

# Localization of the human gene encoding the 13.3-kDa subunit of mitochondrial complex III (UQCRB) to 8q22 by in situ hybridization

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**Abstract.** We have localized the human gene encoding the 13.3-kDa subunit of mitochondrial complex III (UQCRB) to chromosome 8 using both radioactive in situ hybridization and fluorescence in situ hybridization. The additional peak ob-

tained with the former method is attributed to the higher sensitivity of this technique, which results in hybridization of the probe to the less conserved pseudogene. We therefore conclude that the functional gene is most likely located at 8q22.

The mitochondrial respiratory chain in mammals consists of five multisubunit enzyme complexes. In all but one of them, complex I, the subunits are encoded by a combination of mtDNA and nuclear DNA. Complex III consists of at least 11 distinct subunits comprising the mitochondrially encoded cytochrome b, the nuclear encoded cytochrome c, an iron-sulfur protein with an Fe<sub>2</sub>S<sub>2</sub> cluster, and a series of proteins of unknown function, including the 13.3-kDa peptide localized in this paper (Link et al., 1987). Previous disruption of the gene coding for this subunit in yeast homologous to the human 13.3-kDa peptide resulted in a respiratory deficient mutant strain (Schoppink et al., 1989), and proteolytic deletion of the N-terminal seven amino acids of the homologous bovine subunit resulted in decoupling of redox-linked H<sup>+</sup> pumping (Cocco et al., 1991). Although this subunit does not contain a catalytic center, we speculate that it plays a vital role in the development of the mitochondrial membrane potential.

A full-length cDNA of the 13.3-kDa subunit of the mitochondrial ubiquinol cytochrome c reductase (UQCRB) was previously isolated and used to locate genomic clones for UQCRB (Suzuki et al., 1988, 1989). The UQCRB consists of

four exons spanning 4.5–5 kb in length; two other genomic entities were also isolated but had no introns and were thought to be processed pseudogenes (Suzuki et al., 1989). Genomic DNA from the UQCRB gene and a hybrid mapping panel were subsequently used to localize the gene to 8q22 → qter (Hosokawa et al., 1990).

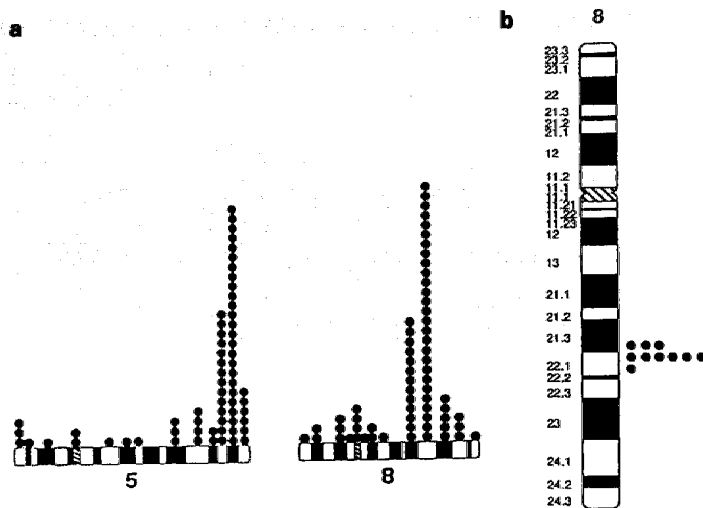
To obtain a more precise localization of the UQCRB gene, the full-length 350-bp cDNA was obtained by PCR from a first-strand cDNA library and used to perform in situ hybridization as described by Duncan et al. (1992). Analysis of the distribution of 400 silver grains following in situ hybridization revealed a significant clustering of grains on the long arms of chromosomes 5 and 8 (Fig. 1a). Thirty-nine silver grains were observed at 5q33 → q34, with a peak at 5q34, and 40 silver grains were observed at 8q21.3 → q32, with a peak at 8q22 ( $P < 0.0001$ ). Examination of metaphase chromosomes showed silver grains located over the 5q34 and 8q22, supporting the localization of UQCRB to one or both of these sites.

Since the cDNA probe was relatively small, fluorescence in situ hybridization (FISH) was performed with the 350-bp clone to confirm the localization (Ali et al., 1993; Heng et al., 1992, 1994; Heng and Tsui 1993). After hybridization and detection, the hybridization efficiency was greater than 50%. Figure 2 shows a typical fluorescence hybridization signal on chromosome 8 obtained with the UQCRB cDNA probe. A total of 10 mitotic figures were photographed, and the data are summarized in Fig. 1b. Each dot in Fig. 1b represents a double fluorescent signal on chromosome 8, suggesting that the gene is located

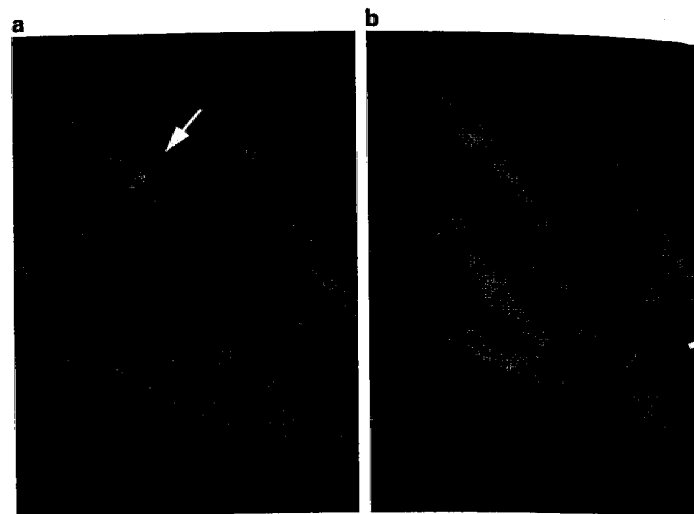
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**Fig. 1.** Localization of the UQCRB gene to 5q34 and 8q22. **(a)** Silver-grain distribution following in situ hybridization. The positions of 400 silver grains observed over well-banded autosomes were recorded on an ISCN-derived ideogram of the human karyotype. In situ hybridization to BrdU-synchronized peripheral blood lymphocytes was performed using the method of Harper and Saunders (1981) with a probe of 348 bp corresponding to the whole coding region of the cDNA and labeled to a specific activity of  $3.3 \times 10^7$  cpm/ $\mu$ g with [ $^3$ H]dTTP and [ $^3$ H]dATP (NEN) using a multiprime labeling system (Amersham). **(b)** An ideogram of UQCRB mapping by FISH to chromosome 8 summarizing the fluorescent signal data. Each dot represents a double fluorescent signal. See legend of Fig. 2 for details.



**Fig. 2.** Localization of the UQCRB gene to 8q22 by FISH. **(a)** An example of FISH mapping for the UQCRB gene. Chromosomes were prepared from synchronized lymphocyte cultures fixed with 3:1 methanol acetic acid, and slides were air dried (Link et al., 1987; Suzuki et al., 1988). Slides aged 7 d were baked at 55°C for 1 h, treated with RNase A, and denatured in 70% formamide for 1 min at 70°C, followed by dehydration with ethanol. The 350-bp UQCRB cDNA probe was biotinylated with dATP, using a BRL BioNick labeling kit, at 15°C for 1 h (Suzuki et al., 1988). The probes were denatured at 75°C for 5 min in a hybridization mixture of 50% formamide and 10% dextran sulfate. After hybridization, slides were washed, and FISH signals were detected and amplified using a modification of the method described by Schoppink et al. (1989). FISH signals and the DAPI banding pattern were visualized in a single operation by simply switching the filters of the microscope (Suzuki et al., 1988). The left panel shows the FISH signal, indicated by an arrow. The right panel **(b)** shows the corresponding chromosomes after DAPI staining.

at 8q22. All of the signals were located between 8q21.3 and 8q22.1, the most frequent location being 8q22.1. No signals were detected with a high frequency above chromosome 5 under the conditions used.

In general, radioactive in situ hybridization is more sensitive than FISH; therefore, it is not surprising to notice the differences in hybridization. In fact, radioactive in situ hybridization generates a much higher background. By comparing mapping results from both methods, we concluded that the most specific hybridization with the UQCRB cDNA occurs on chromosome 8. The most likely explanation for this is that the real gene lies on chromosome 8, whereas a pseudogene, or a related sequence, is located on chromosome 5 and was detected by radioactive in situ hybridization. With the FISH technique, it is not clear whether the probe would hybridize better to the UQCRB pseudogene, which has no introns, rather than the true gene, which has introns (Suzuki et al., 1989). However, the homology between the pseudogene and the cDNA, which is inferior to that between the true gene and its cDNA, may dictate that preferential hybridization should occur with the gene.

It is still a challenge to map small probes by the FISH method, since the hybridization efficiency and the intensity of signals are related to the size of the probes. Recently, small-size

cDNA FISH mapping has greatly improved, such that 1 kb or even smaller sequences can be detected (Ali et al., 1993; Heng et al., 1994). By modifying the probe labeling conditions, as well as those for hybridization and detection, a stronger and larger complex or "probe network" may be formed that is designed to amplify FISH signals for small probe detection. Therefore, the FISH detection method can be reliably used for mapping cDNA probes smaller than 500 bp (Heng et al., manuscript in preparation). The successful mapping of the UQCRB cDNA (350 bp) in the present report is one such example.

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## References

- Ali ST, Duncan AMV, Schappert K, Heng HHQ, Tsui L-C, Chow W, Robinson BH: Chromosomal localization of the human gene encoding the 51-kDa subunit of mitochondrial complex I (NDUFV1) to 11q13. *Genome* 18:435-439 (1993).
- Cocco T, Lorusso M, Sardanelli AM, Minuto M, Ronchi S, Tedeschi G, Papa S: Structural and functional characteristics of polypeptide subunits of the bovine heart ubiquinol-cytochrome-c reductase complex. *Eur J Biochem* 195:731-734 (1991).
- Duncan AMV, Chow W, Robinson BH: Localization of the human 75 kDa Fe-S protein of NADH-coenzyme Q reductase gene (NDUFS1) to 2q33 → q34. *Cytogenet Cell Genet* 60:212-213 (1992).
- Harper M E, Saunders G F: Localization of single copy DNA sequences on G-banded human chromosomes by *in situ* hybridization. *Chromosoma* 83: 431-439 (1981).
- Heng HHQ, Squire J, Tsui L-C: High resolution mapping of mammalian genes by *in situ* hybridization to free chromatin. *Proc natl Acad Sci, USA* 89: 9509-9513 (1992).
- Heng HHQ, Tsui L-C: Modes of DAPI banding and simultaneous *in situ* hybridization. *Chromosoma* 102:325-332 (1993).
- Heng HHQ, Xiao H, Shi X-M, Greenblatt J, Tsui L-C: Genes encoding general initiation factors for RNA polymerase II transcription are dispersed in the human genome. *Hum molec Genet* 3:61-64 (1994).
- Hosokawa Y, Suzuki H, Nishikimi M, Matsukage A, Yoshida MC, Ozawa T: Chromosomal assignment of the gene for the ubiquinone-binding protein of human mitochondrial cytochrome *bc<sub>1</sub>* complex. *Biochem Internat* 21:41-44 (1990).
- Link TA, Schagger H, Von Jagow G: Structural analysis of the BC<sub>1</sub> complex from beef heart mitochondria by the sided hydrophathy plot and by comparison with other BC complexes, in Papa S, Chance B, Ernster L (eds): *Cytochrome Systems*, pp 289-301 (Plenum Publishing, New York 1987).
- Schoppink PJ, Berden JA, Grivell LA: Inactivation of the gene encoding the 14-kDa subunit VII of yeast ubiquinol cytochrome c oxidoreductase and analysis of the resulting mutant. *Eur J Biochem* 181: 475-483 (1989).
- Suzuki H, Hosokawa Y, Toda H, Nishikimi M, Ozawa T: Cloning and sequencing of a cDNA for human mitochondrial ubiquinone-binding protein of complex III. *Biochem biophys Res Commun* 156:987-994 (1988).
- Suzuki H, Hosokawa Y, Toda H, Nishikimi M, Ozawa T: Isolation of a single nuclear gene encoding human ubiquinone-binding protein in complex III of mitochondrial respiratory chain. *Biochem biophys Res Commun* 161:371-378 (1989).