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Rearranged *NFKB-2* Genes in Lymphoid Neoplasms Code for Constitutively Active Nuclear Transactivators

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The *NFKB-2* gene codes for an NF-κB-related transcription factor containing *rel-polyG-ankyrin* domains. Chromosomal rearrangements of the *NFKB-2* locus have been found in various types of lymphoid neoplasms, suggesting that they may contribute to lymphomagenesis. Rearrangements cluster within the 3′-terminal *ankyrin*-encoding domain of the *NFKB-2* gene and lead to the production of C-terminally truncated proteins which, in some cases, are fused to heterologous protein domains. In order to determine the functional consequences of these alterations, we have analyzed the subcellular localization, DNA binding, and transcriptional activity of two representative tumor-associated mutants in which the *ankyrin* domain is either terminally truncated (NFKB-2p85) or truncated and joined to an out-of-frame immunoglobulin Cα domain (lyt-10Cα). Immunofluorescence studies performed on cells transfected with p85 or lyt-10Cα expression vectors showed that both the abnormal proteins were constitutively localized in the nucleus. Immunoprecipitation analysis of UV-cross-linked DNA-protein adducts showed that p85 can bind κB sites in its unprocessed form. Cotransfection of p85 or lyt-10Cα expression vectors with κB-driven reporter plasmids showed that both p85 and lyt-10Cα have retained the ability to mediate transcriptional activation via heterodimerization with Rel-Ap65 but have lost the transrepression activity associated with homodimeric DNA binding. Furthermore, both p85 and lyt-10Cα were capable of independent transactivation of κB-reporter genes and this activity could not be further stimulated by Bcl-3. These findings indicate that the tumor-associated NFKB-2 proteins can override the main mechanisms regulating NFKB-2 function and act as constitutive transactivators without interaction with Rel-Ap65 or Bcl-3. These abnormal proteins may contribute to lymphomagenesis by determining a constitutive activation of the NF-κB system and, in particular, of *NFKB-2* target genes.

The *NFKB-2* gene (previously named Lyt-10 [24], p50B/p97 [3], p55/p98 [20], and p49/p100 [30]) was independently identified via its involvement in a lymphoma-associated chromosomal translocation (24), its homology to *NFKB-1* (20, 30), and its inducible expression in activated T cells (reference 3; for reviews, see references 13, 19, and 32). The *NFKB-2* gene is expressed as a single 3.2-kb mRNA species and a 100-kDa primary translation product (p100) which, as NFKB-1 (5), contains an N-terminal DNA-binding *rel* domain, a poly(G) hinge, and a C-terminal *ankyrin* domain (7, 24). Analogous to NFKB-1, NFKB-2p100 represents a cytoplasmic precursor which, by proteolytic cleavage of the hinge region, is processed into an active molecule, p52, retaining the *rel* domain but losing the *ankyrin* domain (7, 21). NFKB-2p52 is found in the cytoplasm within complexes of Rel-Ap65 (26, 28, 29) and IκB (9) and can be found in the nucleus upon NF-κB activation (7, 21). Although NFKB-2p52 has no intrinsic transcriptional activity, it can stimulate transcription by heterodimerizing with Rel-Ap65 or inhibit it by occupying κB sites as inactive p52-p52 homodimers (4, 7). In addition, p52 can induce transcriptional activation if complexed with the IκB-type protein Bcl-3 (4, 12, 25, 35). Thus, NFKB-2 can regulate κB-mediated transcription either positively or negatively depending on the relative concentration of its heterodimeric partners in the nucleus.

Increasing evidence suggests that structural alterations of the *NFKB-2* gene may be associated with the pathogenesis of certain lymphoid neoplasms. The *NFKB-2* gene was originally cloned by virtue of its juxtaposition to the immunoglobulin heavy chain locus by a lymphoma-associated chromosomal translocation (24). Subsequent screenings of a panel of cases representative of the major subtypes of human lymphoid malignancies indicated that *NFKB-2* rearrangements can be found at low frequency (1 to 2% of cases) in B-cell lymphoma, chronic lymphocytic leukemia, and multiple myeloma and more commonly (14%) in neoplasms derived from mature T cells, such as mycosis fungoides and Sezary syndrome (6, 10, 22). Initial characterizations of these altered *NFKB-2* alleles have indicated that the rearrangements cluster within the 3′ *ankyrin*-encoding domain of the *NFKB-2* gene and lead to production of abnormal proteins retaining the DNA-binding *rel* domain and variable portions of the *ankyrin* domain (10, 22, 24, 33, 36). In some cases, C-terminally truncated proteins are fused to heterologous domains (22, 24). In one tumor case, it has been demonstrated that an abnormal NFKB-2 protein has abnormal transcriptional regulatory properties (33, 36). These observations have led to the hypothesis that NFKB-2 alterations may play a role in tumorigenesis, although no precise information is available on the function of the rearranged *NFKB-2* genes and their protein products.

In order to address these questions, we have performed a detailed analysis of the functional properties of two representative tumor-associated NFKB-2 proteins. The results indicate that these proteins are altered in their subcellular localization, homodimeric-heterodimeric interactions, and transcriptional activity. These results suggest that these abnormal functions

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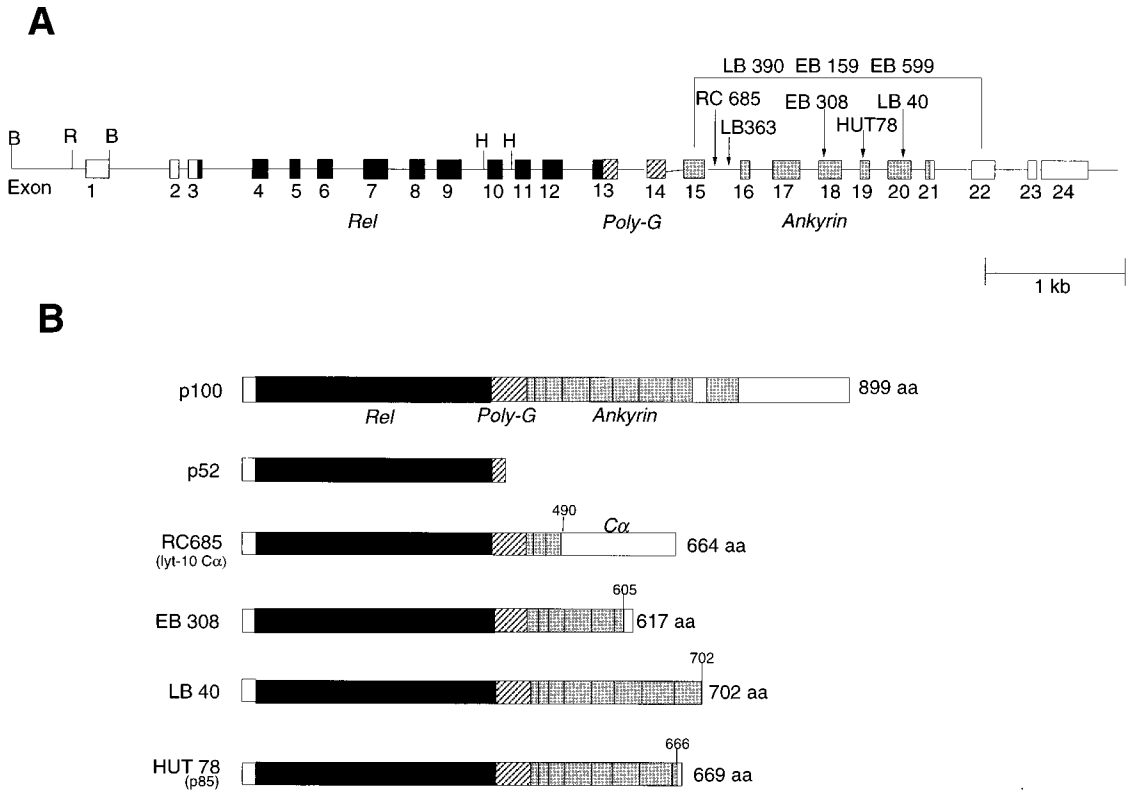

FIG. 1. Position of chromosomal breakpoints within the *NFKB-2* gene and schematic representation of normal and abnormal *NFKB-2* gene products. (A) Position of chromosomal breakpoints within the *NFKB-2* gene. The precise chromosomal breakpoints of cases RC 685, HUT-78, EB 308, LB40, and LB363 were previously reported (10, 22, 36). The breakpoints of cases EB 159, B 390, and EB 599 were estimated from Southern blot analysis with diagnostic genomic DNA probes (10). Coding exons are represented by filled boxes whereas noncoding exons are represented by empty boxes. B, *Bam*HI; R, *Eco*RI; H, *Hin*dIII. (B) Truncated *NFKB-2* genes code for C-terminally truncated proteins lacking variable portions of the *ankyrin* domain. The predicted number of amino acids (aa) of each protein is shown on the right. The number of amino acids derived from the normal *NFKB-2* sequence is also shown for each protein.

may contribute to lymphomagenesis by causing constitutive activation of the NF-κB system.

MATERIALS AND METHODS

Plasmid vectors. The cloning of the cDNAs encoding lyt-10Cα and NFKB-2p85 and the construction of the eukaryotic expression vectors expressing NFKB-2p52 (pMT2T-p52) or NFKB-2p85 (pMT2T-p85) have been previously described (7, 24, 36). To construct pMT2T-Bcl-3, the 1.4-kb *Eco*RI-*Bgl*II fragment from a Bcl-3 cDNA clone (27) was ligated onto *Eco*RI linkers and cloned into the *Eco*RI site of the pMT2T vector. To construct plasmid p52-Tc^r, an 800-bp *Eco*RV-*Nru*I fragment from the tetracycline resistance gene (Tc^r) of plasmid pBR322 was ligated onto *Pst*I linkers and fused in frame to the pMT2T-p52 vector after partial digestion of the vector with *Pst*I. The accuracy of the p52-Tc^r reading frame was verified by DNA sequencing. The reporter plasmid containing tandem repeats of the immunoglobulin kappa chain (IgK)/human immunodeficiency virus (HIV)-κB binding motif linked upstream to the minimal murine c-*fos* promoter and luciferase coding sequences was a gift from K. Saksela and D. Baltimore.

Cell lines and transfection. NTera-2 cells were maintained in Dulbecco's modified Eagle medium supplemented with 10% heat-inactivated fetal bovine serum. For transient transfection assays, cells were plated at 2×10^6/10-cm petri dish and then transfected by a modified calcium phosphate precipitation procedure (15). Luciferase activity was measured by a recommended procedure (Promega).

Electrophoretic mobility shift assay (EMSA). Nuclear extracts were prepared according to the method described by Dignam et al. (8). EMSAs and antibody-mediated supershift assays were performed as previously described (7), except that the gel was prepared in 1× TBE (89 mM Tris, 89 mM boric acid, 19 mM EDTA) and electrophoresed in 0.25× TBE at 200 V for 2 to 3 h.

Indirect immunofluorescence staining. Experimental details have been previously described (7).

UV cross-linking–immunoprecipitation assays. These procedures have been previously described (7). Briefly, a ^{32}P-labeled, bromodeoxyuridine-substituted oligonucleotide probe was incubated with nuclear extracts (4 μg) at 25°C for 25 min. The protein-DNA complexes were digested with DNase I-micrococcus nuclease and immunoprecipitated with an antiserum recognizing the N-terminal peptide of NFKB-2 (antiserum 8892 [7]).

RESULTS

***NFKB-2* genes rearranged within the *ankyrin*-encoding domain produce C-terminally truncated proteins in lymphoma.** Figure 1A provides a schematic representation of the *NFKB-2* genomic locus showing the position of the chromosomal breakpoints in several lymphoma cases as mapped by Southern blot and/or cloning-sequencing (22, 24, 36). These data show that the breakpoints cluster within the 3′ coding portion of the gene corresponding to the *ankyrin* domain. Previous studies have also shown that the sequences 3′ to the breakpoint are substituted by sequences from different chromosomes in different cases (10, 22, 24). Figure 1B provides a schematic representation of the proteins encoded by the rearranged alleles in various cases. In two of four cases, the *NFKB-2* reading frame was found to be interrupted by stop codons at (LB40) or in very close proximity to (9 bp [HUT-78]) the breakpoint, leading to the production of proteins lacking variable portions of the C-terminal portion of the *ankyrin* domain. In the other two cases (RC 685 and EB 308), the reading frame remained open across the breakpoint because of splicing to off-frame immunoglobulin heavy chain Cα coding exons (RC 685) or an unknown locus (EB 308). As a result, fusion proteins produced from these abnormal transcripts will have heterologous tails of

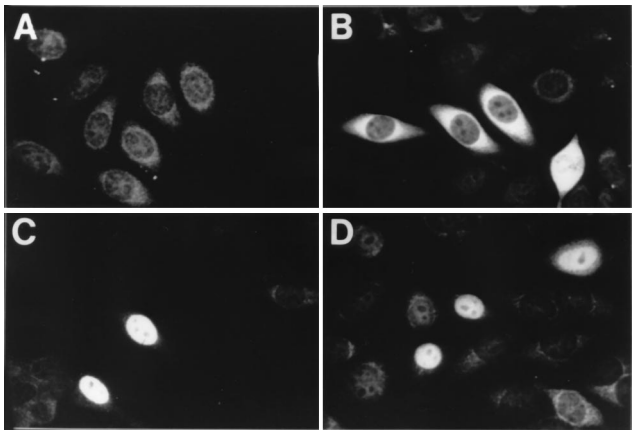

FIG. 2. Abnormal NFKB-2 proteins localize in the nucleus. HeLa cells were transfected with 1 pmol of the indicated NFKB-2 expression vector and analyzed by immunofluorescence with an antiserum recognizing the N-terminal peptide of NFKB-2. (A) Mock transfection; (B) p100; (C) HUT78p85; (D) lyt-10Cα.

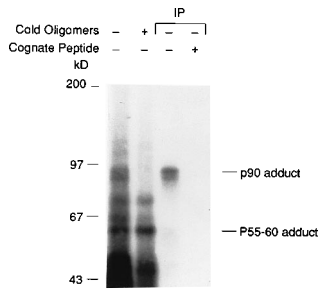

FIG. 3. HUT78p85 binds κB sequences in vitro. Nuclear extracts from HUT-78 cells were incubated with a bromodeoxyuridine-substituted ^{32}P-labeled HIV-κB probe. Protein-DNA complexes were UV cross-linked, and nucleases were digested, and the complexes were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) either directly or after immunoprecipitation (IP) with the anti-NFKB-2 antiserum.

variable length (174 amino acids in RC 685 and 12 amino acids in EB 308).

These observations suggested that the common feature of the rearrangements found in lymphoma was the selection of genes coding for proteins with shortened *ankyrin* domains with or without fusion to heterologous domains. We then chose to investigate the function of proteins lyt-10Cα and p85 as representatives of the two mutant types found in tumor cells, i.e., a fusion protein (lyt-10Cα) and a C-terminally truncated protein (p85).

Abnormal NFKB-2 proteins localize in the nucleus. The normal NFKB-2p100 is localized in the cytoplasm, while p52 can be found either in the cytoplasm complexed with IκB molecules (9) or, upon activation by NF-κB-inducing stimuli, in the nucleus (7, 13, 32). In order to determine whether the abnormal proteins can be regulated in the same fashion, we transfected HeLa cells with lyt-10Cα or p85 vectors (or p100 and p52 as normal controls) and determined the localization of NFKB-2 proteins by immunofluorescence with an antiserum recognizing the N terminus of the protein (7). As shown in Fig. 2, p100 was found mostly in the cytoplasm, whereas p52 (not shown), p85, and lyt-10Cα were detected exclusively in the nucleus. These results demonstrate that both types of tumor-associated NFKB-2 proteins can escape cytoplasmic retention and behave like activated p52.

Abnormal NFKB-2 proteins bind κB sequences in an unprocessed form. The results shown in Fig. 2 suggested the possibility that the abnormal NFKB-2 proteins may translocate to the nucleus and bind DNA in an unprocessed form. To directly test this hypothesis, we examined the DNA-binding ability of the p85 protein in nuclear extracts from the HUT-78 cell line, from which the rearranged *NFKB-2* allele coding for p85 was originally identified (33, 36). HUT-78 nuclear extracts were exposed to a bromodeoxyuridine-substituted ^{32}P-labeled HIV-κB oligonucleotide, UV cross-linked, and then analyzed by gel electrophoresis either directly or after immunoprecipitation with an antiserum against the N terminus of NFKB-2. The results (Fig. 3) showed that, while a number of κB-bound proteins were detectable before immunoprecipitation, the anti-NFKB-2 antiserum identified a protein-DNA adduct (p90) of the size expected for a complex formed by p85 and the oligonucleotide probe. The p90 adduct was not detectable in cell lines carrying normal *NFKB-2* alleles (not shown [7]), indicating its specific derivation from p85 in HUT-78 cells. A much less abundant protein-DNA adduct (p55) was also detectable in HUT-78 cells as well as in other cells (7), consistent with a complex formed by the probe and the normally processed p52 protein. Since this type of experiment can be performed only in cell lines, other tumor cases involving abnormal NFKB-2 products could not be studied because only tumor biopsies were available. Thus, in contrast to NFKBp100, p85 can translocate to the nucleus and bind κB sequences as a primary translation product.

Aberrant NFKB-2 proteins can heterodimerize and transactivate, but not transrepress, κB-driven transcription. By cotransfecting NF-κB effector and κB-driven reporter constructs into cells devoid of endogenous NF-κB activity (NTera-2 [3]), it has been shown that p52 can transactivate κB-linked genes via its heterodimeric association with Rel-Ap65 or, alternatively, repress it when present in excess as p52-p52 homodimers (4, 7). To examine the functional properties of the two abnormal NFKB-2 proteins, we cotransfected NTera-2 cells with an IgK–HIV-κB-driven luciferase gene construct and various amounts of vectors expressing p52, lyt-10Cα, or p85, in combination with a Rel-Ap65-expressing vector. The results (Fig. 4A) showed that, at low input, lyt-10Cα and p85 have similar effects on p65-mediated transcription. However, when present in excess, both abnormal proteins were significantly impaired in their capacity to transrepress p65-mediated transcription. In fact, both abnormal proteins by themselves displayed a transactivating activity which, although lower than the one displayed in the presence of p65, still led to a significant stimulation of reporter gene expression (50- to 100-fold; see below and Fig. 5 for further analysis).

To further investigate the functional properties of the two abnormal proteins, the nature of the NF-κB complexes formed in transfected cells was examined by EMSA with an HIV-κB probe. As shown in Fig. 4B, increasing amounts of p52 and the appearance of transrepressing activity were associated with the appearance of p52-p52 homodimers, consistent with previous findings (7). The two abnormal proteins were both able to form heterodimeric complexes with p65, consistent with their ability to participate in transactivation. However, they both displayed an abnormal behavior in homodimeric complex formation: p85 displayed a significant reduction in homodimeric binding, whereas no homodimeric binding was detectable for lyt-10Cα. The composition of the complexes formed by p65 and the various NFKB-2 proteins was documented by supershift assays with anti-NFKB-2 and anti-p65 antisera (see Fig. 4C for data on p65-p85 complexes; data on p52 and lyt-10Cα are not shown). These results indicate that the two abnormal proteins

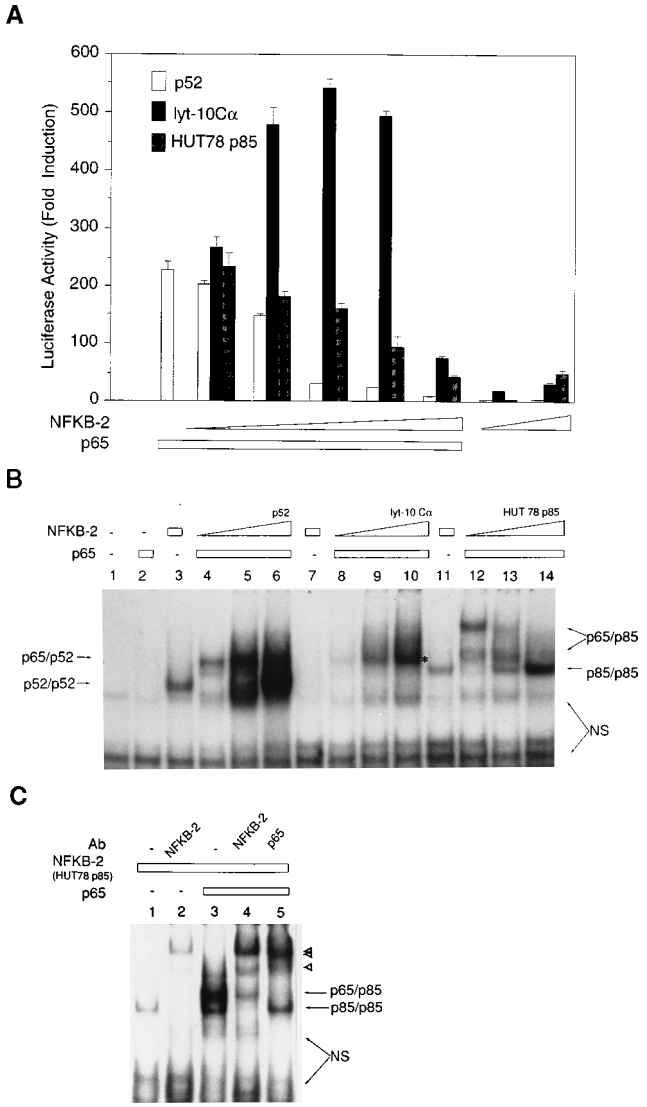

FIG. 4. Abnormal NFKB-2 proteins are defective in transrepressing activity and can form heterodimeric complexes with Rel-Ap65. (A) Abnormal NFKB-2 proteins are defective in the ability to repress Rel-Ap65-mediated transactivation activity. Increasing amounts (0.01, 0.03, 0.1, 0.3, and 1 pmol) of the indicated NFKB-2 plasmids (p52, lyt-10Cα, and p85) and the Rel-Ap65 plasmids (0.1 pmol) were cotransfected with reporter plasmids carrying IgK–HIV-κB sites (0.4 pmol) into NTera-2 cells. Reporter gene (luciferase) activities are expressed as fold induction over values obtained from transfection with target plasmids alone. The results shown here were representative of three to five independent experiments with each transfection performed in triplicate. Similar results were obtained from transfections performed with a reporter construct linked to H2–HLA-κBLUC. (B) Abnormal NFKB-2 proteins can form heterodimeric complexes with Rel-Ap65. NTera-2 cells were cotransfected with increasing amounts of NFKB-2 (0.01, 0.1, and 1 pmol) and Rel-Ap65 (0.1 pmol) plasmids. Nuclear extracts (1 μg) from transfected cells were assayed for binding to HIV-κB sites by EMSA. The various complexes are indicated by arrows, except for the heterodimeric complex p65–lyt-10Cα, which is indicated by an asterisk. NS, nonspecific bands. (C) Identification of DNA-binding complexes in NTera-2 cells transfected with Rel-Ap65 and p85 plasmids by antibody-mediated supershift analysis. Nuclear extracts (1 μg) of NTera-2 cells transfected with the p85 (0.3 pmol) and/or the p65 plasmids (0.1 pmol) were absorbed to 0.5 μl of anti-NFKB-2 or anti-p65 (Santa Cruz) antiserum prior to EMSA with the HIV-κB probe. Supershifted bands are indicated by open triangles. NS, nonspecific bands.

maintained the ability to heterodimerize with p65 and transactivate κB-driven transcription but have lost the transrepressing activity normally associated with the formation of p52 homodimers.

Aberrant NFKB-2 proteins have acquired intrinsic transactivation activities. The surprising observation that lyt-10Cα and p85 were both able to transactivate when transfected alone (Fig. 4A) prompted us to analyze in greater detail the magnitude of this activity and the precise nature of the DNA-binding complexes by which it was mediated. We cotransfected NTera-2 cells with the IgK–HIV-κB-driven reporter plasmid and p52, p85, or lyt-10Cα expression vectors and examined the protein levels by immunoprecipitation analysis, the transcriptional activity by assaying luciferase expression, and the nature of the κB-binding complexes by EMSA. Figure 5A shows that the three transfected plasmids expressed comparable protein levels. Figure 5B shows that, while p52 was devoid of any activity, the two abnormal proteins displayed a clear dose-dependent transactivation activity. Consistent with the preliminary results shown in Fig. 4A, the transcriptional activity of the two proteins reached close to 100-fold, i.e., below the levels displayed by the same proteins in association with p65 but still quite strong. Figure 5C shows that p52 entered into a single abundant DNA-binding complex corresponding to p52-p52 homodimers, whereas p85 and lyt-10Cα entered single complexes detectable at much lower levels. We consider the apparent contrast between the acquisition of the transactivation activity (Fig. 5C) and decreased DNA binding (Fig. 5B) of the two abnormal proteins to be indicative of their altered, rather than decreased, DNA-binding properties. This interpretation is supported by a well-characterized example of p65, which has the strongest transactivation activity yet is barely detectable in standard EMSAs (Fig. 4A) (31, 34). Taken together, these findings demonstrate that the two abnormal proteins have acquired intrinsic transactivation activity and suggest that this abnormal function may be due to altered DNA binding and/or homodimerization.

The transcriptional activity of the aberrant NFKB-2 proteins is not changed by Bcl-3. It has been shown that the IκB-type molecule Bcl-3 (16) can associate with p52 homodimers and convert them into transcriptional transactivators (4). This activity is apparently due to two complementary transactivation domains present in the Bcl-3 protein (4, 25, 27). In order to investigate the consequences of the structural alterations present in p85 and lyt-10Cα for this regulated function of NFKB-2, we tested the effects of cotransfection of the Bcl-3 expression vector on the transcriptional activity of the normal (p52) and abnormal NFKB-2 proteins. Figure 6A shows that, as originally demonstrated (4), coexpression of Bcl-3 with p52 led to transactivation of κB genes but has no (lyt-10Cα) or a very small (p85) effect on the already active abnormal proteins. This notion is corroborated by the data in Fig. 6B, which show that cotransfection of increasing amounts of Bcl-3 has no effect on the activity of the two abnormal proteins, whereas it increased the activity of p52 activity up to 29-fold. These findings indicate that the constitutive transactivation activity acquired by lyt-10Cα and p85 is comparable to the one displayed by the p52–Bcl-3 complexes and cannot be further increased by Bcl-3.

Mechanism of abnormal NFKB-2 activation: linking of heterologous C-terminal tail to p52 is sufficient for activation. The results shown above indicate that an apparently intrinsic transactivation activity is acquired by the two abnormal NFKB-2 proteins via the addition of a C-terminal domain tail. However, these two tails differ completely in their compositions, suggesting that the presence of the tail rather than its structure may determine the acquisition of the transactivation activity. To test this hypothesis, we constructed a vector (p52-Tc^r [Fig. 7A]) in which p52 is fused at its C terminus to residues from the tetracycline resistance gene (Tc^r) of plasmid pBR322,

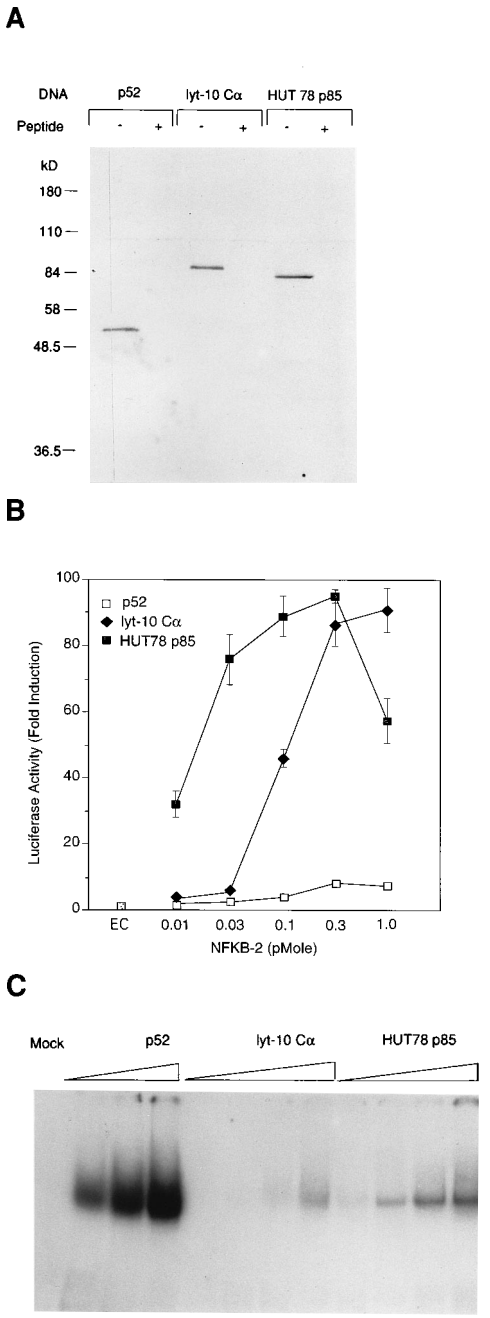

FIG. 5. The DNA binding and transcriptional activity of the NFKB-2 proteins. (A) Expression of NFKB-2 proteins in transfected NTera-2 cells. Cell lysates from NTera-2 cells previously transfected with various NFKB-2 gene constructs (1 pmol) were immunoprecipitated with an anti-NFKB-2 antiserum in the presence or absence of 100 mM cognate peptide and analyzed by SDS-PAGE. (B) lyt-10Cα and HUT78p85, but not p52, are constitutive κB transactivators. NTera-2 cells cotransfected with the IgK–HIV-κB reporter plasmid (0.4 pmol) and increasing concentrations of *NFKB-2* gene constructs (0 to 1.0 pmol as indicated) were assayed for luciferase activity. The experiment shown was representative of results obtained in six to eight independent experiments. The value marked EC (endogenous control) represents the value detected in cells transfected with the reporter construct alone. Similar results were obtained in transfections performed with a reporter gene linked to an H2–HLA-κB site. (C) Reduced homodimeric κB binding by lyt-10Cα and HUT78p85. Nuclear extracts (1 μg) from NTera-2 cells transfected with increasing amounts of NFKB-2 expression vectors (lyt-10Cα and p85, 0.03, 0.1, 0.3, and 1 pmol; p52, 0.03, 0.1, and 0.3 pmol) were assayed for HIV-κB binding by EMSA. Identification of these proteins was performed by antibody-mediated supershift assays with the anti-NFKB-2 antiserum (not shown).

chosen as a totally unrelated domain of a size similar to that of the lyt-10Cα and p85 tails. p52-Tc^r was then compared with p52 and p85 for its ability to transactivate the κB-driven reporter gene in NTera-2 cells. Figure 7B shows that p52-Tc^r is endowed with intrinsic transactivation activity only slightly inferior to that of p85. Thus, three totally unrelated tails were all found to be able to turn p52 into a potent transactivator. These findings strongly suggested that the addition of a tail rather than the addition of specific domains may be critical to confer the capability of p52 to transactivate.

DISCUSSION

Previous reports have provided evidence that structural alterations affecting the 3′ portion of the *NFKB-2* gene are recurrently associated with lymphoid malignancy, particularly with neoplasms derived from mature T cells (6, 10, 22, 33, 36). This study was aimed at characterizing the consequences of these alterations by examining relevant functional features of two abnormal NFKB-2 proteins representative of the two types of altered proteins observed in tumors. The results indicate that the structurally altered proteins display an abnormal behavior in multiple functions including subcellular localization, protein-protein interactions, and ability to regulate transcription. These findings have implications for the understanding of the normal regulation and function of NFKB-2 as well as its role in lymphomagenesis.

Structurally altered proteins accumulate in the nucleus and can bind DNA. The first functional consequence of the heterogeneous alterations affecting NFKB-2 proteins in lymphoid neoplasms is their constitutive localization in the nucleus. Previous reports have shown that NFKB-2 proteins truncated within the *ankyrin* domain could be found almost exclusively in the nucleus of transfected cells (22) or in a cell line expressing abnormal NFKB-2 proteins (33, 36). Our results confirm and extend these findings by showing that a tumor-derived protein representing a C-terminal fusion of NFKB-2 to a heterologous domain (Cα) also localizes in the nucleus. Furthermore, our results indicate that, at least in one instance tested, an abnormal protein, NFKB-2p85, can contact DNA in an unprocessed form, although the mechanism by which this protein can escape processing is not known. The mechanism by which the abnormal proteins localize in the nucleus has not been directly investigated, but the removal of the C-terminal portion of NFKB-2p100 including the seventh *ankyrin* repeat, the common feature of all abnormal proteins (Fig. 1A), may be involved in the proteins' abnormal localization. This hypothesis is supported by the observation that an intact C-terminal domain has been shown to be necessary for its cytoplasmic retention of NFKB-1p105 (1, 17).

Regardless of the mechanism involved, the ability of the tumor-derived proteins to localize in the nucleus should enable them to escape two major mechanisms which normally regulate the levels of NFKB-2 in the nucleus. First, these proteins do not require processing from the primary translation product, a possible rate-limiting step in determining the levels of normal NFKB-2p52 (7, 21). Second, these proteins should be able to escape retention by IκB molecules which normally anchor p52 in the cytoplasm after processing from p100 (2, 9). Since rearrangements of NFKB-2 can be found only in tumors deriving from mature B and T cells (10) in which the *NFKB-2* gene is transcribed at high levels (7), it is conceivable that these alterations synergize in determining constitutively high levels of NFKB-2 in the nucleus.

Tumor-associated NFKB-2 proteins function as constitutive transcriptional activators. The most intriguing result of our

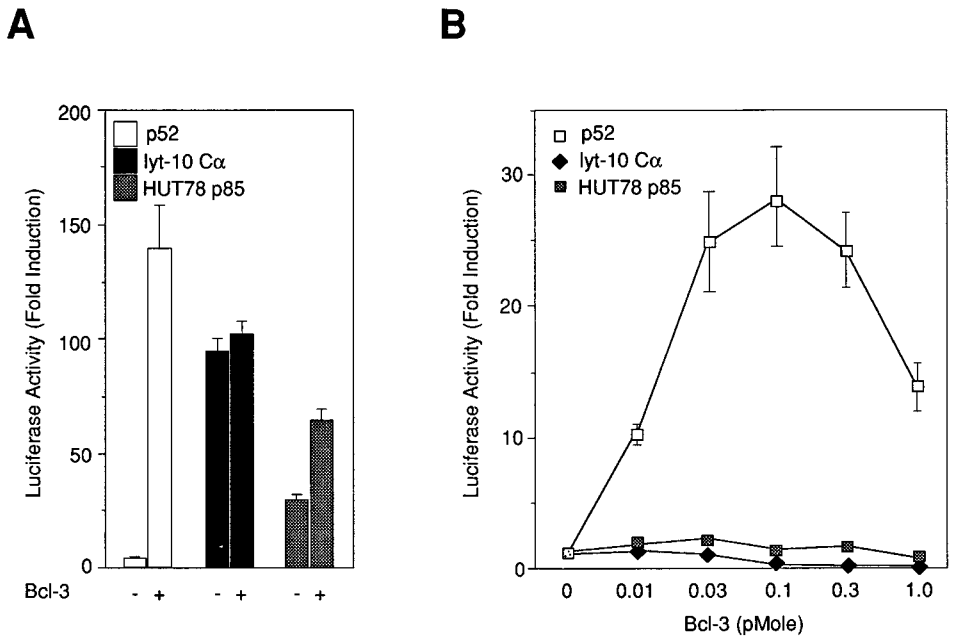

FIG. 6. The transactivation activity of lyt-10Cα or HUT78p85 cannot be increased by Bcl-3. (A) NTera-2 cells were cotransfected with 0.1 pmol of one of the NFKB-2 expression vectors as indicated and the IgK–HIV-κB LUC reporter construct (0.4 pmol) in the presence or absence of a vector expressing Bcl-3 (0.03 pmol) as indicated. Luciferase activity was expressed as fold induction over the levels obtained by transfecting the reporter plasmid alone. (B) NTera-2 cells were transfected as for panel A except that several concentrations of the Bcl-3 expression vector were tested. The values are expressed as fold induction over levels obtained in the absence of cotransfected Bcl-3.

studies is that both the abnormal NFKB-2 proteins studied have lost the ability to repress transcription and appear to have acquired intrinsic transactivation properties. As previously shown and schematically illustrated in Fig. 8, NFKB-2p52 is capable of activating transcription in the context of heterodimers with Rel-Ap65 or when complexed with Bcl-3, while the formation of p52-p52 homodimers is associated with repression of κB-driven transcription. Conversely, our results show that both NFKB-2p85 and lyt-10Cα have acquired the ability to transactivate independently from Rel-Ap65 and Bcl-3. The net effect of these alterations appears to be constitutive transactivation with loss of the transrepression function. This, together with the fact that the abnormal NFKB-2 proteins are present at abnormally high levels in the nucleus (see above), is likely to further contribute to substantial changes in NF-κB function in tumor cells carrying rearranged *NFKB-2* genes.

The mechanism by which the abnormal NFKB-2 proteins have acquired intrinsic transactivation properties is unclear. The possibility that these proteins may acquire the ability to transactivate via complexing with other NF-κB proteins is unlikely since the NTera-2 cells do not contain detectable endo-

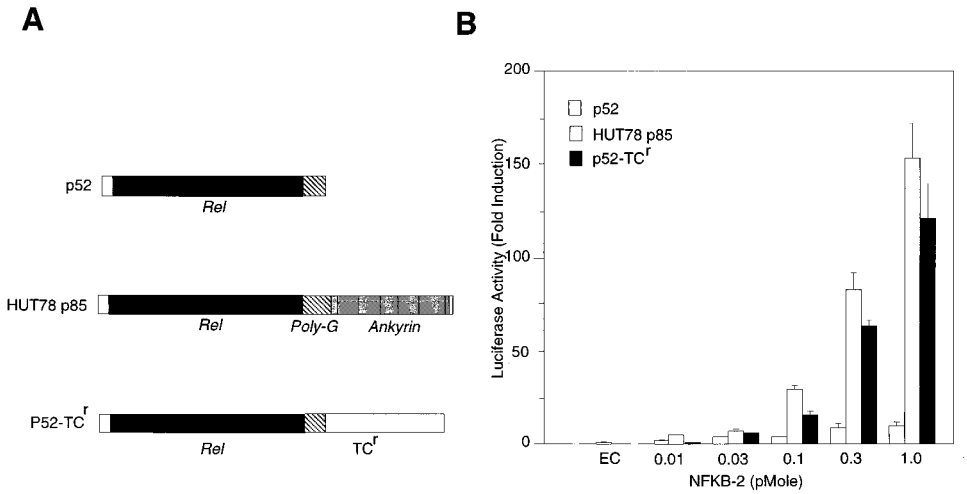

FIG. 7. C-terminal fusion of p52 to a heterologous protein induces its transcriptional activity. (A) Schematic representation of the NFKB-2p52, p85, and p52-Tc^r expression vectors. (B) NTera-2 cells were cotransfected with the IgK–HIV-κB reporter gene construct (0.4 pmol) and one of the NFKB-2 vectors as indicated. EC (endogenous control), reporter activity from cells transfected with the reporter construct alone.

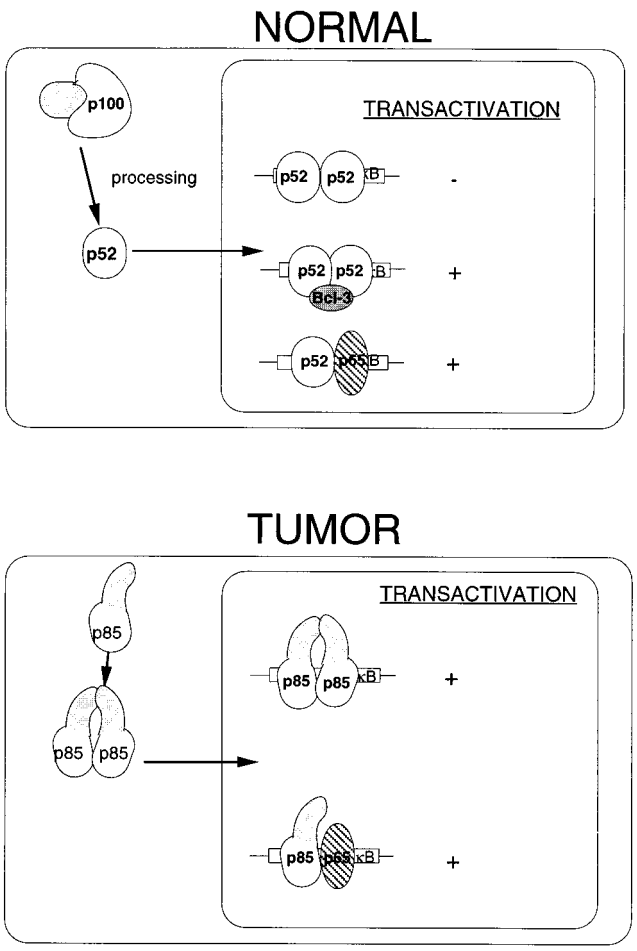

FIG. 8. A model illustrating the transcriptional activities of the normal and tumor-associated NFKB-2 proteins.

genous NF-κB activities (3, 7). On the other hand, the fact that two naturally occurring (NFKB-2p85 and lyt-10Cα) and one experimentally constructed (p52-Tc^r) proteins all have acquired transactivation properties despite the unrelatedness of the heterologous C termini suggests that the addition of a tail, rather than its specific nature, may be critical to confer transactivation properties on p52. We suggest that a tail may change the conformation of the *rel* domain and unmask its transactivation domain, as suggested by at least two observations. First, although neither NFKB-2p52 nor NFKB-1p50 is capable of transactivating Gal-4-driven transcription when linked to a Gal-4 DNA-binding domain, NFKB-1p50 can induce transcription in in vitro (11) and in *Saccharomyces cerevisiae*-based (23) experimental systems, suggesting that NFKB-2p52 may in fact harbor such activity. Second, it has been recently shown that the alternative RNA splicing of the *NFKB-1* gene can generate proteins (p84, p98) which, similarly to lyt-10Cα and p85, have lost the C terminus and have acquired intrinsic transactivation functions (14). The notion that alterations in the C termini of NFKB-1 or NFKB-2 proteins may unmask a transactivation function of their *rel* domains suggests the possibility that the transactivation function of p52–Bcl-3 complexes may also be due to conformational changes rather than the presence of intrinsic transactivation domains within Bcl-3 (4, 25, 27). In vitro transcription assays should provide definitive conclusions on the ability of the tumor-derived proteins to act as direct transactivators of κB-dependent transcription.

Implications for lymphomagenesis. Rearrangements of the *NFKB-2* locus have been found in approximately 2 to 3% of lymphoid malignancies and in 14 to 19% of peripheral T-cell neoplasms (6, 10). In the majority of cases, the rearrangements involve the 3′ portion of the *NFKB-2* gene and lead to truncations within the *ankyrin*-encoding domain analogous to p85, with a minority of cases involving fusion to heterologous coding domains analogous to the one observed in lyt-10Cα (Fig. 1). On the basis of these results, it is conceivable that the common effect of these heterogeneous rearrangements may be the production of NFKB-2 proteins capable of constitutive nuclear localization and transactivation activity. This may lead to quantitative changes in the composition of NF-κB complexes as well as to qualitative consequences for NF-κB function. In fact, it has been shown that different NF-κB complexes (e.g., homodimers versus heterodimers) may have different affinities for various κB target sequences (24, 28). Thus, the overall effect of these alterations should be the constitutive activation of the subset of κB-controlled genes that is preferentially bound by NFKB-2-containing complexes.

Substantial evidence involving the v-*rel* oncogene (18) suggests that an imbalance of NF-κB activity can have oncogenic effects. Toward this end, an oncogenic effect of NFKB-2 has been recently demonstrated in murine fibroblasts in vitro (19a). However, the elucidation of the biological role of NFKB-2 alterations has proven difficult in lymphoid cells. Constitutive overexpression of rearranged NFKB-2 driven by B-cell-specific immunoglobulin enhancer/promoter elements could not be obtained in human lymphoblastoid cells or in transgenic animals, presumably because of the toxic effect of inappropriately high NF-κB levels. This suggests that the fine-tuning of the levels of NFKB-2 expression in specific target cells may be required to prevent toxicity and elicit a biological effect. The use of rearranged *NFKB-2* genes under the control of their own regulatory elements, thus recapitulating the situation found in tumors, may represent the next logical approach for the investigation of the consequences of *NFKB-2* activation in vitro and in vivo.

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