Moloney Murine Leukemia Virus Activates NF- κ B

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Nonacutely transforming retroviruses, such as Moloney murine leukemia virus (M-MuLV), differ from transforming viruses in their mechanisms of tumor induction. While the transforming viruses cause tumors by transduction of oncogenes, the leukemia retroviruses, lacking oncogenes, employ other mechanisms, including promoter insertion and enhancer activation. Although these two mechanisms occur in many tumors induced by leukemia viruses, a substantial proportion of such tumors do not show site-specific proviral insertions. Thus, other, unidentified virus-driven mechanisms may participate in tumorigenesis. In these studies, we show that infection of cells by M-MuLV activates expression of Rel family transcription factors. In murine cells chronically infected with M-MuLV, gel shift analyses with κ B DNA-binding motifs from the murine immunoglobulin kappa light chain enhancer demonstrated induction of at least two distinct κ B enhancer-binding complexes. Supershifting and immunoblotting analyses defined p50, p52, RelB, and c-Rel subunits as constituents of these virus-induced protein complexes. Transient transfections performed with κ B-dependent reporter plasmids showed transcriptional activation in M-MuLV-infected cells relative to uninfected cells. Induction of Rel/NF- κ B transcription factor activity by M-MuLV infection may prove relevant to the mechanism of M-MuLV-induced leukemia.

Moloney murine leukemia virus (M-MuLV), a murine type C nonacute retrovirus, induces T-lymphoblastic lymphoma or myeloid leukemia when inoculated into neonatal NIH Swiss or BALB/c mice. The T-lymphoblastic lymphoma occurs with 100% incidence and a mean latency of 16 to 17 weeks, while M-MuLV-induced myeloid leukemia develops with a 50% incidence and a mean latency of 14 to 15 weeks (24, 61, 64). M-MuLV-induced leukemogenesis may occur through multiple molecular mechanisms, including insertional activation of cellular proto-oncogenes by enhancers or promoters located within the retroviral long terminal repeat, generation of envelope gene recombinants, and a preleukemic state characterized by generalized hematopoietic hyperplasia and splenomegaly (10, 17, 18, 24, 35, 42). Previous studies of the preleukemic state suggest that the generalized hematopoietic hyperplasia could result from the infection of hematopoietic cells, as well as of nonhematopoietic cells, causing increased synthesis of growth factors (10, 18). Molecular mechanisms linking viral infection to regulation of genes controlling proliferation of growth factor production have not been elucidated, however.

The Rel/NF- κ B transcription factors control the expression of a multitude of cellular and viral genes, including many of those involved in immune or acute inflammatory processes, such as the immunoglobulin kappa light chain, interleukin-2, and interleukin-6 genes (29). The vertebrate Rel/NF- κ B family is composed of several proteins related to the proto-oncoprotein c-Rel, including NF- κ B1 (p50), NF- κ B2 (p52), RelA (p65), and RelB (11, 27, 44, 57–59). Each member shares a 300-amino-acid region of homology, the Rel homology domain (RHD), which is located towards the amino terminus. The RHD contains sequences that are essential for DNA binding, homo- and heterodimerization, I κ B binding, and nuclear localization (3, 6, 8, 27, 33, 34, 36, 47, 51). The Rel family can

also be subdivided on the basis of the presence or absence of a transcriptional transactivating domain located in sequences carboxy terminal to the RHD. p65, RelB, and c-Rel each have carboxy-terminal sequences with varying levels of transactivational potential, while p50 and p52 proteins do not contain carboxy-terminal transactivation domains and are derived from larger precursor molecules, p105 and p100, respectively (23, 39, 44, 45).

In most cell types, a majority of the NF- κ B dimers are sequestered within the cytoplasm in an inactive state—that is, they are unable to bind κ B sites and induce transcription—by an inhibitory group of proteins called I κ B (5, 30). The I κ B proteins, i.e., I κ B- α , I κ B- β , I κ B- γ , and Bcl-3, interact and modulate the Rel protein complexes by binding and masking the nuclear localization sequence within the RHD (37, 38). The current model of NF- κ B regulation indicates that upon receiving an activating signal, the I κ B complexed with NF- κ B dimers becomes hyperphosphorylated (16, 19, 20, 25, 43). The hyperphosphorylated I κ B is then selectively degraded by the ubiquitin-dependent proteasome, allowing the NF- κ B dimers to translocate to the nucleus, where the complex activates transcription of Rel/NF- κ B-responsive genes (12, 62).

Various viruses have been shown to activate NF- κ B, such as cytomegalovirus, human immunodeficiency virus type 1, Sendai virus, and adenovirus 5 (2, 7, 13, 53, 55). Additionally, viral gene products, such as the human T-cell lymphotropic virus type I *tax* product, have been reported to increase NF- κ B DNA-binding activity in cells (4, 56). Presumably, the activation of NF- κ B provides a beneficial effect for these viruses by enhancing transcription of viral genes or regulating cellular genes necessary for viral replication and integration (41, 46, 54).

In this report, we demonstrate that NF- κ B is activated by infection or expression of M-MuLV. Using M-MuLV-infected BALB/3T3 cells, we found that M-MuLV infection enhances NF- κ B DNA-binding activity, including that of complexes containing p50, p52, RelB, and c-Rel. Moreover, M-MuLV-in-

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FIG. 1. Transcriptional activity of Rel/NF- κ B members in M-MuLV-infected BALB/3T3 cells (M-MuLV-B). (A) Uninfected and M-MuLV-infected BALB/3T3 cells were plated in 100-mm dishes and fed with fresh media 24 h before transfection by the calcium phosphate coprecipitation technique, as previously described (32). Plasmid pJ16 (1 μ g) consists of two copies of the murine kappa light chain enhancer NF- κ B site (B orientation) cloned into the *Sal*I site of D56, a minimal *c-fos* promoter upstream of the CAT gene (28). Plasmid p2NFBCO consists of four copies of the NF- κ B site from the promoter of the beta interferon gene cloned into the *Xho*I site of plasmid pOBCO, a CAT-containing reporter construct driven by the rabbit β -globin promoter (31, 52). At 48 h after transfection, cells were harvested and lysed by freeze-thawing procedures. Following incubation with 0.5 μ Ci of [14 C]chloramphenicol, extracts were extracted with ethyl acetate, dried, and analyzed by thin-layer chromatography as previously described (32). Cotransfection with the pRSV- β -galactosidase plasmid (0.5 μ g per transfection) and cytofluorometric analysis of β -galactosidase activity were performed to normalize the data for transfection efficiency (49). These data were calculated from four independent assays. Relative CAT activity values ($\pm$ the standard error) were as follows: BALB/c-pJ16, 3.45 ± 2.1 ; BALB/c-p2NFBCO, 4.40 ± 1.6 ; M-MuLV-B-pJ16, 17.30 ± 2.5 ; and M-MuLV-B, 46.25 ± 3.8 . (B) Plasmids p2NFBCO (rabbit β -globin promoter containing two NF- κ B binding sites) and pOBCO (rabbit β -globin promoter lacking two NF- κ B binding sites) were transfected into M-MuLV-B (lanes 1 and 2) or uninfected BALB/3T3 (lanes 3 and 4) cells. (C) Transcriptional activity of Rel/NF- κ B members activated by M-MuLV. Plasmid pE8-CAT, containing the NF- κ B site from the URE of the *c-myc* gene cloned into the *Bam*HI site of the thymidine kinase promoter CAT vector in the sense orientation (21), was cotransfected into BALB/c-3T3 cells with either pMoV9 (lane 1), a biologically active molecular clone of a proviral M-MuLV genome in pBR322 (14); a control plasmid (pBR322) (lane 2); a plasmid containing the CAT gene under the control of the simian virus 40 early promoter (RSV-CAT) (lane 3); or plasmid pE8-CAT, with recombinant tumor necrosis factor alpha (rTNF α) treatment (20 U/ml, 40 min) being done 24 h after transfection (lane 4). This is a representative experiment of five independent assays.

infected BALB/3T3 cells show greater expression of κ B-dependent reporter plasmids than uninfected cells.

To determine whether M-MuLV activated NF- κ B, BALB/3T3 cells were stably infected with M-MuLV and examined for NF- κ B activity by transient introduction of NF- κ B-responsive plasmids. The NF- κ B-dependent reporter construct pJ16, which contains two copies of the murine immunoglobulin kappa light chain enhancer NF- κ B site linked to a minimal *c-fos* promoter, was transiently transfected into uninfected or M-MuLV-infected BALB/3T3 cells. Expression of the NF- κ B-dependent reporter plasmid was increased fivefold in M-MuLV-infected BALB/3T3 cells compared with expression in uninfected BALB/3T3. A different reporter plasmid, p2NFBCO, containing four copies of an NF- κ B from the promoter of beta interferon gene was also induced when transfected into M-MuLV-expressing cells. pJ16 and p2NFBCO reporter constructs exhibit 6- and 15-fold greater transactivation in M-MuLV-infected BALB/3T3 cells than in uninfected BALB/3T3 cells, when values are normalized for transfection efficiency (Fig. 1A). The difference in the transactivation levels of pJ16 and p2NFBCO likely reflects the two additional κ B sites within the p2NFBCO plasmid. The specificity of the NF- κ B-dependent transactivation observed with pJ16 was demonstrated by using pJ32, a size-matched reporter plasmid differing by only two nucleotide alterations in its NF- κ B binding sites. pJ32 is expressed at the same basal levels as pJ16 in

uninfected BALB/c cells. Transfections utilizing p2NFBCO were controlled by transfecting in parallel with pOBCO, a similar reporter plasmid missing the four beta interferon-derived NF- κ B binding sites. Cells transfected with pOBCO exhibited low and similar levels of expression in both uninfected and M-MuLV-infected BALB/c cells (data not shown).

Transient transfections with p2NFBCO and pOBCO also demonstrated that the transactivation seen in the infected cells was NF- κ B dependent (Fig. 1B). Transfection experiments using the NF- κ B-dependent reporter construct pE8 (containing the κ B binding site from the upstream regulatory element *c-myc* promoter linked to a minimal thymidine kinase promoter) showed that levels of transactivation were quantitatively similar in infected and uninfected cells (data not shown).

Chronic or stable M-MuLV infection was not required for induction of NF- κ B activity. As shown in Fig. 1C, transient cotransfection of pMoV9 (which contains the M-MuLV provirus) transactivated the κ B-dependent reporter pE8. Additional cotransfections with pMoV9 and pJ16 showed similar results (data not shown). The magnitude of the induction of expression was comparable to that generated by treatment of the cells with recombinant tumor necrosis factor alpha. Altogether, these data show that M-MuLV-infected or -transfected BALB/3T3 cells exhibit activation of κ B enhancer-binding proteins which potentially transactivate various κ B-dependent reporter constructs.

FIG. 2. M-MuLV-infected BALB/3T3 cells (M-MuLV-B) exhibit Rel/NF- κ B DNA-binding activity. EMSAs were performed with a probe containing the NF- κ B binding site present in the murine immunoglobulin kappa light chain enhancer (5'-AGTTGAGGGGACTTTCCAGGC-3') (60). Probes were end labeled with [α - 32 P]dCTP by using T4 polynucleotide kinase by standard procedures. Nuclear proteins from uninfected BALB/3T3 cells (BALB/c) and M-MuLV-B were used in each assay (lanes 1 and 2 to 10, respectively; 10 mg per sample). Nuclear proteins from M-MuLV-B cells were preincubated with a 40-fold excess of unlabeled oligonucleotides as indicated (lanes 3 and 4). Where indicated (+), nuclear extracts were preincubated for 15 min with the indicated antibody prior to the addition of the probe. Supershift analyses were performed with antibodies to p50, p52, p65, RelB, c-Rel, and normal rabbit serum (NRS) (lanes 5 through 10, respectively). Peptide antisera to Rel family members were kindly provided by N. Rice (National Cancer Institute, Bethesda, Md.) and previously described (50). The compositions of the bands are indicated at the right. ns, nonspecific.

To verify that M-MuLV induces κ B enhancer-binding proteins and to determine which κ B proteins are involved, we examined NF- κ B DNA-binding activity in nuclear extracts of M-MuLV-infected BALB/3T3 cells by an electrophoretic mobility shift assay (EMSA). The labeled oligonucleotide probe used in these assays contained the κ B site from the murine immunoglobulin kappa light chain enhancer (60). In uninfected BALB/3T3 cell nuclear extracts, in the absence of exogenous stimulation, weak κ B-like complexes were visible (Fig. 2, lane 1). In comparison, nuclear extracts of M-MuLV-infected BALB/3T3 cells contained significantly higher levels of NF- κ B DNA-binding activity (lane 2). To determine whether the observed κ B DNA-binding complexes were specific toward κ B sites, competitions with two nonradiolabeled oligonucleotides were performed. While competition with a 40-fold excess of a nonradiolabeled oligonucleotide probe containing an AP2 motif did not affect the complexes (lane 4), an excess of nonradiolabeled κ B-containing oligonucleotide abolished a majority of the inducible complexes (lane 3). Since nuclear extracts of M-MuLV-infected BALB/3T3 cells showed in-

creased NF- κ B DNA-binding activity, we examined whether activation of other transcription factors occurred. Binding assays with Oct1 and Sp1 motifs and uninfected and M-MuLV-infected BALB/3T3 cell nuclear extracts showed no significant change in DNA-binding activity (data not shown). These results demonstrate that the M-MuLV-induced changes in nuclear expression of κ B enhancer-binding proteins are relatively specific.

These EMSA results demonstrate that the formation of NF- κ B/Rel complexes have a functional effect on promoters containing an NF- κ B site. This effect was verified earlier with the chloramphenicol acetyltransferase (CAT) reporter genes expressed from NF- κ B-responsive promoters, namely, the murine immunoglobulin kappa light chain enhancer and the beta interferon promoters, in cells infected with M-MuLV.

To identify the composition of the κ B DNA-binding complexes induced in M-MuLV-infected BALB/3T3 cells, supershift analyses were performed by EMSA with specific antisera to specific Rel subunits. As shown in Fig. 2, antisera specific to p50, p52, and RelB resulted in supershifts of DNA-binding protein complexes. These supershift assays demonstrated that the middle complex was supershifted by the p52 and RelB antibodies while the lower complex was supershifted only by p50 antibody. The addition of the neutralizing anti-c-Rel antibody completely ablated the most slowly migrating protein-DNA complex and, although less clearly evident, the middle complex. However, immunoreactions with antisera against p65 (RelA), which was previously shown to supershift p65 (RelA)-containing complexes under similar experimental conditions (data not shown), did not shift any M-MuLV-inducible complexes.

These data demonstrate that the M-MuLV-inducible complexes consist of p50, p52, RelB, and c-Rel. Specifically, the middle complex contains p52, RelB, and c-Rel and the lower complex contains p50. Interestingly, no immunoreaction with a single antibody completely disrupted any inducible complex.

To examine the nature of the increased κ B site DNA-binding activity and increased κ B-dependent transactivation caused by M-MuLV infection, we examined steady-state protein expression of the Rel family members in cytoplasmic and nuclear fractions of uninfected and M-MuLV-infected BALB/3T3 cells by using immunoblotting techniques. As shown in Fig. 3A, while uninfected BALB/3T3 cells exhibit barely detectable levels of c-Rel, M-MuLV-infected BALB/3T3 cells contain higher levels of c-Rel, especially in the cytoplasm. The greater steady-state cytoplasmic levels of c-Rel could be due to its retention within the cytoplasm by I κ B- β protein levels or by the precursor proteins, p105 and p100; however, this remains unproven. Similar to the findings for c-Rel expression, RelB and p52 steady-state protein levels were substantially higher in M-MuLV-infected BALB/3T3 cell extracts than in those of uninfected BALB/3T3 cells, particularly in the nuclear compartment. It is important to rule out contamination of the nuclear extract preparation by the cytoplasmic compartment. Therefore, immunoblotting experiments examining levels of p100 were incorporated since this molecule is found solely in the cytoplasm. Immunoblots showed no detectable levels of p100 in the nuclear extracts (data not shown).

The induction of c-Rel, RelB, and p52 correlated with their presence as constituents of the inducible DNA-protein complexes observed in M-MuLV-infected BALB/3T3 cell nuclear extracts (see above). Although gel shift analyses showed a p50-containing complex induced by M-MuLV, the cytoplasmic p50 protein levels were similar in both cells. Interestingly, the nuclear compartments of M-MuLV-infected BALB/3T3 cells contained slightly less p50 than those of uninfected cells. The

FIG. 3. Immunoblot analyses of Rel/NF- κ B family members in M-MuLV-infected BALB/3T3 cells. (A) Nuclear and cytoplasmic proteins were extracted by a modification of the method of Andrews and Faller (1). Cytoplasmic and nuclear proteins were electrophoresed on sodium dodecyl sulfate-polyacrylamide denaturing gels and transferred to nitrocellulose. The filters were blocked overnight at 4°C in Tris-buffered saline (pH 7.5) containing 1% nonfat milk and then incubated for 3 h in Tris-buffered saline (pH 7.5) containing 3% nonfat milk and 0.05% Tween-20 with anti-p50 (1263), anti-p52 (1495), anti-relB (1318), anti-p65 (1266), or anti-c-Rel (1207) at a 1:2,000 dilution. After being washed, the filters were incubated with horseradish peroxidase-conjugated goat anti-rabbit antibody (Boehringer Mannheim, Indianapolis, Ind.) at a 1:8,000 dilution. Later, the filters were washed and proteins were detected by enhanced chemiluminescence (Amersham Corporation, Arlington Heights, Ill.). Lanes 1 and 3, uninfected BALB/3T3 cell cytoplasmic and nuclear proteins, respectively; lanes 2 and 4, M-MuLV-infected BALB/3T3 cell cytoplasmic and nuclear proteins, respectively. (B) Immunoblot analysis of p100 and p52 from whole-cell extracts of uninfected (lane 1) and M-MuLV-infected (lane 2) BALB/3T3 cells. Equal amounts of protein were run in all lanes on the basis of protein concentration determinations with the Bradford reagent (Bio-Rad). The identity of each band is indicated on the right.

effect of this difference is uncertain. Gel shift analyses did not show augmented NF- κ B binding activity of p65 (RelA), although p65 (RelA) levels were significantly higher in the M-MuLV-infected BALB/3T3 cell cytoplasmic and nuclear compartments. Despite increases in expression of p65 (RelA), however, we observed no increases in its contribution to the κ B complex DNA-binding activity. Recently, similar observations were made with transgenic mice overexpressing p65 (RelA) in thymocytes, in which neither enhancement nor alteration of κ B DNA-binding activity was observed despite high levels of p65 (RelA) (thought to be mainly due to an increase in endogenous I κ B- α levels preventing p65 [RelA] access and binding to κ B sites) (48). To examine if a nuclear function of I κ B- α is present, immunoblotting was conducted with nuclear extracts of uninfected and infected BALB/c cells. Immunoblots showed no identifiable nuclear I κ B- α .

Although little is known about how infection by M-MuLV chronically activates NF- κ B, studies examining the activation of NF- κ B in human immunodeficiency virus-infected macrophages have implicated the processing of I κ B molecules, such as p105 and p100, in the regulation of NF- κ B (15). This was based on the observation that chronic human immunodeficiency virus infection increases processing of p105; presumably, this would allow the generation of active DNA-binding

heterodimers, such as p52:RelB, which are able to translocate into the nucleus, where they could affect transcription of NF- κ B-responsive genes. Therefore, increased processing of I κ B molecules could serve as a mode of regulation whereby external stimuli like viruses could activate NF- κ B. To examine whether an increase in the p52 protein level was related to increased levels of its precursor protein, p100, immunoblot analyses were performed with whole-cell extracts from uninfected and M-MuLV-infected cells. Although M-MuLV-infected and uninfected cells exhibit similar levels of p100, the levels of p52 and the ratio of p52 to p100 are greater in M-MuLV-infected cells (Fig. 3B). This suggests that the up-regulation of p52 could be a result of increased stability of the processed form or increased processing of p100, in which case the upregulated levels of p52 are not simply due to increased levels of its precursor protein. This suggests that increased processing of p100 may participate in M-MuLV-induced activation of NF- κ B. However, it remains unclear how infection by M-MuLV could affect the processing of the precursor protein, p100, or its transcription.

In this study, we have shown that M-MuLV-infected or -transfected BALB/3T3 cells show increased NF- κ B site DNA-binding and transactivation activities. We have previously reported that cells infected with M-MuLV exhibit upregulated cell surface expression of class I and II major histocompatibility complex antigens and the T-cell receptor (TcR) β -chain, both of which are known to be regulated by Rel/NF- κ B (22, 26, 40, 63). Most of the molecules found to be upregulated are members of the immunoglobulin gene superfamily, and all can serve as activation antigens for T cells. We and others have proposed that the induction of cell surface expression of T-cell activation antigens by M-MuLV, and the resulting T-cell proliferation, may contribute to the chronic immunostimulation and thymic hyperplasia observed in the preleukemic state (63). The ability of M-MuLV to activate NF- κ B could subvert the homeostatic regulation of immune-associated signaling pathways and augment growth factor-dependent processes such as preleukemic hematopoietic hyperplasia or other aspects of leukemogenesis (9). Studies are currently under way to examine whether M-MuLV-infected leukemia cells express constitutive NF- κ B activity or whether this constitutive NF- κ B activity is present only in distinct transformation stages of M-MuLV-infected cells.

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REFERENCES

- Andrews, N. C., and D. V. Faller. 1991. A rapid micropreparation technique for extraction of DNA-binding proteins from limiting numbers of mammalian cells. *Nucleic Acids Res.* **19**:2499.
- Bachelier, F., J. Alami, F. Arenzana-Seisdedos, and J. L. Virelizier. 1991. HIV enhancer activity perpetuated by NF- κ B induction on infection of monocytes. *Nature (London)* **350**:709-712.
- Baeuerle, P. A. 1991. The inducible transcription activator NF- κ B: regulation by distinct protein subunits. *Biochim. Biophys. Acta* **1072**:63-80.
- Ballard, D. W., E. Bohnlein, J. W. Lowenthal, Y. Wano, B. R. Franza, and W. C. Greene. 1988. HTLV-I tax induces cellular proteins that activate the κ B element in the IL-2 receptor α gene. *Science* **241**:1652-1655.
- Beg, A. A., and A. Baldwin, Jr. 1993. The I κ B proteins: multifunctional regulators of Rel/NF- κ B transcription factors. *Genes Dev.* **7**:2064-2070.

6. Blank, V., P. Kourilsky, and A. Israel. 1992. NF-kappa B and related proteins: Rel/dorsal homologies meet ankyrin-like repeats. *Trends Biochem. Sci.* **17**:135-140.
7. Borellini, F., Y. F. He, A. Aquino, G. Yu, S. F. Josephs, and R. I. Glazer. 1992. Increased DNA binding and transcriptional activity associated with transcription factor Sp1 in K562 cells transfected with the myeloid-specific c-fes tyrosine kinase gene. *Pharmacol. Ther.* **55**:303-318.
8. Bose, H., Jr. 1992. The Rel family: models for transcriptional regulation and oncogenic transformation. *Biochim. Biophys. Acta* **1114**:1-17.
9. Bours, V., G. Franzoso, K. Brown, S. Park, V. Azarenko, M. Tomita-Yamaguchi, K. Kelly, and U. Siebenlist. 1992. Lymphocyte activation and the family of NF-kappa B transcription factor complexes. *Curr. Top. Microbiol. Immunol.* **182**:411-420.
10. Brightman, B. K., B. R. Davis, and H. Fan. 1990. Preleukemic hematopoietic hyperplasia induced by Moloney murine leukemia virus is an indirect consequence of viral infection. *J. Virol.* **64**:4582-4584.
11. Brownell, E., N. Mittereder, and N. R. Rice. 1989. A human rel proto-oncogene cDNA containing an Alu fragment as a potential coding exon. *Oncogene* **4**:935-942.
12. Chen, Z., J. Hagler, V. J. Palombella, F. Melandri, D. Scherer, D. Ballard, and T. Maniatis. 1995. Signal-induced site-specific phosphorylation targets I kappa B alpha to the ubiquitin-proteasome pathway. *Genes Dev.* **9**:1586-1597.
13. Cherrington, J. M., and E. S. Mocarski. 1989. Human cytomegalovirus ie1 transactivates the α promoter-enhancer via an 18-base-pair repeat element. *J. Virol.* **63**:1435-1440.
14. Chumakov, I., H. Stuhlmann, K. Harbers, and R. Jaenisch. 1982. Cloning of two genetically transmitted Moloney leukemia proviral genomes: correlation between biological activity of the cloned DNA and viral genome activation in the animal. *J. Virol.* **42**:1088-1098.
15. Conant, K., W. J. Atwood, R. Traub, C. Tornatore, and E. O. Major. 1994. An increase in p50/p65 NF- κ B binding to the HIV-1 LTR is not sufficient to increase viral expression in the primary human astrocyte. *Virology* **205**:586-590.
16. Cordle, S. R., R. Donald, M. A. Read, and J. Hawiger. 1993. Lipopolysaccharide induces phosphorylation of MAD3 and activation of c-Rel and related NF-kappa B proteins in human monocytic THP-1 cells. *J. Biol. Chem.* **268**:11803-11810.
17. Cuypers, H. T., G. Selten, W. Quint, M. Zijlstra, E. R. Maandag, W. Boelens, P. van Wezenbeek, C. Melief, and A. Berns. 1984. Murine leukemia virus-induced T-cell lymphomagenesis: integration of proviruses in a distinct chromosomal region. *Cell* **37**:141-150.
18. Davis, B. R., B. K. Brightman, K. G. Chandy, and H. Fan. 1987. Characterization of a preleukemic state induced by Moloney murine leukemia virus: evidence for two infection events during leukemogenesis. *Proc. Natl. Acad. Sci. USA* **84**:4875-4879.
19. Devary, Y., C. Rosette, J. A. DiDonato, and M. Karin. 1993. NF-kappa B activation by ultraviolet light not dependent on a nuclear signal. *Science* **261**:1442-1445.
20. Diaz-Meco, M. T., E. Berra, M. M. Municio, L. Sanz, J. Lozano, I. Dominguez, V. Diaz-Golpe, M. T. Lain de Lera, J. Alami, C. V. Payá, F. Arenzana-Seisdedos, J.-L. Virelizier, and J. Moscat. 1993. A dominant negative protein kinase C ζ subspecies blocks NF- κ B activation. *Mol. Cell. Biol.* **13**:4770-4775.
21. Duyao, M. P., A. J. Buckler, and G. E. Sonenshein. 1990. Interaction of an NF-kappa B-like factor with a site upstream of the c-myc promoter. *Proc. Natl. Acad. Sci. USA* **87**:4727-4731.
22. Faller, D. V., L. D. Wilson, and D. C. Flyer. 1988. Mechanism of induction of class I major histocompatibility antigen expression by murine leukemia virus. *J. Cell. Biochem.* **36**:297-309.
23. Fan, C. M., and T. Maniatis. 1991. Generation of p50 subunit of NF-kappa B by processing of p105 through an ATP-dependent pathway. *Nature (London)* **354**:395-398.
24. Fan, H. 1990. Influences of the long terminal repeats on retroviral pathogenicity. *Semin. Virol.* **1**:164-174.
25. Finco, T. S., and A. Baldwin, Jr. 1993. Kappa B site-dependent induction of gene expression by diverse inducers of nuclear factor kappa B requires Raf-1. *J. Biol. Chem.* **268**:17676-17679.
26. Flyer, D. C., S. J. Burakoff, and D. V. Faller. 1985. Expression and CTL recognition of cloned subgenomic fragments of Moloney murine leukemia virus in murine cells. *Surv. Immunol. Res.* **4**:168-172.
27. Ghosh, S., A. M. Gifford, L. R. Riviere, P. Tempst, G. P. Nolan, and D. Baltimore. 1990. Cloning of the p50 DNA binding subunit of NF-kappa B: homology to rel and dorsal. *Cell* **62**:1019-1029.
28. Gilman, M. Z., R. N. Wilson, and R. A. Weinberg. 1986. Multiple protein-binding sites in the 5'-flanking region regulate c-fos expression. *Mol. Cell. Biol.* **6**:4305-4316.
29. Gilmore, T. D. 1992. Role of rel family genes in normal and malignant lymphoid cell growth. *Cancer Surv.* **15**:69-87.
30. Gilmore, T. D., and P. J. Morin. 1993. The I kappa B proteins: members of a multifunctional family. *Trends Genet.* **9**:427-433.
31. Godowski, P. J., D. Picard, and K. R. Yamamoto. 1988. Signal transduction and transcriptional regulation by glucocorticoid receptor-LexA fusion proteins. *Science* **241**:812-816.
32. Gorman, C. M., L. F. Moffat, and B. H. Howard. 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. *Mol. Cell. Biol.* **2**:1044-1051.
33. Grilli, M., J. J. Chiu, and M. J. Lenardo. 1993. NF-kappa B and Rel: participants in a multifunctional transcriptional regulatory system. *Int. Rev. Cytol.* **143**:1-62.
34. Grimm, S., and P. A. Baeuerle. 1993. The inducible transcription factor NF-kappa B: structure-function relationship of its protein subunits. *Biochem. J.* **290**:297-308.
35. Hayward, W. S., B. G. Neel, and S. M. Astrin. 1981. Activation of a cellular onc gene by promoter insertion in ALV-induced lymphoid leukemia. *Nature (London)* **290**:475-480.
36. Henkel, T., U. Zabel, K. van Zee, J. M. Muller, E. Fanning, and P. A. Baeuerle. 1992. Intramolecular masking of the nuclear location signal and dimerization domain in the precursor for the p50 NF-kappa B subunit. *Cell* **68**:1121-1133.
37. Inoue, J., L. D. Kerr, D. Rashid, N. Davis, H. Bose, Jr., and I. M. Verma. 1992. Direct association of pp40/I kappa B beta with rel/NF-kappa B transcription factors: role of ankyrin repeats in the inhibition of DNA binding activity. *Proc. Natl. Acad. Sci. USA* **89**:4333-4337.
38. Kerr, L. D., J. Inoue, N. Davis, E. Link, P. A. Baeuerle, H. Bose, Jr., and I. M. Verma. 1991. The rel-associated pp40 protein prevents DNA binding of Rel and NF-kappa B: relationship with I kappa B beta and regulation by phosphorylation. *Genes Dev.* **5**:1464-1476.
39. Kieran, M., V. Blank, F. Logeat, J. Vandekerckhove, F. Lottspeich, O. Le Bail, M. B. Urban, P. Kourilsky, P. A. Baeuerle, and A. Israel. 1990. The DNA binding subunit of NF-kappa B is identical to factor KBF1 and homologous to the rel oncogene product. *Cell* **62**:1007-1018.
40. Koka, P., K. van de Mark, and D. V. Faller. 1991. *trans*-Activation of genes encoding activation-associated human T lymphocyte surface proteins by murine retroviral sequences. *J. Immunol.* **146**:2417-2425.
41. Leung, K., and G. J. Nabel. 1988. HTLV-1 transactivator induces interleukin-2 receptor expression through an NF-kappa B-like factor. *Nature (London)* **333**:776-778.
42. Li, Q.-X., and H. Fan. 1991. Bone marrow depletion by ^{89}Sr complements a preleukemic defect in a long terminal repeat variant of Moloney murine leukemia virus. *J. Virol.* **65**:4442-4448.
43. Li, S., and J. M. Sedivy. 1993. Raf-1 protein kinase activates the NF-kappa B transcription factor by dissociating the cytoplasmic NF-kappa B-I kappa B complex. *Proc. Natl. Acad. Sci. USA* **90**:9247-9251.
44. Mercurio, F., J. DiDonato, C. Rosette, and M. Karin. 1992. Molecular cloning and characterization of a novel Rel/NF-kappa B family member displaying structural and functional homology to NF-kappa B p50/p105. *DNA Cell Biol.* **11**:523-537.
45. Mercurio, F., J. A. DiDonato, C. Rosette, and M. Karin. 1993. p105 and p98 precursor proteins play an active role in NF-kappa B-mediated signal transduction. *Genes Dev.* **7**:705-718.
46. Nabel, G., and D. Baltimore. 1987. An inducible transcription factor activates expression of human immunodeficiency virus in T cells. *Nature (London)* **326**:711-713. (Erratum, **344**:178, 1990.)
47. Nolan, G. P., and D. Baltimore. 1992. The inhibitory ankyrin and activator Rel proteins. *Curr. Opin. Genet. Dev.* **2**:211-220.
48. Perez, P., S. A. Lira, and R. Bravo. 1995. Overexpression of RelA in transgenic mouse thymocytes: specific increase in levels of the inhibitor protein I κ B α . *Mol. Cell. Biol.* **15**:3523-3530.
49. Renfranz, P. J., M. G. Cunningham, and R. D. McKay. 1991. Region-specific differentiation of the hippocampal stem cell line HiB5 upon implantation into the developing mammalian brain. *Cell* **66**:713-729.
50. Rice, N. R., T. D. Copeland, S. Simek, S. Oroszlan, and R. V. Gilden. 1986. Detection and characterization of the protein encoded by the v-rel oncogene. *Virology* **149**:217-229.
51. Rice, N. R., M. L. MacKichan, and A. Israel. 1992. The precursor of NF-kappa B p50 has I kappa B-like functions. *Cell* **71**:243-253.
52. Richardson, P. M., and T. D. Gilmore. 1991. vRel is an inactive member of the Rel family of transcriptional activating proteins. *J. Virol.* **65**:3122-3130.
53. Riviere, Y., V. Blank, P. Kourilsky, and A. Israel. 1991. Processing of the precursor of NF-kappa B by the HIV-1 protease during acute infection. *Nature (London)* **350**:625-626.
54. Ross, E. K., A. J. Buckler-White, A. B. Rabson, G. Englund, and M. A. Martin. 1991. Contribution of NF- κ B and Sp1 binding motifs to the replicative capacity of human immunodeficiency virus type 1: distinct patterns of viral growth are determined by T-cell types. *J. Virol.* **65**:4350-4358.
55. Roulston, A., M. D'Addario, F. Boulterice, S. Caplan, M. A. Wainberg, and J. Hiscott. 1992. Induction of monocytic differentiation and NF-kappa B-like activities by human immunodeficiency virus 1 infection of myelomonoblastic cells. *J. Exp. Med.* **175**:751-763.
56. Ruben, S., H. Poteat, T. H. Tan, K. Kawakami, R. Roeder, W. Haseltine, and C. A. Rosen. 1988. Cellular transcription factors and regulation of IL-2 receptor gene expression by HTLV-I tax gene product. *Science* **241**:89-92.
57. Ruben, S. M., P. J. Dillon, R. Schreck, T. Henkel, C. H. Chen, M. Maher, P. A. Baeuerle, and C. A. Rosen. 1991. Isolation of a rel-related human

- cDNA that potentially encodes the 65-kD subunit of NF-kappa B. *Science* **251**:1490–1493. (Erratum, **254**:11.)
58. **Ryseck, R.-P., P. Bull, M. Takamiya, V. Bours, U. Siebenlist, P. Dobrzanski, and R. Bravo.** 1992. RelB, a new Rel family transcription activator that can interact with p50-NF- κ B. *Mol. Cell. Biol.* **12**:674–684.
 59. **Schmid, R. M., N. D. Perkins, C. S. Duckett, P. C. Andrews, and G. J. Nabel.** 1991. Cloning of an NF-kappa B subunit which stimulates HIV transcription in synergy with p65. *Nature (London)* **352**:733–736.
 60. **Sen, R., and D. Baltimore.** 1986. Inducibility of kappa immunoglobulin enhancer-binding protein NF-kappa B by a posttranslational mechanism. *Cell* **47**:921–928.
 61. **Shen-Ong, G. L. C., and L. Wolff.** 1987. Moloney murine leukemia virus-induced myeloid tumors in adult BALB/c mice: requirement of *c-myb* activation but lack of *v-abl* involvement. *J. Virol.* **61**:3721–3725.
 62. **Traenckner, E. B., S. Wilk, and P. A. Baeuerle.** 1994. A proteasome inhibitor prevents activation of NF-kappa B and stabilizes a newly phosphorylated form of I kappa B-alpha that is still bound to NF-kappa B. *EMBO J.* **13**:5433–5441.
 63. **Wilson, L. D., D. C. Flyer, and D. V. Faller.** 1987. Murine retroviruses control class I major histocompatibility antigen gene expression via a *trans* effect at the transcriptional level. *Mol. Cell. Biol.* **7**:2406–2415.
 64. **Wolff, L., J. F. Mushinski, G. L. Shen-Ong, and H. Morse.** 1988. A chronic inflammatory response. Its role in supporting the development of *c-myb* and *c-myc* related promonocytic and monocytic tumors in BALB/c mice. *J. Immunol.* **141**:681–689.