

## Localization of the Gene Encoding R $\kappa$ B (*NFRKB*), A Tissue-Specific DNA Binding Protein, to Chromosome 11q24-q25

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Although NF (nuclear factor)- $\kappa$ B binds *in vitro* to several of the  $\kappa$ B regulatory elements found in cellular and viral genes, another DNA binding protein, R $\kappa$ B, also binds to a related variant of the  $\kappa$ B site that regulates interleukin-2 receptor  $\alpha$ -chain gene expression, a critical event in T cell activation. Southern blot analysis of a human-mouse somatic cell hybrid panel and *in situ* hybridization using a fluorescent genomic R $\kappa$ B probe have allowed assignment of the R $\kappa$ B gene (*NFRKB*) to 11q24-q25. The *NFRKB* locus is in close proximity to the chromosomal breakpoint implicated in Ewing sarcoma, but it does not appear to span this region. Nonetheless, *NFRKB* may be particularly useful as the most telomeric marker thus far assigned to 11q. © 1992

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### INTRODUCTION

Cellular proteins that bind specifically to *cis*-acting regulatory sequences can modulate gene expression by enhancing or repressing transcriptional initiation. Highly conserved elements may be common to the regulatory regions of several genes, and these elements may participate in both coordinate regulation and differential regulation of gene expression. One such conserved regulatory element is the 11-bp  $\kappa$ B site found in the immunoglobulin (Ig) light chain enhancer (Sen and Baltimore, 1986), human immunodeficiency viruses 1 and 2 (HIV-1, HIV-2) (Nabel and Baltimore, 1987; Markovitz *et al.*, 1990), and several cellular and viral genes (Leonardo and Baltimore, 1989). Considerable structural and functional heterogeneity of  $\kappa$ B-binding proteins has been demonstrated (Kieran *et al.*, 1990; Ghosh *et al.*, 1990; Nolan *et al.*, 1991; Baldwin *et al.*, 1990; Fan and Maniatis, 1990; Perkins *et al.*, 1992), including heterogeneity in the components of NF- $\kappa$ B, the nuclear protein complex that binds to the Ig/HIV  $\kappa$ B site (Schmid *et al.*, 1991).

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A  $\kappa$ B site has been identified in the enhancer region of the interleukin-2 receptor  $\alpha$ -chain gene (IL-2R $\alpha$ ), differing by 2 of 11 bp from the Ig/HIV  $\kappa$ B site (Leung and Nabel, 1988; Bohnlein *et al.*, 1988; Ruben *et al.*, 1988). Expression of the inducible IL-2R  $\alpha$ -chain in response to stimulation by antigen or mitogen produces high-affinity IL-2 receptors on the cell surface. These receptors mediate the physiologic interleukin-2 (IL-2) response and participate in an autostimulatory IL-2 circuit that mediates clonal expansion of activated T cells (for review, see Waldmann, 1989). IL-2R $\alpha$  expression is regulated primarily at the transcriptional level, in part by a positive regulatory region between nucleotides -299 and -243 (Lin *et al.*, 1990) that contains the variant IL-2R $\alpha$   $\kappa$ B site (Leung and Nabel, 1988). Despite the ability of IL-2R $\alpha$   $\kappa$ B to bind NF- $\kappa$ B, functional assays can distinguish the two related sites on the basis of their responses to phorbol esters and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) (Leung and Nabel, 1988; Cross *et al.*, 1989; Freimuth *et al.*, 1989; Adams *et al.*, 1991). In addition, we have identified a specific binding activity in nuclear extracts that preferentially recognizes the IL-2R $\alpha$   $\kappa$ B site (Adams *et al.*, 1991). These results suggested that a protein distinct from NF- $\kappa$ B recognizes the  $\kappa$ B-like sequence in the IL-2R $\alpha$  enhancer.

A cDNA encoding a nuclear factor that preferentially binds to the IL-2R $\alpha$   $\kappa$ B site was isolated by probing a  $\lambda$ gt11 expression library with radiolabeled multimerized IL-2R $\alpha$   $\kappa$ B sites (Adams *et al.*, 1991). This factor, designated R $\kappa$ B, is expressed preferentially in lymphoid cells of T and B lineages in brain and in testis (Adams *et al.*, 1991). R $\kappa$ B exhibits no similarity to previously cloned  $\kappa$ B-binding proteins and is unrelated to a known class of DNA-binding proteins. It is possible that R $\kappa$ B may play a role in directing tissue-specific expression through the conserved  $\kappa$ B element. These mapping studies were undertaken to define the chromosomal location of the R $\kappa$ B gene (HGM symbol *NFRKB*).

### MATERIALS AND METHODS

*Isolation of human genomic R $\kappa$ B clones.* A human genomic HBP-ALL library in Charon 30 (Rimm *et al.*, 1980) (kindly provided by Dr.

Jeffrey Leiden) was screened at high stringency with a probe containing RκB cDNA sequence from nucleotide 2703 to 2976 (*EcoRI/SphI* fragment) (Adams *et al.*, 1991) labeled with <sup>32</sup>P by random hexamer-primed synthesis (Feinberg and Vogelstein, 1983). Three independent positive clones were isolated from screening approximately 700,000 phage plaques. DNA from plaque-purified positive genomic clones was characterized by restriction mapping. *EcoRI* and *BamHI* fragments were subcloned into the Bluescript plasmid (Stratagene) for partial sequencing.

**Isolation of human cosmid clones.** Cosmid clones containing the gene encoding RκB were isolated from an arrayed chromosome 11q-specific cosmid library that has been previously described (Evans and Lewis, 1989). This library is archived as individual bacterial cultures in 96-well microtiter plates. A 403-bp fragment from a human genomic RκB clone, corresponding to cDNA sequences from nt 4470 to 4472, was labeled with <sup>32</sup>P by random hexamer-primed synthesis (Feinberg and Vogelstein, 1983) and hybridized to replicas of the arrayed clone libraries on nylon-based filter membranes. Cosmid clones 1B11 and 8H6 were identified as containing RκB coding sequences by hybridization and by analysis with specific PCR primer sets derived from human RκB sequences from nt 4406 to 4435 (sense) and from nt 4852 to 4881 (antisense). Additional characterization by Southern blot analysis confirmed the identity of the clones.

**Somatic cell hybrids.** DNA from the NIGMS Human Genetic Mutant Cell Repository human/rodent somatic cell hybrid mapping panel No. 1 (Coriell Institute, Camden, NJ) was digested with *EcoRI* for Southern blot analysis with the 403-bp human RκB genomic probe described above. Hybridization was concordant with the presence of human chromosome 11, although the highly conserved RκB sequences of the human probe also hybridized with rodent sequences. Chromosomal location was subsequently confirmed in this panel and in a second somatic cell hybrid panel (previously described in Grissmer *et al.*, 1990) by PCR analysis with the RκB-specific PCR primer set described above. The primers were derived from human RκB sequences corresponding to nt 4406 to 4435 (sense primer) and nt 4852 to 4881 (antisense primer); PCR of rodent parent DNA with these primers was negative. Primer annealing and PCR extension phases were carried out at 72°C.

**Fluorescence in situ hybridization (FISH).** An *EcoRI* fragment of approximately 9.5 kb from RκB genomic clone 11a was biotin-labeled by nick-translation (Bio-Nick Kit, BRL, Gaithersburg, MD) for use as a probe in FISH. Metaphase chromosomes were prepared from peripheral blood lymphocytes by mitotic blockage. FISH was carried out as previously described (Pinkel *et al.*, 1986) with DAPI counterstaining.

Cosmid clones 1B11 and 8H6 were utilized for fluorescence *in situ* hybridization as previously described (Lichter *et al.*, 1990; Selleri *et al.*, 1991). Cosmid DNA was labeled by random hexamer-primed synthesis with biotinylated dCTP and dUTP, and hybridization was carried out to human metaphase chromosomes prepared from a human fibroblast cell line. Hybridization was detected with FITC-labeled avidin; a Bio-Rad MRC600 confocal microscope was used for imaging. Chromosome position was determined by band location, as well as fractional chromosome length (Lichter *et al.*, 1990).

## RESULTS

### *Chromosomal Localization Using Somatic Cell Hybrid Panels*

RκB sequences were initially localized to human chromosome 11 by using a set of somatic cell hybrids carrying various human chromosomes. Southern blot analysis was carried out with a probe containing a 403-bp fragment of the human genomic clone. For confirmation, PCR analysis of the set of somatic cell hybrids was carried out using RκB-specific PCR primers. Complete

concordance was found between human chromosome 11 and the presence of RκB sequences (Table 1).

### *Chromosomal Localization of RκB by Fluorescence in Situ Hybridization*

Further specification of RκB position on chromosome 11 was defined by FISH analysis using a 9.5-kb *EcoRI* fragment from RκB genomic clone 11A. Eighteen cells were examined, in which fluorescence signals were observed on 49/72 chromatids. All 49 of the specific hybridization signals localized to band 11q24–q25 (Fig. 1). Cosmid clones 1B11 and 8H6 were also used for FISH analysis. Thirty metaphases were examined for each probe and an FLpter location of 0.98 was determined. Comparison of the hybridization position with the visible banding pattern seen on some of the metaphases confirmed a band position of 11q24–q25. Two positions of hybridization were seen on each chromosome (one on each sister chromatid) in >90% of metaphases examined.

### *Chromosomal Localization with Regard to the Ewing Sarcoma (ES) Breakpoint*

To confirm the localization of RκB to chromosome 11q24 and to determine the orientation relative to the ES translocation breakpoint, an additional set of somatic cell hybrids was screened by PCR. Hybrid J1 (Kao *et al.*, 1977) contains the entire chromosome 11. Cell lines MC-1 and TG5D1-1 (Glaser *et al.*, 1989; Evans and Lewis, 1989) have been described previously (Glaser *et al.*, 1989); these contain breakpoints in 11q23. Cell lines 66/23 and 66/36 are somatic cell hybrids constructed in this laboratory (G. Hermanson and G. Evans, unpublished) that carry derivative chromosomes 11 and 22 isolated from a Ewings sarcoma cell line. Cell line 66/23 represents a somatic cell hybrid carrying the der11 from a ES cell line, representing the region from 11pter to the 11q23–q24 ES breakpoint, isolated in a CHO background. Cell line 66/36 carries the der22, representing the region from the 11q23–q24 ES breakpoint to 11qter from the same ES cell line isolated in a CHO background. Analysis of this set of hybrids (shown in Fig. 2A) demonstrates that 66/36, TG5D1-1, and J1 are positive and that 66/23 and MC-1 are negative for RκB. Analysis was also carried out using previously described cosmids flanking the ES breakpoint, denoted 5.8 and 23.2 (Selleri *et al.*, 1991). This maps the RκB gene distal to the t(11) breakpoint of ES in the vicinity of the 5.8 marker. Additionally, hybridization and PCR analysis established that the RκB gene was not contained in any of the recently isolated YAC and cosmid clones that span about 1.5 Mb surrounding the ES breakpoint (Selleri *et al.*, manuscript in preparation). Thus, RκB maps outside of the ES breakpoint region and telomeric to the 5.8 cosmid marker (Fig. 2B).

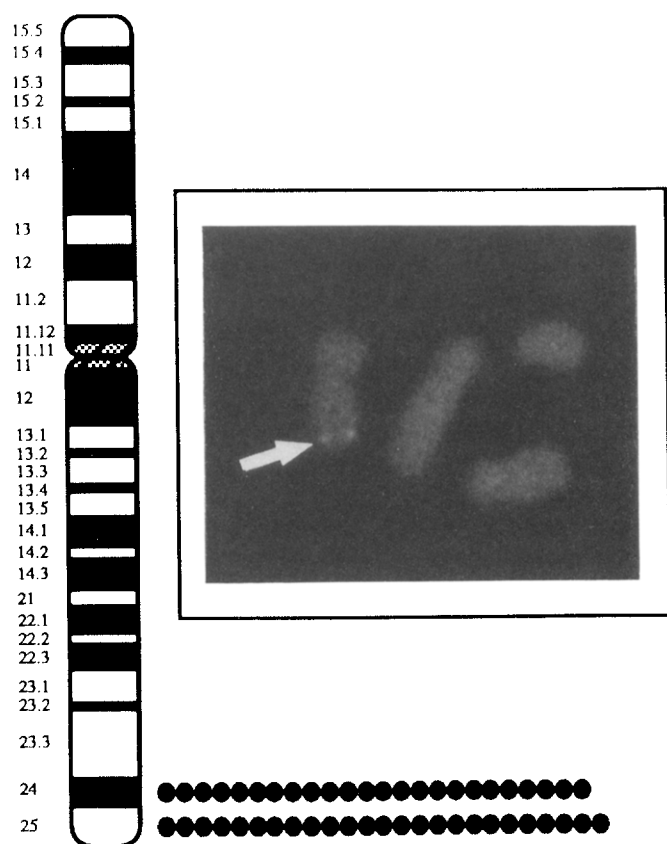
## DISCUSSION

Chromosomal location of a gene may suggest the biologic significance of its product, particularly where disruption of the gene by deletion or translocation is

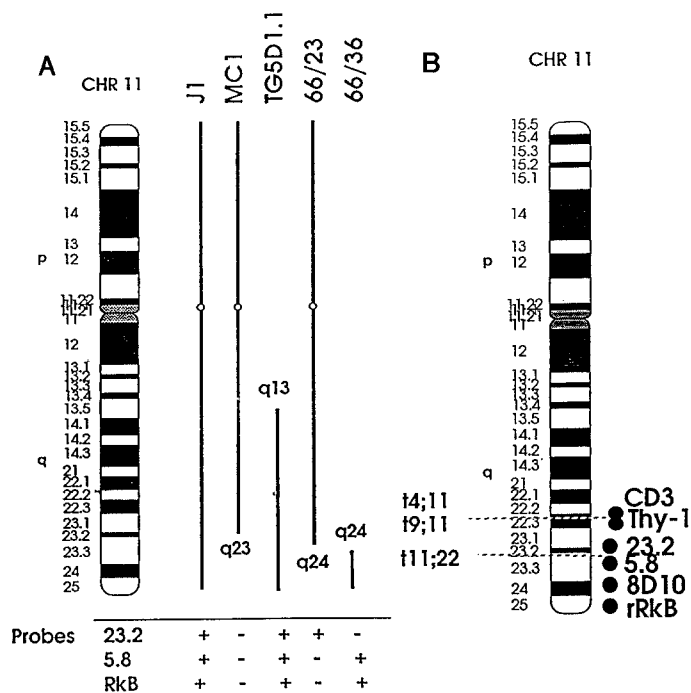


associated with identifiable clinical disease. Human chromosome 11 contains several "hot spots" of chromosomal rearrangement that are associated with solid tumors and leukemias, suggesting that chromosomal disruption in these regions may result in dysregulation of cell growth or differentiation. Because  $R\kappa B$  binds to the regulatory region of a growth factor receptor gene, we determined its chromosomal position and its relation to known disease-associated markers.

The  $R\kappa B$  locus, which we designate *NFKB*, is located on 11q24-q25; this places it in the proximity of markers for several known diseases. Currently, the most distal marker that has been mapped to chromosome 11 is a translocation breakpoint that occurs in virtually all cases of Ewing sarcoma and peripheral neuroepithelioma (Selleri *et al.*, 1991). High-resolution mapping of  $R\kappa B$  by fluorescence *in situ* hybridization with an ordered panel of cosmid clones containing previously identified DNA markers flanking the Ewing sarcoma breakpoint indicates that  $R\kappa B$  is telomeric to the translocation breakpoint. Thus, loss of  $R\kappa B$  function does not appear to be involved in the pathogenetic processes of Ewing sarcoma or peripheral neuroepithelioma. With the paucity of chromosomal markers in this region, however,  $R\kappa B$ 's telomeric position may prove especially useful in mapping other genes to 11qter.



**FIG. 1.** Localization of  $R\kappa B$  to chromosome 11q24-q25 by FISH. The idiogram of chromosome 11 summarizes the results of *in situ* hybridization with the  $R\kappa B$  genomic probe. Each dot indicates a FISH signal. The inset illustrates a representative chromosome 11 with hybridization to both chromatids at 11q24-q25.



**FIG. 2.** Idiogram of chromosome 11. (A) Summary of PCR screening of cell hybrids containing 11q23 or 11q23-q24 breakpoints, using  $R\kappa B$ -specific PCR primers. The portion of chromosome 11 carried in each cell hybrid is indicated by the solid bars. The presence or absence of specific  $R\kappa B$  sequences and sequences flanking the ES breakpoint is indicated by a + or a -, respectively. (B) Summary of the position of  $R\kappa B$  in relation to other markers on 11q.

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