1986; Scambler et al., 1987; Zengerling et al., 1987) have been based on hybridization analysis with a patient cell line (GM1059) with an interstitial deletion spanning 7q22 and 7q32 or with a human × rodent somatic cell hybrid containing this del(7) chromosome (Rommens et al., 1988); neither MET nor D7S8 are present on this chromosome, suggesting that these DNA sequences and CF map between 7q22 and 7q32. A more recent assignment of MET to 7q32 was derived from the analysis of a translocated chromosome 7 in a MNNG-transformed human osteosarcoma (HOS) cell line (Park et al., 1988).

In view of these mapping data, we have examined the chromosome location of D7S122 and D7S340, two recently identified DNA segments closely linked to the CF locus (Rommens et al., 1988), by in situ hybridization. These two DNA segments have been localized between MET and D7S8 by linkage (Rommens et

al., 1988) and long range restriction enzyme mapping studies (Rommens et al., in preparation). Mapping D7S122 and D7S340 is therefore important to furthering our knowledge of the chromosomal location of CF.

D7S122 and D7S340 are recognized by unique DNA probes, pH131 and TM58, respectively; pH131, a 3.4-kb *Eco*RI insert in pUC13, and TM58, a 2.6-kb *Eco*RI insert in pUC9, have been described (Rommens et al., 1988). The two plasmids were nick-translated with [³H]dTTP and [³H]dCTP to a specific activity of 2–4 × 10⁷ cpm/μg DNA for use as hybridization probes (Rigby et al., 1977).

In situ hybridization was carried out according to the method of Harper and Saunders (1981) on BrdU-synchronized peripheral blood lymphocytes (Lin et al., 1985). Briefly, metaphase chromosomes were denatured on slides in 70% formamide, 30% 2 × SSC for 2 min at 70 °C. The slides were quickly transferred to 70% ethanol at –20 °C and then dehydrated with 80% and 95% ethanol at room temperature (2 min per wash). The probe was denatured at 70 °C for 5 min in a hybridization mix consisting of 50% formamide, 10% dextran sulphate, 2 × SSC (phosphate buffered to pH 6.0), 0.2 μg/ml probe, and 20 μg/ml carrier DNA (sonicated salmon sperm). To each slide were added 50 μl of denatured hybridization mix. The slides were covered with slips, sealed with rubber cement, incubated overnight at 37 °C, and washed three times in 50% formamide, 2 × SSC, and five times in 2 × SSC (3 min per wash). The slides were then dehydrated sequentially in 70%, 80%, and 95% ethanol (2 min per wash). The slides were coated with NTB-2 emulsion, exposed for four wk at 4 °C, and developed by conventional methods (Harper and Saunders, 1981). The slides were stained using a modification of the fluorescence plus Giemsa technique (Lin et al., 1985). The silver grains over well-banded metaphase chromosomes were scored. Sublocalization of TM58 was carried out with less condensed chromosomes to resolve the sub-bands q31.1, q31.2, and q31.3.

Analysis of the silver grain distribution following in situ hybridization of TM58 and pH131 revealed significant hybridization in the 7q31 → q32 region (Figs. 1 and 2). These cytogenetic bands have been estimated to represent, respectively, 9.3% and 5% of the length of human chromosome 7 (Daniel, 1985). The hybridization observed in more highly banded chromosomes 7 with TM58 suggested that this probe recognizes a sequence which maps to the 7q31.3 → q32 region (Fig. 1). The pH131 probe hybridized somewhat less specifically owing to some repetitive elements in its sequence (Tsui, unpublished data). However, since the results of chromosome walking experiments indicate that TM58 and pH131

SILVER GRAIN DISTRIBUTION

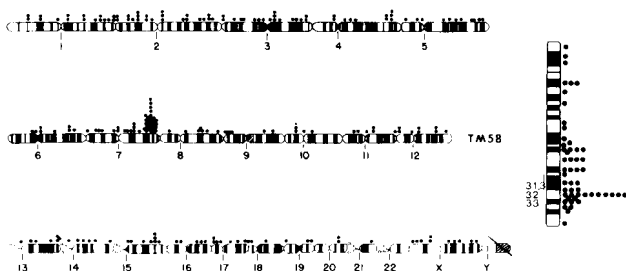


Fig. 1. Histogram of silver grain distribution following in situ hybridization with probe TM58. The positions of 200 silver grains were mapped at the 450-band resolution level of the human karyotype. Significant hybridization, 15% (29 grains), was observed over the 7q31 → qter region. Less condensed chromosomes 7 were then analyzed selectively in order to refine the sublocalization of the TM58 sequence. An idiogram of silver grain distribution observed over chromosome 7 banded at the 550-band level of resolution is shown to the right.

SILVER GRAIN DISTRIBUTION

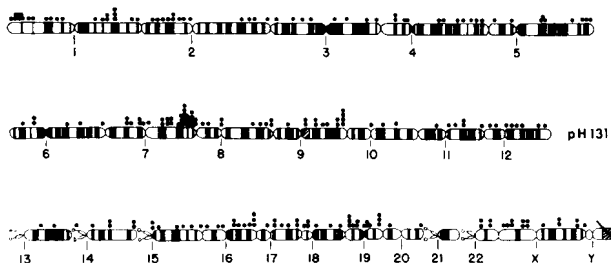


Fig. 2. Histogram of silver grain distribution following in situ hybridization with probe pH131. The positions of 220 silver grains were mapped at the 450-band resolution level of the human karyotype. Significant hybridization, 10% (22 grains), was observed over the 7q31 → qter region.

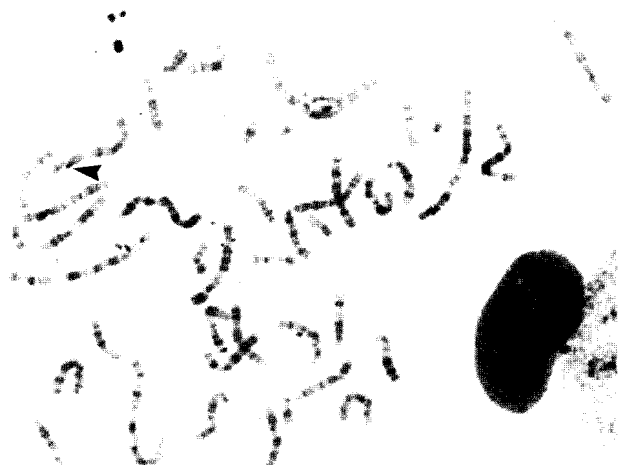


Fig. 3. Partial metaphase spread. Chromosomes were G-banded after development. The arrow indicates a large silver grain over the 7q31.3 → q32 region.

are separated by only 8 kb (Rommens et al., in preparation), it is reasonable to conclude from the grain distribution obtained for pH131 (Fig. 2) that this sequence also maps to the 7q31.3 → q32 region.

These results, coupled with the genetic linkage information (Rommens et al., 1988), suggest that the CF locus probably maps between the distal one-third of 7q31 and the proximal half of 7q32. This conclusion is consistent with the previous high resolution in situ mapping data which showed that D7S13 (B79a) and D7S18 (7C22) (two DNA markers mapped centromeric to MET) are located in bands 7q22.3 → q31.1 and 7q31.1 → q31.2, respectively (Buckle et al., 1987a, b), and with the physical order of these markers based on pulsed field gel analysis (Poustka et al., 1988; Rommens et al., in preparation). This information on the localization of the CF locus should be useful in identifying translocation break-points and other cytogenetic markers for more refined mapping of genetic loci on the long arm of chromosome 7 and for isolation of the CF gene.

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