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BRIEF REPORT — GENE MAPPING

Naohiko Seki · Akiko Hayashi · Masumi Abe
Ryoko Araki · Akira Fujimori · Ryutaro Fukumura
Atsushi Hattori · Sumie Kozuma · Miki Ohhira
Tada-aki Hori · Toshiyuki Saito

Chromosomal assignment of the gene for human DNA-PKcs interacting protein (KIP) on chromosome 15q25.3–q26.1 by somatic hybrid analysis and fluorescence *in situ* hybridization

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Abstract We report the chromosomal location of the gene for DNA-PKcs interacting protein KIP. Based on fluorescence *in situ* hybridization and polymerase chain reaction (PCR)-based analyses with both a human/rodent monochromosomal hybrid cell panel and a radiation hybrid mapping panel, this gene was mapped to q25.3–q26.1 region on chromosome 15.

Key words DNA-PKcs · KIP · Chromosome 15q25.3–q26.1 · FISH · Radiation hybrid mapping

DNA-dependent protein kinase (DNA-PK) has a role in the repair of double-strand DNA breaks and in the related process of V(D)J recombination during lymphoid development (Jackson and Jeggo 1995; Jeggo et al. 1995). DNA-PK is composed of a large catalytic subunit of approximately 470kDa (DNA-PKcs) and DNA-binding protein, Ku (Anderson and Carter 1996; Jackson 1996; Lees-Miller 1996). The catalytic subunit, DNA-PKcs, belongs to the phosphatidylinositol-3 kinase family of proteins (Hartley et al. 1995) that includes ATM (Savitsky et al. 1995), FRAP (Brown et al. 1994; Sabatini et al. 1994) and ATR (Cimprich et al. 1996; Enoch and Norbury 1995). Recently, a novel DNA-PKcs interacting protein, KIP (kinase interacting protein), was isolated by the use of a two-hybrid analysis which showed a significant homology to calcineurin B (Wu and Lieber 1997). The possibility is discussed that the protein might regulate the phosphorylation pathway induced in DNA end joining (Wu and Lieber 1997).

Chromosomal assignment of the human *KIP* gene was made by polymerase chain reaction (PCR) analysis of a human/rodent somatic cell hybrid panel and a radiation hybrid panel. The PCR primer sets were designed for the 3′ untranslated region of the gene. The specific amplified human PCR product was detected only from the hybrid containing human chromosome 15 (Fig. 1a). We performed further mapping analysis using a radiation hybrid panel (Genebridge 4, Research Genetics, Huntsville, AL, USA) with the same primers used in the assay for the human/rodent somatic cell hybrid panel. Statistical analysis of the radiation hybrid mapping data was carried out using the RHMAPPER software package (http://www genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl). The data vector for the *KIP* gene was 0000010100 0100011100 0100000110 0100100100 0110001000 1100000100 0000110000 1011000000 1010011010 101, and the consequent report indicated the gene was mapped between markers GCT14H07 and WI-6947, both of which have been cytogenetically mapped to 15q25–q26. The position of the gene is 1.31 cR distal from GCT14H07.

To confirm the PCR-based chromosome mapping by an independent approach, we performed R-banding fluorescence *in situ* hybridization (FISH) using the P1 phase DNA as described previously (Saito et al. 1995, 1997; Seki et al. 1997). The P1 clone was isolated by the method described previously (Ohira et al. 1997). Clear doublet signals were consistently demonstrated at the q25.3–q26.1 position of chromosome 15. The typical pattern of the FISH experiment is represented in Fig. 1b,c. Thus, the gene was judged to map on 15q25.3–q26.1.

Our precise chromosomal positioning data of such the gene will contribute toward ongoing positional candidate approaches for the disease genes linked to this genomic locus.

N. Seki · A. Hayashi · A. Hattori · S. Kozuma · T. Hori · T. Saito (✉)
Genome Research Group, National Institute of Radiological Sciences, Anagawa, 4-9-1, Inage-ku, Chiba 263-8555, Japan
Tel. 81-43-206-3135; Fax 81-43-251-9818
e-mail: t_saito@nirs.go.jp

M. Abe · R. Araki · A. Fujimori · R. Fukumura
Division of Biology and Oncology, National Institute of Radiological Sciences, Anagawa, Inage-ku, Chiba, Japan

M. Ohhira
Division of Biochemistry, Chiba Cancer Center Research Institute, Nitona, Chuoh-ku, Chiba Japan

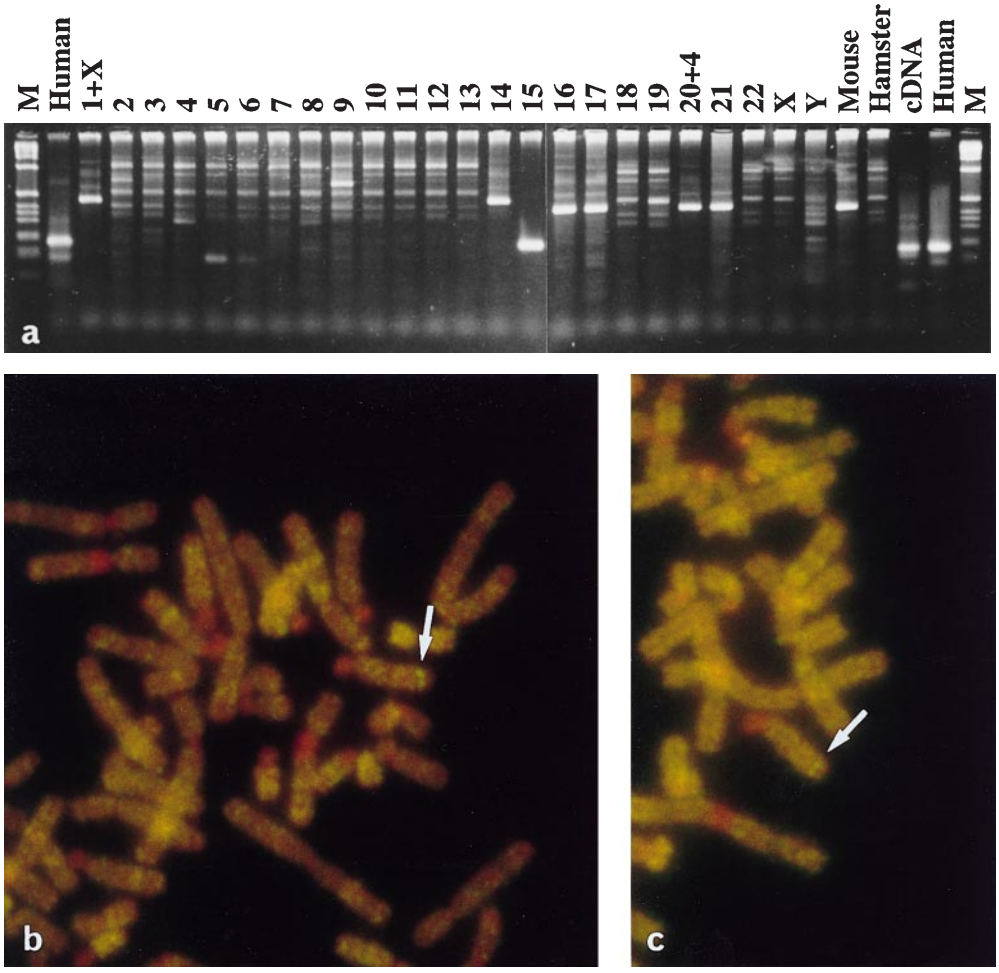

Fig. 1a–c Chromosome mapping of the *KIP* gene. **a** PCR analysis of human/rodent hybrid cell panel. A PCR screening of a human/rodent somatic cell hybrid panel was performed to map the KIP gene to human chromosome 15. DNA of the human/rodent somatic cell hybrid panel was purchased from the National Institute of General Medicine Service, Coriell Cell Repositories (Palo Alto, CA, USA). Human, mouse, and hamster genomic DNAs were also included as controls in the assay. Primers used for PCR amplification correspond to (5′-TGACAGCAGCCCCAGCGTGTGTCCT-3′) and (5′-GACAACAGCAGTGAGGAGAGGCCC-3′) (PCR product size: 260 bp). PCR was carried out in a final volume of 10 μl containing 1 × LA-PCR buffer (Takara, Kyoto, Japan), 2 μM of each primer, 200 μM of each dNTP, 50 ng of template DNA, and 0.01 unit of LA-Taq DNA polymerase (Takara). Temperature and time profiles were 30 cycles of 95°C for 20 s and 66°C for 1 min. Numbers on the top of each lane indicate the human chromosome contained in each somatic cell hybrid. **b,c** Fluorescence *in situ* hybridization (FISH) of the *KIP* gene. FISH was carried out using a bioinylated hybridization probe made from P1 phage clone harboring the KIP gene. *Arrows* indicate the hybridization signals on human chromosome 15q25.3–q26.1. The metaphase spreads were photographed with a Nikon B-2A filter.

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