Identification of Interleukin 1 Receptor-associated Kinase as a Conserved Component in the p75-Neurotrophin Receptor Activation of Nuclear Factor-κB*

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The neurotrophin nerve growth factor (NGF) supports neuronal survival by activating the transcription factor nuclear factor-κB (NF-κB). We report here, for the first time, the identification of p75-associated kinase that mediates NGF-driven NF-κB activation. Using co-immunoprecipitation, we demonstrate an NGF-dependent association of interleukin 1 receptor-associated kinase (IRAK) with the p75 neurotrophin receptor in PC12 cells. Our results reveal that IRAK is recruited to the p75-NGF receptor leading to formation of a complex between IRAK, atypical protein kinase C interacting protein, p62, and TRAF6. Activation of NF-κB occurs predominantly through the p75 receptor, and TrkA activity suppresses NF-κB activation and retards IκBβ degradation. In addition, we observe a requirement for the kinase activity of IRAK in mediating NGF-induced NF-κB activation, recruitment of the adapter protein p62 to the p75 receptor, and cell survival. Moreover, p75-IRAK-mediated IκBβ activation and the recruitment of IKKδ, but not IKKα, to the receptor require p62. Altogether, our data provide novel information regarding the proximal components involved in p75 receptor signaling and underscore the importance of the atypical PKC interacting protein p62 in this process.

The transcription factor, nuclear factor-κB (NF-κB) regulates the expression of a wide variety of genes involved in immunity, inflammation, apoptosis, and other cellular processes (1–3). NF-κB resides in the cytoplasm in an inactive form bound to an inhibitory protein of the IκB family. Activation of NF-κB by an external stimulus involves phosphorylation and rapid degradation of IκB proteins by the IκB kinase (IKK) complex, leading to nuclear translocation of NF-κB. The pro-inflammatory cytokines IL-1β and TNFα are the most well characterized stimuli that lead to the activation of NF-κB.

Binding of interleukin-1 (IL-1) to its receptor promotes the association of IL-1 receptor-associated kinase (IRAK) with the receptor (4), through the adapter protein MyD88 (5). IRAK then gets highly phosphorylated and leaves the receptor complex to interact with TRAF6, a member of the TNF receptor associated factor family (6). The IRAK-TRAF6 interaction triggers kinase cascades that lead to the activation of NF-κB (6–8).

IRAK is a serine/threonine-specific protein kinase that shares 25% sequence identity with human mixed-lineage kinase, 30–33% sequence identity with a protein kinase that is a product of the pto gene of tomato plant, and 32% identity to the kinase domain of Drosophila pelle, which is involved in the activation of Dorsal, the Drosophila equivalent of NF-κB (4). These kinases define a subgroup of cytoplasmic kinases called the serine/threonine innate immunity kinases (SIIK) group (9). IRAK is a multidomain protein containing an N-terminal death domain of 120 amino acids, a central 300-amino acid kinase domain, and an undetermined C-terminal domain that is absent in pelle (10). Two other IRAK-related proteins IRAK-2 and IRAK-M (for its higher levels of expression in cells of monocytic lineage), have been found in humans (8, 11). Properties of IRAK resemble the serine/threonine kinase RIP, implicated in TNF signaling. TNF-R1 signaling to NF-κB activation is in part reported to be mediated by recruitment of mPLK, which, based upon chromosomal location and sequence identity, is the mouse homologue of the human IRAK (12).

In addition to IL-1 and TNFα, treatment of neuronal cells with nerve growth factor (NGF) promotes NF-κB activation (13), by binding to two distinct receptors, TrkA and p75. We demonstrated previously that in the NGF NF-κB activation pathway, the atypical PKC binding protein p62 serves as a scaffold by linking p75-TRAF6 and TrkA receptor components (13). Although TrkA has intrinsic tyrosine kinase activity (14), the p75 receptor, a member of the tumor necrosis factor family, has no known intrinsic kinase activity (15–17). Moreover, p75 has been shown to signal independently of TrkA in the NF-κB pathway and enhance the survival response of sensory neurons to NGF (18). In this study, we searched for a p75-associated kinase that mediates NGF-induced NF-κB signaling. We report here, for the first time, an NGF-dependent association of IRAK with p75 in PC12 cells leading to NF-κB activation. We demonstrate that IRAK is a critical component of this pathway. Overexpression of catalytically active IRAK in PC12 cells enhances cell survival, whereas catalytically inactive IRAK blocks activation of NF-κB through a mechanism whereby the association of the p62 scaffold was blocked from its association with the p75 receptor.

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¶ The abbreviations used are: NF-κB, nuclear factor-κB; IL-1, interleukin-1; TNF, tumor necrosis factor; PKC, protein kinase C; mPLK, mouse pelle-like kinase; BDNF, brain-derived neurotrophic factor; NT, neurotrophin; NTR, neurotrophin receptor; NGF, nerve growth factor; IRAK, interleukin-1 receptor-associated kinase; TRAF, tumor necrosis factor receptor-associated factor; IKK, IκB kinase; RIP, receptor interacting protein; MBP, myelin basic protein; E3, ubiquitin-protein isopeptide ligase.
Co-immunoprecipitations were conducted as previously described (13) with 750 μg of cell extract, incubated for 3 h with 3 μg of antibody, followed by 2-h incubation with 30 μl of protein A or G beads. After incubation, the beads were washed five times with lysis buffer (20 mM Tris-Cl, pH 7.4, 150 mM NaCl, 1% Triton X-100, 2 mM p-nitrophenyl phosphate, 1 μg/ml leupeptin, and 2 mM sodium orthovandate) followed by immunoblotting. For kinase assays, immunoprecipitates were incubated with 20 μl of kinase buffer (20, 21) with 10 μCi of [γ-32P]ATP for 30 min at 30 °C and analyzed by SDS-PAGE followed by autoradiography.

Measurement of NF-κB Activity—NF-κB activation was measured by reporter gene assay. HEK 293, IRAK-I1A, and PC12 cells were transfected with a b-galuciferase reporter gene plasmid, 3EconA-luc, pGOL-3, or Renilla luciferase (used as internal control for transfection efficiency) (13, 22). In all cases the amount of DNA was kept constant by transfecting empty vector. After 24 or 46 h, the cells were stimulated with NGF and the activity was determined using a Promega luciferase assay system. Individual constructs were transfected in duplicate, and each assay was measured in triplicate. Values are reported as the mean ± S.E. of four individual experiments.

Cell Survival Assay—PC12, HEK 293, and IRAK-deficient I1A cells were incubated for 2 h with MTS reagent (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxzybenzyloxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) (Promega). For each treatment, six wells were incubated in the presence of MTS and converted to a water-soluble formazan by dehydrogenase enzyme found in metabolically active cells. The quantity of formazan product was determined by spectrophotometry (Dynatech microplate reader) at 490 nm. Values are the mean and S.E. (n = 2–4).

RESULTS

Identification of IRAK as a p75-associated Kinase—Previous studies have revealed the presence of a 104-kDa serine/threonine kinase activity that co-associates with the p75-NGF receptor (23). Given the similarities in size as well as biochemical properties and the fact that p75 has been shown to bind TRAF6 (24, 25), we hypothesized that the 104-kDa kinase might be IRAK.

To test this idea, a series of co-immunoprecipitation experiments were performed. IRAK co-immunoprecipitated with the p75 receptor only upon stimulation with NGF (Fig. 1A). The association of IRAK with the p75 receptor peaked by 1 min post stimulation with NGF and rapidly declined thereafter to a point where IRAK was hardly detectable after 30 min. To examine if NGF led to activation of IRAK, we examined the activity of IRAK in an immunocomplex kinase assay employing the exogenous substrate myelin basic protein (MBP). In PC12
cells, the activity of IRAK peaked within 1–2 min of NGF stimulation, however, by 15 min the activity declined to the basal levels (Fig. 1B). We also analyzed autophosphorylation of IRAK. As shown in Fig. 1C, NGF stimulated autophosphorylation of IRAK, which paralleled activation of the enzyme and efficient phosphorylation of MBP, as indicated by the heavy \[^{32}P\]ATP labeling with kinetics identical to that of its activity (Fig. 1B). These results demonstrate that IRAK is heavily phosphorylated and concomitantly activated upon NGF treatment of PC12 cells. Comparison of kinetics of association between IRAK and p75 indicate that IRAK recruitment to the p75 receptor parallels its activation. We then set out to determine if the previously reported 104-kDa serine/threonine kinase that associates with the p75-NGF receptor (23) is IRAK. We performed immunoblotting experiments and in vitro kinase reactions with PC12 cell lysates immunodepleted of IRAK and observed that immunodepletion of IRAK resulted in a disappearance of autophosphorylated 104-kDa protein kinase that associates with the p75 receptor (data not shown).

To determine if MyD88 functions as an adapter (25) in an NGF signaling pathway by recruiting IRAK to p75 receptor, we examined whether endogenous MyD88 could interact with p75 and IRAK (Fig. 2). PC12 cell lysates were immunoprecipitated with p75 or MyD88 antibodies and immunoblotted for MyD88 (Fig. 2A) or IRAK (Fig. 2B) with or without NGF treatment. In the absence of NGF, there was little association of MyD88 with p75 or IRAK. However, addition of NGF promoted robust association of these proteins. The amount of MyD88 that associated with p75 peaked at 1 min of NGF treatment and declined thereafter. By comparison, the association of MyD88 with IRAK reached a maximum at an earlier time period (0–0.5 min) and declined steeply after 1 min of NGF treatment.

NGF-inducible Interactions Exist among IRAK, TRAF6, and p62—TNF receptor-associated factors (TRAFs) and the atypical PKC interacting protein, p62, have been shown to be important components of IL-1 and TNF signaling pathways that control NF-κB activation (22). Moreover, we have recently demonstrated that p62 serves as a molecular bridge via its ability to directly bind TrkA and is recruited to the p75 receptor through its interaction with TRAF6 (13). To test the possibility that p62 interacts with IRAK, we immunoprecipitated TRAF6 or p62 from PC12 cells at different times of NGF treatment and Western blotted with IRAK antibody. In this assay, IRAK co-associated with both p62 and TRAF6, however, IRAK interaction with TRAF6 preceded (1 min) its interaction with p62 (5 min) (Fig. 3A). The association of IRAK with either TRAF6 or p62 was dependent upon NGF dose (Fig. 3B).

Although the tyrosine kinase receptor TrkA can bind only NGF, the p75 receptor binds NGF, BDNF, NT-3, NT-4/5 with the same affinity but different kinetics (26, 27). We explored the ability of different neurotrophins to promote interaction between IRAK with either p62 or TRAF6. There was some degree of variation in the ability of the neurotrophins to promote association between IRAK and TRAF6/p62 (Fig. 3C). For example, treatment with NT-3 produced a small degree of association of IRAK with p62, whereas NT-4/5 and BDNF promoted a slightly greater (~3-fold) degree of association. Almost similar amounts of TRAF6 co-immunoprecipitated with IRAK.
later, K252a (250 nM) was added or not, followed by addition of NGF (50 ng/ml), as indicated (minutes). The expression of I
activity was determined (relative light units (RLU)/addition of 50 ng/ml NGF for 4 h. The cells were lysed, and luciferase experiments.

These findings are similar to those obtained in two other independent

FIG. 4 . Inhibition of TrkA potentiates NGF-induced activation of NF-κB. A, PC12 cells were co-transfected with either IRAK+/− (1.25 μg) along with NF-κB reporter (25 ng). Thirty-six hours post-transfection the cells were treated with K252a (250 nM) for 1 h, followed by addition of 50 ng/ml NGF for 4 h. The cells were lysed, and luciferase activity was determined (relative light units (RLU/μg)). The mean and S.E. from three independent experiments, conducted in triplicate, is shown. B, PC12 cells were transfected with NF-κB reporter (25 ng), 36 h later, K252a (250 nM) was added or not, followed by addition of NGF (50 ng/ml) as indicated. NF-κB activity was measured by luciferase activity (RLU/μg). The results are shown as mean ± S.E. C, PC12 cells were treated with K252a (250 nM) or not, followed by addition of NGF (50 ng/ml), as indicated (minutes). The expression of IκB was examined by SDS-PAGE/Western blotting of (50 μg) of the cell lysate (inset). The blots were scanned, and the relative change in intensity of IκB is shown. These findings are similar to those obtained in two other independent experiments.

upon treatment with NT-4/5 or BDNF. In addition, NGF was also capable of stimulating a complex with IRAK and either TRAF6 or p62 in 3T3 cells that exclusively express the p75 receptor (data not shown). By comparison, NT-3 promoted greater (−4-fold) association between TRAF6 and IRAK. Collectively, these results reveal that activation of p75 can stimulate the formation of a complex between IRAK-TRAF6 and p62, although the magnitude of the response varies with respect to different neurotrophins. This effect may be due to the formation of distinct signaling molecules (28, 29). Interestingly, treatment of PC12 cells with NGF3T, a mutant form of NGF that fails to bind p75 but still retains the ability to bind TrkA (30), did not completely impair the interaction between p62 and IRAK. However, the interaction between TRAF6 and IRAK stimulated by NGF3T was greatly diminished compared with NGF (Fig. 3C). Some variance in the response between the purified neurotrophins and NGF3T might also be due to the relative activity of NGF3T in the culture supernatant compared with use of purified neurotrophins.

Collectively, these findings suggest that the TrkA receptor may play some role in engaging the NF-κB activation pathway, because neurotrophins that bind exclusively to p75 fail to promote maximal responses, and the NGF3T stimulated a small, but significant interaction of p62 with IRAK and TRAF6. To test the contribution of the TrkA in the activation of NF-κB, PC12 cells were co-transfected with control plasmid in the presence or absence of IRAK active (+) or inactive constructs (−) (Fig. 4A), followed by treatment with K252a in the presence or absence of NGF. Inhibition of TrkA, by K252a, enhanced NGF-induced NF-κB activity. To test whether TrkA modulated the kinetics of NGF-induced κB activation, PC12 cells were treated with K252a, followed by addition of NGF for different periods of time (Fig. 4B). Inhibition of TrkA shifted the kinetics of κB activation from a late 3-h period to an early 1-h period, with a greater magnitude in the response. The degradation of the inhibitory component of the κB complex, IκB, was also examined in the absence or presence of K252a (Fig. 4C). Inhibition of TrkA likewise potentiated NGF-induced IκB degradation but had no effect on degradation of IκBα (data not shown). The degradation of IκB (Fig. 4C) parallels enhancement of NGF-κB activation via suppressing TrkA (Fig. 4, A and B). Collectively, these findings reveal that TrkA suppresses activation of NF-κB, and thus, p75 plays a primary role in activation of the NF-κB pathway.

IRAK Activity Is Required for NGF-mediated NF-κB Activation—In the IL-1 system, the kinase activity of IRAK is not required for its function (12, 31). In contrast, the catalytic activity of IRAK has been reported to be crucial for TNF signaling (12). To investigate the role of IRAK in NGF signaling, we determined if overexpression of IRAK would enhance NF-κB activation in PC12 cells. Cells transfected with mPLK, the mouse homologue of human IRAK (12), displayed enhanced basal NF-κB responsiveness, which was dependent upon the dose of the construct expressed (Fig. 5A). cimPLK was created by the substitution of an asparagine for an aspartic acid residue (D358N) in the Mg-ATP binding site of mPLK (12). This substitution impairs the catalytic activity of IRAK, and over-expression of this construct serves to inhibit the activity of IRAK in a dominant negative manner. This mutation was previously used to demonstrate that the kinase activity of IRAK/mPLK is not required for its function in IL-1 signaling but is required for TNF-R1 signaling (12). To assess the role of IRAK kinase activity in NGF-mediated NF-κB activation, PC12 cells transfected with increasing doses of cimPLK/IRAK were assayed for NF-κB activation post-NGF stimulation. The IRAK−mediated decrease in the NF-κB response of NGF was
likewise dose-dependent (Fig. 5B). Moreover, IRAK− at 1.0 μg concentration completely abrogated NGF-induced NF-κB activation in PC12 cells (Fig. 5B). Thus, the NGF NF-κB pathway can be enhanced by overexpressing wild type IRAK− (Fig. 5A) or inhibited by overexpressing catalytically inactive IRAK− (Fig. 5B).

To determine if p75-mediated NF-κB activation requires the catalytic activity of IRAK, we examined the ability of p75 receptor to activate NF-κB in IRAK-deficient 293 cells (11A) by transfecting increasing concentrations of the p75 receptor along with IRAK−. 11A cells lack IRAK mRNA and protein and have been used as a model system to study the function of IRAK in IL-1 signaling (31). Expression of p75 alone failed to promote a significant increase in NF-κB activity in IRAK-deficient 11A cells (Fig. 5C) by comparison, expression of IRAK− along with p75 restored NGF-induced NF-κB activation. Moreover, the magnitude of the NGF-dependent response was much greater in the absence of TrkA (Fig. 5C) than compared with the response when catalytically active IRAK levels were modulated in cells expressing both p75 and TrkA. Collectively, these results demonstrate that the kinase activity of IRAK is essential for p75-mediated NF-κB activation and further confirm that TrkA modulates the NF-κB pathway.

p62 has been isolated independently by two groups as an atypical PKC-interacting protein (32, 33). We recently have shown that the recruitment of p62 to the NGF receptor is critical for the activation of NF-κB (13). Because our current findings reveal that IRAK activity is required for NGF-mediated NF-κB activation (Fig. 5A and B), we examined if catalytically inactive IRAK− could block the recruitment of p62 to the p75 receptor complex in PC12 cells. In untransfected cells, p62 was recruited to the p75 receptor upon stimulation with NGF (Fig. 5D). Interestingly in cells overexpressing IRAK−, p62 failed to co-immunoprecipitate with the p75 receptor upon stimulation with NGF. These results demonstrate that the catalytic activity of IRAK is required for recruitment of the atypical PKC interacting protein p62 to the p75 receptor in PC12 cells. Moreover, this finding is consistent with the IRAK-p75 activation complex forming (Fig. 1, A and B) prior to association of p62 with IRAK (Fig. 3A).

**IRAK Signals through p62 in the NGF-mediated NF-κB Pathway**—To examine the signaling pathways that couple IRAK to NF-κB activation, we tested whether an antisense construct of p62 (13, 22) could block IRAK-mediated NF-κB activation in HEK 293 (Fig. 6A) and PC12 cells (data not shown). p62 is an important intermediary in IL-1-, TNFα-, and NGF-mediated NF-κB activation pathways (13, 22, 34, 35). Because p62 is capable of binding TRAF6, it is a likely candidate to connect IRAK to NF-κB activation. Expression of p75 alone failed to activate NF-κB, whereas, either IRAK or p62 were capable of modest NF-κB activation (Fig. 6A). Co-transfection of p62 potentiated NGF-stimulated activation of NF-κB by p75/IRAK, whereas depleting the levels of p62 abolished the activation (Fig. 6B). These results demonstrate that, in the p75/NGF-mediated NF-κB activation pathway, IRAK signals through the atypical PKC interacting protein p62.

The key event in NF-κB activation involves phosphorylation and degradation of IκB by IκB kinase (36). Two IκB kinase (IKK) activities (IKKα and IKKβ) that phosphorylate residues 32 and 36 of IκBα, the best studied IκB, have been cloned and identified (36–39). Both IKKα and IKKβ are activated by NGF (40). Additionally, we have previously shown that NGF-stimu-
IRAK is a Component of the p75-mediated NF-κB Pathway

**Fig. 6.** Antisense p62 blocks IRAK-mediated NF-κB activation. A, HEK 293 cells were co-transfected with either myc-p75 (0.15 μg), IRAK (0.25 μg), p62 (0.5 μg), or with (1.0 μg) of antisense p62 (ASp62) in the presence of NF-κB reporter as indicated. NF-κB activity was determined by luciferase assay. The mean and S.E. from four different experiments is shown. The expression of p62 protein was also examined in each of the cell lysates (50 μg) by Western blotting as shown. B, p62 is required for formation of the IKK receptor complex. PC12 cells transfected with either pcDNA or with antisense construct of p62 were either untreated (–) or treated with 50 ng/ml NGF for 2 min. Cell lysates were immunoprecipitated with anti-p75 antibody and Western blotted with antibody to IRAK, IKKα, IKKβ, and p62.

**Fig. 7.** Overexpression of IRAK enhances NGF-mediated cell survival. A, six individual wells of PC12 cells (1.5 × 10^4) were co-transfected with either IRAKα (1.0 μg), IRAKβ (1.0 μg), or with antisense p62 (1.0 μg) as indicated. The following day, the cells were washed five times with serum-free media followed by addition of NGF (50 ng/ml) or not, and cell survival was assessed by MTS reduction 24 h later. The mean and S.E. of three experiments is shown. Although IRAK enhanced survival, treatment with K252a and NGF significantly enhanced IRAK-mediated survival. Antisense p62 significantly inhibited IRAK-mediated survival of PC12 cells (p < 0.05). B, IRAK-deficient I1A cells (IRAK−) were transfected with either 0.25 μg of myc-p75, IRAKα (0.5 μg), or IRAKβ (0.5 μg) as indicated. The following day, the cells switched to a serum-free environment. NGF (50 ng/ml) was added or not, and cell survival was assessed by MTS reduction 24 h later. These findings are the mean ± S.E. of three experiments where each assay was conducted in triplicate.

*IRAK Promotes Survival Signaling—*Altogether our studies...
growth. Transfection of increasing concentrations of IRAK did not have a significant effect on NGF-mediated neurite outgrowth (data not shown).

The p75 receptor has been shown to induce both cell survival and cell death signals (43, 44). To examine if the catalytic activity of IRAK plays a role in modulating neurotrophin apoptosis, we overexpressed the p75 receptor in IRAK-deficient IIA cells (19), in the presence or absence of catalytically active IRAK, followed by serum withdrawal and treatment or not with NGF (Fig. 7B). NGF-induced cell survival was enhanced by catalytically active IRAK. This observation is consistent with a requirement for IRAK in regulating NF-κB activation (Fig. 5C) and results obtained in PC12 cells (Fig. 7A). Taken together, these findings underscore the requirement for IRAK activity in regulation of p75-mediated NF-κB activation (Fig. 5, A–C) and p75 survival signaling (Fig. 7, A and B).

**DISCUSSION**

A number of protein kinases have been known to associate with p75 (46). A 120/104-kDa kinase has been reported as a predominant protein kinase that co-immunoprecipitates with the p75 receptor in PC12 cells upon NGF stimulation (23). However, the identity of the kinase remained elusive. We report here the identification of a 100/104-kDa serine/threonine kinase that co-immunoprecipitates with p75 in NGF-treated PC12 cells. In addition, the similarity in molecular weight and the ability of the kinase to co-immunoprecipitate with p75 receptor upon NGF stimulation suggests that the 104-kDa kinase previously reported by Canossa et al. (23) could likely be the 104-kDa kinase identified by us as IRAK.

IRAK was initially shown to be a critical player in the IL-1 signaling pathway of NF-κB. The N-terminal region (residues 1–198) and the C-terminal region (residues 523–618) have been shown to be required for the IL-1-induced binding of IRAK to TRAF6 (31). Kinase activity of IRAK is not required for its function in the IL-1 system (12, 31). In contrast to the IL-1 signaling pathway, the catalytic activity of mPLK/IRAK has been reported to be critical for TNF signaling (12). In this study we show for the first time that overexpression of mPLK/IRAK can constitutively induce NGF-mediated NF-κB activation. Our results also demonstrate that, in NGF signaling, the kinase activity of mPLK/IRAK is required for NF-κB activation. Evolutionary conservation of IRAK structure and function reported between different species (4, 47) and receptor systems may cause an even stronger overlap of their biological functions. Functional studies performed by us revealed that the catalytic activity of IRAK is required for p75-mediated NF-κB activation as well as NGF-mediated activation of NF-κB. In parallel, the catalytic activity of IRAK appears to be required for NGF-mediated survival of PC12 cells.

Six different TNF receptor-associated factors (TRAFs) have been identified so far of which TRAF2 and TRAF5 have been implicated in activating NF-κB in response to TNF-α. TRAF6 has been shown to be a signal transducer for the IL-1 receptor superfamily (6, 48). Although TRAF2 is recruited by receptor interacting protein (RIP) in TNF signaling, TRAF6 is recruited by IRAK (6). In addition, p62/ZIP interacts with TRAF6 and RIP in IL-1 and TNF signaling pathways, respectively (22, 34). Previous reports have confirmed an interaction of TRAF6 with p75 (24). Here we extend this observation and show an NF-κB-mediated association of TRAF6 and the atypical PKC interacting protein, p62, with IRAK. Furthermore, we find that IRAK-mediated NF-κB activation and cell survival could be blocked in NGF signaling by the antisense construct of p62, thus suggesting that p62 is a downstream transducer of the p75/NGF-IRAK-mediated NF-κB activation pathway. In addition, we demonstrate here that p62 is necessary for coupling of IκB kinase, IκKB, with the p75-NGF receptor. The mechanisms whereby the IκBs are regulated are not yet completely understood. Ubiquitination, in addition to phosphorylation, has been shown to play a regulatory role in IκK activation. It was shown recently that TRAF6 functions as a ubiquitin ligase (E3), which, together with the E2 Ubc13/Uev1A, mediates the assembly of K63-linked polyUb chains required for IκK activation (49). It has been suggested that the ubiquitin binding property of p62 may also be relevant in regulating IκK activation.

The NF-κB signaling pathway is conserved between invertebrates and vertebrates (8, 50). The components of Toll signaling pathway, Toll, tube, pelle, dorsal, and cactus, which function to form the dorsal-ventral axis in the Drosophila embryo, show significant homology with IL-1R, MyD88, IRAK, NF-κB, and IκB of the IL-1 signaling pathway (51). A Drosophila homologue of TRAF protein (DTRAF-1) was recently identified (52). Our present study demonstrating the presence of a homologous signaling pathway in the NGF system reinforces the notion that the NF-κB signaling pathway is evolutionarily conserved. The most striking feature that distinguishes IL-1- and NGF-mediated NF-κB activation seems to be the requirement of IRAK kinase activity in NGF signaling but not in IL-1 signaling. In this context p75-NGF receptor-mediated signaling shares greater homology with TNF signaling than IL-1 signaling, which could be due to the high degree of sequence similarity between TNF and p75 receptors (15, 17, 53). It is interesting to note the requirement for the catalytic activity of IRAK exhibited by p75 for activation of NF-κB as well as survival signaling. In cells deficient in IRAK, NGF failed to activate NF-κB (Fig. 5C) with a parallel reduction in NGF-induced survival (Fig. 7B). Collectively, these findings support a role for the catalytic activity of IRAK as a bifunctional switch for p75-mediated survival in PC12 cells.

Based upon these findings we propose a model for how IRAK signals NF-κB activation in the NGF system (Fig. 8). Under basal conditions, IRAK is a cytoplasmic inactive kinase not bound to the receptor. Upon stimulation with NGF, an
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MyD88-IRAK complex forms that is recruited to the p75 receptor. After recruiting IRAK, MyD88 leaves the receptor complex. At the time of recruitment to the p75 receptor, IRAK is rapidly phosphorylated and activated by an unknown mechanism. NGF-induced activation of IRAK leads to recruitment of TRAF6 followed by binding of p62. Moreover, the kinase activity of IRAK is necessary for recruitment of p62 to the receptor complex to engage the NF-κB pathway. Our findings reveal that p75 is capable of activating NF-κB (Figs. 4, A–C, and 5C). Co-expression of TrkA with p75 appears to suppress the kinetics and magnitude of the NF-κB response (Fig. 4B), which is suppressed when TrkA is inhibited as noted by increased degradation of IκBα. These findings further explain previous NGF-induced activation kinetics for the kB pathway (54, 55). The ability of p75 to engage the survival signaling pathway (Fig. 7, A and B) is consistent with its ability to activate NF-κB (Figs. 4 and 5). Additionally, usage of the NGF 9/13 mutant, which fails to activate TrkA autophosphorylation, reveals that p75 is able to activate NF-κB (Figs. 4 and 5). Additionally, use of the NGF 9/13 mutant, which fails to activate TrkA autophosphorylation, reveals that p75 is able to activate NF-κB (Figs. 4 and 5). Furthermore, use of the NGF 9/13 mutant, which fails to activate TrkA autophosphorylation, reveals that p75 is able to activate NF-κB (Figs. 4 and 5).

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Note Added in Proof—We have recently determined that k252a blocks NGF internalization in a dose-dependent manner, therefore prolonging p75-mediated signaling at the cell surface. These findings are consistent with survival signaling taking place at the cell surface (61).

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