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Fluorescence in situ hybridization mapping of the cystic fibrosis transmembrane conductance regulator (CFTR) gene to 7q31.3

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Abstract. We have used the fluorescence in situ hybridization (FISH) technique to refine the localization of the cystic fibrosis transmembrane conductance regulator (CFTR) gene on human chromosome 7. The result shows that the gene is most likely located within band q31.3.

The cystic fibrosis transmembrane conductance regulator (CFTR) gene has been studied intensively for mutations causing the disease (Tsui, 1992a,b). As a consequence, a large number of mutations as well as sequence variations have been detected. Some of the sequence polymorphisms, particularly several of the dinucleotide and oligonucleotide repeats (Chaheb et al., 1991; Moral et al., 1991; Zielenski et al., 1991), are in fact excellent markers for genetic mapping studies. The chromosome localization of the CFTR gene has, however, only been implicated through studies with linked DNA markers to band q31→q32 of chromosome 7 (Dean et al., 1985; Wainwright et al., 1985; Buckle et al., 1987; Duncan et al., 1988). Therefore, in order to obtain a more precise localization of this useful genetic marker, we have performed fluorescence in situ hybridization (FISH) of the CFTR gene directly on DAPI-banded chromosomes.

The chromosome spreads were prepared according to the method of Lin et al. (1985), except that the slides were aged at – 20 °C for at least 1 wk (up to 1 yr) and baked at 60 °C for 1 h before use. After RNase A treatment, the slides were denatured in 70% formamide in 2 × SSC for 1 min at 70 °C followed by dehydration with ethanol. The FISH procedure was performed essentially as previously described (Heng et al., 1992). The cosmid clone (cW10-20) containing exon 4, 5, 6a, 6b and 7 of the gene (Rommens et al., 1989) was used as the hybridization probe; the cosmid DNA was isolated from the *E. coli* DNA by the QIAGEN™ column and labeled with biotinylated dUTP with the BioNick kit (BRL) and purified with the use of the Nick column (Pharmacia). The probes were denatured at 75 °C for 5 min in a hybridization mix consisting of 50% formamide and 10% dextran sulphate. Repetitive sequence was suppressed by incubating the probes with human cotI DNA (BRL) for 15–30 min at 37 °C. After hybridization for 16–24 h at 37 °C, the hybridized probes were detected by an amplification procedure as described (Heng et al., 1992).

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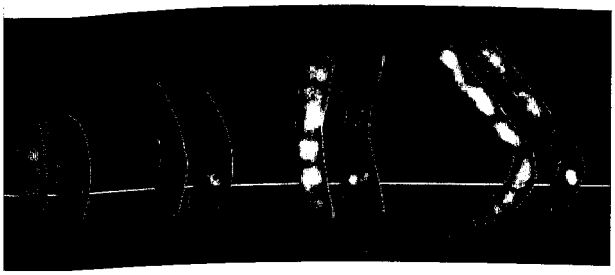
To obtain chromosome banding, a heating step was applied for 20–60 s at 65–70 °C after staining with 4′,6-diamidino-2-phenylindole (DAPI). The probe signals and the DAPI-banding (similar to G- or Q-banding) patterns were visualized in the microscope with different optical filters. The detailed protocol for DAPI-banding was presented elsewhere (Heng and Tsui, submitted).

Two hundred chromosome 7 structures were examined for positive probe hybridization signals. Under the condition used, the majority (174/200 = 87%) of the structures showed double hybridization signals, one on each of the two sister chromatids. Forty of the mitotic figures were photographed and the signals were localized by superimposing with the DAPI-banding patterns; chromosomes with hybridization signals from only one of the two sister chromatids and those with unclear DAPI-banding patterns were not scored. Examples of the FISH data are shown in Fig. 1 and the locations of the probe-hybridization signals are schematically summarized in Fig. 2. It is clear from these data that the CFTR gene is most likely located in band q31.3.

Previous mapping studies with the use of closely flanking markers showed that the CFTR gene was located between bands q22 and q32 (Dean et al., 1985; Wainwright et al., 1985; Buckle et al., 1987; Duncan et al., 1988). Since radioactively labeled probes were used in those studies, the localization of hybridization signals might have been affected by emulsion shifting and signal scattering. On the other hand, while FISH mapping is generally considered to be more precise than that offered by radioactive labeling (Trask, 1991), our data show that the position of FISH signals could be greatly affected by overbaking or over-denaturization of the metaphase chromosomes. These undesirable results are probably due to destruction of the higher order structure of the chromatin fibers. Good DAPI-banding pattern seems to be an excellent indicator of good chromosome integrity required for consistent localization of FISH signals.

The more precise regional assignment of CFTR to 7q31.3 provides an excellent reference marker for future mapping of genes on human chromosome 7 and towards the construction of a complete physical and genetic map of this chromosome.



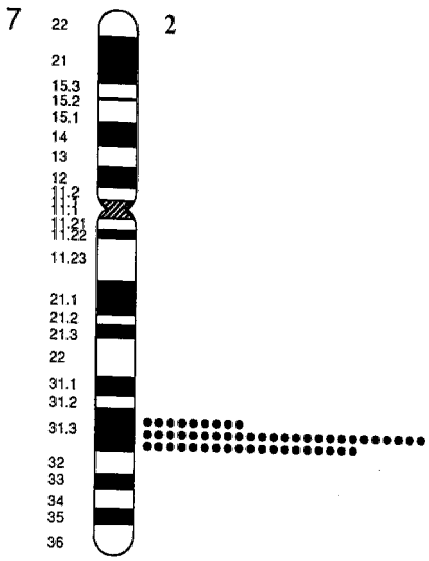


Fig. 1. Examples of FISH detection of the CFTR gene on human chromo-
le 7. Four metaphase chromosome structures of different resolutions are
wn, each with the DAPI-banding pattern on the left and the fluorescent
al from the labeled cW10-20 probe on the right.
Fig. 2. Summary of FISH data for CFTR on human chromosome 7. Each
represents a double fluorescent signal on clear DAPI-banded chromo-
es.

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