PERIODICUM BIOLOGORUM
VOL. 113, No 1, 1–6, 2011

UDC 57:61
CODEN PDBIAD
ISSN 0031-5362

Spiridion Brusina lecture

Interplay between ubiquitin networks and NF-κB signaling

JANOŠ TERZIĆ[1]
IVAN ĐIKIĆ[1,2]

[1]Department of Immunology and Medical Genetics, University of Split, School of Medicine, Šoltanska 2, 21 000 Split, Croatia
E-mail: janos.terzic@mefst.hr

[2]Frankfurt Institute for Molecular Life Sciences and Institute of Biochemistry II Goethe University School of Medicine Theodor-Stern-Kai 7, D-60590 Frankfurt (Main), Germany
E-mail: Ivan.Dikic@biochem2.de

Key words: ubiquitin, NF-κB

Received March 14, 2011.

Abstract

NF-κB transcription factors were discovered 25 years ago and since then they have been in the focus of biomedical research. Their important role in immune system development is recognized and NF-κB is becoming more attractive because of its role in cancer biology. Understanding the molecular mechanisms controlling NF-κB activity appear important as possible therapeutic targets of numerous human diseases. Here we summarize how ubiquitin networks control the NF-κB pathway and specifically focus on recent findings implicating linear ubiquitin chains as critical components in this process.

The ubiqutin system

Ubiquitin is highly conserved and ubiquitously expressed, 76 amino acid long protein present in all eukaryotic cells. Its C-terminal end can be covalently attached to other proteins in a stepwise enzymatic reaction called ubiquitination. Ubiquitination is catalyzed by three enzymes E1 (ubiquitin-activating enzyme), E2 (ubiquiting-conjugating enzyme) and E3 (ubiquitin ligase) *(5, 25, 31, 40)*. E1 hydrolyses ATP and transfers activated ubiquitin to E2. Once charged, E2 enzyme transfers ubiquitin to E3 ligase, which facilitates ligation of ubiquitin carboxyl terminus to the amino terminus of lysine (K) residue of substrate. Enormous potential of biological processes regulation by ubiquitin system can be viewed through number of genes involved in enzymatic reactions underlying conjugation of ubiquitin to substrate proteins; there are only two E1 genes, around 50 E2 genes and over 600 known E3 genes. In addition, there are over 100 enzymes that are removing conjugated ubiquitin from protein substrates named deubiquitinases (DUB's) *(37)*. Furthermore, the involvement of ubiquitin in numerous cellular processes is additionally complexed through existence of more than 20 different structural families of ubiquitin-binding domains (UBD) that are recognizing ubiqutinated proteins *(6)*. All above options opens many possibilities for fine-tuning and precise regulation of diverse biological networks ranging from signal transduction, protein activation and degradation, to developmental processes and cancer.

Seven lysines in ubiquitin (K6, K11, K27, K29, K33, K48 and K63) are enabling the formation of different ubiquitin chains, e.g. K48 or K63 chains, through conjugation of one ubiquitin to another via lysine linkage that then mediate different functions *(15)*. Moreover, the formation of non-lysine-linked chains where amino terminus of one ubiquitin is bound to carboxyl terminus of another to form linear ubiquitin chain has been recently described. Signal transmission in

cells can also be achieved through the attachment of a single ubiquitin to the target molecule in the process known as monoubiquitination *(15)*. The type of ubiquitin modification of the target is mainly determined by E2 and E3 enzymes. For example, E2 enzyme UBC13 together with E2 variant UEV1 and TRAF3 create K63--polyubiquitin chains, while UCB5/ROC1/$SCF^{\beta TrCP}$ complex promotes formation of K48 chains *(36)*. Different types of chains are recognized by specific ubiquitin binding domains (UBDs) that serve as receptors thus determining fate of ubiquitinated protein *(6)*, e.g. proteasomal Rpn13 ubiquitin associated UBA-domain binds K48 chains labeling substrates for degradation via proteasome, while UBAN domain of NEMO binds K63 chains and promotes signaling via NF-κB *(15)*.

Similar to phosphorylation, ubiquitin modifications on target proteins can be removed by deubiquitinating enzymes (DUBs), a papain-like cysteine proteases or metalloproteases *(37, 38)*. DUBs are critical regulators in ubiqutin system, which is nicely demonstrated by genetic ablation of deubiquitinase A20 that results in excessive inflammation in many organs due to unlocking of NF-κB activity; its alterations are found in lymphomas and autoimmune disorders *(19, 21)*. The same applies for another NF-κB DUB regulator – CYLD which is clearly demonstrating that ubiquitination and deubiquitination cycle in NF-κB pathway is important for cellular homeostasis. Ubiquitin system within itself has potential of initiating numerous cellular processes by ubiqutinating particular substrates as well as controlling the same process by deubiqutination. If we consider hundreds of different enzymes involved in a ubiquitination process, hundred of different DUBs, many different ubiquitin chains and many different ubiquitin binding domains, it is not hard to imagine the complexity and specificity by which processes in our cells are controlled through ubiquitin system *(14)*.

The NF-κB signaling pathway

NF-κB transcription factors were described 25 years ago *(32)* as a proteins that bind to the regulatory region of antibody kappa light chain gene. There are five NF-κB transcription factors (the NF-κB family) and they include: RelA (p65), RelB, c-Rel, NF-κB1 (p50; p105), NF-κB2 (p52;p100) *(22)*. As a transcription factor, all NF-κB proteins possess DNA binding, dimerization and nuclear localization domains (NLD) located within Rel homology domain (RHD). Some of them, RelA, RelB and c-Rel, also have transcriptional activation domain (TAD), while others (NF-κB1, NF-κB2) make dimers with TAD-possessing and rely on their TAD to positively regulate gene transcription. The NF-κB1 and NF-κB2 transcription factors are secreted as precursors (p105 and p100) and are proteolyticaly processed into smaller, transcriptionaly active units. The NF-κB transcription factors act as dimers and among 15 different possibilities, 12 dimers bind to DNA, with p65/p50 dimer being most commonly employed *(13)*. This pathway is centered on trimeric IKK (IκB kinase) complex containing two catalytic units IKKα and IKKβ and one regulatory subunit IKKγ also called NEMO (NF-κB essential modulator). After activation, IKKβ subunit phosphorylates I-κBα on Ser32 and Ser36, which is followed by its ubiquitination at K19 by K48-linked ubiquitin chains. Phosphorylated and ubiquitinated I-κBα is degraded by proteasome, while NF-κB dimer p65/p50 is free to enter nucleus and activate transcription of their target genes. Upon nuclear translocation, NF-κB transcription factors bind to the regulatory unit of their target genes which includes proinflammatory cytokines (TNF-α, IL-6, IL-1β, BAFF), survival genes ($Bcl\text{-}X_L$), adhesion molecules (VCAM, ICAM-1, E-selectin), chemokines (IL-8, RANTES, BLC), enzymes like iNOS and COX-2 and others *(18, 24)*. Such diversity of NF-κB target genes suggests its possible involvement in many biological processes including immune system development, inflammatory response and control of cell differentiation as well as cancer development.

The main mechanism of controlling NF-κB activity is to hold NF-κB transcription factors in the cytoplasm bound to their inhibitors IκB (inhibitors of κB) *(32)*. There are six different IκB's; α, β, ε, δ, ζ and Bcl-3. They are characterized by several ankyrin repeats that mediate their binding to different NF-κB dimers. C-terminal end of NF-κB1 and NF-κB2 carry an ankyrin repeat that allows them to serve an IκB-like function *(36)*. The best understood member of IκB family is IκBα that controls the activation of classical p65/p50 dimer. Upon TNF-α or lipopolysaccharide (LPS) stimulation, IκBα undergoes rapid degradation in proteasome, liberating p65/p50 dimer to be shifted to the nucleus in order to activate its target genes, among which is gene IκBα itself. Newly synthesized IκBα goes to the nucleus where is associated with DNA-bound p65/p50 dimer and brings it back to cytoplasm thus, by forming negative feedback loop, terminates its action *(4)*. This is common pattern of IκB's action and other inhibitors (β and ε) show similar but slower kinetics then IκBα or may have different functions (IκBζ and Bcl-3) *(36)*. In order to allow NF-κB dimers to translocate to the nucleus, their inhibitors – IκB's need to be degraded. Usually, upon cellular stimulation, IκB's are modified (phosphorylated and ubiqutinated) and degraded by proteasome thus liberating NF-κB transcription factors and allowing their nuclear accumulation.

NF-κB activation can be achieved in classical (canonical) and alternative (non-canonical) pathway. Classical NF-κB pathway is activated after TNF-α, IL-1β or LPS stimulation, while alternative pathway is started after engagement of CD40 ligand with CD40. Binding of potent proinflammatory cytokine TNFα to its receptor TNFR1 induces receptor trimerization and recruits adaptor protein TRADD (TRNFR1-associated DEATH domain) to the receptor. Other proteins, like RIP1 (receptor-interacting protein kinase 1), TRAF2, TRAF5 (TNFR-associated factor) and E3 ubiquitin ligase cIAP (that ubiquitinates RIP1) join receptor and together with TRADD form complex 1. cIAP polyubiquitinates RIP1 and is being polyubiquitinated. Polyubiquitin chains on RIP1 are binding

TAB2, which mediates recruitment and activation of TAK1 (TGF-b activated kinase 1), while ubiquitin chains on cIAP are binding proteins HOIL-1 and HOIP that additionally stabilize *complex 1* and are recruiting and activating IKK. HOIL-1 and HOIP form LUBAC (Linear Ubiquitin Assembly Complex) that together with our recently discovered member of LUBAC complex – SHARPIN (Figure 1) are capable of introducing linear ubiqutin-modification on NEMO and subsequent activation of NF-κB *(14)*. Activated IKK leads to phosphorylation and degradation of IκBα followed by nuclear translocation of NF-κB transcription factors. Following NF-κB activation, TRADD, RIP1 and TRAF2 dissociate from the receptor and together with FADD and procaspase-8 form cytosolic, death-inducing, *complex II*. This allows procaspase-8 to mature into caspase-8 that subsequently activates caspase-3 that triggers apoptosis. In most cells, rapid TNFα signaling via complex I induces several anti-apoptotic genes including cIAP, Bcl-xL, cFLIP thus preventing cell apoptosis by various mechanisms. For instance, c-FLIP binds to procaspase-8 and prevents its activation and apoptosis while inhibition of RIP1 polyubiqutination promotes cIAP degradation complex II formation, procaspase-8 activation and apoptosis *(23, 27)*.

Another NF-κB signaling pathway activator is citokine IL-1β that together with LPS potently activate the expression of plethora of pro-inflammatory genes. Receptors for IL-1β – IL1R and LPS – TLR-4 (Toll like receptor) have similar cytoplasmic domain called TIR (Toll like IL1R), which recruit another TIR containing protein to the receptor complex. Adaptor protein MyD88 (myeloid differentiation primary gene 88) is attracted to activated receptor IL1R and TLR-4 and in turn recruits two other proteins: IRAK 4 and IRAK 1 (IL-1 Receptor-associated kinase). IRAK1 binds to E3 ligase TRAF6 that catalyze K63 ubiqutination of TAB2, TAB3 and NEMO leading to autophosphorylation of kinase TAK1 that is part of the complex *(16)*. Activated TAK1 complex phosphorylates IKKβ that leads to subsequent phosphorylation and ubiquitination of IκBα, targeting it for proteosomal degradation again leading to NF-κB nuclear translocation.

In B cells, NF-κB signaling pathway can be activated through alternative or non-canonical pathway. Following stimulation of CD40 and BAFF-R (B-cell activating factor receptor) receptors three E3 ligases, TRAF2, TRAF3 and cIAP, are recruited to receptor complex. TRAF2 activates cIAP by catalyzing its K63 polyubiquitination, which in turn promotes TRAF3 degradation via K48 polyubiquitination. Without TRAF3, NIK (nuclear factor κB-inducing kinse) kinase is stabilized and is free to phosphorylate NF-κB2 (p52;p100) precursor which is then polyubiquitinated and processed by proteasome into its mature p52 subunit *(39)*. p52 forms a complex with Rel-B and together they translocate to the nucleus to regulate expression of the genes relevant for B cell maturation and activation *(36)*.

Physiological roles played by NF-κB

The NF-κB signaling pathway plays important role in immune system development, inflammation and cancer development. During the process of T-cell maturation, T cells undergo positive and negative selection, in order to eliminate potentially autoreactive, as well as unresponsive, T cells. Although mice lacking different components of NF-κB did not exhibit big developmental abnormalities, $Relb^{-/-}$ mice show severe abnormality in the process of T cell negative selection leading to dermatitis *(2)*. Classical NF-κB signaling activated by pre-B cell receptor is responsible for early stages of B cell development while BAFF-initiated alternative NF-κB signaling is involved in later stages of B cell development. BAFF transgenic mice accumulate large number of B cells in the spleen leading to autoimmunity *(7)*. Alternative NF-κB pathway components NIK and RelB are required for development of marginal zone B cells and proper expression of their integrins *(33)*. Deletion of TRAF2 and TRAF3 impairs early B cell development as well as exhibits abnormalities in peripheral B cells leading to autoimmunity *(9)*. Proper differentiation of natural killer (NK) cells responsible for eradication of infectious agents and transformed cells is dependent on proper NF-κB signaling. Furthermore, cytotoxicity of NK cells is compromised in patients with mutations in NEMO protein *(28, 30)*. Mayority of evidence present is obtained from mouse models and it still partially but nicely underscores important role of NF-κB signaling in normal development of various immune elements. Regulating synthesis of many cytokines involved in inflammatory response, including TNFα, IL-1β, IL-6 and many more, NF-κB signaling pathway is considered to be the most important regulator of adequate inflammatory response to different biological, physical or chemical agents *(36)*.

NF-κB in cancer

Physiological activation of NF-κB signaling leads to activation of a group of genes regulating basic cellular properties like proliferation, survival, involvement in inflammatory response and resistance to infection. De-regulation of those processes can result in uncontrollable cell division, resistance to apoptosis or chronic inflammation, which are characterizing malignant growth. First clue that NF-κB can play important role in cancer came from sequencing of RelA (p65) that instantly suggested the close relationship to its oncogenic homologue v-Rel *(10)*. It seems that NF-κB supports cancer development in two ways. Firstly, in lymphoid malignancies NF-κB pathway can gain oncogenic mutation and secondly, in most solid tumors NF-κB is activated without any mutations in itself, but its activity is creating inflammatory microenvironment through up-regulation of proliferative (IL-6, TNF-α) and anti-apoptotic ($Bcl\text{-}X_L$) cytokines *(17, 18)*. Initial search for oncogenic NF-κB mutations led to discovery of Bcl-3 (the IκB member) activation by chromosomal translocation *(8)* and to a discovery of activated NF-κB2 gene due to its gene rearrangement in B and T cell lymphomas *(26)*. After this initial success,

only a few more NF-κB mutations were found including mutation in IκBα gene in Hodgkin's lymphoma *(3)*. Multiple myeloma is another lymphoid malignancy associated with high NF-κB activity. Extensive search for genetic abnormalities in this condition revealed mutations in many genes whose proteins are part of NF-κB signaling cascade, this include NF-κB1, NF-κB2, TRAF2, cIAP, CYLD and NIK with the most common abnormality of TRAF3 inactivation *(20)*.

Although mutations in NF-κB pathway are very rare in solid tumors, it's increased activity is detected in almost all solid tumors. Chronic inflammation accompanied with high NF-κB activity is making fertile soil for tumor initiation and development. Furthermore, inflammatory processes are often accompanying the tumors that were not initiated on inflammatory background but it is shown that it supports tumor growth and metastasis *(1, 34)*. It is well known that chronic inflammatory bowel disease (IBD) are predisposing factors to colon cancer development and more then 20% IBD patients will develop colon cancer 30 years after IBD diagnosis *(35)*. By conditional ablation of IKKβ in mouse intestinal epithelial cells, it was demonstrated that NF-κB signaling is playing key role in colon tumorigenesis *(11)*. Our and other recent work shows that elevated NF-κB activity, either present in IBD or accompanying sporadic or genetic colon cancer, is driving high expression of IL-6 and

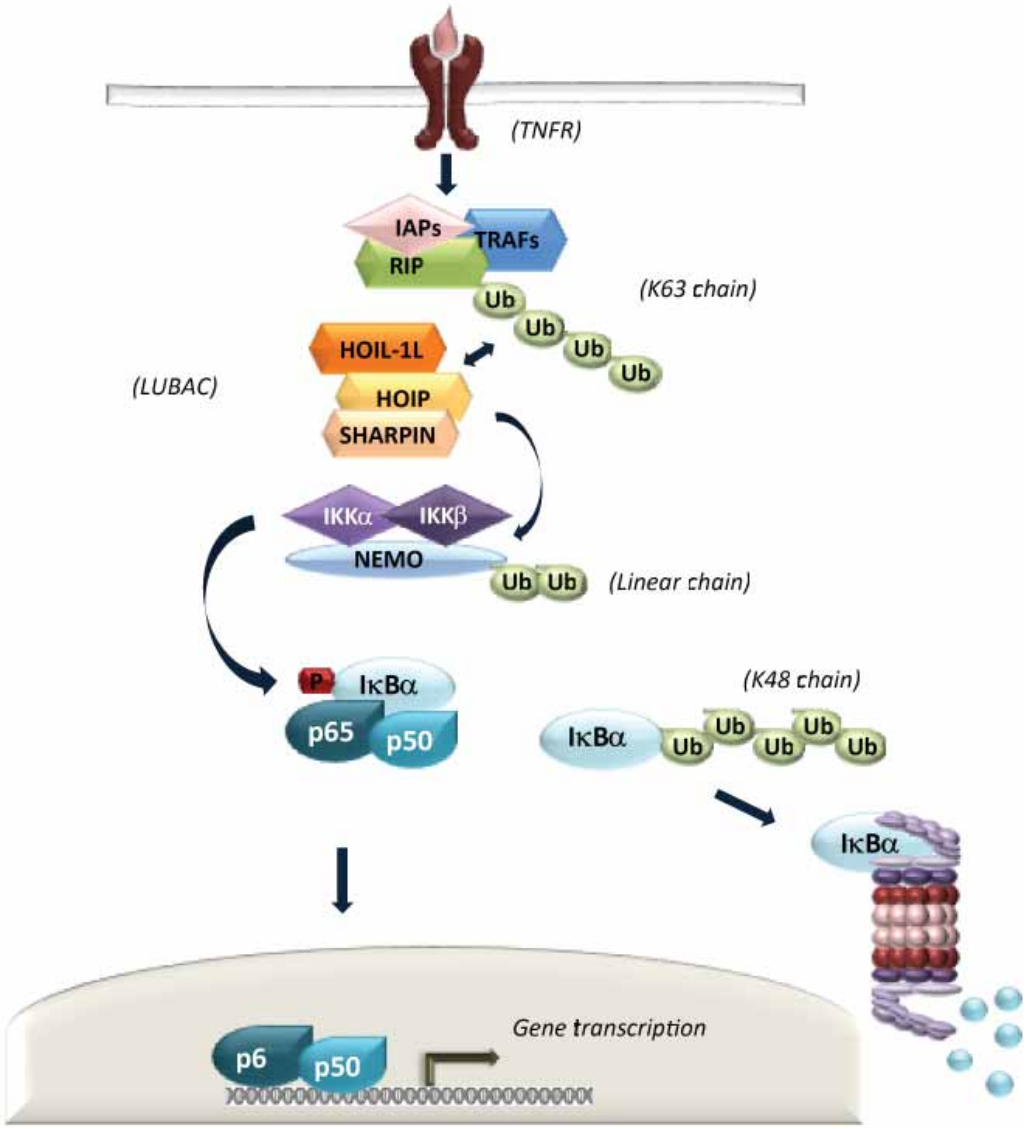

Figure 1. *Activation of the NF-κB pathway via ubiquitin signals. Activated cell surface receptors, such as TNF receptor, recruit a number of molecules with scaffolding and catalytic activities that are ubiquitinated by K63 ubiquitin chains. This leads to the increased complex formation with the LUBAC that creates linear ubiquitin chain on NEMO and subsequent trans binding of NEMO molecules and activation of the IKK. Following activation, the IKKs phosphorylate the inhibitory IκBα protein, subsequently triggering the Lys48-dependent proteasomal degradation of IκBα. Released from its inhibitor, p65/RelA (NF-κB) dimers translocate into the nucleus and activate transcription of genes promoting cell survival and immune responses.*

TNF-α which in turn stimulate proliferation and survival of tumor cells *(12, 29)*. In other solid tumors (hepatocellular, prostate, breast and others) mechanism by which NF-κB activation exerts its pro-tumorigenic potential is different but high NF-κB activity in tumor and accompanying immune cells is equally important *(18)*.

High NF-κB activity is one of the main denominators of cancer development, which is opening possibility to use pharmacological means to alter its pro-tumorigenic potential either by directly targeting its component or by influencing their ubiquitin or phosphate modifications. It is reasonable to expect that future will bring manipulations of NF-κB signaling pathway as a part of anti-tumor strategy as well as some surprises in mechanisms regulating this pathway.

Acknowledgements: *We apologize to all scientists whose important contribution was not referenced in this review due to space limitations. Research in the J.T. laboratory is supported by Ministry of Science, Education and Sport of the Republic of Croatia. Research in the I.D. laboratory is supported by the Deutsche Forschungsgemeinschaft, the Cluster of Excellence «Macromolecular Complexes« of the Goethe University Frankfurt (EXC115) and European Research Council Advanced Grant. We thank Ivana Novak for critical reading of the manuscript.*

REFERENCES

1. BALKWILL F, MANTOVANI A 2001 Inflammation and cancer: back to Virchow? *Lancet 357*: 539–545
2. BARTON D, HOGENESCH H, WEIH F 2000 Mice lacking the transcription factor RelB develop T cell-dependent skin lesions similar to human atopic dermatitis. *Eur J Immunol 30:* 2323–2332
3. CABANNES E, KHAN G, AILLET F, JARRETT R F, HAY R T 1999 Mutations in the IkBa gene in Hodgkin's disease suggest a tumour suppressor role for IkappaBalpha. *Oncogene 18*: 3063–3070
4. CHEN L, FISCHLE W, VERDIN E, GREENE W C 2001 Duration of nuclear NF-kappaB action regulated by reversible acetylation. *Science 293*: 1653–1657
5. DESHAIES R J, JOAZEIRO C A 2009 RING domain E3 ubiquitin ligases. *Annu Rev Biochem 78:* 399–434
6. DIKIC I, WAKATSUKI S, WALTERS K J 2009 Ubiquitin-binding domains – from structures to functions. *Nat Rev Mol Cell Biol 10*: 659–671
7. ENZLER T, BONIZZI G, SILVERMAN G J, OTERO D C, WIDHOPF G F, ANZELON-MILLS A, RICKERT R C, KARIN M 2006 Alternative and classical NF-kappa B signaling retain autoreactive B cells in the splenic marginal zone and result in lupus-like disease. *Immunity 25*: 403–415
8. FRANZOSO G, BOURS V, PARK S, TOMITA-YAMAGUCHI M, KELLY K, SIEBENLIST U 1992 The candidate oncoprotein Bcl-3 is an antagonist of p50/NF-kappa B-mediated inhibition. *Nature 359*: 339–342
9. GARDAM S, SIERRO F, BASTEN A, MACKAY F, BRINK R 2008. TRAF2 and TRAF3 signal adapters act cooperatively to control the maturation and survival signals delivered to B cells by the BAFF receptor. *Immunity 28*: 391–401
10. GILMORE T D 2003 The Re1/NF-kappa B/I kappa B signal transduction pathway and cancer. *Cancer Treat Res 115*: 241–265
11. GRETEN F R, ECKMANN L, GRETEN T F, PARK J M, LI Z W, EGAN L J, KAGNOFF M F, KARIN M 2004 IKKbeta links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. *Cell 118*: 285–296
12. GRIVENNIKOV S, KARIN E, TERZIC J, MUCIDA D, YU G Y, VALLABHAPURAPU S, SCHELLER J, ROSE-JOHN S, CHEROUTRE H, ECKMANN L *et al.* 2009 IL-6 and Stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. *Cancer Cell 15*: 103–113.
13. HOFFMANN A, NATOLI G, GHOSH G 2006 Transcriptional regulation via the NF-kappaB signaling module. *Oncogene 25*: 6706–6716
14. IKEDA F, CROSETTO N, DIKIC I What determines the specificity and outcomes of ubiquitin signaling? *Cell 143*: 677–681
15. IKEDA F, DIKIC I 2008 Atypical ubiquitin chains: new molecular signals. 'Protein Modifications: Beyond the Usual Suspects' review series. *EMBO Rep 9*: 536–542
16. KANAYAMA A, SETH R B, SUN L, EA C K, HONG M, SHAITO A, CHIU Y H, DENG L, CHEN Z J 2004 TAB2 and TAB3 activate the NF-kappaB pathway through binding to polyubiquitin chains. *Mol Cell 15*: 535–548
17. KARIN M 2006 Nuclear factor-kappaB in cancer development and progression. *Nature 441*: 431–436
18. KARIN M 2009 NF-kappaB as a critical link between inflammation and cancer. *Cold Spring Harb Perspect Biol 1*: a000141
19. KATO M, SANADA M, KATO I, SATO Y, TAKITA J, TAKEUCHI K, NIWA A, CHEN Y, NAKAZAKI K, NOMOTO J *et al.* 2009 Frequent inactivation of A20 in B-cell lymphomas. *Nature 459*: 712–716
20. KEATS J J, FONSECA R, CHESI M, SCHOP R, BAKER A, CHNG W J, VAN WIER S, TIEDEMANN R, SHI C X, SEBAG M *et al.* (2007). Promiscuous mutations activate the noncanonical NF-kappaB pathway in multiple myeloma. *Cancer Cell 12*: 131–144
21. LEE E G, BOONE D L, CHAI S, LIBBY S L, CHIEN M, LODOLCE J P, MA A 2000 Failure to regulate TNF-induced NF--kappaB and cell death responses in A20-deficient mice. *Science 289*: 2350–2354
22. I Q, VERMA I M 2002 NF-kappaB regulation in the immune system. *Nat Rev Immunol 2*: 725–734
23. LIU S, CHEN Z J Expanding role of ubiquitination in NF-kappaB signaling. *Cell Res 21*: 6–21
24. LUO J L, KAMATA H, KARIN M 2005 IKK/NF-kappaB signaling: balancing life and death-a new approach to cancer therapy. *J Clin Invest 115*: 2625–2632
25. NAGY V, DIKIC I Ubiquitin ligase complexes: from substrate selectivity to conjugational specificity. *Biol Chem 391*: 163–169
26. NERI A, CHANG C C, LOMBARDI L, SALINA M, CORRADINI P, MAIOLO A T, CHAGANTI R S, DALLA-FAVERA R 1991 B cell lymphoma-associated chromosomal translocation involves candidate oncogene lyt-10, homologous to NF-kappa B p50. *Cell 67*: 1075–1087
27. O'DONNELL M A, LEGARDA-ADDISON D, SKOUNTZOS P, YEH W C, TING A T 2007 Ubiquitination of RIP1 regulates an NF-kappaB-independent cell-death switch in TNF signaling. *Curr Biol 17*: 418–424
28. ORANGE J S, BRODEUR S R, JAIN A, BONILLA F A, SCHNEIDER L C, KRETSCHMER, R, NURKO S, RASMUSSEN W L, KOHLER J R, GELLIS S E *et al.* 2002 Deficient natural killer cell cytotoxicity in patients with IKK-gamma/NEMO mutations. *J Clin Invest 109*: 1501–1509
29. POPIVANOVA B K, KITAMURA K, WU Y, KONDO T, KAGAYA T, KANEKO S, OSHIMA M, FUJII C, MUKAIDA N 2008 Blocking TNF-alpha in mice reduces colorectal carcinogenesis associated with chronic colitis. *J Clin Invest 118*: 560–570
30. SAMSON S I, MEMET S, VOSSHENRICH C A, COLUCCI F, RICHARD O, NDIAYE D, ISRAEL A, DI SANTO J P 2004) Combined deficiency in IkappaBalpha and IkappaBepsilon reveals a critical window of NF-kappaB activity in natural killer cell differentiation. *Blood 103*: 4573–4580
31. SCHULMAN B A, HARPER J W 2009 Ubiquitin-like protein activation by E1 enzymes: the apex for downstream signalling pathways. *Nat Rev Mol Cell Biol 10*: 319–331
32. SEN R, BALTIMORE D 1986 Inducibility of kappa immunoglobulin enhancer-binding protein Nf-kappa B by a posttranslational mechanism. *Cell 47*: 921–928
33. SIEBENLIST U, BROWN K, CLAUDIO E 2005 Control of lymphocyte development by nuclear factor-kappaB. *Nat Rev Immunol 5*: 435–445
34. TAN W, ZHANG W, STRASNER A, GRIVENNIKOV S, CHENG J Q, HOFFMAN R M, KARIN M Tumour-infiltrating regulatory T cells stimulate mammary cancer metastasis through RANKL-RANK signalling. *Nature 470*: 548–553

35. TERZIC J, GRIVENNIKOV S, KARIN E, KARIN M Inflammation and colon cancer. *Gastroenterology 138*: 2101–2114 e2105
36. VALLABHAPURAPU S, KARIN M 2009 Regulation and function of NF-kappaB transcription factors in the immune system. *Annu Rev Immunol 27*: 693–733
37. WERTZ I E, DIXIT V M Signaling to NF-kappaB: regulation by ubiquitination. *Cold Spring Harb Perspect Biol 2*: a003350
38. WILKINSON K D 2009 DUBs at a glance. *J Cell Sci 122*: 2325–2329
39. XIAO G, HARHAJ E W, SUN S C 2001 NF-kappaB-inducing kinase regulates the processing of NF-kappaB2 p100. *Mol Cell 7*: 401–409
40. YE Y, RAPE M 2009 Building ubiquitin chains: E2 enzymes at work. *Nat Rev Mol Cell Biol 10*: 755–764