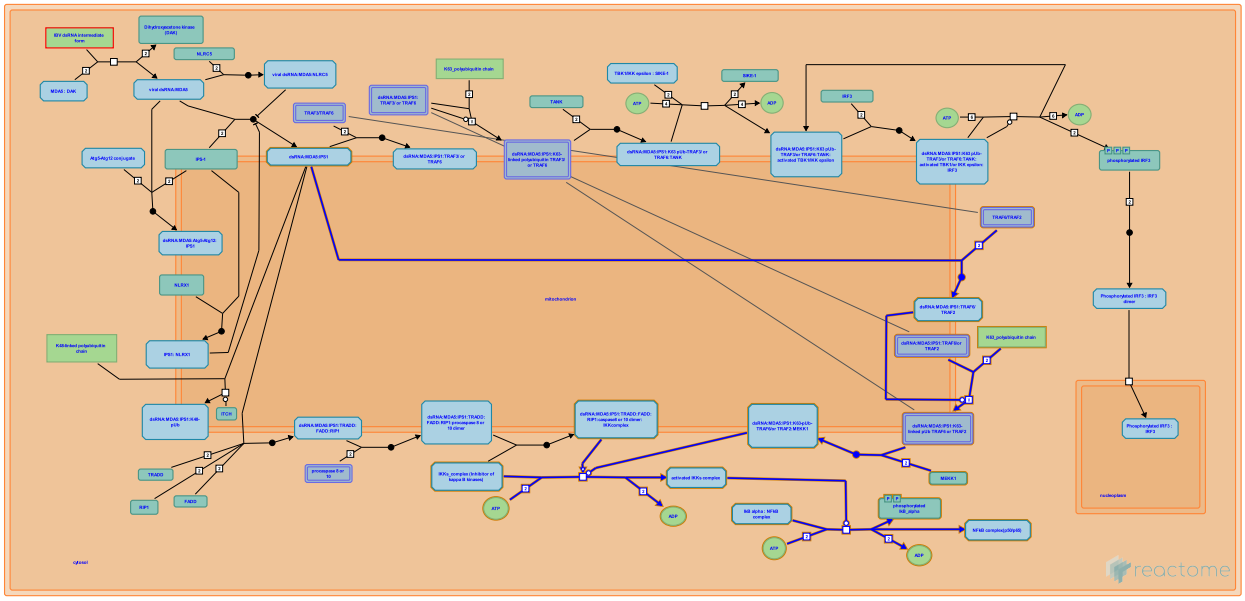
# TRAF6 mediated NF-kB activation



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# Introduction

Reactome is open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.
The development of Reactome is supported by grants from the US National Institutes of Health (P41 HG003751), University of Toronto (CFREF Medicine by Design), European Union (EU STRP, EMI-CD), and the European Molecular Biology Laboratory (EBI Industry program).

## Literature references

Fabregat, A., Sidiropoulos, K., Viteri, G., Forner, O., Marin-Garcia, P., Arnau, V. et al. (2017). Reactome pathway analysis: a high-performance in-memory approach. *BMC bioinformatics, 18*, 142. ↗

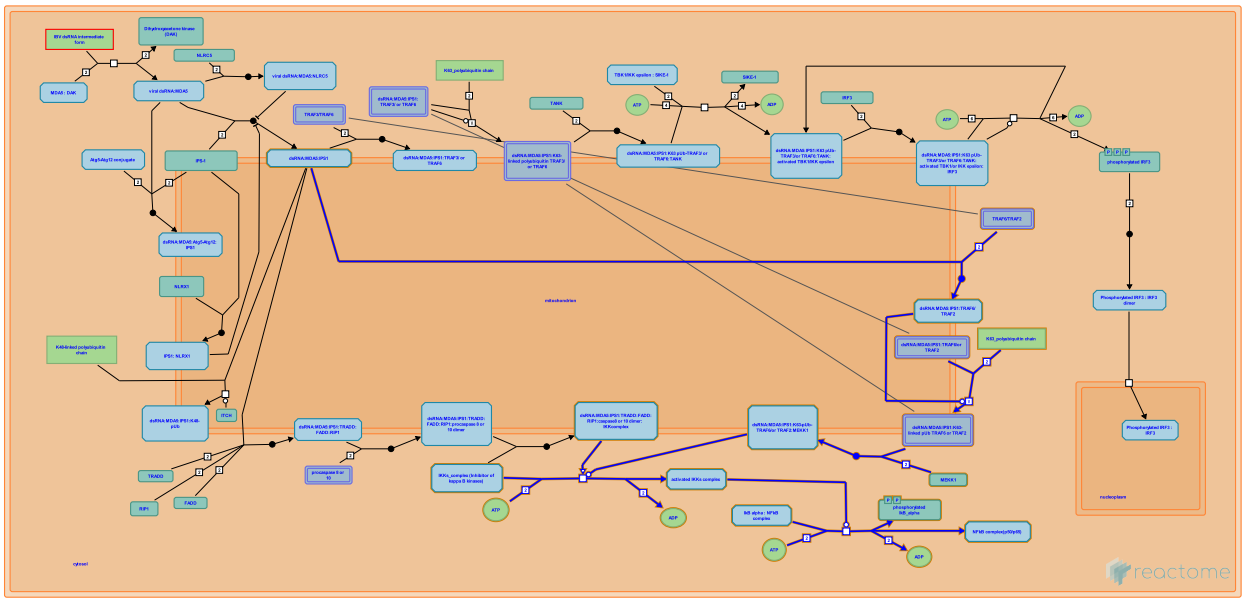
Sidiropoulos, K., Viteri, G., Sevilla, C., Jupe, S., Webber, M., Orlic-Milacic, M. et al. (2017). Reactome enhanced pathway visualization. *Bioinformatics, 33*, 3461-3467. ↗

Fabregat, A., Jupe, S., Matthews, L., Sidiropoulos, K., Gillespie, M., Garapati, P. et al. (2018). The Reactome Pathway Knowledgebase. *Nucleic Acids Res, 46*, D649-D655. ↗

Fabregat, A., Korninger, F., Viteri, G., Sidiropoulos, K., Marin-Garcia, P., Ping, P. et al. (2018). Reactome graph database: Efficient access to complex pathway data. *PLoS computational biology, 14*, e1005968. ↗

## TRAF6 mediated NF-kB activation ↗

**Stable identifier:** R-GGA-1227892



Although RLR-mediated signaling to NFkB and MAPK shares similarity with better-characterized TLR and TNF signaling pathways, many of the details need to be clarified. Thus, TGF-beta activated kinase 1(TAK1, also known as MAP3K7) - an essential mediator in TLR signaling downstream of TRAF6, was shown to associate with IPS-1 [Xu LG et al 2005]. However, there are conflicting reports on the role of TAK1 in RIG-1/IPS-1 mediated NFkB activation [Konno H et al 2009, Yoshida R et al 2008, Mikkelsen SS et al 2009].

Another MAP3K, MEKK1, has been reported to induce IKK and MAPK activation in RIG1 signaling pathway in response to dsRNA.[Yoshida R et al 2008].

TNF receptor associated factor (TRAF) protein family members are E3 ligases that have been implicated in various signal transduction pathways including RLR signaling and Toll-like receptors (TLRs) leading to activation of NFkB, MAPK and IRF family members [Oganesyan G et al 2006, Xia et al 2009, Sasai M et al 2010].

## Literature references


Xu, LG., Shu, HB., Han, KJ., Wang, YY., Zhai, Z., Li, LY. (2005). VISA is an adapter protein required for virus-triggered IFN-beta signaling. *Mol Cell, 19*, 727-40. ↗


## Editions

| | | |
|---|---|---|
| 2011-01-05 | Authored | Shamovsky, V. |
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| 2011-05-16 | Reviewed | Garapati, P V. |

## TRAF6/or TRAF2 interacts with IPS-1 ↗

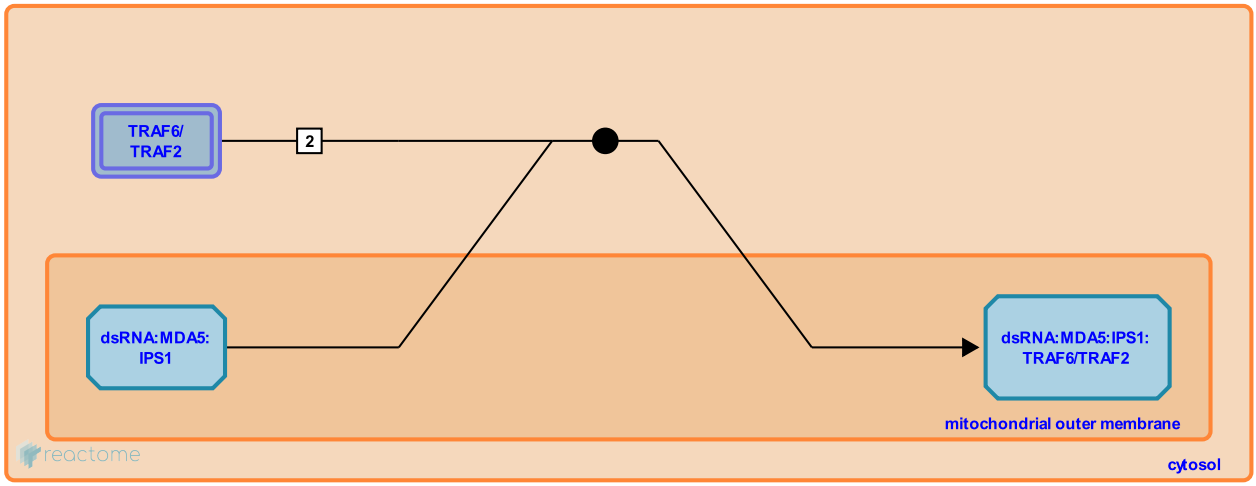
**Location:** TRAF6 mediated NF-kB activation

**Stable identifier:** R-GGA-1227775

**Type:** binding

**Compartments:** cytosol, mitochondrial outer membrane

**Inferred from:** Recruitment of TRAF6/TRAF2 to IPS-1 (Homo sapiens)




In mammals, the response to viral infection is mediated through direct and specific interaction between a TRAF-interaction motif (TIM) of IPS-1 and TRAFs, which can recruit other molecules into signaling complex [Ye H et al 2002, Xu LG et al 2005, Saha SK et al 2006, Konno H et al 2009]. IPS-1 harbors few distinct TIMs. One of the motifs is located at aa 143-PVQET-147 and binds both TRAF2 and TRAF3, while TRAF6 exclusively binds to aa 153-PGENSE-158 & 455-PEENEY-460 motifs. IPS-1 mediated response to viral infection in mammals requires:

- TRAF6 to activate NFkB, MAPK and IRF7, but not IRF3.[Xu LG et al 2005, Yoshida R et al 2009, Konno H et al 2009].
- TRAF3 to activate IRF3 and IRF7 [Saha SK et al 2006, Oganesyan G et al 2006].
- TRAF2 to activate p38 MAPK and contribute to NFkB induction [Mikkelsen SS et al 2009, Xu LG et al 2005].

Predicted chicken TRAF2, TRAF3 and TRAF6 proteins show 75, 82 and 73% amino acid sequence identity to their human counterparts respectively. In this project we assume that chicken TRAF proteins function similar to mammalian TRAFs upon viral infection.

**Followed by:** TRAF6/or TRAF2 ubiquitination within dsRNA:Mda5:Ips1:Traf6/ or Traf2 complex

## Literature references


Xu, LG., Shu, HB., Han, KJ., Wang, YY., Zhai, Z., Li, LY. (2005). VISA is an adapter protein required for virus-triggered IFN-beta signaling. *Mol Cell, 19*, 727-40. ↗

Takeuchi, O., Kato, A., Goto, H., Tsunetsugu-Yokota, Y., Su, B., Yamazaki, K. et al. (2009). TRAF6 establishes innate immune responses by activating NF-kappaB and IRF7 upon sensing cytosolic viral RNA and DNA. *PLoS One, 4*, e5674. ↗

Kobayashi, T., Kawai, T., Yoshioka, T., Yoshida, H., Takaesu, G., Yoshida, R. et al. (2008). TRAF6 and MEKK1 play a pivotal role in the RIG-I-like helicase antiviral pathway. *J Biol Chem, 283*, 36211-20. ↗

Melchjorsen, J., Chiliveru, S., Arthur, JS., Ghosh, S., Mikkelsen, SS., Gaestel, M. et al. (2009). RIG-I-mediated activation of p38 MAPK is essential for viral induction of interferon and activation of dendritic cells: dependence on TRAF2 and TAK1. *J Biol Chem, 284*, 10774-82. ↗

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## TRAF6/or TRAF2 ubiquitination within dsRNA:Mda5:Ips1:Traf6/ or Traf2 complex ↗

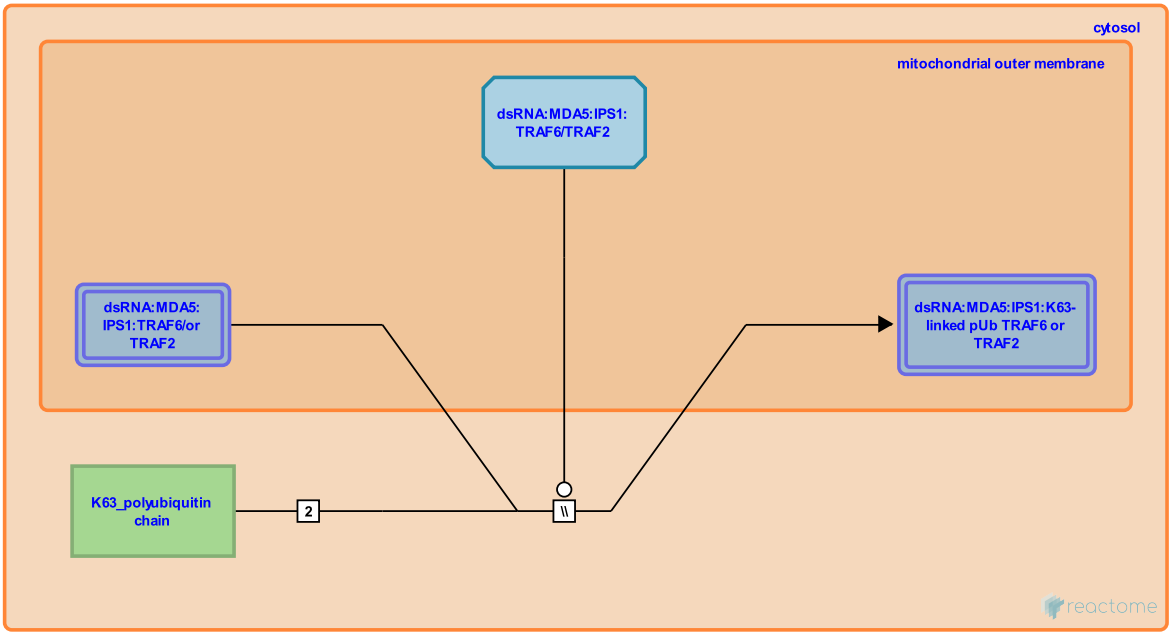
**Location:** TRAF6 mediated NF-kB activation

**Stable identifier:** R-GGA-1227769

**Type:** omitted

**Compartments:** cytosol, mitochondrial outer membrane

**Inferred from:** Recruitment of TRAF6/TRAF2 to IPS-1 (Homo sapiens)




TRAFs are E3 ubiquitin ligases that bind to an E2 - ubiquitin thioester and catalyse Lys63-ubquitination on the associated target proteins and possibly on themselves [Lamothe B et al 2007, Mao AP et al 201]. Although TRAF2 failed to interact with a number of E2 ligases related to Ubc13 and showed considerable difference in its RING domain structure from the known TRAF6 RING structure [Yin Q et al 2009], yet TRAF2 is believed to act as an E3 ubiquitin ligase. Sphingosine-1-phosphate (S1P), which is synthesized during inflammatory responces, was shown to bind to TRAF2 and stimulate TRAF2-mediated K63-linked polyubiquitination [Alvarez SE et al 2010; Napolitano G and Karin M 2010]

**Preceded by:** TRAF6/or TRAF2 interacts with IPS-1

**Followed by:** MEKK1 binds TRAF6

## Literature references


Lamothe, B., Wu, H., Darnay, BG., Besse, A., Campos, AD., Webster, WK. (2007). Site-specific Lys-63-linked tumor necrosis factor receptor-associated factor 6 auto-ubiquitination is a critical determinant of I kappa B kinase activation. *J Biol Chem, 282*, 4102-12. ↗

Deng, L., Pickart, C., Spencer, E., You, J., Wang, C., Slaughter, C. et al. (2000). Activation of the IkappaB kinase complex by TRAF6 requires a dimeric ubiquitin-conjugating enzyme complex and a unique polyubiquitin chain. *Cell, 103*, 351-61. ↗

Teng, C., Mao, AP., Yan, J., Li, S., Li, Q., Shu, HB. et al. (2010). Virus-triggered ubiquitination of TRAF3/6 by cIAP1/2 is essential for induction of interferon-beta (IFN-beta) and cellular antiviral response. *J Biol Chem, 285*, 9470-6. ↗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## MEKK1 binds TRAF6 ↗

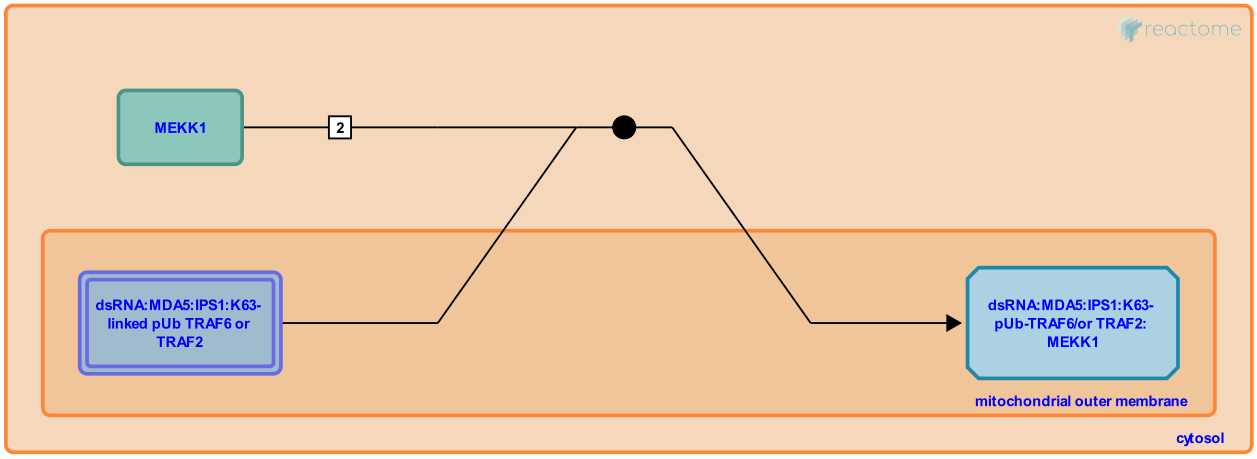
**Location:** TRAF6 mediated NF-kB activation

**Stable identifier:** R-GGA-1227778

**Type:** binding

**Compartments:** cytosol, mitochondrial outer membrane

**Inferred from:** Interaction of MEKK1 with TRAF6 (Homo sapiens)




Mammalian MEKK1 was reported to be involved to IPS-1/TRAF6 signaling pathway leading to NFkB activation, however the mechanism of virus-triggered MEKK1 activation remains unclear.

Predicted chicken MEKK1 shows 77% amino acid sequence identity to its human counterpart.

**Preceded by:** TRAF6/or TRAF2 ubiquitination within dsRNA:Mda5:Ips1:Traf6/ or Traf2 complex

**Followed by:** Phosphorylation of IKKs complex mediated by MEKK1

## Literature references


Dang, LC., Lee, FS., Peters, RT., Maniatis, T. (1998). MEKK1 activates both IkappaB kinase alpha and IkappaB kinase beta. *Proc Natl Acad Sci U S A, 95*, 9319-24. ↗

Kobayashi, T., Kawai, T., Yoshioka, T., Yoshida, H., Takaesu, G., Yoshida, R. et al. (2008). TRAF6 and MEKK1 play a pivotal role in the RIG-I-like helicase antiviral pathway. *J Biol Chem, 283*, 36211-20. ↗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## Phosphorylation of IKKs complex mediated by MEKK1 ↗

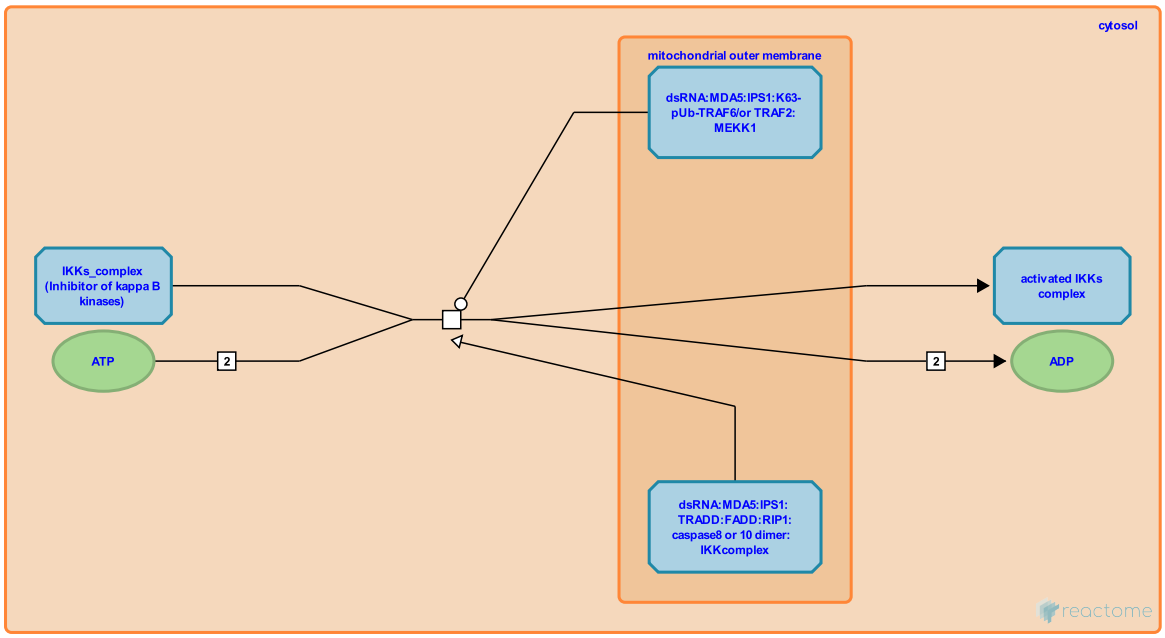
**Location:** TRAF6 mediated NF-kB activation

**Stable identifier:** R-GGA-1227783

**Type:** transition

**Compartments:** cytosol, mitochondrial outer membrane

**Inferred from:** Activation of IKK by MEKK1 (Homo sapiens)




MEKK1 can activate both IKK-alpha and IKK-beta in vivo[Lee FS et al 1998]. IKKs are activated through phosphorylation of Ser-176 & Ser-180 in IKKA and Ser-177 & Ser-181 in IKKB in their activation loops, leading to the IkB alpha phosphorylation and NF-kB activation [Ling L et al 1998, Delhase M et al 1999, Kamata H et al 2002] The IKKs - IkB kinase (IKK) complex serves as the master regulator for the activation of NF-kB by various stimuli. It contains two catalytic subunits, IKK alpha and IKK beta, and a regulatory subunit, IKKgamma/NEMO. The activation of IKK complex and the NFkB mediated antiviral response are dependent on the phosphorylation of IKK alpha/beta at its activation loop and the K63-linked ubiquitination of NEMO[Solt et al 2009; Li et al 2002]. NEMO ubiquitination by TRAF6 is required for optimal activation of IKKalpha/beta. This basic trimolecular complex is referred to as the IKK complex.

Each catalytic IKK subunit has an N-terminus kinase domain, a leucine zipper (LZ) motif, a helix-loop-helix (HLH) and a C-terminus NEMO binding domain (NBD). IKK catalytic subunits are dimerized through their LZ motifs.

IKK beta is the major IKK catalytic subunit for NF-kB activation. Phosphorylation in the activation loop of IKK beta requires Ser177 and Ser181 (data for human proteins) and thus activates the IKK kinase activity, leading to the IkB alpha phosphorylation and NF-kB activation.

Chicken IKK alpha and IKK beta showed 81% and 68% identity to the corresponding human IKK kinases (UniProtKB sequence data using NCBI BLASTP 2.2.20). Multiple sequence alignment of the proteins by ClustalW demonstrated that chicken IKK alpha serine residues 190 and 194 correspond to human Ser176 and Ser180, that are required for the activation. It is unclear what serine residues of chicken IKK beta are involved into the phosphorylation.

Chicken IKK gamma or NEMO is considered as avian lost.[Cormican et al 2009].

**Preceded by:** MEKK1 binds TRAF6

**Followed by:** activated IKKs phosphorylates IkB alpha causing the NFkB complex to dissociate from the phospho-IkB

## Literature references

Karin, M., Hacker, H. (2006). Regulation and function of IKK and IKK-related kinases. *Sci STKE, 2006*, re13.

Dang, LC., Lee, FS., Peters, RT., Maniatis, T. (1998). MEKK1 activates both IkappaB kinase alpha and IkappaB kinase beta. *Proc Natl Acad Sci U S A, 95*, 9319-24.

Garcia-Sastre, A., Chua, MA., Ng, SL., McWhirter, SM., Tenoever, BR., Maniatis, T. (2007). Multiple functions of the IKK-related kinase IKKepsilon in interferon-mediated antiviral immunity. *Science, 315*, 1274-8.

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## activated IKKs phosphorylates IkB alpha causing the NFkB complex to dissociate from the phospho-IkB

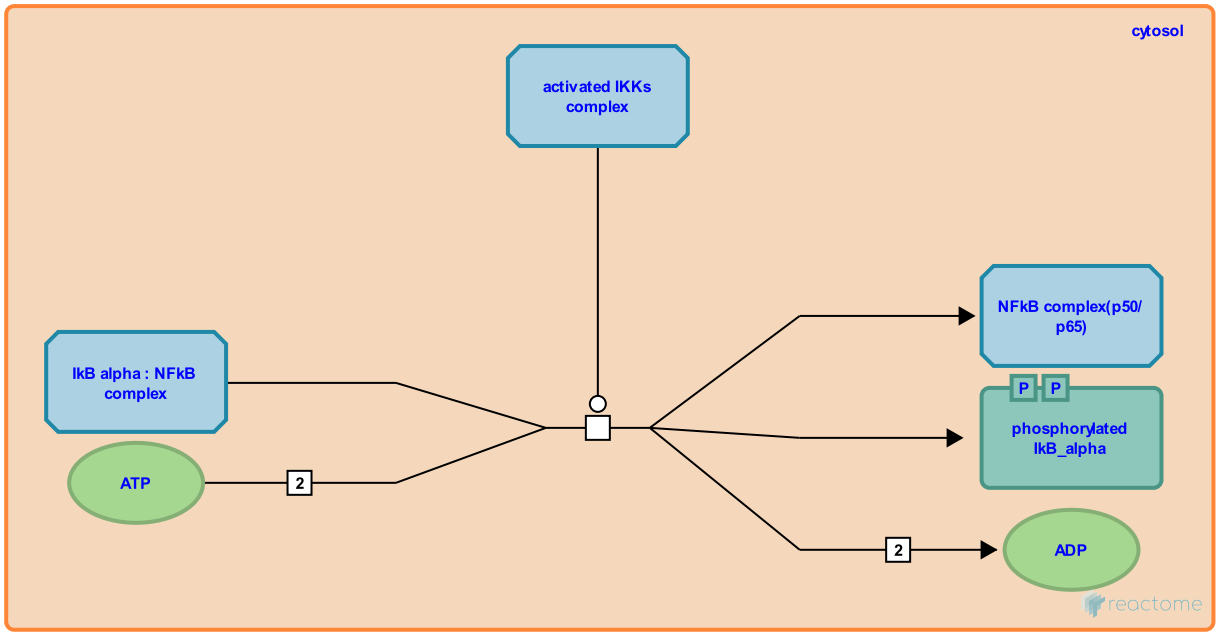
**Location:** TRAF6 mediated NF-kB activation

**Stable identifier:** R-GGA-433986

**Type:** transition

**Compartments:** cytosol

**Inferred from:** IKKbeta phosphorylates IkB causing NF-kB to dissociate (Homo sapiens)




The phosphorylation and ubiquitination of IkB kinase complex is mediated by two distinct pathways, either the classical or alternative pathway. In the classical NF-kB signaling pathway, the activated IKK complex, predominantly acting through IKK beta in an IKK gamma-dependent manner, catalyzes the phosphorylation of IkBs (at sites corresponding to Ser32 and Ser36 of human IkB-alpha or Ser19 and Ser22 of human IkB-beta), polyubiquitination (at sites corresponding to Lys21 and Lys22 of human IkB-alpha) and subsequent degradation by the 26S proteasome. The K-48 ubiquitination is mediated by the E2 ubiquitin ligases (or SCFs) formed by three subunits: Skp1, Cul A (Cdc53), and one of many F-box proteins.

Chicken IkB-alpha shows 71% identity to the human IkB alpha kinase. Multiple sequence alignment of the human and chicken IKB-alpha showed that chicken Ser36 and Ser40 correspond to human Ser32 and Ser 36 mentioned above.

There is insufficient data for chicken IkB beta to make a comparison with other species.

**Preceded by:** Phosphorylation of IKKs complex mediated by MEKK1

## Literature references

Karin, M., Hacker, H. (2006). Regulation and function of IKK and IKK-related kinases. *Sci STKE, 2006*, re13.

## Editions

| | | |
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| 2009-08-27 | Authored | Shamovsky, V. |
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# Table of Contents