Hypothesis

At the crossroads of SUMO and NF-κB

Martin P Kracklauer*1 and Christian Schmidt*2

Address: 1Section of Molecular Cell and Developmental Biology, Institute for Cellular and Molecular Biology, The University of Texas at Austin, 1 University Station, A4800, 78712, Austin, TX, USA and 2Department of Surgical Oncology and Molecular Oncology, University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, Texas 77030, USA

Email: Martin P Kracklauer* - mordechai30@hotmail.com; Christian Schmidt* - christian.schmidt@molecular-cancer.org

* Corresponding authors

Abstract

Background: Recognition of pathogens by immune receptors leads to activation of macrophages, dendritic cells, and lymphocytes. Signals are communicated to enhance expression of target molecules such as cytokines and adhesion molecules, depending on activation of various inducible transcription factors, among which the family NF-κB transcription factors plays an evolutionarily conserved and critical role. Classical activation of NF-κB involves phosphorylation, polyubiquitination and subsequent degradation of the inhibitor molecules of NF-κB, referred to as IκB. Modification of IκBα, one of the mammalian IκB isoforms, with the small ubiquitin-like modifier (SUMO) results its protection from degradation.

Presentation of the hypothesis: SUMO-IκBα localizes in the nucleus. The nuclear SUMO-IκBα pool may be dynamic. SUMO-IκBα functions as synergy control factor.

Testing the hypothesis: Immunoprecipitation from cellular fractions, 35S methionine pulse-chase, and FRET assays should reveal the localization of SUMO-IκBα and the dynamics of the pool. Expression of SUMOylation defective IκBα in an IκBα-/- background should yield insights into the function of SUMO-IκBα.

Implication of the hypothesis: IκBα contains the required SUMOylation motif but IκBβ does not. The suggested study would provide evidence whether or not IκBα and IκBβ can substitute each other. In addition, the suggested assays would reveal a possible redundancy in controlling transcriptional activity of NF-κB.

Background

For nearly two decades, the NF-κB activation pathway has been the focus of experimental investigation. This pathway is highly conserved among metazoans and plays key roles in immune responses [1], cell proliferation [2], inflammation [3], apoptosis [4], early embryonic development [5], and many other processes.

Five members of mammalian NF-κB are described: NF-κB1 (p50 and its precursor p105), NF-κB2 (p52 and its precursor p100), c-Rel, RelA (p65) and RelB [6–9], each of which has a 300-residue Rel homology domain (RHD) [1,10–14]. The C-terminal domains are responsible for dimerization with other Rel proteins, but sequence-spe-
specific interactions come primarily from loops in the N-terminal domain [15].

Interaction of c-Rel, RelA and RelB with its inhibitors, referred to as IκB, results in inactive complexes in the cytoplasm by masking the nuclear localization signal, which is located at the C terminal end of the Rel homology domain [1,10–14]. Currently, the inhibitor proteins IκBα, IκBβ, IκBγ, IκBε, Bcl-3, and the Drosophila protein Cactus are described and characterized [1,10–14].

In most cell types, NF-κB proteins are sequestered in the cytoplasm in an inactive form through their non-covalent association with the inhibitor IκB [7]. This association masks the nuclear localization signal of NF-κB, thereby preventing NF-κB nuclear translocation and DNA binding activity [16]. NF-κB is activated through complex signaling cascades that are integrated by activation of IκB kinase complex (IKK) [17–21], which phosphorylates IκB bound to NF-κB complexes as its substrates [22]. Consequently, NF-κB proteins are translocated into the nucleus, where they activate transcription of their target genes [11,12]. One of the key target genes regulated by NF-κB is its inhibitor IκBα. A feedback inhibition pathway for control of IκBα gene transcription and down-regulation of transient activation of NF-κB activity is described [23–25].

Signal-induced degradation of IκB family members has traditionally been considered to regulate NF-κB activity. However, accumulating evidence suggests that NF-κB activity is also controlled by transcriptional repression mediated by Rel-family members [26], phosphorylation of RelA [27–29], NF-κB [30], and interaction of RelA and NF-κB with histone deacetylases (HDACs) [31–33]. In addition, modification of Dorsal by Smt3, the Drosophila melanogaster orthologs of NF-κB and SUMO-1, respectively, has been demonstrated to regulate Dorsal-mediated activation [34]. Indeed, modification of proteins at lysine residues by ubiquitination, SUMOylation and acetylation has been suggested as a means by which control of transcriptional responses in general can be fine-tuned [35].

Small ubiquitin-like modifier (SUMO) is the best-characterized member of a growing family of ubiquitin-related proteins. SUMO is conjugated to target proteins using an enzyme conjugation system similar to that of ubiquitin [36,37]. The targets of SUMOylation suggest roles for protein SUMOylation in TNFR-mediated apoptosis [38] and, paradoxically, protection from cell death [39]; nucleocytoplasmic transport [40–44] and subnuclear [45] localization; and modulation of transcription factor activity [46,47].

Among the perhaps more intriguing targets for SUMO modification is IκBα. Polyubiquitination of IκBα chiefly at lysine residues 21 and 22 targets it for proteasomal degradation, whereas SUMOylation at lysine 21 appears to protect IκBα from proteasome-mediated degradation [48]. Furthermore, current data suggest that SUMOylation of IκBα is an event that occurs in the nucleus, as pyruvate kinase-IκBα fusion protein constructs that lack a functional NLS fail to be modified by SUMO [49].

Presentation of the hypothesis
Desterro et al showed that a modified form of IκBα is found in whole cell extracts from HEK 293, COS7, Jurkat, and HeLa cell lines. Reciprocal immunoprecipitation experiments with anti-IκBα and anti-SUMO-1 antibodies demonstrated that IκBα is SUMOylated. Further experimentation revealed that the level of SUMO-IκBα in whole cell lysates from TNFα-stimulated COS7 cells remains constant over time, whereas levels of unmodified IκBα diminish and are replenished over time, consistent with a previous study [23].

In parallel, cellular fractionation was carried out on these cell lines. Cytoplasmic extracts showed little to no SUMO-IκBα. Roff et al suggest that SUMO-IκBα is located in the nucleus [50]. Supporting these data are data by Rodriguez et al that suggest that IκBα retained in the nucleus after LMB treatment is impervious to signal-induced degradation [52].

We therefore hypothesize that SUMO-IκBα localizes in the nucleus, creating a nuclear pool of SUMO-IκBα, which functions as synergy control factor.

Testing the hypothesis
Data from reporter gene assays suggest a role for SUMO-IκBα in inhibiting NF-κB-dependent transcription [48]. What may however be complicating assessment of function is the fact that the reporter gene data derive from cells in which SUMO is overexpressed. Given that little free SUMO-1 is detectable in mammalian cells [53], and that other members of the NF-κB pathway can crosstalk with Ubc9, the SUMO E2 conjugating enzyme [54], overexpression of SUMO-1 may result in phenotypes that do not correspond to in vivo situations. Moreover, all extant data regarding SUMO-IκBα stem from transformed and/or oncogenic cell lines, in which NF-κB activity cannot necessarily be assumed normal. To date, no data show the existence of SUMO-IκBα in primary cell lines. The following experiments are proposed in order to assess the presence of SUMO-IκBα in primary cells, and the physiological relevance of SUMO-IκBα.

Dynamics of the SUMO-IκBα pool
Hay et al stated that levels ranging from barely detectable to almost 50% of the total IκBα can be found in the cell types examined in their experiments [48,55]. The cell lines
investigated were HEK 293, COS7, Jurkat and HeLa. In order to establish the extent of IκBα SUMOylation in non-transformed/non-oncogenic cell lines, the following experiments might prove to be of considerable interest, and should not entail significant technical difficulties. From wild-type mice, isolate fibroblast and B- and T-cell populations. These primary cells could be directly used in the subsequent experiments. However, strategies for creating genetically stable, nontumorigenic immortalized cell lines have been described [56,57]. The use of these or comparable strategies for creating immortalized, genetically stable and nontumorigenic fibroblast, B- and T-cell populations may therefore be useful for keeping these primary cells in culture for repeat experiments. With these cell lines in hand, prepare cytoplasmic and nuclear fraction of cells according to Roff et al [50], and whole cell extracts as described by Desterro et al [48]. Reserve some of the whole cell extracts for immunoprecipitation experiments. Fractionate the cytoplasmic, nuclear and whole cell extracts on a denaturing, reducing polyacrylamide gel and transfer to membranes for immunoblot analysis with an anti-IκBα antibody. In parallel, immunoprecipitate with anti-SUMO-1 antibody as described [58], followed by an IκBα Western analysis, and vice versa [48].

Fluorescence resonance energy transfer (FRET) may present an additional means by which a nuclear association of SUMO-1 and IκBα could be demonstrated. CFP-IκBα and SUMO-YFP have both been described [47,59], as has the use of FRET microscopy to assess protein interactions in living cell nuclei [60]. As stated previously, overexpression experiments are potentially problematic due both to interactions and localization that may occur only when proteins are overexpressed. Therefore, the use of a targeted knock-out/knock-in strategy to make CFP-IκBα and SUMO-YFP double mutant mice might provide the specificity needed in this experimental system. The creation of such mice may be time consuming, yet the techniques for making the mice are established.

Two different options suggest potential (and mutually inclusive) roles for the pool in controlling NF-κB responses and in nucleocytoplasmic transport. To address the issue of static vs. dynamic pools of SUMO-IκBα, the following experiment is suggested: A pulse-chase 35Smethionine assay using cytoplasmic, nuclear and whole extracts from cells at time points described above and by Desterro et al [48,61].

Both nucleocytoplasmic trafficking and nuclear translation [62] would result in radiolabeled proteins being found in the nucleus. However, most of these proteins are not expected to associate with IκBα pulled down in the immunoprecipitation. If, contrary to expectation, radiolabeled proteins other than Rel family members are found to associate with IκBα, the likelihood that any of these would have the same molecular weight as SUMO-IκBα is assumed to be manageable small. If no radiolabel is detected at 36 kD, this suggests that the SUMO-IκBα pool does not turn over appreciably. If radiolabel is detected at 36 kDa, this would suggest that, while the total pool of SUMO-IκBα remains constant, this pool turns over. Changing levels of radioactivity would suggest that SUMO-IκBα turns over in the nucleus. This in turn may indicate a key role in nucleocytoplasmic transport to ensure a constant nuclear pool of IκBα.

**SUMO-IκBα – a Synergy Control Factor**

Cheng et al created IκBα/IκBβ knockout/knock-in mice, and no abnormalities in viability or reproduction were observed in these animals [63]. This suggests a functional redundancy of these two IκB family members. However, IκBα is rapidly degraded upon stimuli that trigger NF-κB responses, whereas IκBβ is degraded at a significantly slower rate [64]. In addition, the observation that deletion of IκBα or IκBβ from the genome appears to be compensated by other IκB family members [65,66], but that deletion of IκBα results in neonatal lethality [67], together hint at distinct roles for IκBα and IκBβ in NF-κB responses. Inspection of the amino acid sequence of IκBβ shows it lacks the Ψ KXE amino acid motif characteristic of proteins that are SUMOylated. Therefore, IκBα/IκBβ knockout/knock-in mice may phenocopy mice in which wild-type IκBα is replaced with IκBαK21R, a mutant IκBα for which SUMOylation, but not ubiquitination, is compromised.

The creation of IκBα/IκBβ knockout/knock-in mice indicates that the creation of IκBαK21R mutant mice is feasible. Whether or not such mutant mice phenocopy IκBα/IκBβ knockout/knock-in mice, the viability of the IκBα/IκBβ knockout/knock-in mice might provide the specificity needed in this experimental system. The following suggested experiments should prove to be informative with respect to SUMO-IκBα function: Create IκBα/IκBαK21R mutant mice using established protocols [68,69]. Assess viability. Challenge immune systems of mutant, wild-type and IκBα/IκBβ knockout/knock-in mice as described [70,71], and assess immune responses at the level of the organism. Establish immortalized cell lines from the IκBα/IκBαK21R mutant mice as described above. Stimulate an NF-κB response using TNF-α in fibroblasts and lymphoid immortalized cell lines from IκBαK21R mutant, wild type, and IκBα/IκBβ knockout/knock-in mice and analyze the response.

The Ψ KXE amino acid sequence element required for SUMOylation strongly resembles what is referred to as a synergy control (SC) motif. SC motifs in certain transcription factors (TFs) have been shown to confer onto TFs the property of downregulating transcription at promoters
with multiple binding sites for that TF, while promoters with single TF binding sites are unaffected. When the SC motifs are disrupted, transcription from promoters with multiple sites is enhanced. SUMOylation at TFs at SC motifs has been demonstrated in at least two cases [34,72], suggesting role for SUMOylation in transcriptional control.

Does SUMOylation of IκBα play a role in transcriptional control? A nuclear pool of SUMO-IκBα may help titrate NF-κB and influence transcriptional responses. As stated previously, NF-κB transcriptional responses can be regulated at the level of repression by certain Rel-family homodimers [26], by phosphorylation [27–29], and by interaction with histone deacetylases [31–33]. Therefore, titration of NF-κB by nuclear IκBα, whether SUMOylated or not, may be a redundant mechanism to control NF-κB transcriptional activity. Given the general importance of the NF-κB pathway, redundancy in controlling transcriptional responses by ensuring a nuclear pool of IκBα may be useful.

**Implications of the hypothesis**

IκBα contains the required SUMOylation but IκBβ does not. The suggested study may demonstrate whether or not IκBα and IκBβ can substitute each other. In addition, the suggested assays would reveal a possible redundancy in controlling transcriptional activity of NF-κB.

**Authors’ contributions**

MPK drafted the paper. CS provided suggestions for its finalization. Both authors read and approved the final manuscript.

**Acknowledgements**

CS acknowledges support from the German Research Foundation.

**References**

1. Ghosh S, May MJ and Kopp EB: NF-κB and Rel proteins: evolutionarily conserved mediators of immune responses. *Annu Rev Immunol* 1998, 16:225-260.
2. Luque I and Gelinas C: Rel/NF-κB and IκB factors in oncogenesis. *Semin Cancer Biol* 1997, 8:103-111.
3. Barnes PJ and Karin M: Nuclear factor-κB: a pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med* 1997, 336:1066-1071.
4. Sonenshein GE: Rel/NF-κB transcription factors and the control of apoptosis. *Semin Cancer Biol* 1997, 8:113-119.
5. Reach M, Galindo RL, Towb P, Allen JL, Karin M and Wassermann SA: A gradient of cactus protein degradation establishes dorso-ventral polarity in the Drosophila embryo. *Dev Biol* 1996, 180:353-364.
6. Sen R and Baltimore D: Multiple nuclear factors interact with the immunoglobulin enhancer sequences. *Cell* 1986, 46:705-716.
7. Sen R and Baltimore D: Inducibility of immunoglobulin enhancer-binding protein NF-κB by a posttranslational mechanism. *Cell* 1986, 47:921-928.
8. Lenardo MJ and Baltimore D: NF-κB: a pleiotropic mediator of inducible and tissue-specific gene control. *Cell* 1989, 58:227-229.
9. Lenardo MJ, Fan CM, Maniatis T and Baltimore D: The involvement of NF-κB in β-interferon gene regulation reveals its role as widely inducible mediator of signal transduction. *Cell* 1989, 57:287-294.
10. Siebenlist U, Franzeno G and Brown K: Structure, regulation and function of NF-κB. *Annu Rev Cell Biol* 1994, 10:405-455.
11. Verma IM, Stevenson JK, Schwarz EM, Van Antwerp D and Miyamoto S: Rel/NF-κB/IκB family: intimate tales of association and dissociation. *Genes Dev* 1995, 9:2723-2735.
12. Baldwin AS Jr: The NF-κB and IκB proteins: new discoveries and insights. *Annu Rev Immunol* 1996, 14:649-683.
13. Karin M and Ben-Neriah Y: Phosphorylation meets ubiquitination: the control of NF-κB activity. *Annu Rev Immunol* 2000, 18:621-663.
14. Ghosh S and Karin M: Missing pieces in the NF-κB puzzle. *Cell* 2002, 109(Suppl):S81-S96.
15. Jacobs MD and Harrison SC: Structure of an IκBα/NF-κB complex. *Cell* 1998, 95:749-759.
16. Baeuerle PA and Baltimore D: A 65-kD subunit of active NF-κB is required for inhibition of NF-κB by IκB. *Genes Dev* 1989, 3:1689-1698.
17. Brown K, Gerstberger S, Carlson L, Franzeno G and Siebenlist U: Control of IκBα proteolysis by site-specific, signal-induced phosphorylation. *Science* 1995, 267:1485-1488.
18. Chen ZJ, Parent L and Maniatis T: Identification and characterization of an IκB kinase. *Cell* 1997, 90:373-383.
19. Zandi E, Rothwarf DM, Delhase M, Hayakawa M and Karin M: The IκB kinase complex (IKK) contains two kinase subunits, IKKα and IKKβ, necessary for IκB phosphorylation and NF-κB activation. *Cell* 1997, 91:243-252.
20. DiDonato JA, Hayakawa M, Rothwarf DM, Zandi E and Karin M: A cytokine-responsive IκB kinase that activates the transcription factor NF-κB. *Nature* 1997, 380:548-554.
21. Zandi E, Chen Y and Karin M: Direct phosphorylation of IκB by IKKα and IKKβ: discrimination between free and NF-κB-bound substrate. *Science* 1998, 281:1360-1363.
22. Chiao PJ, Miyamoto S and Verma IM: Autoregulation of IκBα activity. *Proc Natl Acad Sci USA* 1994, 91:28-32.
23. Sun SC, Ganchi PA, Ballard DW and Greene WC: NF-κB controls expression of inhibitor IκBα: evidence for an inducible autoregulatory pathway. *Science* 1993, 259:1912-1915.
24. Rice NR and Ernst MC: In vivo control of NF-κB activation by IκBα. *EMBO J* 1993, 12:4685-4695.
25. May MJ and Ghosh S: Rel/NF-κB and IκBα proteins: an overview. *Semin Cancer Biol* 1997, 8:63-73.
26. Sakurai H, Chiba H, Miyoshi H, Sugita T and Toriumi W: IκBα kinases phosphorylate NF-κB p65 subunit on serine 536 in the transactivation domain. *J Biol Chem* 1999, 274:30353-30356.
27. Wang D, Westerheide SD, Hansson JL and Baldwin AS Jr: Tumor necrosis factor α-induced phosphorylation of RelA/p65 on Ser529 is controlled by casein kinase II. *J Biol Chem* 2000, 275:32592-32597.
28. Zhong H, Voll RE and Ghosh S: Phosphorylation of NF-κB p65 by PKA stimulates transcriptional activity by promoting a novel bivalent interaction with the coactivator CBP/p300. *Mol Cell* 1998, 1:661-671.
29. Koul D, Yao Y, Abbuzzese JL, Yung WK and Reddy SA: Tumor suppressor MMAC/PTEN inhibits cytokine-induced NF-κB activation without interfering with the IκB degradation pathway. *J Biol Chem* 2001, 276:14102-14108.
30. Chen LF, Mu Y and Greene WC: Acetylation of RelA at discrete sites regulates distinct nuclear functions of NF-κB. *EMBO J* 2000, 21:6539-6548.
31. Ashburner BP, Westerheide SD and Baldwin AS Jr: The p65 (RelA) subunit of NF-κB interacts with the histone deacetylase (HDAC) corepressors HDAC1 and HDAC2 to negatively regulate gene expression. *Mol Cell Biol* 2001, 21:12740-12747.
34. Bhaskar V, Smith M and Courrey AJ: Conjugation of Smt3 to dor-sal may potentiate the Droshophilia immune response. Mol Cell Biol 2002, 22:492-499.
35. Freiman RN and Tijan R: Regulating the regulators: lysine mod-iifications make their mark. Cell 2003, 112:11-17.
36. Hochstrasser M: Evolution and function of ubiquitin-like pro-tein-conjugation systems. Nat Cell Biol 2000, 2:E153-157.
37. Melchior F: SUMO – nonclassical ubiquitin. Annu Rev Cell Dev Biol 2000, 16:591-626.
38. Liou ML and Liou HC: The ubiquitin-homology protein, DAP-1, associates with tumor necrosis factor receptor (p60) death domain and induces apoptosis. J Biol Chem 1999, 274:10145-10153.
39. Hanania U, Furman-Matarasso N, Ron M and Avni A: Isolation of a novel SUMO protein from tissue that suppresses EIS-induced cell death. Plant J 1999, 19:533-541.
40. Panse VG, Kuster B, Gerstberger T and Hurt E: Unconventional shuttling of the SUMO family of nuclear acid transport channel of the nuclear pore complex by karyopherins. Nat Cell Biol 2003, 5:21-27.
41. Pichler A and Melchior F: Ubiquitin-related modifier SUMO1 and nucleocytoplasmic transport. Traffic 2002, 3:381-388.
42. Stade K, Vogel F, Schwienhorst I, Meusser B, Volkwein C, Nentwig B, Dohmen RJ and Sommer T: A lack of SUMO conjugation affects cNLS-dependent nuclear protein import in yeast. J Biol Chem 2002, 277:49554-49561.
43. Wood LD, Irvin B, Nuñofira G, Liuc KS and Hibert SW: Small ubiquitin-like modifier conjugation regulates nuclear export of TEL, a rearranged trans virtue suppressor. Proc Natl Acad Sci USA 2003, 100:3257-3262.
44. Girwood D, Bumpass D, Vaughan OA, Thain A, Anderson LA, Snowden AW, Garcia-Wilson E, Perkins ND and Hay RT: SUMO-1 modification represses Sp3 transcriptional activity and modulates its subnuclear localization. Mol Cell 2002, 10:831-842.
45. Gill G: Post-translational modification by the small ubiquitin-related modifier SUMO has big effects on transcription factor activity.Curr Opin Genet Dev 2003, 13:108-113.
46. Girdwood D, Bumpass D, Vaughan OA, Thain A, Anderson LA, Snowden AW, Garcia-Wilson E, Perkins ND and Hay RT: p300 Transcriptional Repression Is Mediated by SUMO Modification. Mol Cell 2004, 11:1043-1054.
47. Desterro JM, Rodriguez MS and Hay RT: SUMO-1 modification of IkBa inhibits NF-kB activation. Mol Cell Biol 1998, 23:233-239.
48. Rodriguez MS, Dargenomt C and Hay RT: SUMO-1 conjugation in vivo requires both a consensus modification motif and a novel targeting signal. J Biol Chem 2001, 276:12654-12659.
49. Roff M, Thompson J, Rodriguez MS, Jacque JM, Baleux F, Arenzana-Seisdedos F and Hay RT: Role of IkBa ubiquitination in signal-induced activation of NF-kB in vivo. J Biol Chem 1996, 271:7844-7852.
50. Huang TT and Miyamoto S: Postrepression activation of NF-kB requires the amino-terminal nuclear export signal specific to IkB. Mol Cell Biol 2001, 21:4737-4747.
51. Rodriguez MS, Desterro JM, Lain S, Midgley CA, Lane DP and Hay RT: SUMO-1 modification activates the transcriptional response of p53. EMBO J 1999, 18:45-61.
52. Saitoh H and Hinchey J: Functional heterogeneity of small ubiquitin-related protein modifiers SUMO-1 versus SUMO-2/3. J Biol Chem 2000, 275:6252-6258.
53. Saltzman A, Searfoss G, Marcireau C, Stone M, Resner R, Munro R, Franks C, D’Alonzo J, Tocque B, Jaye M and Hirschborn Y: NUBC associates with MEKK1 and type I TNF-α receptor and stimulates NF-κB activity. FEBs Lett 1998, 425:431-435.
54. Hay RT, Vuillard L, Desterro JM and Rodriguez MS: Control of NF-kB transcriptional activation by signal-induced proteolysis of IκBα. Philos Trans R Soc Lond B Biol Sci 1999, 354:1601-1609.
55. Cascar SM: Novel strategies for immortalization of human hepatocytes. Artif Organs 2001, 25:529-538.
56. Mendonca MS, Antoniono RJ, Latham KM, Stanbridge EJ and Redpath JL: Characterization of intestinal alkaline phosphatase expression and the tumorigenic potential of gamma-irradiated HeLa x fibroblast cell hybrids. Cancer Res 1991, 51:4455-4462.
57. Arenzana-Seisdedos F, Thompson J, Rodriguez MS, Bacherie F, Thomas D and Hay RT: Inducible nuclear expression of newly synthesized IkBa negatively regulates DNA-binding and transcriptional activities of NF-κB. Mol Cell Biol 1995, 15:2689-2696.
58. Birbach A, Gold P, Binder BR, Hofer E, de Martin R and Schmid JA: Signaling molecules of the NF-κB pathway shuttle constitutively between cytoplasm and nucleus. J Biol Chem 2002, 277:10842-10851.
59. Day RN, Periasamy A and Schaaffele F: Fluorescence resonance energy transfer microscopy of localized protein interactions in the living cell nucleus. Methods 2001, 25:4-18.
60. Biermann E, Baack M, Kreutz S and Knippers R: Synthesis and turn-over of the replicative Cdc6 protein during the HeLa cell cycle. Eur J Biochem 2002, 269:1040-1046.
61. Iborra FJ, Jackson DA and Cook PR: Coupled transcription and translation within nuclei of mammalian cells. Science 2001, 293:1139-1142.
62. Cheng JD, Ryseck RP, Actar RM, Dambach D and Bravo R: Functional redundancy of the nuclear factor κB inhibitors IκBα and IκBβ. J Exp Med 1998, 188:1055-1062.
63. Velasco M, Diaz-Guerra MJ, Martin-Sanz P, Alvarez A and Bosca L: Rapid Up-regulation of IκBα and abrogation of NF-κB activity in peritoneal macrophages stimulated with lipopolysaccharide. J Biol Chem 1997, 272:23025-23030.
64. Hoffmann A, Lechvenko A, Scott ML and Baltimore D: The IκBα NF-κB signaling module: temporal control and selective gene activation. Science 2002, 298:1241-1245.
65. Whiteside ST, Epistat JC, Rice NR and Israel A: IκBα, a novel member of the IκB family, controls RelA and cRel NF-κB activity. EMBO J 1997, 16:1413-1426.
66. Beg AA, Sha WC, Bronson RT and Baltimore D: Constitutive NF-κB activation, enhanced granulopoiesis, and neonatal lethality in IκBα-deficient mice. Genes Dev 1995, 9:2736-2746.
67. Cohen-Tannoudji M and Babinet C: Beyond ‘knock-out’ mice: new perspectives for the programmed modification of the mammalian genome. Mol Hum Reprod 1998, 4:929-938.
68. Muller U: Ten years of gene targeting: targeted mouse mutants, from vector design to phenotype analysis. Molec Dev 1999, 92:3-21.
69. Parmely MJ, Wang F and Wright D: -interferon prevents the inhibitory effects of oxidative stress on host responses to Escherichia coli infection. Infect Immun 2001, 69:2621-2629.
70. Cochran JR, Khan AM, Eidemir O, Xue H, Cua B, Fullmer J, Luersen GL and Colasurdo GN: Influence of lipopolysaccharide exposure on airway function and allergic responses in developing mice. Pediatr Pulmonol 2002, 34:267-277.
71. Tian S, Poukka H, Palvimo JJ and Jänne OA: Small ubiquitin-related modifier-1 (SUMO-1) modification of the glucocorticoid receptor. Biochem J 2002, 367:907-911.