

Localization of the Human I- κ B kinase- β (IKBKB) to Chromosome 8p11.2 by Fluorescence *in Situ* Hybridization and Radiation Hybrid Mapping

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Received March 11, 1998; accepted June 9, 1998

Functional gene description: The transcription factor NF- κ B controls gene expression in a wide range of host-defense and immune responses, including T-cell-mediated and inflammatory reactions (1). In endothelial cells, it governs the inducible expression of genes encoding cell adhesion molecules, chemokines, and factors of the coagulation system, thus controlling distinct steps of adhesion, activation, and transmigration of cells of the immune system across the endothelial monolayer. Upon stimulation, NF- κ B is liberated from an inactive cytoplasmic complex by the inducible phosphorylation of its inhibitor I κ B α on two serine residues, S32 and S36, and subsequent degradation via the ubiquitin-proteasome pathway. Two kinases that phosphorylate I κ B α have been identified, IKK-1/IKK- α /CHUK and IKK-2/IKK- β /IKBKB, that are composed of helix-loop-helix, leucine zipper, and kinase domains (3, 5). We report here the localization of IKBKB to chromosome 8p11.2.

Name of clone or DNA source: IKBKB5 was used.

Description of clone or DNA: PAC clones were obtained by screening a human PAC library from male white blood cells in the vector pADsacBII (Genome Systems, St. Louis, MO) with a 1.8-kb IKBKB-specific cDNA probe containing the entire coding region (5). The clone address of IKBKB5 is PACH-70d13.

Method used to validate gene identity: Using IKBKB-specific primers (IKBKB/3, 5'-TACAGGAGACTAAGGGAA-3', and IKBKB/10, 5'-TCATCCTCATTATTAAGCT-3'), a 490-bp PCR fragment was amplified from clone IKBKB5. Upon sequencing, this fragment contained the correct

The authors thank M. Raidl for excellent technical assistance. This work was supported by Austrian Science Foundation Grant SFB 005-12 (to R.M.).

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IKBKB sequence including two introns of 108 and 193 bp at positions 1738 and 1838, respectively, of the published sequence (GenBank Accession No. AF029684). The same fragment was obtained when total human genomic DNA was used as template.

Flanking markers used: No flanking markers were used.

Methods of mapping: DNA from IKBKB5 was labeled with biotin-16-dUTP by nick-translation (Boehringer Mannheim, Germany), and fluorescence *in situ* hybridization (FISH) was performed as described (2). The hybridization product was visualized using a sandwich technique consisting of mouse anti-biotin and TRITC-conjugated rabbit anti-mouse antibodies (Dakopatts, Denmark). Subsequent DAPI banding was performed to determine the exact position of the gene in the human genome. Examination of 22 metaphase spreads showed in all cells strong paired signals on at least one identifiable copy of chromosome 8, localizing to 8p11.2 (Fig. 1). Radiation hybrid mapping using the GeneBridge 4 radiation hybrid mapping panel (Research Genetics, Huntsville, AL) was performed by PCR using the primers described above. The results of the PCR screening were submitted to the Whitehead Institute, Center for Genome Research (<http://www.genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl>).

Results: The gene mapped to 8p11.2, at 11.99 cR below CHLC.GAAT2F03 (LOD > 3.0), between SGC31558 and D8S268.

Additional comments: 8p11.2 has been associated with a number of T cell and myeloproliferative disorders, similar to 10q24-q25, the region to which IKK-1 has been localized (4).

Homologies: Human IKK-1 shares 52% amino acid identity with IKBKB and has been localized to chromosome 10q24-q25.

References

- Baeuerle, P. (1998). Pro-inflammatory signaling: Last pieces in the NF- κ B puzzle? *Curr. Biol.* **8**: R19-R22.
- Lichter, P., Chang Tang, C. J., Call, K., Hermanson, G., Evans, G. A., Housman, D., and Ward, D. C. (1990). High-resolution mapping of human chromosome 11 by *in situ* hybridization with cosmid clones. *Science* **247**: 64-69.
- Mercurio, F., Zhu, H., Murray, B. W., Shevchenko, A., Bennet, B. L., Li, J. W., Young, D. B., Barbosa, M., Mann, M., Manning, A., and Rao, A. (1997). IKK-1 and IKK-2: Cytokine-activated I κ B kinases essential for NF- κ B activation. *Science* **278**: 860-866.
- Mock, B. A., Connelly, M. A., McBride, O. W., Kozak, C. A., and Marcu, K. B. (1995). CHUK, a conserved helix-loop-helix ubiquitous kinase, maps to human chromosome 10 and mouse chromosome 19. *Genomics* **27**: 348-351.
- Woronicz, J. D., Gao, X., Cao, Z., Rothe, M., and Goeddel, D. V. (1997). I κ B kinase- β : NF- κ B activation and complex formation with I κ B kinase- α and NIK. *Science* **278**: 866-869.