Protein Kinase C-associated Kinase Can Activate NFκB in Both a Kinase-dependent and a Kinase-independent Manner*

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Protein kinase C-associated kinase (PKK, also known as RIP4/DIK) activates NFκB when overexpressed in cell lines and is required for keratinocyte differentiation in vivo. However, very little is understood about the factors upstream of PKK or how PKK activates NFκB. Here we show that certain catalytically inactive mutants of PKK can activate NFκB, although to a lesser degree than wild type PKK. The deletion of specific domains of wild type PKK diminishes the ability of this enzyme to activate NFκB; the same deletions made on a catalytically inactive PKK background completely ablate NFκB activation. PKK may be phosphorylated by two specific mitogen-activated protein kinase kinases, MEKK2 and MEKK3, and this interaction may in part be mediated through a catalytically inactive PKK background completely ablate NFκB activation. PKK may be phosphorylated by these two specific mitogen-activated protein kinase kinase kinases, MEKK2 and MEKK3. Catalytically inactive PKK mutants that block phorbol ester-induced NFκB activation do not interfere with, but unexpectedly enhance, the activation of NFκB by these two catalytically active protein kinase kinase kinases. Taken together, these data indicate that PKK may function in both a kinase-dependent as well as a kinase-independent manner to activate NFκB.

Protein kinase C-associated kinase (PKK), also known as RIP4/DIK, is an ankyrin repeat domain containing serine/threonine kinase that can activate NFκB when expressed in cell lines (1, 2) and is required for keratinocyte differentiation in vivo (3). PKK was originally identified on the basis of its association with PKCβ (4) and PKCδ (5) in yeast two-hybrid screens. Although PKCβ can phosphorylate PKK, there is no evidence for the catalytic activation of PKK by this or other PKCs (4).

The kinase domain of PKK shares a high degree of homology with the catalytic domains of members of the receptor-interacting protein (RIP) family of protein kinases (1, 2). RIP is a death domain containing serine/threonine kinase that was first described as a consequence of its association with TNFR1 (tumor necrosis factor-α receptor 1) and FAS (CD95) (6, 7) and has since been shown to be recruited by TRADD (TNFR1-associated death domain protein) (6–8) and TRAF2 (TNF receptor associated factor 2) (8) following TNF-α signaling. RIP has also been shown to associate with other TNFR family members such as RAIDD and DR3 (9, 10). Two other kinases, RIP2 and RIP3, were designated on the basis of the homology of their kinase domains to the corresponding domain of RIP, although other segments of these proteins are highly divergent. RIP2 (RICK/CARDIAK) is a caspase-associated recruitment domain-containing kinase that associates with TNFR1 and the TRAF1, TRAF5, and TRAF6 adaptors (11). It has been implicated in Toll-like receptor signaling and has been shown to be important for both innate and adaptive immune responses (12, 13). The C terminus of RIP3 has no homology to any known functional domain, although this segment is critical for RIP3 to interact with and negatively regulate RIP (14).

RIP, RIP2, and RIP3 have all been shown to both activate NFκB and to induce apoptosis in a kinase-independent manner when overexpressed (7, 8, 11, 15, 16), although RIP3 has also been shown to inhibit NFκB induction in some cases (14, 17). Following TNFR stimulation, both RIP and RIP2 have been shown to recruit IKK-γ and to consequently contribute to the activation of the IKK complex, suggesting that these RIP family members function as scaffold-like molecules rather than as protein kinases when they activate NFκB (18–20). As with other RIP kinases, PKK can activate NFκB when overexpressed in cell lines, although the catalytic activity of PKK has been considered to be required for NFκB activation (1, 2). In addition, PKK can activate NFκB in IKK-γ-deficient cell lines (1), further suggesting that it induces the activation of NFκB by a distinct mechanism from that employed by other RIP kinases.

Given our limited understanding of how PKK is activated and how it activates NFκB, we wished to further explore these issues biochemically. There is an appropriately positioned SXXS motif in the activation segment of the catalytic loop of murine and human PKK (in kinase subdomains VII and VIII) that is identical to a motif in members of the MAP2K family that is phosphorylated by MAP3Ks (21). Although we have established that these serines are not required for the catalytic activity of PKK, we have identified two MAP3Ks, MEKK2 and MEKK3, that can phosphorylate PKK in a specific manner. Surprisingly, we have also determined that certain catalytically inactive mutants of PKK can activate NFκB, whereas other enzymatically compromised mutants cannot. Catalytically inactive mutants of PKK that are incapable of activating NFκB can block PMA-induced NFκB activation. These mutants...
do not abrogate but surprisingly enhance MEKK2- and MEKK3-induced activation of NFκB. Taken together, these data support a model in which PKK can participate in the activation of NFκB in both a kinase-dependent and a kinase-independent manner.

MATERIALS AND METHODS

Plasmids and Cell Lines—FLAG-tagged PKK has been described previously (4). Plasmids for c-Raf, ASK1, MEKK1, and MEKK4 were kindly provided by Dr. John Kyriakis. Plasmids for MEKK2 and MEKK3 were kindly provided by Dr. Michael Karin and Dr. Gary Johnson. PCR-based site-directed mutagenesis was used to generate FLAG-tagged versions of all PKK mutants. 293T cells were maintained as described previously (4). U2OS cell lines stably expressing M68G-PKK were generated by co-transfection of pCMV-FLAG-M68G-PKK in conjunction with pBabePURO and maintained in complete medium supplemented with puromycin (1 µg/ml).

Assays for Kinase Activity and Phosphorylation—293T cells were transiently transfected, and assays were performed as described previously (4). Briefly, 293T cells were transfected using the calcium phosphate method. After 48 h the cells were lysed in 1% Triton X-100, and the post-mitochondrial supernatant was immunoprecipitated using anti-FLAG (M2 monoclonal; Sigma) antibodies. Equal amounts of each immunoprecipitate were analyzed using an anti-FLAG immunoblot assay and an in vitro kinase assay. Histone H1 (Sigma) was used as a substrate in in vitro kinase assays.

Luciferase Assays—The luciferase assay was performed using the dual luciferase reporter assay system (Promega). The pBInLuc NFκB reporter construct, containing four NFκB-binding sites upstream of a firefly luciferase reporter gene, was kindly provided by Dr. Sankar Ghosh (22). A Renilla luciferase reporter construct, pRL-TK (Promega), was used to normalize for transfection.

RESULTS

Thr<sup>184</sup> Is a Critical Residue within the Activation Loop of PKK—A notable feature of the activation loop of PKK is an appropriately positioned SXXXS motif (Fig. 1A), characteristically seen in MAP2Ks, which is typically phosphorylated by MAP3Ks when MAP2Ks are activated. Mutation of these serine residues typically results in a catalytically inactive kinase, and we have also shown that the catalytic activity of PKK is altered migration of PKK on a Western blot. Interestingly, the band when FLAG-tagged PKK is over expressed in 293T cells, and we have also shown that the catalytic activity of PKK is measured in an anti-FLAG immunoblot assay (to assess protein levels). The catalytic activity of PKK was measured by examining the autophosphorylation of PKK and the phosphorylation of histone H1 in an in vitro kinase assay. We have previously shown that PKK migrates as two major species: a hyperphosphorylated 110-kDa band and an underphosphorylated 97-kDa band when FLAG-tagged PKK is over expressed in 293T cells, and we have also shown that the catalytic activity of PKK is required for it to become hyperphosphorylated (4). Therefore, a third criterion for assessing catalytic activity is the decreased altered migration of PKK on a Western blot. Interestingly, the mutation of Ser<sup>173</sup>, Ser<sup>177</sup>, or both to alanines does not affect the catalytic activity of PKK (data not shown), although similar mutants frequently, but not invariably, contribute to the activation of many MAP2Ks (21). Taken together, these data strongly suggest that the SXXXS motif is not of relevance for the catalytic activation of PKK.

We subsequently mutated additional serines and threonines that were proximal to the SXXXS motif (Fig. 1A). The S171A mutation in concert with the S173A/S177A mutation does not have any affect on the catalytic activity of PKK as measured in our assays (Fig. 1, B and C, lane 5). Thr<sup>184</sup> which is conserved across all species (Fig. 1A), was mutated to an alanine, either alone or in conjunction with the S171A/S173A/S177A triple mutation. This latter combination, including T184A, is referred to as quadruple mutant (QM). Both T184A-PKK and QM-PKK exhibit markedly compromised catalytic activity as measured by autophosphorylation as well as the ability of PKK to phosphorylate histone H1 in an in vitro kinase assay. In addition, the T184A and QM mutants migrate largely as 97-kDa species, further suggesting that their catalytic activity has been compromised (Fig. 1, B and C, lanes 6 and 7). Although the mutation of Thr<sup>184</sup> to a glutamic acid (T184E) did not restore the catalytic activity of PKK (Fig. 1, B and C, lanes 8), these data suggest that Thr<sup>184</sup> is a critical residue within the activation loop of PKK with respect to catalytic activity.

MEKK2 and MEKK3 Can Phosphorylate PKK—As stated above, SXXXS motifs are characteristically found in the activation loops of MAP2Ks, and serine residues in these motifs are typically phosphorylated by MAP3Ks. Based on this knowl-

![Figure 1](http://www.jbc.org/content/doi/10.1074/jbc.M106014200/fig1)
edge, we surveyed a panel of MAP3Ks as to whether or not they could phosphorylate PKK. Because wild type PKK has been shown to be able to presumably phosphorylate itself in in vitro kinase assays (Fig. 1), a previously described catalytically inactive mutant of PKK, K51R-PKK (4), was used in this assay (see Fig. 4). The MAP3Ks surveyed included MEKK1, MEKK2, MEKK3, MEKK4, ASK-1, c-Raf, and NIK, but only MEKK2 and MEKK3 were found to be able to phosphorylate PKK when co-transfected in 293T cells. This was demonstrated by the phosphorylation of K51R-PKK in an in vitro kinase assay and the decreased mobility of K51R-PKK on an anti-FLAG Western blot (Fig. 2
A and data not shown).

We initially predicted that PKK would be phosphorylated by MAP3Ks on the serine residues within the SXXXS motif. However, we have already shown that these serines, when mutated to alanines, have no affect on the catalytic activity of PKK. Therefore, as expected in light of the data from Fig. 1, a previously described catalytically inactive mutant of PKK, K51R-PKK (4), was used in this assay (see Fig. 4). The MAP3Ks surveyed included MEKK1, MEKK2, MEKK3, MEKK4, ASK-1, c-Raf, and NIK, but only MEKK2 and MEKK3 were found to be able to phosphorylate PKK when co-transfected in 293T cells. This was demonstrated by the phosphorylation of K51R-PKK in an in vitro kinase assay and the decreased mobility of K51R-PKK on an anti-FLAG Western blot. Some information regarding the sites that may be critical for the interaction of PKK with MEKK2 and MEKK3 came from the analysis of the quadruple mutant, QM-PKK (S171A/S173A/S177A/T184A). When MEKK3 (Fig. 2C) or MEKK2 (data not shown) is co-transfected with QM-PKK into 293T cells, a portion of QM-PKK becomes phosphorylated as evidenced by the appearance of a more slowly migrating PKK species on an anti-FLAG Western blot. However, neither MEKK3 nor MEKK2 can phosphorylate QM-PKK in an in vitro kinase assay, whereas they can phosphorylate K51R-PKK. In addition, the proportion of the slower migrating QM-PKK species in cells co-transfected with MEKK3 or MEKK2 is less than what is seen with K51R-PKK (co-transfected with MEKK3 or MEKK2) on an anti-FLAG Western blot (compare Fig. 2,
B, lane 2, and C, lane 4). These results suggest that Thr
184 may participate in the interaction between PKK and MEKK2/MEKK3.

Some Catalytically Inactive Mutants of PKK Can Activate NFkB—It has recently been reported that PKK can activate NFkB when overexpressed in cell lines. In contrast to RIP, RIP2, and RIP3, this activity can be abolished when a point mutation (K51R or D143A) is made within the predicted ATP-binding pocket to catalytically inactivate this kinase (1, 2). Moreover, these catalytically inactive mutants can function in a dominant negative manner, blocking the induction of NFkB by a variety of different stimuli, including phorbol ester. Surprisingly, when the catalytically inactive mutants, K51R-PKK, QM-PKK (Figs. 1 and 2), and T184A-PKK (Fig. 1) were co-transfected with an NFkB reporter plasmid into 293T cells, we were able to detect a significant amount of NFkB activation, albeit to a lesser degree than with wild type PKK (Fig. 3).

To further investigate whether the catalytic activity is in fact dispensable for PKK to activate NFkB, we generated three additional catalytically inactive mutants: M96G-PKK, D143A-PKK, and D143N-PKK. M96G-PKK represents a mutation within the predicted ATP-binding pocket that is thought to enlarge the pocket so that specially engineered ATP analogs may bind. Analogous mutations render some kinases unable to bind ATP and thus catalytically inactivate them (26). D143A-
PKK mutants that are able to activate NFkB. PKK and the various mutants described in the text (1 μg) were co-transfected with pBIIxLuc (0.1 μg) and pRL-TK (0.02 μg) into 293T cells. The upper panel depicts a typical luciferase assay. All of the values have been normalized to luciferase values obtained from pCMV5 mock transfected cells, and each assay was performed in triplicate. The error bars denote one standard deviation in this experiment. In the lower panel, an aliquot from each of the cell lysates used above was loaded on an 8% SDS-PAGE gel and subjected to an anti-FLAG Western blot to determine the protein levels.

PKK is an ATP-binding pocket mutation that has previously been shown not to activate NFkB (1). D143N-PKK represents a more conservative mutation of the aspartate residue similar to one that has been shown to catalytically inactivate other kinases (33). In contrast to the catalytically inactive kinases described above, when these mutants were co-transfected with an NFkB reporter construct into 293T cells, no NFkB activation was detected (Fig. 3). Therefore, we have identified two groups of catalytically inactive PKK mutants. One group (K51R-PKK, QM-PKK, and T184A-PKK) can activate an NFkB reporter construct when expressed in cell lines, whereas another group (M96G-PKK, D143A-PKK, and D143N-PKK), cannot.

To determine whether there was a difference between these two groups of PKK mutants that might account for their distinct abilities to activate NFkB, we closely examined the catalytic activities of each of the mutants. All six of the catalytically inactive mutants and wild type PKK were individually expressed in 293T cells and immunoprecipitated by virtue of their FLAG tag. As before, half the immunoprecipitate was used in an in vitro kinase assay, with the other half being utilized in an anti-FLAG Western blot in order to determine the protein levels.

Some catalytically inactive PKK mutants are phosphorylated in an in vitro kinase assay. The same mutants tested in Fig. 3 were transfected into 293T cells. The upper panel depicts an in vitro kinase assay, similar to that seen in Fig. 1. The lower panel is an anti-FLAG Western blot, similar to that seen in Fig. 1. The asterisk represents unidentified proteins also seen in mock transfected lysates. The arrow shows the specific, phosphorylated, PKK band.

Intriguingly, all of the truncation and deletion mutants surveyed that also contained the K51R mutation were unable to activate NFkB in our assay (Fig. 5B). We have already seen that these truncations significantly decrease the ability of wild type PKK to activate NFkB. These results suggest that the K51R mutation does indeed compromise the catalytic activity of this kinase. Because full-length K51R-PKK has only a fraction of the NFkB activating ability of wild type PKK, these assays further reveal the essential role of the ankyrin repeat region and the intermediate domain of PKK in NFkB activation.

FIG. 3. Some catalytically inactive PKK mutants can activate NFkB. The mutants tested in Fig. 3 were transfected into 293T cells. The upper panel depicts an in vitro kinase assay, similar to that seen in Fig. 1. The lower panel is an anti-FLAG Western blot, similar to that seen in Fig. 1. The asterisk represents unidentified proteins also seen in mock transfected lysates. The arrow shows the specific, phosphorylated, PKK band.
PKK Mutants That Cannot Activate NFκB Act as Dominant Negative Mutants and Block PMA-induced Activation of NFκB—It has been recently reported that catalytically inactive mutants of PKK can block PMA-induced activation of NFκB (1, 2). We wished to determine whether specific domains of PKK were required in a catalytically inactive context to compromise PMA-induced activation of NFκB. As expected, full-length M96G-PKK, which we have shown is catalytically inactive and cannot activate NFκB (Figs. 3 and 4), significantly blocks PMA-induced NFκB activation (Fig. 6). We then examined whether the truncation mutants K51R/442-PKK and K51R/304-PKK can also act in a dominant negative manner. As can be seen in Fig. 6, both of these mutants block PMA-induced activation of NFκB. This is an interesting observation given that the catalytically active forms of these mutants, 304-PKK and 442-PKK, are both able to activate NFκB (Fig. 5). Although it might have been predicted that inactive kinases containing the ankyrin repeat or intermediate domains or both might have been required to compete with endogenous PKK, it is clear that the catalytically compromised kinase domain of PKK alone suffices to abrogate PMA-mediated activation of NFκB.

Dominant Negative PKK Does Not Block the NFκB Activating Ability of MEKK2 and MEKK3—Aside from PMA-induced activation of NFκB, we were interested in identifying other pathways in which PKK may contribute to NFκB activation. Transient co-transfection-based luciferase assays for NFκB activation, in which a dominant negative form of PKK is used in conjunction with other expression constructs, have been used to examine whether PKK lies downstream of specific receptors or signaling molecules. In our hands these kinds of transient assays give highly variable results most likely because of varying expression levels and transfection efficiencies. We therefore generated U2OS cell lines that stably express M96G-PKK and chose three stable transfectants that expressed increasing amounts of mutant PKK (Fig. 7A) to examine whether specific MAP3Ks may be functionally linked to PKK.

We have already shown that two specific MAP3Ks, MEKK2 and MEKK3, can phosphorylate PKK (Fig. 2). These two kinases have been reported to activate NFκB (27, 28). We reasoned that if PKK were required for the activation of NFκB by MEKK2 or MEKK3, then M96G-PKK would be expected to...
compromise the activation of NF\(\kappa\)B by these MAP3Ks in our stable U2OS transfectants. It is clear from Fig. 7B that MEKK2 and MEKK3 do not activate NF\(\kappa\)B in a PKK-dependent manner, implying that PKK does not functionally lie downstream of these MAP3Ks. Surprisingly, the ability of MEKK2 and MEKK3 to activate NF\(\kappa\)B was enhanced in U2OS cells expressing intermediate levels of M96G-PKK (\(p < 0.05\)) as well as in cells expressing higher levels of M96G-PKK (\(p < 0.01\); Fig. 7B). In contrast, M96G-PKK did not significantly influence the activities of MEKK1, which activates NF\(\kappa\)B (Ref. 29 and Fig. 7B) but does not phosphorylate PKK (Fig. 2A and data not shown), or MEKK4, which neither phosphorylates PKK (data not shown) nor activates NF\(\kappa\)B (Ref. 30 and Fig. 7B).

To test the possibility that M96G-PKK contributes to MEKK2- and MEKK3-mediated activation of NF\(\kappa\)B as a result of being phosphorylated by these MAP3Ks, we initially asked whether MEKK2 or MEKK3 can phosphorylate M96G-PKK. Surprisingly, MEKK2 (data not shown) and MEKK3 (Fig. 7C) are unable to phosphorylate M96G-PKK as measured by in vitro kinase assay and Western blot. Although we do not understand the relevance of the interaction between PKK and these MAP3Ks, these data lend some support to the view that PKK may participate in the activation of NF\(\kappa\)B in a kinase-independent manner.

**DISCUSSION**

Very little is understood about the mechanisms by which PKK is activated or about the process by which PKK activates NF\(\kappa\)B. We have presented evidence that Thr\(^{184}\), located within the activation loop, is critical with respect to the catalytic activity of PKK. In addition, we have shown that specific MAP3Ks, MEKK2 and MEKK3, can phosphorylate PKK, and this phosphorylation event is influenced by Thr\(^{184}\). Every domain of PKK, including the kinase domain, the intermediate domain, and the ankyrin repeat region, contributes to its ability to activate NF\(\kappa\)B. Finally, we have provided evidence that supports a model that PKK can act in both a kinase-dependent and a kinase-independent manner to activate NF\(\kappa\)B, and this is discussed here.

The role for the catalytic activity of PKK in the activation of NF\(\kappa\)B is most evident when we consider that wild type PKK activates NF\(\kappa\)B to levels that are significantly higher than those induced by catalytically inactive mutants. Identification of proteins phosphorylated by PKK may be crucial to obtaining a better understanding of this pathway. Perhaps this kinase-dependent step requires the autophosphorylation of PKK and/or the phosphorylation of a downstream target. Indeed, other RIP-like kinases have been reported to phosphorylate factors involved in cell signaling events. RIP has been shown to phosphorylate and catalytically activate MEKK1 (31), and RIP2 has been shown to phosphorylate and catalytically activate ERK2, thereby activating AP-1 (32). In an analogous fashion, PKK may phosphorylate and thereby activate a downstream factor that can activate NF\(\kappa\)B.

More surprising are the results that suggest PKK may participate in the activation of NF\(\kappa\)B in a kinase-independent manner. The observation that K51R-PKK, QM-PKK, and T184A-PKK, three presumably catalytically inactive mutants, can activate NF\(\kappa\)B when overexpressed in cell lines was unexpected, given that it has been reported that the catalytic activity of PKK is absolutely required for the activation of NF\(\kappa\)B (1, 2). The question obviously arose as to whether or not these mutants are truly catalytically inactive. Although we cannot rule out the possibility that QM-PKK and T184A-PKK retain a small amount of catalytic activity, there is considerable evidence to suggest that K51R-PKK is catalytically inactive. The mutation of this critical lysine within the ATP-binding domain has been well characterized as one that ablates the ability of a kinase to associate with ATP (33–36). Additional evidence comes from the analysis of truncation mutants that carry the K51R mutation. All of the catalytically active truncations of wild type PKK can activate NF\(\kappa\)B to some degree in our hands, whereas truncation mutants that carry the K51R mutation act as dominant negative inhibitors, blocking PMA-induced NF\(\kappa\)B activation. If the K51R mutation retained some catalytic activity, it would have been expected that these truncation mutants would not have acted in a dominant negative fashion. We
therefore believe that many pieces of evidence suggest that K51R-PKK is catalytically inactive and that these data illustrate that PKK can activate NFκB in a kinase-independent manner.

How then do K51R-PKK, QM-PKK, and T184A-PKK activate NFκB in a kinase-independent manner? Some evidence comes from in vitro kinase studies, where we have observed that these PKK mutants become slightly phosphorylated. It could be that phosphorylated PKK can recruit other proteins that contribute to the activation of NFκB. As stated earlier, RIP and RIP2 are thought to recruit IKK-γ and assemble the IKK complex in a kinase-independent manner (18–20). Because PKK can activate NFκB in IKK-γ-deficient cells (1), it is possible that PKK recruits IKK-β, or some other unidentified protein in the IKK complex, to activate NFκB. In addition, PKK may possibly recruit other kinases, such as MEKK2 or MEKK3, to facilitate the activation of NFκB. Why three additional catalytically inactive PKK mutants, M96G-PKK, D143A-PKK, and D143N-PKK, cannot activate NFκB when expressed at high levels is still unclear. It may be that these mutations may contribute to a more global distortion of PKK, blocking their association with other factors, possibly other kinases. This is evidenced by the observation that these mutants are not phosphorylated in an in vitro kinase assay. Functional studies avoiding the use of PKK overexpression may reveal further insights into this pathway.

We do not fully understand why K51R-PKK activates NFκB in our studies, whereas it has been shown to act as a dominant
natural resolution by others (2). One possible explanation is that we used mouse PKK with a K51R mutation in our studies, whereas the other study used a human K51R-PKK mutant. There are a number of small structural differences between human and murine PKK, some of which might contribute to species-specific differences in PKK function. A more detailed analysis might provide further insights regarding NFκB activation by PKK.

We have also provided evidence that PKK is phosphorylated by two MAP3Ks, MEKK2 and MEKK3. This phosphorylation appears to be specific, because other MAP3Ks were tested and were not found to phosphorylate PKK, although it should be noted that this panel was far from exhaustive, and there may still be other MAP3Ks that can phosphorylate PKK. Ser173 and Ser177, the serines within the SXXS motif; this interaction is important for the activation of ERK2 (32). Although the interaction between MEKK2 and MEKK3 with PKK may be other MAP3Ks that can phosphorylate PKK. Ser173 and Ser177, the serines within the SXXS motif; this interaction is important for the activation of ERK2 (32). Although the interaction between MEKK2 and MEKK3 with PKK remains unclear. Interestingly, RIP2 has been reported to be phosphorylated by the MAP3K, c-Raf, even though it does not contain the canonical SXXS motif; this interaction is important for the activation of ERK2 (32). Although the interaction between MEKK2 and MEKK3 with PKK may have parallels when one considers other RIP kinases and MAP3Ks, the biological significance of this interaction remains to be determined.

It is clear from these data that PKK can activate NFκB in both a kinase-dependent and a kinase-independent manner. The relative contributions of each of these mechanisms, which may not be mutually exclusive, remains to be ascertained. Further studies involving PKK-deficient cells may prove useful in this regard.

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