SHORT COMMUNICATION

Chromosomal Localization of the Genes Encoding the p50/p105 Subunits of NF-κB (*NFKB2*) and the IκB/MAD-3 (*NFKBI*) Inhibitor of NF-κB to 4q24 and 14q13, Respectively

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The regulation of expression of a variety of genes involved in immune function, inflammation, and cellular growth control, as well as control of expression of certain viruses such as the human immunodeficiency virus (HIV), is dependent on the transcription factor NF-κB. In many cells, NF-κB is found in the cytoplasm where it is associated with an inhibitor protein known as IκB. Recently the genes encoding the p50 and p65 subunits of NF-κB, as well as one form of IκB/MAD-3 (NFKBI), have been cloned. As part of our goal to determine the chromosomal organization of members of the *REL/NFKB* family, as well as their inhibitors, we localized the NFKBp50/p105 (*NFKB2*) and IκB/MAD-3 (*NFKBI*) genes to human chromosome bands 4q24 and 14q13, respectively.

NF-κB is a transcription factor involved in the regulation of expression of numerous genes, including immunoglobulin κ, IL-2 receptor, and class I major histocompatability genes, as well as HIV gene expression [see (9) for review]. NF-κB has been characterized as a heterodimeric complex of two proteins, p50 and p65 (3). cDNAs encoding both the p50 and p65 subunits have been cloned, and the predicted proteins were shown to be related to the *REL* protooncogene product and to the product of the *dorsal* maternal effect gene of Drosophila (5, 8, 13, 16). Interestingly, the p50 DNA-binding subunit of NF-κB is apparently processed from a 105-kDa precursor protein that has six C-terminal repeats of the ankyrin/cdc10 consensus sequence (5, 8). Recently a novel subunit of NF-κB, p49 or LYT-10, has been identified and may be a spliced form of a larger protein, p100, that also contains ankyrin repeats (11, 17).

In many cells, NF-κB is found in the cytoplasm where it is associated with its inhibitor, IκB (1, 2). Recently a clone for one form of IκB has been identified (6) and has been shown to encode a protein with multiple repeats of the ankyrin homology. Interestingly, the oncogene *BCL3* also contains multiple copies of the ankyrin homology (14) and has been found to have IκB-like activity (7).

A human genomic library was screened with restriction fragments of cDNAs encoding NFKB2 and NFKBI. Several positive phage were identified from each screen and were purified as single isolates. Phage DNA was restriction mapped and probed with *NFKB2* or *NFKBI* cDNA fragments to identify positive genomic restriction fragments. Subsequently, regions of subcloned DNA were sequenced by the dideoxy method to demonstrate identity with the relevant cDNA clone.

To localize the *NFKBI* and *NFKB2* genes, we performed fluorescence *in situ* hybridization (FISH) of biotin-labeled probes to normal human metaphase chromosomes. All hybridizations were repeated twice and gave similar results. Human metaphase cells were prepared from phytohemagglutinin-stimulated peripheral blood lymphocytes. FISH was performed as described previously (15). Biotin-labeled probes were prepared by nick-translation using Bio–11-dUTP (Enzo Diagnostics, New York). Hybridization was detected with fluorescein-conjugated avidin (Vector Laboratories, Burlingame, CA), and chromosomes were identified by staining with 4,6-diamidino-2-phenylindole-dihydrochloride (DAPI).

Hybridization of the *NFKBI* probe resulted in specific labeling of chromosome 14 only (Figs. 1A–1C). Specific labeling of 14q13 was observed on two (6 cells), three (5 cells), or all four (14 cells) chromatids of the chromosome 14 homologues in 25 cells examined. In hybridizations of the *NFKB2* probe, we observed specific labeling of chromosome 4 only (Figs. 1D–1F). Specific labeling of 4q24 was observed on one (1 cell), two (9 cells), three (11 cells), or all four (4 cells) chromatids of the chromosome 4 homologues (25 cells examined). Thus, the *NFKBI* and *NFKB2* genes are localized to 14q13 and 4q24, respectively.

As described earlier, *NFKB2* is a member of the *NFKB/REL* family; NFKBI belongs to the family of NF-κB inhibitors. These families can be further divided into subfamilies on the basis of the presence (NFKBI,

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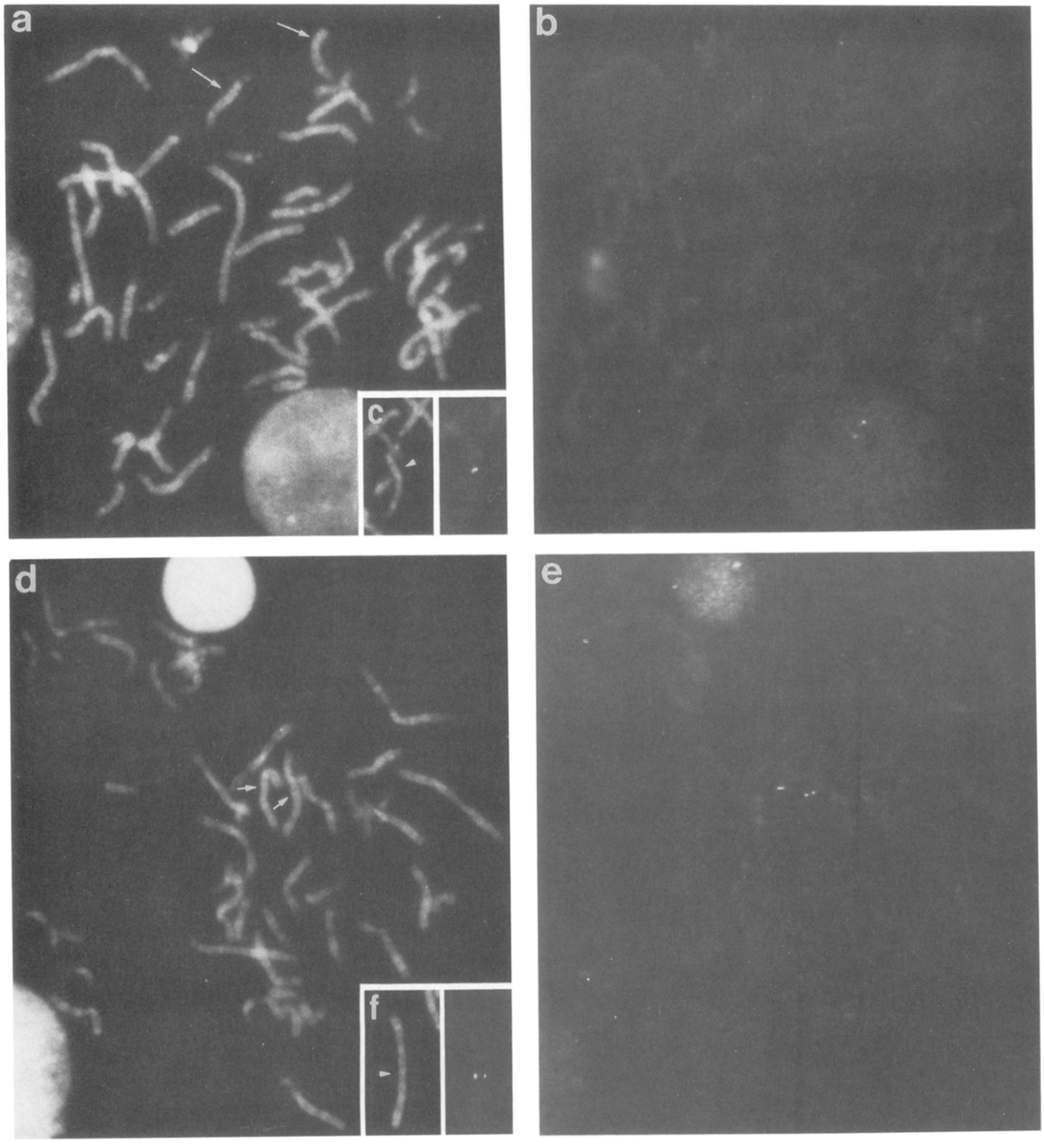

FIG. 1. *In situ* hybridization of biotin-labeled *NFKBI* (**a–c**) or *NFKB2* (**d–f**) probes to human metaphase cells from mitogen-stimulated lymphocytes. (**a, d**) Metaphase cells that are counterstained with DAPI; (**b, e**) the detection of the biotin-labeled probes with FITC-conjugated avidin. (**c, f**) Partial karyotypes of labeled chromosomes from additional metaphase cells. (**a–c**) Hybridization of the *NFKBI* probe; specific labeling was observed at 14q13 (arrows). (**d–f**) Hybridization of the *NFKB2* probe; specific labeling was observed at 4q24 (arrows).

14q13; NFKB2, 4q24; p49/LYT10/NFKB1, 10q24; BCL3, 19q13.1–q13.2) or the absence (REL, 2p12–p13; p65) of ankyrin repeats. Thus, although members of these families have functional and sequence similarities, the genes encoding these proteins are dispersed to different human chromosomes.

Experimental evidence obtained recently suggests that these proteins may be involved in growth control and, in some instances, in the proliferation of neoplastic cells. *BCL3* and *NFKB1* were initially identified as transforming genes in B cells and are located at the breakpoints of the t(14;19) and t(10;14) in B cell chronic lymphocytic leukemia and B cell lymphomas, respectively (11, 14). *REL* is the cellular homologue of the transforming gene v-*rel*; alterations of the *REL* locus have been identified recently in lymphomas (10). To

date, bands 14q13 and 4q24 have not been identified as being involved in recurring chromosomal abnormalities in human tumors. Although genes on chromosomes 4 and 14 have been implicated in cell growth (12, 18, 19), whether the *NFKBI* or *NFKB2* genes are involved in the pathogenesis of human tumors remains to be determined.

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