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Precise Localization of the Excision Repair Gene, ERCC5, to Human Chromosome 13q32.3-q33.1 by Direct R-Banding Fluorescence *in situ* Hybridization

Ei-ichi Takahashi, Naoko Shiomi and Tadahiro Shiomi[1]

Division of Genetics, National Institute of Radiological Sciences, Anagawa 4-9-1, Inage-ku, Chiba 263

The genomic DNA fragment encoding the excision repair gene, ERCC5, was mapped by direct R-banding fluorescence *in situ* hybridization. The signals were localized to human chromosome 13q32.3-q33.1. This result was in agreement with previous reports, and the gene was assigned to a narrower region.

Key words: Human excision repair gene ERCC5 — Mapping by FISH — 13q32.3-q33.1

Nucleotide excision repair removes chemical adducts and UV-induced photoproducts from DNA. Patients with the genetic disorder xeroderma pigmentosum (XP) are sensitive to sun exposure and develop skin cancers in exposed regions. Assays for repair synthesis and incision activity reveal cells from the classical form of XP to be defective in performing the incision step of nucleotide excision repair. To facilitate studying this repair process, UV-sensitive rodent cell mutants have been isolated and classified into ten different genetic complementation groups.[1,2] The human nucleotide excision repair genes which complement the defects of the mutants have been cloned[3–7] and designated as the ERCC (excision repair cross complementing rodent repair deficiency) genes; a number is added to refer to the particular rodent complementation group that is corrected by the gene. Recently, we have cloned the human ERCC5 gene and its cDNA (Shiomi *et al.*, manuscript in preparation) which complements the defect of the mouse UV-sensitive mutant, XL216, belonging to complementation group 5.[8]

Two genomic DNA fragments encoding ERCC5, which were cloned in EMBL3 phage, were used as DNA probes in the present study. 2R10-1 (a 15-kb insert) covers the 5′-side of the gene and Rb (a 16-kb insert) covers the 3′-side with a 6.6-kb overlap. A direct mapping system, which is based on fluorescence *in situ* hybridization (FISH) combined with replicated prometaphase R-bands,[9,10] has been applied. This system allows the precise localization of the fluorescent signals on the R-banded prometaphase chromosomes stained with propidium iodide. For the suppression of repetitive sequences contained in the probes, 50-fold excess amounts of total human placenta DNA were added in a routine manner.[10,11] Procedures of DNA labeling, hybridization and detection have been described elsewhere.[10]

We have examined 100 typical R-banded (pro)metaphase chromosomes for both 2R10-1 and Rb probes. The hybridization efficiencies of the two probes were similar. Of them, 48% exhibited complete double spots on both homologs, and 52% were incomplete single and/or double spots on either or both homologs. The fluorescent signals for 2R10-1 were observed in the following locations of the long arm of chromosome 13; the interphase between the distal part of R-positive q32.3 band and the proximal part of the q33.1 band, 68%, and the proximal part of the q33.1 band, 32%. No double spots were observed on other chromosomes. The location of Rb was the same as that of 2R10-1. Thus, the ERCC5 gene could be assigned to band 13q32.3-q33.1 (Fig. 1a–c). On careful inspection of the prometaphase R-bands, however, the signals seemed to be localized to the R-positive 13q32.3 band (Fig. 1c). In mammals, R-positive (early replicating) bands contain housekeeping genes, which are characterized by GC-rich DNA sequences.[12,13] ERCC5 is a typical housekeeping gene functioning in removal of DNA damage. The localization of the ERCC3 gene recently analyzed by FISH was the R-positive 2q21 band.[14] These facts support the assignment of the ERCC5 gene to the R-positive q32.3 band.

As for the assignments of relevant genes, ERCC1 and 2 (19q13.3), and ERCC4 (16p13.11-p13.3) have been reported based on analyses of somatic cell hybrids, and isotopic *in situ* hybridization.[15] With the modern FISH system, ERCC3 and ERCC6 have been recently localized to 2q21 and 10q11-q21, respectively,[14,16] confirming the previous assignments.

The ERCC5 gene has been assigned to chromosome 13 by the analysis of somatic cell hybrids,[17] and to band 13q22-q34 by a hybrid-cell deletion panel.[18–20] Very recently, Warburton *et al.* have reported the localization of this ERCC5 gene to 13q32-q33 by FISH.[21] The present result by direct R-banding FISH is in agreement with these assignments and the gene was localized to a narrower region.

[1] To whom correspondence should be addressed.

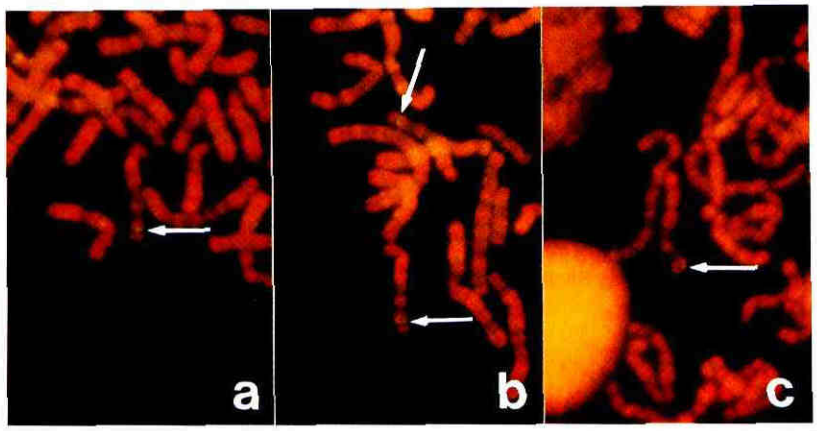

Fig. 1. a–c. Partial R-banded (pro)-metaphases after FISH with the biotinylated ERCC5 gene (a and b; 500-band stage, c; 700-band stage). Arrows indicate signals on 13q32.3-q33.1.

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