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The NF-κB transcription factor and cancer: high expression of NF-κB- and IκB-related proteins in tumor cell lines

Abstract—NF-κB is a pleiotropic transcription factor which controls the expression of many genes and viruses. To date, there is good evidence, but no difinitive proof, for its role in tumor formation and development of metastasis. To investigate the possibility that members of the NF-κB family could participate in the molecular control of the transformed and invasive phenotype, we examined the expression of these proteins in a variety of human tumor cell lines. The expression of p50, p65, p52 and IκB was quantified at the protein level using western immunoblot and mobility shift assay and at the RNA level by northern blot. We observed high expression of the NF-κB inhibitor IκB in the ovarian carcinoma cell line OVCAR-3 together with constitutive nuclear NF-κB activity. We also studied the colon carcinoma cell line HT-29 and its metastatic counterpart HTM-29 and we observed specific expression of the p52 NF-κB-related protein in the metastatic cells. Our data confirm that NF-κB could be involved in the genesis of a variety of cancers including solid tumors and provide us with interesting models to explore the exact role of these transcription factors in cancer.

NF-κB is a pleiotropic transcription factor complex which controls the expression of numerous genes and viruses through binding to specific DNA sequences (named κB sites) inside the gene's promoters or enhancers (for recent reviews see Refs. 1, 2). Among the long list of genes whose expression is controlled by NF-κB are those coding for the immunoglobulin κ light chain, the major histocompatibility complex, cytokines and cytokine receptors (IL-2,* IL-2 receptor, IL-6, IL-8, TNF-α, TNF-β, GM-CSF, G-CSF, etc.), acute phase reaction proteins, adhesion molecules (VCAM-1 ICAM-1, ELAM-1), oncogenes, etc. Several viruses contain a (or several) κB site in their promoter [2]. The most studied is HIV-1 [3, 4] but CMV, SV40, adenoviruses and others also have their expression stimulated by NF-κB.

A family of proteins forms the NF-κB complexes [5, 6]. All the members of this family share a similar but non-identical domain related to the v-Rel viral oncoprotein and its cellular homolog c-Rel; this domain is thus referred to as the Rel homology domain (RelHD) and is necessary for DNA-binding and dimerization. These Rel-related proteins can be classified into two classes according to their structure and function.

The first class contains the p50 and p52 (or p50B) proteins and their precursors [7–13]. These two proteins do not harbor any transactivation domains and, since they can bind DNA as homodimers, function as inhibitors of the NF-κB-mediated transactivation [14]. They are derived from distinct cytoplasmic precursors which need to be processed in order to release the nuclear DNA-binding proteins [13, 15, 16]. These precursors contain in their carboxy-terminal portion a domain composed of seven partially conserved repeats. Similar repeated sequences, usually called Ankyrin Repeats, were described in a number of proteins from various origins, including human ankyrin and several yeast cell cycle proteins, and they are thought to mediate specific protein–protein interactions [5, 6, 17]. The Ankyrin Repeats domain of the p50 and p52 precursors inhibit nuclear translocation and DNA-binding by the full-length protein.

The second class of Rel-related proteins is formed from three proteins (p65, c-Rel and RelB) which contain unrelated transactivation domains [18–25]. p65 (recently renamed relA) and c-Rel can bind DNA and transactivate as homodimers. However, the most potent NF-κB complexes are composed of heterodimers containing one protein of each group.

The function of these homo or heterodimeric NF-κB complexes is controlled by a second family of proteins: the IκB family [5, 6]. The different members of this family (IκB α, β and γ, and Bcl-3) are Ankyrin Repeats-containing proteins which interact specifically with NF-κB-proteins of class I or II [14, 26–30]. The three IκB (IκB α, β and γ) proteins inhibit NF-κB DNA-binding and transactivation. We have demonstrated that the oncoprotein Bcl-3 can, through specific interaction with p50 or p52 homodimers, induce a transactivation of κB-dependent genes [14, 31].

Several reports suggest that proteins from the NF-κB or IκB families are involved in the development of cancer. v-Rel containing viruses are highly oncogenic and cause agressive tumors in young birds [32, 33] while mutated c-Rel is transforming *in vitro* [34]. The genes for c-Rel, p65, p50, p52 and Bcl-.3 are located at sites of recurrent translocations and genomic rearrangements in cancer [11, 29, 35–37] and we have shown that p52 (p50B) and Bcl-3 can form a potent activating complex [31]. A naturally occurring mutant of p65 was shown to be transforming when over-expressed in rat embryo fibroblasts [38]. Finally, the Tax protein from the leukemogenic virus HTLV-1 is a potent activator of NF-κB [39, 40]; it was recently demonstrated that Tax-induced tumors in mice could be suppressed by antisense NF-κB constructs [41].

NF-κB controls the expression of several proteins important for cellular adhesion (ICAM-1, ELAM-1, VCAM-1) and of extracellular matrix proteases (type IV collagenase 92) [42–46]. A recent report confirmed the importance of NF-κB for cellular adhesion [47]. Also, down-regulation of the expression of MHC Class I molecules in tumors is associated with reduced immunogenicity and metastatic phenotype. The promotor of MHC Class I genes contains an NF-κB site and it was demonstrated that p50 homodimers repress H-2K^b expression in metastatic tumor cells [48]. Taken together, all these data suggest that a dysregulation of NF-κB activity in tumor cells could lead to a modification of the cellular adhesion properties and to the development of metastases.

Nevertheless, the exact role of NF-κB in cancer development and metastasis remains obscure. We screened several solid tumors cell lines for expression of NF-κB and IκB-related genes and proteins.

* Abbreviations: IL, interleukin; TNF, tumor necrosis factor; DMEM, Dulbecco's modified Eagle's medium; GM–CSF, granulocyte macrophage colony-stimulating factor; CAM, cell adhesion molecule; VCAM, vascular cell adhesion molecule; PVDF, polyvinylidenefluoride.

Materials and Methods

Cell lines and culture. The human ovarian carcinoma cell lines OVCAR-3 and CaOV3 and the colon carcinoma cell line WIDR were obtained from the American Type Culture Collection (Rockville, MD, U.S.A.) (ATCC); the Jurkat T cell line was a gift from U. Siebenlist. HT-29 and its metastatic subclone HTM-29 were derived from a human colon carcinoma and were obtained from Dr E. Kohn (NIH, Bethesda, MD, U.S.A.). Cells were grown in RPMI 1640 (Jurkat) or in DMEM (all other cells) supplemented with 10% fetal calf serum.

mRNA extraction and northern blots. Total cellular RNA was isolated from the cells using the guanidine isothiocyanate extraction procedure and cesium chloride gradient centrifugation as described [49]. For each sample, RNA concentration was determined by spectrophotometric analysis at 260 nm. RNA (5 μg) from each sample was electrophoresed on a 1.2% agarose-formaldehyde gel and transferred to nitrocellulose membrane. The probes were P32 labeled by nick translation and hybridized to the blots at 40°.

Immunoblots. Protein extracts were prepared from cultured cells and run on a 10% SDS–PAGE gel. After transfer to an Immobilon PVDF membrane (Millipore) and blocking in 5% dry milk, the membrane was incubated for 2 hr with the first antibody, washed and incubated with the second peroxidase-conjugated antibody. The reactions were revealed with the enhanced chemoluminescence detection method (ECL kit, Amersham).

Electrophoretic mobility shift assays. OVCAR-3 cells were lysed in a Dounce homogenizer and the resulting nuclei were salt-extracted [14]. κB DNA probes, mobility shift assays and supershifting experiments were as described previously [8, 14, 31].

Antibodies. Antibodies used were a polyclonal IκB antibody [50], antibodies directed against p50 or p65 amino-terminal peptides [14] and a p52 monoclonal antibody [31].

Results and Discussion

Northern blots demonstrated that several ovarian carcinoma cell lines express high levels of IκB-α messenger RNA (Fig. 1A, right panel) while p65 message is quite uniformly present in all cell lines (Fig. 1A, left panel). The IκB message is particularly abundant in OVCAR-3 cells and this cell line was thus investigated further. OVCAR-3 cells contain substantial amounts of IκB-α protein as demonstrated by immunoblot analysis (Fig. 1B). However, electrophoretic mobility shift assays performed with nuclear extracts from the same cells also showed substantial amounts of NF-κB proteins in the nucleus (Fig. 1C). Two clearly separated bands were observed (Fig. 1C, lane 1) and supershifting experiments with specific antibodies revealed that the lower faint band contained mainly p50 homodimers while the upper band was constituted from p50/p65 heterodimers (lanes 2–3). The specificity of the observed bandshifts was confirmed by competition experiments (data not shown).

IκB-α is an inhibitor of NF-κB DNA-binding and it has been shown to sequester NF-κB complexes in the cytoplasm [26, 51–54]. Several cell types contain high levels of IκB-α protein in their cytoplasm where it forms a complex with NF-κB. Following cellular stimulation by agents such as phorbol esters, TNF-α or IL-1, IκB-α is phosphorylated and degraded within a few minutes [50, 55, 56]. Simultaneously, the NF-κB complexes migrate to the nucleus. The activation of NF-κB will in turn increase transcription of the IκB-α gene. Newly synthesized IκB-α protein could possibly enter the nucleus and inhibit NF-κB-mediated DNA-binding and transactivation [54]. It is therefore somewhat surprising to find in a cell type a high expression of IκB and a constitutive substantial intranuclear NF-κB binding activity. One might envisage continuous stimulation of the signal transduction pathway leading to phosphorylation and inactivation of IκB-α. However such a reaction, in the cases described so far, is accompanied by rapid degradation of IκB-α. To explain the fact that OVCAR-3 cells retain a substantial amount of IκB-α protein, we would have to hypothesize that the intracellular NF-κB activity generates very high transcription of the IκB gene (as assessed by the northern blot) with neosynthesis of the protein at a rate that would insure the presence of the protein despite the continuous stimulation of the signal transduction pathway.

Another possibility would be that OVCAR-3 cells express a non functional IκB-α protein. Hypo or hyperphosphorylation could lead to IκB loss of function [56–58], but IκB-α from OVCAR-3 cells comigrates with the protein from unstimulated Jurkat cells which does not favor, but does not formally rule out, important post-translational differences. Another IκB-α domain, not involved in phosphorylation could also be defective, impairing the function of the protein.

Finally, IκB-α could have a novel unexpected function in OVCAR-3 cells. The Bcl-3 protein, a member of the IκB family, has been shown to behave quite differently in various cell lines. When transfected in Cos7 cells, Bcl-3 remains confined to the cytoplasm and prevents p50 nuclear translocation while in NTera-2, L929 and NIH3T3 cells, Bcl-3 is predominantly in the nucleus and can associate on DNA with p50 or p52 homodimers to mediate κB-dependent transactivation [30, 31, 59]. We demonstrated that Bcl-3 indeed contains two cooperating transactivation domains active in eukaryotic cells [31]. Others reported that the Ankyrin Repeat domains of the p50 precursor and pp40 (the chicken equivalent of IκB) contain potential transactivation domains [60]. Moreover IκB-α and pp40 can translocate to the nucleus [54, 60]. Can we thus envisage nuclear activating function for IκB-α as for Bcl-3 in some cell types? We do not have any evidence to substantiate this hypothesis and are currently investigating the IκB-α cellular localization and function in OVCAR-3 cells.

We also studied expression of NF-κB and IκB-related proteins in cell lines with various invasive and metastatic potentials. We investigated the non-invasive colon carcinoma cell line HT-29 and its metastatic counterpart HTM-29. We detected a specific high expression of p52 messenger RNA and protein in the invasive cells (Fig. 2). The mRNA for other Rel-related (p65, p50) proteins is not differentially expressed in the two cell lines (Fig. 1A and data not shown). Also, HTM-29 cells express slightly more IκB-α mRNA than do HT-29 cells.

The importance of NF-κB for cellular adhesion is now well established. Several adhesion molecules have their expression controlled by NF-κB and antisense oligonucleotides of p65 sequences dramatically alter cellular adhesion [42–45, 47]. On the other hand, p50 homodimers were shown to inhibit the expression of MHC Class I proteins in metastatic tumors and thus to favor tumor invasiveness [48]. Finally, expression of extracellular proteases associated with tumor invasive phenotype is also controlled by NF-κB [46]. These observations suggest that NF-κB could play an important role in the development of metastasis and cancer invasiveness. Excessive NF-κB activity could upregulate the expression of extracellular proteases leading to a degradation of the extracellular matrix and the basal membrane and thus to cancer invasion. Alternatively, over-expression of inhibiting p50 (or p52) homodimers could block the transcription of genes coding for adhesion molecules or MHC Class I proteins, generating again increased invasiveness.

The function of p52 is largely unknown. Its gene was cloned at a site of recurrent chromosomal translocations in human lymphomas suggesting a possible involvement in tumor formation [11]. p52 tissue expression is more restricted than that of p50 and so far we don't know of any gene promoters or κB sites that are specifically bound and regulated by p52-containing complexes. It is clear, however, that p52 can form transactivating hetereodimers with p65,

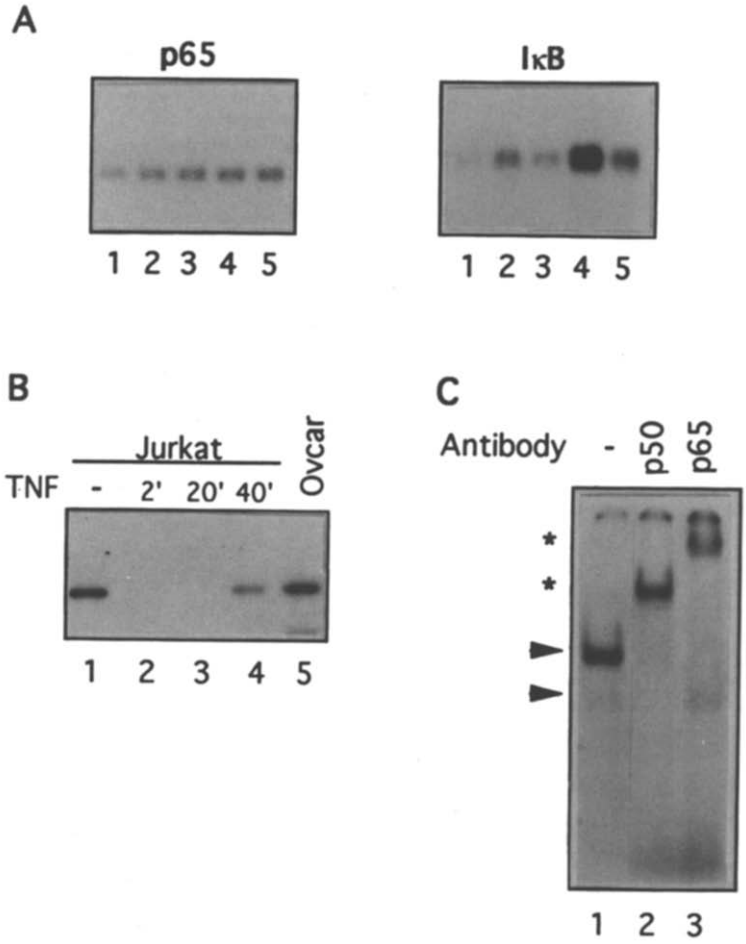

Fig. 1. (A) High expression of IκB-α message in OVCAR-3 cells. mRNAs from various cell lines were prepared and analysed on northern blots with p65 or IκB-α probes. The cell lines were: (1) HT-29; (2) HTM-29; (3) WIDR; (4) OVCAR-3; (5) CaOV3. (B) Expression of IκB-α protein in OVCAR-3 cells. Cellular extracts were prepared and analysed on an Immunoblot and revealed with anti-IκB-α antibody. Jurkat cell extracts were used as a reference and show, as described previously [50, 55], a high expression of IκB-α in resting cells (lane 1) and, after treatment with TNF-α a disappearance of the protein (lanes 2–3) followed by a neosynthesis (lane 4). OVCAR-3 IκB-α comigrates with the protein expressed in Jurkat cells (compare lanes 1, 4 and 5). (C) OVCAR-3 cells nuclei contain significant amounts of NF-κB. Nuclear extracts from OVCAR-3 cells were run on an electrophoretic mobility shift assay with a labeled probe corresponding to a synthetic palindromic κB site. Two bands are seen on the gel (lane 1) and are indicated by arrowheads. Antibodies directed against p50 supershifted both bands (lane 2) while p65 antibodies supershifted only the upper band (lane 3). Asterisks show the supershifted complexes.

RelB or c-Rel [8, 12, 13]. On the other hand, we demonstrated that p52 homodimers, when expressed in excess, can inhibit NF-κB-mediated transactivation (V. Bours, G. Franzoso and U. Siebenlist, unpublished observations). p52 can also form a potent activating complex with Bcl-3 [31]. Therefore, p52 could play a role in the development of metastasis either through upregulation of κB-dependent genes (in cooperation with a partner) or through inhibition of transcription. Further studies are needed to determine the function of p52 in HTM-29 cells and the relationship between p52 expression and invasiveness. Interestingly, we detected high p52 expression in other tumor cell lines, either lymphoid cell lines (U266, HUT78) or solid tumor cell lines.

Our results, although only preliminary, support the concept of an important role for NF-κB in the development of cancer and metastasis. They also suggest that NF-κB could be involved in the development of many different types of cancers, including solid tumors.

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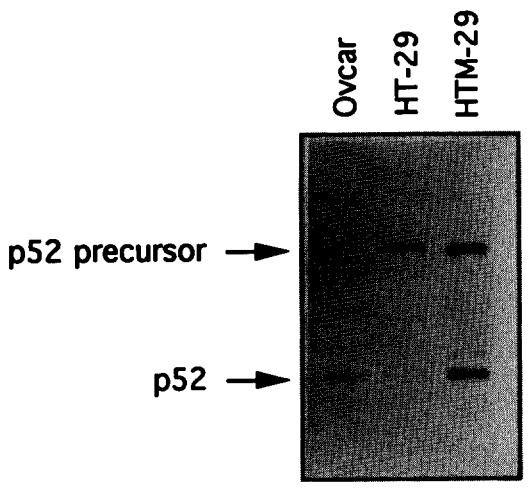

Fig. 2. High expression of p52 in HTM-29 cells. p52 expression in OVCAR-3, HT-29 and HTM-29 cells was analysed on an Immunoblot performed with a monoclonal anti p52 antibody. The higher molecular weight band (100 kDa) corresponds to the p52 precursor and the lower band to p52 itself.

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REFERENCES

1. Baeuerle PA, The inducible transcription factor NF-κB: regulation by distinct protein subunit. *Biochim Biophys Acta* **1072**: 63–80, 1991.
2. Grilli M, Chiu JJ-S and Lenardo MJ, NF-κB and Rel: participants in a multiform transcriptional regulatory system. *Int Rev Cytol* **148**: 1–63, 1993.
3. Greene WC, Regulation of HIV-1 gene expression. *Annu Rev Immunol* **8**: 453–475, 1990.
4. Nabel G and Baltimore D, An inducible transcription factor activates expression of human immunodeficiency virus in T cells. *Nature* **326**: 711–713, 1987.
5. Blank V, Kourilsky P and Israel A, NF-kappa B and related proteins: Rel/dorsal homologies meet ankyrin-like repeats. *Trends Biochem Sci* **17**: 135–140, 1992.
6. Nolan GP and Baltimore D, The inhibitory ankyrin and activator Rel proteins. *Curr Opin Genet Dev* **2**: 211–200, 1992.
7. Bours V, Villalobos J, Burd PR, Kelly K and Siebenlist U, Cloning of a mitogen-inducible gene encoding a κB DNA-binding protein with homology to the *rel* oncogene and to cell cycle motifs. *Nature* **348**: 76–80, 1990.
8. Bours V, Burd PR, Brown K, Villalobos J, Park S, Ryseck R-P, Bravo R, Kelly K and Siebenlist U, A novel mitogen-inducible gene product related to p50/p105-NF-κB participates in transactivation through a κB site. *Mol Cell Biol* **12**: 685–695, 1992.
9. Ghosh S, Gifford AM, Riviere LR, Tempst P, Nolan GP and Baltimore D, Cloning of the p50 binding subunit of NF-κB: homology to *rel* and *dorsal*. *Cell* **62**: 1019–1029, 1990.
10. Kieran M, Blank V, Logeat F, Vandekerckhove J, Lottspeich F, Bail OL, Urgan MB, Kourilsky P, Baeuerle PA and Israël A, The DNA-binding subunit of NF-κB is identical to factor KBF1 and homologous to the *rel* oncogene product. *Cell* **62**: 1007–1018, 1990.
11. Neri A, Chang C-C, Lombardi L, Salina M, Corradini P, Maiolo AT, Chaganti RSK and Dalla-Favera R, B cell lymphoma-associated chromosomal translocation involves candidate oncogene *lyt*-10, homologous to NF-κB p50. *Cell* **67**: 1075–1087, 1991.
12. Schmidt RM, Perkins ND, Duckett CS, Andrews PC and Nabel GJ, Cloning of an NF-κB subunit which stimulates HIV transcription in synergy with p65. *Nature* **352**: 733–736, 1991.
13. Mercurio F, Didonato J, Rosette C and Karin M, Molecular cloning and characterization of a novel rel/NF-κB family member displaying structural and functional analogy to NF-κB p50/p105. *DNA Cell Biol* **11**: 523–537, 1992.
14. Franzoso G, Bours V, Park S, Tomita-Yamaguchi M, Kelly K and Siebenlist U, The candidate oncoprotein Bcl-3 is an antagonist of p50/NF-κB-mediated inhibition. *Nature* **359**: 339–342, 1992.
15. Mercurio F, DiDonato JA, Rosette C and Karin M, p105 and p98 precursor proteins play an active role in NF-κB-mediated signal transduction. *Gene Dev* **7**: 705–718, 1993.
16. Fan C-M and Maniatis T, Generation of p50 subunit of NF-κB by processing of p105 through an ATP-dependent pathway. *Nature* **354**: 395–398, 1991.
17. Lux SE, John KM and Bennett V, Analysis of cDNA for human erythrocyte ankyrin indicates a repeated structure with homology to tissue-differentiation and cell-cycle control proteins. *Nature* **344**: 36–42, 1990.
18. Ballard DW, Dixon EP, Peffer NJ, Bogerd H, Doerre S, Stein B and Greene WC, The 65-kDa subunit of human NF-κB functions as a potent transcriptional activator and a target for v-Rel-mediated repression. *Proc Natl Acad Sci USA* **89**: 1875–1879, 1992.
19. Nolan GP, Ghosh S, Liou H-C, Tempst P and Baltimore D, DNA binding and IκB inhibition of the cloned p65 subunit of NF-κB, a *rel*-related polypeptide. *Cell* **64**: 961–969, 1991.
20. Ruben SM, Dillon PJ, Schreck R, Henkel T, Chen CH, Maher M, Baeuerle PA and Rosen CA, Isolation of a rel-related human cDNA that potentially encodes the 65-kD subunit of NF-kappa B. *Science* **251**: 1490–1493, 1991.
21. Ryseck P-P, Bull P, Takamiya M, Bours V, Siebenlist U, Dobrzanski P and Bravo R, RelB, a new rel family transcription activator that can interact with p50-NF-κB. *Mol Cell Biol* **12**: 674–684, 1992.
22. Ruben SM, Klement JF, Coleman TA, Maher M, Chen C-H and Rosen CA, I-Rel: a novel *rel*-related protein that inhibits NF-κB transcriptional activity. *Gene Dev* **6**: 745–760, 1992.
23. Dobrzanski P, Ryseck R-P and Bravo R, Both N- and C-terminal domains of RelB are required for full transactivation: role of the N-terminal leucine zipper-like motif. *Mol Cell Biol* **13**: 1572–1582, 1993.
24. Brownell E, Mitereder N and Ricer NR, A human rel proto-oncogene cDNA containing an Alu fragment as a potential coding exon. *Oncogene* **4**: 935–942, 1989.
25. Kamens J, Richardson P, Mosialos G, Brent R and Gilmore T, Oncogenic transformation by v-Rel requires

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an amino-terminal activation domain. *Mol Cell Biol* **10**: 2840–2847, 1990.
26. Haskill S, Beg AA, Tompkins SM, Morris JS, Yurochko AD, Sampson-Johannes A, Mondal K, Ralph P, and Baldwin ASJ, Characterization of an immediate-early gene induced in adherent monocytes that encodes I kappa B-like activity. *Cell* **65**: 1281–1289, 1991.
27. Inoue J, Kerr LD, Kakizuka A and Verma IM, I kappa B gamma, a 70 kd protein identical to the C-terminal half of p110 NF-kappa B: a new member of the I kappa B family. *Cell* **68**: 1109–1120, 1992.
28. Liou HC, Nolan GP, Ghosh S, Fujita T and Baltimore D, The NF-kappa B p50 precursor, p105, contains an internal I kappa B-like inhibitor that preferentially inhibits p50. *EMBO J* **11**: 3003–3009, 1992.
29. Ohno H, Takimoto G and McKeithan TW, The candidate proto-oncogene *bcl-3* is related to genes implicated in cell lineaage determination and cell cycle control. *Cell* **60**: 991–997, 1990.
30. Naumann M, Wulczyn FG and Scheidereit C, The NF-κB precursor p105 and the proto-oncogene product Bcl-3 are IκB molecules and control nuclear translocation of NF-κB. *EMBO J* **12**: 213–222, 1993.
31. Bours V, Franzoso G, Azarenko V, Park S, Kanno T, Brown K and Siebenlist U, The oncoprotein Bcl-3 directly transactivates through κB motifs via association with DNA-binding p50B homodimers. *Cell* **72**: 729–739, 1993.
32. Moore BE and Bose HR, Transformation of avian lymphoid cells by reticuloendotheliosis virus. *Mutation Res* **195**: 79–80, 1988.
33. Moore BE and Bose HRJ, Expression of the v-*rel* oncogene in reticuloendotheliosis virus-transformed fibroblasts. *Virology* **162**: 377–387, 1988.
34. Sylla BS and Temin HM, Activation of oncogenicity of the c-*rel* proto-oncogene. *Mol Cell Biol* **6**: 4709–4716, 1986.
35. Liptay S, Schmid RM, Perkins ND, Meltzer P, Altherr MR, McPherson JD, Wasmuth JJ and Nabel GJ, Related subunits of NF-kappa B map to two distinct loci associated with translocations in leukemia, NFKB1 and NFKB2. *Genomics* **13**: 287–292, 1992.
36. Lu D, Thompson JD, Gorski GK, Rice NR, Mayer MG and Yunis JJ, Alterations of the *rel* locus in human lymphoma. *Oncogene* **6**: 1235–1241, 1991.
37. Mathew S, Murty VVVS, Dalla-Favera R and Chaganti RSK, Chromosomal localization of genes encoding the transcription factors c-*rel*, NF-κBp50, NF-κBp65 and *lyt*-10 by fluorescence *in situ* hybridization. *Oncogene* **8**: 191–193, 1993.
38. Narayanan R, Klement JF, Ruben SM, Higgins KA and Rosen CA, Identification of a naturally occurring transforming variant of the p65 subunit of NF-kappa B. *Science* **256**: 367–370, 1992.
39. Ruben S, Poteat H, Tan T-H, Kawakami K, Roeder R, Haseltine W and Rosen CA, Cellular transcription factors and regulation of IL-2 receptor gene expression by HTLV-1 *tax* gene product. *Science* **241**: 89–91, 1988.
40. Ballard DW, Böhnlein E, Lowenthal JW, Wano Y, Franza BR and Greene WC, HTLV-1 tax induces cellular proteins that activate the κB element in the IL-2 receptor α gene. *Science* **241**: 1652–1655, 1988.
41. Kitajima I, Shinohara T, Bilakovics JDAB, Xu X and Nerenberg M, Ablation of transplanted HTLV-I Tax-transformed tumors in mice by antisense inhibition of NF-κB. *Science* **258**: 1792–1795, 1992.
42. Ghersa P, Hooft van Hiujsduinen R, Whelan J and DeLamarter JF, Labile proteins play a dual role in the control of Endothelial Leukocyte Adhesion Molecule-1 (ELAM-1) gene regulation. *J Biol Chem* **267**: 19226–19232, 1992.
43. Iademarco MF, McQuillan JJ, Rosen GD and Dean DC, Characterization of the promoter for vascular cell adhesion molecule-1 (VCAM-1). *J Biol Chem* **267**: 16323–13329, 1992.
44. Voraberger G, Schäfer R and Stratowa C, Cloning of the human gene for intercellular adhesion molecule 1 and analysis of its 5′-regulatory region. *J Immunol* **147**: 2777–2786, 1991.
45. Whelan J, Ghersa P, Hooft van Huijsduijnen R, Gray J, Chandra G, Talabot F and DeLamarter JF, An NF-κB-like factor is essential but not sufficient for cytokine induction of endothelial leukocyte adhesion molecule 1 (ELAM-1) gene transcription. *Nucleic Acids Res* **19**: 2645–2653, 1991.
46. Sato H and Seiki M, Regulatory mechanisms of 92 kDa type IV collagenase gene expression which is associated with invasiveness in tumor cells. *Oncogene* **8**: 395–405, 1993.
47. Narayanan R, Higgins KA, Perez JR, Coleman TA and Rosen CA, Evidence for differential functions of the p50 and p65 subunits of NF-κB with a cell adhesion model. *Mol Cell Biol* **13**: 3802–3810, 1993.
48. Plaskin D, Baeuerle PA and Eisenbach L, KBF1 (p50 NF-κB homodimers) acts as a repressor of H-2K gene expression in metastatic tumor cells. *J Exp Med* **177**: 1651–1662, 1993.
49. Glisin V, Crkvenjakov R and Byus C, Ribonucleic acid isolated by cesium chloride centrifugation. *Biochemistry* **13**: 2633–2637, 1974.
50. Brown K, Park S, Kanno T, Franzoso G and Sibenlist U, Mutual regulation of the transcriptional activator NF-κB and its inhibitor, IκB-α. *Proc Natl Acad Sci USA* **90**: 2532–2536, 1993.
51. Baeuerle PA and Baltimore D, IκB: a specific inhibitor of the NF-κB transcription factor. *Science* **242**: 540–546, 1988.
52. Beg AA, Ruben SM, Scheinman RI, Haskill S, Rosen CA and Baldwin ASJ, IκB interacts with the nuclear localization sequences of the subunits of NF-κB: a mechanism for cytoplasmic retention. *Gene Dev* **6**: 1899–1913, 1992.
53. Schmitz ML, Henkel T and Baeuerle PA, Proteins controlling the nuclear uptake of NF-κB, Rel and dorsal. *Trends Cell Biol* **1**: 130–137, 1991.
54. Zabel U, Henkel T, Dos Santo Silva M and Baeuerle PA, Nuclear uptake control of NF-κB by MAD-3, an IκB protein present in the nucleus. *EMBO J* **12**: 201–211, 1993.
55. Sun S-C, Ganchi PA, Ballard DW and Greene WC, NF-κB controls expression of inhibitor IκBα: evidence for an inducible autoregulatory pathway. *Science* **259**: 1912–1915, 1993.
56. Beg AA, Finco TS, Nantermet PV and Baldwin ASJ, Tumor necrosis factor and interleukine-1 lead to phosphorylation and loss of IκBα: a mechanism for NF-κB activation. *Mol Cell Biol* **13**: 3301–3310, 1993.
57. Link E, Kerr LD, Schreck R, Zabel U, Verma I and Baeuewrle PA, Purified I kappa B-beta is inactivated upon dephosphorylation. *J Biol Chem* **267**: 239–246, 1992.
58. Ghosh S and Baltimore D, Activation *in vitro* of NF-κB by phosphorylation of its inhibitor IκB. *Nature* **344**: 678–682, 1990.
59. Fujiita T, Nolan GP, Liou H-C, Scott ML and Baltimore D, The candidate proto-oncogene *bcl3* encodes a transcriptional coactivator that activates through NF-κB p50 homodimers. *Gene Dev* **7**: 1354–1363, 1993.
60. Morin PJ and Gilmore TD, The C terminus of the NF-kappa B p50 precursoe and an I kappa B isoform contain transcription activation domains. *Nucleic Acids Res* **25**: 2453–2458, 1992.