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Short Communication

ASSIGNMENT OF THE HUMAN GENE FOR KBF2/RBP-Jk TO CHROMOSOME 9p12-13 AND 9q13 BY FLUORESCENCE *IN SITU* HYBRIDIZATION

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Summary The transcription factor KBF2 has been characterized as a factor that binds to the NFkB site of mouse major histocompatibility complex (MHC) class I genes and its amino acid sequence has been shown to be identical to those of members of the recombination signal-sequence binding protein (RBP-Jk) family. Previous studies by Amakawa *et al.* (Genomics **17**, 306-315, 1993) demonstrated that the functional gene is localized at human chromosome 3q25. However, in the present study we showed by *in situ* hybridization with the functional KBF2/RBPJk cosmid clone that the gene is localized at 9p12-13 and 9q13, namely, at the same loci as pseudogenes that were reported previously (Zhang *et al.*, Jpn J Human Genet **39**, 391-401, 1994).

Key Words KBF2, RBP-Jk, cosmid, *in situ* hybridization

The major histocompatibility complex (MHC) class I antigens specified by MHC class I genes play a key role in a number of immunological processes, particularly in the recognition of foreign antigens by cytotoxic T cells and natural killer cells of the host (Zinkernagel and Doherty, 1979). In the mouse, the promoters of the K^b and L^d class I MHC genes have been used as models in studies of transcription (Israël *et al.*, 1989). A factor designated KBF2, which can bind as a monomer to conserved sequence element (TGGGGATTCCCCA) located 165 bp upstream of the initiation site of the H-$2K^b$ class I gene. Interestingly, the same

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control element is also recognized by NF-kB (Nuclear factor kappa B; Singh *et al.*, 1988) or KBF-1 (Israël *et al.*, 1989). The KBF2 protein has been purified and the corresponding cDNA has been isolated (Brou *et al.*, 1994). A search of databases revealed perfect homology with proteins that belong to the recombination signal-sequence binding protein (RBP-Jk) family. RBP-Jk was originally purified on the basis of its specific binding to the recombination signal of the Jk immunoglobulin gene (Hamaguchi *et al.*, 1989; Matsunami *et al.*, 1989). Recent studies showed that the amino acid sequences of RBP-Jk and the Su (H) proteins of *Drosophila* are 82% homologous and 94% similar over most of their length, strongly suggesting that RBP-Jk acts as a transcriptional factor and interacts directly with the Notch receptor that is involved in cell lineage commitment (Furukawa *et al.*, 1991). These observations also imply the existence of a uniquely simple mode of communication between the surface receptor and the nucleus (Honjo, 1996). KBF2/RBP-Jk makes direct contact with the EBNA2 protein of Epstein-Barr virus (EBV) and recruits EBNA2 for binding to its cognate DNA sequence. Thus, KBF2/RBP-Jk seems likely to mediate the EBNA2-dependent transactivation of both cellular and viral genes (Zimble *et al.*, 1994). Since the location of the functional gene for RBP-Jk was reported at human chromosome 3q25 (Amakawa *et al.*, 1993), while the location was chromosome 9p13 or 9q13 in the case of pseudogenes (Zhang *et al.*, 1994), we were interested in determining the site(s) of other functional gene(s) for KBF2/RBP-Jk. We found, by FISH (fluorescence *in situ* hybridization), that the functional gene for KBF2/RBP-Jk was located at chromosome 9p12-13 and 9q13.

In situ chromosome hybridization was carried out by the standard method, with some modifications, as follows. Chromosome preparations for FISH were made from cultures of peripheral lymphocytes from healthy donors after synchronization with bromodeoxyuridine (BrdU) (10^{-7} M, for 17 hr) that was followed by incorporation of BrdU (30 μg/ml, 6 hr) (Hori *et al.*, 1990). We isolated a cosmid clone that corresponded to human KBF2/RBP-Jk from a human peripheral lymphocyte pWE15 (Toyobo, Tokyo) cosmid library. A total of 4×10^6 cosmid clones was screened by colony hybridization with a probe of 1.7-kb *Eco*R1 digested DNA fragment of KBF2/RBP-Jk cDNA. Then, 13 positive clones were obtained. We confirmed that one of cosmid clones (no. 8) was the correct functional clone corresponded to a PCR-3 clone by nucleotide sequencing as described by Tang *et al.* (1993) (data not shown; Amakawa *et al.*, 1993; Brou *et al.*, 1994; Hamaguchi *et al.*, 1989; Matsunami *et al.*, 1989). A cosmid clone (no. 8) for KBF2/RBP-Jk (40 kb) was labeled with biotin-16-dUTP by nick translation by the standard method (Ozawa *et al.*, 1992). To eliminate the cross hybridization by repetitive sequences in the probe, a 50-fold excess of sonicated human placental DNA was added to the probe. The prehybridization procedures for detection of fluorescent signals were essentially the same as described previously (Hori *et al.*, 1990). The resulting hybridization signals on chromosomes that had been counter-

stained with propidium iodide (PI) were observed under fluorescent microscope (FXA Nikon, Tokyo) with a B-2A filter for signals and a UV-2A filter for the G-banding pattern. Ektachrome 100 film (Eastman Kodak Company, Rochester, NY) was used for microphotography.

Fluorescence hybridization signals were detected exclusively on chromosomes 9. A total of 45 chromosomes 9 from preparations of 45 human peripheral lymphocytes was observed, all of which showed a doublet fluorescent spot in the chromosome 9p12-13 region, and 40 (89%) of the 45 chromosomes 9 had another somewhat weaker but distinct single or double signals on band 9q13 (Fig. 1). Amakawa *et al.* (1993) reported that the functional human gene for KBF2/RBP-Jk was assigned to chromosome 3q25 and two pseudogenes IGKJRBP 1 and IGKJRBP 2 were mapped to 9p13 and 9q13, respectively. We also reported the similar intense signals of KBF2/RBP-Jk pseudogenes on two sites of chromosome 9p13 and 9q13 (Zhang *et al.*, 1994). Thus, more than four or at least four different genomic pseudogenes of humen KBF2/RBP-Jk may be present in human chromosome. Surprisingly, we also found that the functional gene for human KBF2/RBP-Jk was assigned to both regions such as chromosome 9p12-13 and 9q13. However no fluorescent signals were detected on chromosome 3. Therefore, our results are inconsistent with those of Amakawa *et al.* (1993). We do not know this discrepancy why we found no fluorescent signals on human chromosome 3. We

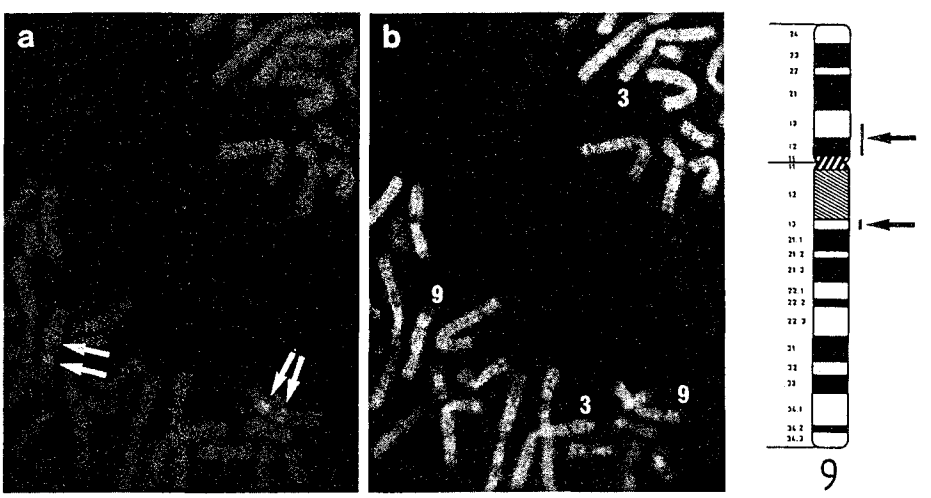

Fig. 1. FISH mapping with a genomic KBF2/RBP-Jk clone, no. 8, on human chromosomes. a: Fluorescent signals (arrows) on propidium iodide-stained chromosomes, as detected with the B-2A filter (Nikon). b: G-banding patterns on the same chromosomes as shown in a, visualized with the UV-2A filter (Nikon). Numerals indicate chromosomes 3 and 9. Right: ideogram of chromosome 9 illustrated the distribution of hybridization signals (arrows: 9p12-13 and 9q13).

have reexamined this result carefully by fluorescence *in situ* hybridization using the restriction DNA fragments derived from cosmid clone no. 8, which were excluded from the positive DNA fragments after southern hybridization with KBF2/RBP-Jk cDNA as a probe. In that case, the fluorescent signals were also detected on chromosome 9p12-13 and 9q13 (data not shown). Moreover, we obtained similar fluorescent signals on both loci of chromosome 9p12-13 and 9q13 using a cDNA clone of KBF2/RBP-Jk (data not shown). Thus, under our experimental conditions, we did not detect the fluorescent spots of KBF2/RBP-Jk in human chromosome 3q25. Furthermore, we were not able to separate the fluorescent signals on each loci of human chromosome 9. Thus, we can't rule out a possibility that the similar functional gene encoding KBF2/RBP-Jk is present in two discrete loci such as 9p12-13 and 9q13. Identification of the yeast artificial chromosome recombinant clone for KBF2/RBP-Jk will provide the further insight to discriminate both loci on human chromosome 9. However, at least, under our experimental conditions, the present results of fluorescence *in situ* hybridization using cosmid clone no. 8 demonstrate that the human functional KBF2/RBP-Jk gene is located at the same loci of 9p13 and 9q13 on which its pseudogenes located.

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Note added in proof Recent paper (Minoguchi *et al.*, Mol Cell Biol **17**: 2679-2687, 1997) described the RBP-L transcription factor related in RBP-Jk.