SINK Is a p65-interacting Negative Regulator of NF-κB-dependent Transcription

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The transcription factor NF-κB plays important roles in inflammation and cell survival. In this study, we identified SINK, an NF-κB-inducible protein. Overexpression of SINK inhibited NF-κB-dependent transcription induced by tumor necrosis factor (TNF) stimulation or its downstream signaling proteins but did not inhibit NF-κB translocation to the nucleus and binding to DNA. Co-immunoprecipitation and in vitro kinase assays indicated that SINK specifically interacted with the NF-κB transactivator p65 and inhibited p65 phosphorylation by the catalytic subunit of protein kinase A, which has previously been shown to regulate NF-κB activation. Consistent with its role in inhibition of NF-κB-dependent transcription, SINK also sensitized cells to apoptosis induced by TNF and TRAIL (TNF-related apoptosis-inducing ligand). Taken together, these data suggest that SINK is critically involved in a novel negative feedback control pathway of NF-κB-induced gene expression.

NF-κB is one group of important transcription factors involved in a broad range of physiological and pathological processes. For example, NF-κB plays critical roles in immune regulation and inflammation through induction of a large set of downstream genes, including cytokines, chemokines, adhesion molecules, and effectors (1–5). Recent studies suggest that NF-κB is also critically involved in regulation of cell death and survival through transcriptional activation of genes important for apoptosis and cell proliferation, such as Caspase-FLIP, c-IAPs, TRAF1, TRAF2, Bfl-1/c-IAP1, Bcl-xL, Bcl-2 (p49 and its precursor p100) (4). The major cellular form of NF-κB is a heterodimer consisting of the DNA-binding subunit p50 and the transactivator p65. Normally, NF-κB is retained in the cytoplasm through association with its inhibitor, IκB. Upon stimulation by various NF-κB activating signals, IκB is phosphorylated and degraded through an ubiquitin-dependent process. This process frees NF-κB, which is then translocated into the nucleus to activate transcription of downstream genes.

Regulation of NF-κB activation does not rely entirely on IκB phosphorylation and degradation. Various studies have shown that phosphorylation of p65 by serine/threonine protein kinases, particularly the catalytic subunit of protein kinase A (PKAc), is critical for its interaction with the transcriptional co-activator p300/CBP (CREB-binding protein) and the transcriptional competence of nuclear NF-κB (4, 9–13). Gene knock-out studies of the serine/threonine protein kinases GSK-3β, NAK/T2K/TBK, and NIK provide additional evidence of a role for nuclear NF-κB modification in its transcriptional competence (14–17). These studies indicate that a deficiency of these kinases does not affect IκB degradation, NF-κB translocation into the nucleus, or binding to DNA, but it severely impairs NF-κB-mediated transcription induced by various stimuli.

TNF is one of the major proinflammatory cytokines (18). This effect of TNF is mediated through activation of NF-κB (18). The signaling pathway that is responsible for TNF-induced NF-κB activation has been resolved for the most part. Upon TNF stimulation, TNF receptor 1 (TNF-R1) trimerizes and recruits the downstream death domain-containing protein, TRADD (TNF-receptor-associated death domain), to the receptor (19). TRADD, in turn, functions as an adaptor to recruit several downstream proteins, including FADD (Fas-associated death domain), TRAP2, and RIP to the TNF-R1 signaling complex (20–22). Although FADD is critically involved in TNF-R1-mediated apoptosis, TRAF2 and RIP recruit IKK, the IκB kinase, to the TNF-R1 complex (23, 24). IKK contains three subunits, the catalytic subunits IKKα and IKKβ and the regulatory subunit IKKγ (4). Once IKK is recruited to the TNF-R1 complex, it is activated and subsequently leads to IκB phosphorylation and NF-κB activation (2, 4).

In addition to TNF-R1, most members of the TNF receptor family can also activate NF-κB (18). Recently, we and others (25–29) identified a new member of the TNF family of ligands, TALL-1, also called BAFF, Blys, THANK, and zTNF4, which is expressed by macrophages and monococytes and is critically involved in peripheral B cell survival. TALL-1 signals through NF-κB, which is then translocated into the nucleus to activate transcription of downstream genes.

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FIG. 1. Amino acid sequence analysis of SINK. A, sequence alignment of human SINK and two uncharacterized or hypothetical proteins, GS3955 and SKIP1. These proteins contain a kinase-like domain (as indicated by the angle brackets), which corresponds to the C-terminal half of a conserved kinase domain. B, sequence alignment of the kinase-like domain of human SINK and the kinase domains of human PKAc and protein kinase C α (PKC-A). The conserved subdomains of the kinases are indicated. C, Northern blot analysis of SINK. Human tissue mRNA blots were hybridized with SINK probe.
I reveal a novel negative feedback control pathway of NF-κB. The sequence homology to serine/threonine protein kinases. Surprisingly, our results suggest that SINK interacts with p65 and inhibits NF-κB-dependent transcription through blocking p65 phosphorylation by the protein kinase PKA. Our findings reveal a novel negative feedback control pathway of NF-κB-dependent gene expression.

EXPERIMENTAL PROCEDURES

Reagents—Mouse monoclonal antibody against FLAG (Sigma) and HA (Covance, Berkeley, CA) epitopes, goat polyclonal antibody against p65, rabbit polyclonal antibody against p50 and IκB (Santa Cruz Biotechnology, Santa Cruz, CA), Texas Red-conjugated Affinipure goat anti-mouse IgG (Molecular Probes, Eugene, OR), and recombinant human TNF and IL-1 (R & D Systems Inc., Minneapolis, MN) were purchased from the indicated manufacturers. Recombinant soluble TRAIL was produced in our laboratory (37). The rabbit and serum against human SINK was raised against the following peptide: LSRKKLRLEL-DNDLITERPVQKRARSGPQ.

Yeast Two-hybrid Screening—To construct a BCMA bait vector, a cDNA fragment encoding amino acids 119–184 of BCMA was inserted in-frame into the Gal4 DNA-binding domain vector pGBT (Clontech, Palo Alto, CA). The human B cell cDNA library (ATCC, Manassas, VA) was screened as described (19–21).

Northern Blot Hybridization—Human multiple tissue mRNA blots were purchased from Clontech. The blots were hybridized with labeled SINK cDNA (spanning the coding region) in Rapid Hybridization Buffer (Clontech) under high stringency conditions.

Vectors and Transfection—Mammalian expression vectors for HA- or FLAG-tagged SINK and PKA were constructed by PCR amplification of the corresponding cDNA fragments and subsequently cloned into CMV promoter-based vectors containing an HA or FLAG tag.

Expression plasmids for HA-BCMA and HA-BAFF-R were previously described (40). Expression plasmids for TNF-R1, RIP, TRAF2, TRAF5, IKKγ, p65, p50, IκBα, and IκBα/SS/AA (provided by David Goeddel), MEKK1 (provided by Gary Johnson), and NF-κB luciferase reporter plasmids (provided by Gary Johnson) were provided by the indicated investigators. Transfection of 293 cells was performed using the standard calcium phosphate precipitation method (40).

Reported Gene Assays—293 cells (2 × 10^5) were seeded on 6-well (35-mm) dishes and were transfected the following day with standard calcium phosphate precipitation. Within the same experiment, each transfection was performed in triplicate, and where necessary, a sufficient amount of empty control plasmid was added to ensure that each transfection continued to receive the same amount of total DNA. To normalize for transfection efficiency, 0.3 μg of RSV-β-galactosidase plasmid was added to each transfection. Luciferase reporter assays were performed using a luciferase assay kit (BD PharMingen) and following the manufacturer’s protocols. β-Galactosidase activity was measured using the Galacto-Light chemiluminescent kit (Tropix, Bedford, MA). Luciferase activities were normalized on the basis of β-galactosidase expression levels.

RNAi Assays—We have used the RNAi system purchased from Oligoengine Inc. A cDNA fragment containing the SINK targeting sequence (AAAGCTGTGCGCTTGTCTTC) was cloned into the pSuper.Retro retroviral vector, which contains a puromycin selection marker. The resulting vector or control empty vector was transfected into 293-10A1 packaging cells. The retrovirus-containing supernatant was collected to infect 293 cells. The infected 293 cells were selected by puromycin (2 μg/ml) for 3 days. The puromycin-resistant cells were amplified. NF-κB luciferase reporter gene assays were performed as described above.

Apoptosis Assay—β-Galactosidase co-transfection assays for determination of cell death were performed as described previously (19–21). Briefly, 293 cells (~2 × 10^5) were seeded on 6-well (35-mm) dishes and were transfected the following day by calcium phosphate precipitate with 0.1 μg of pCMV-β-galactosidase plasmid and the indicated transfection plasmids. Within the same experiment, each transfection was performed in triplicate, and where necessary, a sufficient amount of empty control plasmid was added to keep each transfection receiving the same amount of total DNA. Approximately 14 h after transfection, the cells were treated with TNF (20 ng/ml) or TRAIL (200 ng/ml) or left untreated for 12 h. Cells were then stained with X-gal as described previously (19–21, 41). The numbers of survived blue cells from five representative viewing fields were determined microscopically. Data shown are the averages and standard deviations of one representative experiment in which each transfection was performed in triplicate.

Electrophoretic Mobility Shift Assay—The electrophoretic mobility shift assay was performed as described previously (37). Briefly, 293 cells stably transfected with SINK or empty control plasmid were treated with TNF for 30 min. Cells were then harvested and incubated in 500 μl Buffer A (10 mM Hepes, 15 mM NaCl, 5 mM EDTA, 1.5 mM MgCl2, 10 μg/ml aprotinin, 1 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride). For each immunoprecipitation, 0.4-ml aliquots of lysates were incubated with 0.5 μg of the indicated antibody or control IgG and 25 μl of a suspension of GammaBind Plus (Amersham Biosciences) for at least 1 h. The Sepharose beads were washed three times with 1 ml of lysis buffer containing 500 μM NaCl. The precipitates were fractionated on SDS-PAGE, and subsequent Western blot analyses were performed as described (19–21, 39, 40). Endogenous co-immunoprecipitation was performed as described previously (20–22).

DNA Transfection—293 cells were transfected with p50, p65, and the protein kinase PKA receptors (upper panel). The same blot was reprobed with anti-β-tubulin antibody. The sample loaded into lane 1 was one-fifth of that loaded into lanes 2–8. The band in lane 1 in the upper panel is FLAG-tagged SINK, which is slightly larger than endogenous SINK (lanes 2–8).
FIG. 3. SINK inhibits NF-κB-mediated transcription but not NF-κB translocation into the nucleus and binding to DNA. A, SINK inhibits NF-κB-dependent transcription induced by various proteins. 293 cells (2 × 10⁶) were transfected with 0.3 μg of NF-κB-luciferase and 0.3 μg of RSV-β-galactosidase plasmids together with 2 μg of each of the indicated plasmids. 15 h after transfection, luciferase reporter gene assays were performed. Data shown are the averages and standard deviations of relative luciferase activities (normalized by β-galactosidase activities) from one representative experiment in which each transfection was performed in triplicate. B, SINK does not inhibit MEKK1-induced AP1 activation. 293 cells (2 × 10⁶) were transfected with 0.3 μg of AP1-luciferase and 0.3 μg of RSV-β-galactosidase plasmids together with 2 μg of each of the indicated plasmids. Luciferase reporter gene assays were performed as described in A. C, SINK inhibits NF-κB-dependent transcription induced by IL-1 and TNF in a dose-dependent manner. 293 cells (2 × 10⁶) were transfected with 0.3 μg of NF-κB-luciferase and 0.3 μg of RSV-β-galactosidase plasmids together with the indicated amounts of expression plasmid for SINK. 14 h after transfection, cells were treated with 10 ng/ml IL-1 or 20 ng/ml TNF or left untreated for 6 h. Luciferase reporter gene assays were then performed as described in A. D, SINK RNAi inhibits SINK but not β-tubulin protein expression induced by Cd²⁺. SINK RNAi and control transfected 293 cells (2 × 10⁶) were induced by 5 or 10 mM CdCl₂ for 24 h. Cell lysates were then analyzed by Western blot with anti-SINK (upper panel) or anti-β-tubulin antibody (lower panel). E, SINK RNAi potentiates basal and TNF-induced NF-κB activation. SINK RNAi and control transfected 293 cells (2 × 10⁶) were transfected with 0.3 μg of NF-κB-luciferase and 0.3 μg of RSV-β-galactosidase plasmids. 14 h after transfection, cells were treated with 20 ng/ml TNF or left untreated for 18 h. Luciferase reporter gene assays were then performed as in A. F, SINK does not inhibit NF-κB translocation and binding to DNA. 293 cells (2 × 10⁶) stably transfected with an expression plasmid for SINK (pool, pooled stable cells; c1, clone 1; c2, clone 2) or an empty control plasmid (vector) were treated with TNF or left untreated for 30 min. Nuclear extract was incubated with a consensus NF-κB probe in the presence or absence of 100× excess of cold competitor. Supershift was performed with goat anti-p65 antibody or normal goat IgG. G, SINK is localized in the nucleus. 293 cells were transfected with an expression plasmid for FLAG-tagged SINK. Immunofluorescent staining was performed to visualize SINK (in red) and DNA (in blue).
I FLAG-SINK together with p65, p50, or transfected with expression plasmids for SINK antibody. Cell lysates were immunoprecipitated with control mouse IgG or monoclonal anti-FLAG antibody. The immunoprecipitates were analyzed by Western blot with anti-p65 (lanes 1 and 2), anti-p50 (lanes 3 and 4), and anti-IκBα antibodies (lanes 5 and 6), respectively. Expression levels of p65, p50, and IκBα are shown in lanes 7–9. Expression of SINK is similar in all three transfections (data not shown). B, reverse co-immunoprecipitation between SINK and p65. 293 cells (2 × 10⁶) were transfected with expression plasmids for FLAG-SINK and p65. Cell lysates were immunoprecipitated with control goat IgG or goat anti-p65 antibody. The immunoprecipitates and lysate (lys) were analyzed by Western blot with anti-FLAG. C, endogenous interaction between SINK and p65. 293 cells (2 × 10⁶) were treated with CdCl₂ (10 μM) for 24 h. Cell lysate was immunoprecipitated with goat anti-p65 antibody or normal goat IgG. The immunoprecipitates and lysate were analyzed by Western blot with anti-SINK antibody.

Red-conjugated AffiniPure goat anti-mouse IgG (1:200 dilution) for 45 min at room temperature. The cells were then rinsed with PBS containing Hercul and mounted in Prolong Antifade (Molecular Probes). The cells were observed with a Leica DMR/XA immunofluorescent microscope using a 100× plan objective.

RESULTS

Identification and Cloning of SINK—To identify BCMA-associated molecules, we performed yeast two-hybrid screening with a cytoplasmic fragment (amino acids 119–184) of BCMA as bait. We screened a total of ~2 × 10⁸ clones from a human B cell library and obtained two β-galactosidase-positive clones. Sequencing analysis and GenBankTM data base searches suggest that one of the clones encodes for an uncharacterized protein, which we designated SINK (for p65-interacting inhibitor of NF-κB; see below). Sequence analysis of 10 SINK expressed sequence tag clones suggested that SINK encodes for a 358-amino acid protein (Fig. 1A). The SINK amino acid sequence we obtained is full-length because there is an in-frame stop codon at the 5′ end (data not shown).

BLAST searches of the GenBankTM data bases suggested that the sequence of full-length SINK is identical to that of an uncharacterized or hypothetical protein called SKIP3 (GenBankTM accession numbers AF250310, AK026945, NM021156, BC027484, AL034548, and BC019363). It seems that SKIP3 is the human ortholog of the rat protein NIPK, which was identified as a protein up-regulated during neuronal apoptosis induced by NGF withdrawal (42), an uncharacterized mouse protein TRB3 (GenBankTM accession number AF538868), and the Drosophila protein Tribbles, which is critically involved in inhibition of cell cycle progression (43–46) (data not shown). Data base searches also identified two uncharacterized or hypothetical human proteins, GS3955 (GenBankTM accession number NP_067675) and SKIP1 (GenBankTM accession number AA58174), which are highly homologous to human SINK (Fig. 1A). Taken together, these data suggest that SINK is a member of an evolutionarily conserved protein family.

Sequence analysis suggests that SINK contains a kinase-like domain and shares significant homology to serine/threonine protein kinases such as PKAc and PKA-C (Fig. 1B). However, SINK is probably not a functional kinase because it contains only 5 of the 12 subdomains found in most serine/threonine protein kinases and lacks a conserved ATP-binding site (47).

Northern blot analysis suggests that human SINK mRNA is weakly expressed in spleen, thymus, prostate, liver, and pancreas (Fig. 1C) and is undetectable in other examined tissues, including testis, ovary, small intestine, colon, leukocyte, heart, brain, placenta, lung, skeletal muscle, and kidney.

Expression of SINK Is Inducible—To detect endogenous human SINK protein, we raised a peptide-directed rabbit polyclonal anti-SINK antibody. Human SINK protein was barely detectable in several tested cell lines by Western blot analysis (Fig. 2 and data not shown). Because rat NIPK is induced during neuronal apoptosis (42), we reasoned that human SINK is probably also an inducible protein. As shown in Fig. 2, SINK protein expression could be induced potently by stimulation of 293 cells with the heavy metal ion Cd²⁺, TNF, and phorbol 12-myristate 13-acetate plus ionomycin. Also, overexpression of BCMA in 293 cells could induce SINK expression, and this was inhibited by blocking NF-κB activity with an undegradable IκBα mutant (Fig. 2). Because TNF and phorbol 12-myristate 13-acetate plus ionomycin can also activate NF-κB in 293 cells (data not shown), collectively these data suggest that SINK can be induced through an NF-κB-dependent process.

SINK Does Not Interact with BCMA in Mammalian Cells—To determine whether SINK interacts with BCMA in
mammalian cells, we transfected expression plasmids for C-terminal HA-tagged BCMA and N-terminal FLAG-tagged SINK into 293 cells. We then performed co-immunoprecipitation with anti-FLAG antibody and Western blot analysis with anti-HA antibody. This experiment indicated that SINK could interact with BCMA (data not shown). However, reverse co-immunoprecipitation experiments failed to detect an interaction between BCMA and SINK (data not shown). In combination with data shown below, we conclude that SINK does not interact specifically with BCMA.

**SINK Inhibits NF-κB-dependent Transcription but Not NF-κB Translocation into the Nucleus or Binding with DNA**—Because SINK is homologous to PKAc, a serine/threonine kinase involved in p65 phosphorylation and NF-κB activation, we examined the effect of SINK on NF-κB activation by NF-κB luciferase reporter gene assays. The results indicated that SINK inhibits NF-κB-induced transcription triggered by overexpression of BCMA, TNF-R1, and its downstream signaling proteins RIP, TRAF2, IKKβ, and p65. These experiments indicate that SINK inhibits NF-κB activation induced by overexpression of all the tested proteins (Fig. 3A). SINK also inhibited NF-κB activity induced by TRAF5 and TRAF6 (Fig. 3A), two signaling proteins involved in NF-κB activated by multiple TNF receptor family members (18). However, SINK did not significantly inhibit MEKK1-induced AP1 activation (Fig. 3B), suggesting that SINK specifically inhibits NF-κB-dependent transcription.

We also tested whether SINK could inhibit NF-κB activation triggered by TNF and IL-1 stimulation. The data suggest that SINK can inhibit TNF- and IL-1-triggered NF-κB activation in a dose-dependent manner (Fig. 3C).

To determine whether SINK has a physiological role in NF-κB-induced transcription, we obtained 293 cell lines stably expressing SINK RNAi. Western blot analysis indicated that SINK RNAi decreased SINK protein expression induced by Cd²⁺ (Fig. 3D). We then determined the effect of SINK RNAi on TNF-induced NF-κB activation. The results indicated that SINK RNAi expression could potentiate basal and TNF-induced NF-κB activation (Fig. 3E). These data suggest that SINK is a physiological inhibitor of NF-κB activation.

Because SINK can inhibit NF-κB activation induced by downstream signaling proteins, including the NF-κB transactivator p65, we reasoned that SINK functions in the nucleus to inhibit nuclear NF-κB transcriptional competence. To test this hypothesis, we made 293 cell lines that stably express SINK and then examined the effect of overexpression of SINK on TNF-induced NF-κB nuclear translocation and binding to DNA. These experiments indicated that SINK does not affect TNF-induced NF-κB translocation into the nucleus or binding to DNA (Fig. 3F). We also transfected SINK into 293 cells and performed immunofluorescent staining experiments. The results indicated that SINK was localized primarily in the nucleus (Fig. 3G). Taken together, our results suggest that SINK...
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inhibits NF-κB-dependent transcription but not NF-κB translocation and DNA binding.

SINK Interacts with p65—Because SINK inhibited the transcriptional competence but not translocation and DNA binding of NF-κB, we reasoned that SINK might directly bind to NF-κB. To test this possibility, we co-transfected SINK and p65, p65, or λExo into 293 cells and performed immunoprecipitation experiments. The results suggest that SINK interacts with p65 but not with p50 or λExo (Fig. 4, A and B).

To determine whether SINK interacts with p65 under physiological condition, we induced SINK expression by Cd²⁺ and then performed endogenous co-immunoprecipitation. The results indicated that the induced SINK could interact constitutively with endogenous p65 (Fig. 4C).

SINK Inhibits p65 Phosphorylation by PKAc—The next question we asked is why SINK binding to p65 affects NF-κB transcriptional competence. We first determined whether SINK could disrupt p50-p65 interaction. The results indicated that overexpression of SINK did not affect p50-p65 interaction (data not shown). Previously, it has been shown that p65 phosphorylation by PKAc is important for the transcriptional competence of nuclear NF-κB (4, 9–13). Because SINK shares homology with PKAc (Fig. 1B) and interacts with p65, we tested whether SINK affects the phosphorylation of p65 by PKAc in vitro kinase assays. As shown in Fig. 5, PKAc could potently phosphorylate both p65 (lanes 4 and 6) and SINK (lanes 7 and 9–11) in these assays. Moreover, p65 phosphorylation by PKAc was significantly inhibited by SINK (compare lane 3 with lane 4), and this inhibition was dose-dependent (lanes 8–11). These data are consistent with the conclusion that SINK inhibits NF-κB transcription competence through blocking p65 phosphorylation by PKAc.

SINK Sensitizes Cells to TNF- and TRAIL-induced Apoptosis—NF-κB promotes cell survival through transcriptional induction of anti-apoptotic genes (3–8). Because SINK inhibits NF-κB-dependent transcription, we determined whether SINK affects NF-κB-dependent cell survival. Therefore, we transfected 293 cells with SINK and examined its effect on TNF- and TRAIL-induced apoptosis. The results suggest that SINK can significantly sensitize 293 cells to TNF- and TRAIL-induced apoptosis (Fig. 6).

DISCUSSION

In a yeast two-hybrid screening using the TNF receptor family member B cell membrane BCA as bait, we identified SINK. However, SINK is probably not a BCMA-interacting protein in mammalian cells, because SINK is a nuclear protein, whereas BCA is a membrane receptor. In addition, we failed to detect an interaction between BCMA and SINK in the reverse co-immunoprecipitation experiments.

SINK shares significant sequence homology with serine/threonine protein kinases. However, SINK may not be a functional kinase because it contains only 5 of 12 conserved kinase subdomains and lacks a conserved ATP-binding site found in all serine/threonine kinases. We transfected SINK into 293 cells and found that it was weakly phosphorylated in vitro kinase assays (Fig. 5). It is possible that SINK has intrinsic kinase activity, or, alternatively, SINK is phosphorylated by an unidentified kinase. In this context, we found that SINK could be phosphorylated by PKAc (Fig. 5). We also mutated several conserved amino acids critical for kinase activity of many well studied serine/threonine kinases, including K97A, K118A, D182A, K184A, and found that mutation of the sites did not affect its phosphorylation in vitro kinase assays (data not shown). Based on these data, we believe that SINK is not an authenticated kinase.

Rat NIPK was identified in a study of genes up-regulated during nerve growth factor withdrawal-induced neuronal apoptosis (42). It was also found that rat NIPK was up-regulated by other apoptotic stimuli, such as serum depletion or DNA damage in PC6-3 and NIH3T3 cells (42). These studies suggest that rat NIPK is involved in either the promotion or inhibition of apoptosis. In human cells, SINK is weakly expressed or undetectable. Interestingly, we found that various stimuli, including Cd²⁺, TNF, phosphatase inhibitors, and overexpression of BCMA, could induce expression of SINK protein. Because λExo could inhibit BCMA-induced SINK expression, we conclude that SINK cannot be induced in a non-NF-κB-dependent manner.

Our results suggest that overexpression of SINK inhibits NF-κB-dependent transcription but not NF-κB translocation and DNA binding, whereas SINK RNAi potentiates basal and TNF-induced NF-κB activation. Co-immunoprecipitation experiments suggest that SINK interacts directly with p65 and inhibits p65 phosphorylation by PKAc. Previously, it has been well documented that PKAc-mediated p65 phosphorylation is critically involved in NF-κB transcriptional competence. Thus, we reason that SINK inhibits NF-κB-dependent transcription through blocking p65 phosphorylation by PKAc or its related kinases. Consistent with its role in inhibiting NF-κB-dependent transcription, SINK also sensitizes cells to TNF and TRAIL-induced apoptosis, probably through inhibition of expression of NF-κB activated anti-apoptotic genes.

In conclusion, we have identified an NF-κB-inducible protein that can inhibit NF-κB-dependent transcription. Our findings reveal a novel negative feedback control pathway for NF-κB-dependent transcription and cell survival.

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