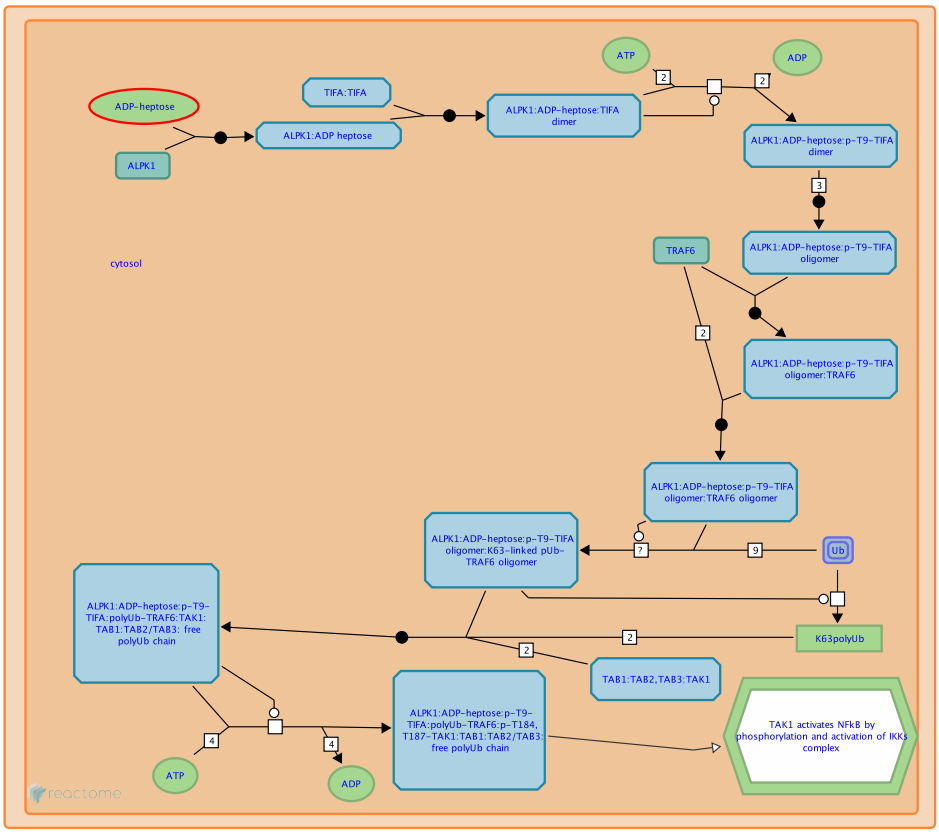
Alpha-protein kinase 1 signaling pathway



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11/09/2021

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. ↗

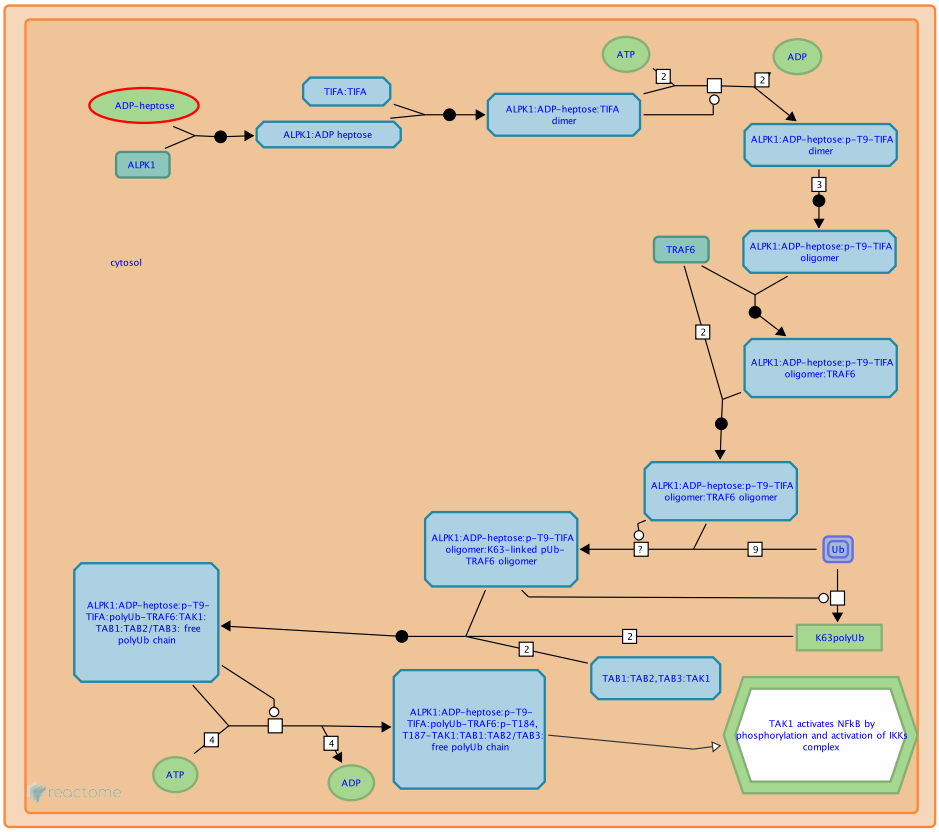
Fabregat, A., Jupe, S., Matthews, L., Sidiropoulos, K., Gillespie, M., Garapati, P. et al. (2018). The Reactome Pathway Knowledgebase. *Nucleic Acids Res, 46*, D649-D655. ↗

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. ↗

Alpha-protein kinase 1 signaling pathway ↗

Stable identifier: R-HSA-9645460

Compartments: cytosol



Immune recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRR) often activates proinflammatory nuclear factor kappa B (NF-κB) signalling. Lipopolysaccharide (LPS) is a well-known PAMP produced by gram-negative bacteria. LPS is recognized by toll like receptor 4 (TLR4) and is a strong activator of NF-κB inflammatory responses (Akashi S et al. 2003). LPS is also recognized in the cytosol by mouse caspase-11 and related human caspase-4 and caspase-5, which stimulate pyroptosis, a proinflammatory form of cell death (Kayagaki N et al. 2011; Shi J et al. 2015). Key metabolic intermediates in LPS biosynthesis, d-glycero-β-d-manno-heptose 1,7-bisphosphate (HBP) and ADP L-glycero-β-d-manno-heptose (ADP-heptose) were reported to activate the NF-κB pathway and trigger the innate immune responses (Milivojevic M et al. 2017; Zimmermann S et al. 2017; Zhou P et al. 2018; García-Weber D; 2018). ADP-heptose but not HBP can enter host cells autonomously (Zhou P et al. 2018). During infection, ADP-heptose or HBP translocate into the host cytosol where their presence is sensed by alpha-protein kinase 1 (ALPK1) (Zimmermann S et al. 2017; Zhou P et al. 2018). ADP-heptose directly binds and activates ALPK1 (Garcia-Weber D et al. 2018; Zhou P et al. 2018); instead, HBP is converted by host-derived adenylyltransferases, such as nicotinamide nucleotide adenylyltransferases, to ADP-heptose 7-P, a substrate which can then activate ALPK1 (Zhou P et al. 2018). The ADP-heptose binding to ALPK1 is thought to trigger conformational changes and stimulate the kinase domain of ALPK1 (Zhou P et al. 2018). ALPK1 kinase activity in turn leads to the phosphorylation-dependent oligomerization of the tumor necrosis factor (TNF-α) receptor–associated factor (TRAF)–interacting protein with the forkhead-associated domain (TIFA) (Zimmermann S et al. 2017; Zhou P et al. 2018). This process activates TRAF6 oligomerization and ubiquitination, and the recruitment of transforming growth factor β-activated kinase 1 (TAK1)-binding protein 2 (TAB2), a component of the TAK1 (MAP3K7) complex (Ea CK et al. 2004; Gaudet RG et al. 2017). This TIFA oligomer signaling platform was given the term: TIFAsome. TIFAsome-activ-

ated TAK1 induces NF-κB nuclear translocation and proinflammatory gene expression. The ALPK1-TIFA signaling pathway has been identified in human embryonic kidney cells, intestinal epithelial cells, gastric cells and cervical cancer cells (Gaudet RG et al. 2015, 2017; Stein SC et al. 2017; Gall A et al. 2017; Zimmermann S et al. 2017; Milivojevic M et al. 2017; Zhou P et al. 2018). In vivo studies demonstrate that ADP-heptose and Burkholderia cenocepacia trigger massive inflammatory responses with increased production of several NF-κB-dependent cytokines and chemokines in wild type (WT), but not in Alpk1-/- mice (Zhou P et al. 2018).

This Reactome module describes ALPK1 as a cytosolic innate immune receptor for bacterial ADP-heptose.

Literature references

Hu, X., Yang, C., Wang, PG., Zhang, GL. (2019). ADP-heptose: A new innate immune modulator. *Carbohydr. Res., 473*, 123-128. ↗

Zhou, P., She, Y., Dong, N., Li, P., He, H., Borio, A. et al. (2018). Alpha-kinase 1 is a cytosolic innate immune receptor for bacterial ADP-heptose. *Nature, 561*, 122-126. ↗

Editions

2019-05-17	Authored	Shamovsky, V.
2019-06-03	Reviewed	Gillespie, ME.
2019-07-08	Reviewed	Shao, F.
2019-08-09	Edited	Shamovsky, V.

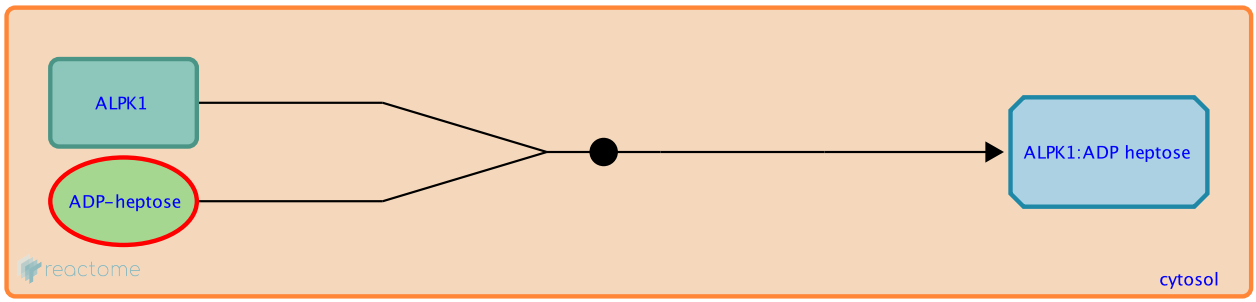
ALPK1 binds ADP-heptose ↗

Location: Alpha-protein kinase 1 signaling pathway

Stable identifier: R-HSA-9645428

Type: binding

Compartments: cytosol



A transposon mutagenesis study of 21,000 mutants induced in the Gram-negative bacterium Yersinia pseudotuberculosis (Y. pseudotuberculosis) revealed that a bacterial transposon carrying a hldE mutation had an impaired ability to induce activation of the nuclear factor kappa B (NF-κB) pathway in the human embryonic kidney 293T cell line (HEK293T) (Zhou P et al. 2018). The gene hldE is essential for the biosynthesis of ADP L-glycero-β-d-manno-heptose (ADP-heptose), a bacterial metabolic intermediate in lipopolysaccharide (LPS) biosynthesis, which is present in all Gram-negative and some Gram-positive bacteria (Tang W et a. 2018). Moreover, deletion of related genes required for the biosynthesis of the ADP-heptose-related metabolite, d-glycero-β-d-manno-heptose 1,7-bisphosphate (HBP), prevented activation of NF-κB by Y. pseudotuberculosis (Zhou P et al. 2018). Further, synthetic ADP-heptose but not HBP, when added to the

extracellular space, induced NF-κB activation and interleukin 8 (IL8 or CXCL8) secretion in HEK293T cells (Zhou P et al. 2018). A fluorescence-activated cell sorting (FACS)-based, genome-wide CRISPR-Cas9 screen on a HEK293T NF-κB reporter cell line identified alpha protein kinase 1 (ALPK1), tumor necrosis factor (TNF-α) receptor-associated factor (TRAF)-interacting protein with the forkhead-associated domain (TIFA) and TRAF6 as mediators of NF-kB activation induced by ADP-heptose or Y. pseudotuberculosis (Zhou P et al. 2018). ALPK1-/- or TIFA-/- HEK293T cells showed abolished NF-κB activation and cytokine expression. Defective NF-κB activation in ADP-heptose-treated ALPK1-/- HEK293T cells was restored by wild-type ALPK1 but not by its kinase-inactive K1067M mutant (Zhou P et al. 2018). Moreover, ADP-heptose stimulated coimmunoprecipitation of TIFA with ALPK1 (Zhou P et al. 2018). Further, ALPK1 kinase activity was required for ADP-heptose-induced phosphorylation of TIFA in HEK293T cells, thus indicating that ALPK1 acts upstream of TIFA (Zhou P et al. 2018). Similarly, ADP-heptose sensing was ALPK1-dependent during S. flexneri infection (Garcia-Weber D et al. 2018). These findings are supported by studies showing that cytosolic HBP, found in the bacteria Neisseria meningitidis, Shigella flexneri, Salmonella enterica serovar Typhimurium and Helicobacter pylori (H. pylori), induced activation of ALPK1-TIFA-dependent NF-κB signaling in host cells (Zimmermann S et al. 2017; Milivojevic M et al. 2017; Gaudet RG et al. 2017). In addition, HBP failed to directly activate ALPK1 (Garcia-Weber D et al. 2018; Zhou P et al. 2018); instead, HBP is converted by host-derived adenylyltransferases, such as nicotinamide nucleotide adenylyltransferases, to ADP-heptose 7-P, a substrate which activates ALPK1 and the downstream NF-κB response (Zhou P et al. 2018). ALPK1 contains a kinase domain (KD) and an α-helical domain linked by an unstructured region (Zhou P et al. 2018). Co-expression of the N-terminal domain (NTD) and KD of ALPK1 (ALPK1-NTD (1–473) and ALPK1-KD (959–1244), respectively) in HEK293T cells was sufficient to allow ADP-heptose or Y. pseudotuberculosis to induce activation of NF-κB and phos-

phorylation of TIFA (Zhou P et al. 2018). High-performance liquid chromatography (HPLC)- mass spectrometry (MS) fractionation of small-molecule extracts from His6–ALPK1-NTD purified from wild-type E. coli coupled with anti-pT9-TIFA immunoblotting and NF-κB luciferase reporter assays in HEK293T identified one active fraction that contained the presumed ADP-heptose ion. Direct binding of E. coli ΔhldE-derived apo-ALPK1-(NTD+KD) complex to ADP-heptose was detected by BioLayer Interferometry (BLI) assay in vitro (Zhou P et al. 2018). The structural studies revealed that a narrow pocket in ALPK1-NTD directly binds ADP-heptose (Zhou P et al. 2018). The ADP-heptose-binding residues are conserved in ALPK1 of other vertebrates. Introducing several mutations in respective residues at the NTD of ALPK1 impaired the ability of ALPK1 to activate NF-κB in response to ADP-heptose (Zhou P et al. 2018). The ADP-heptose binding to ALPK1 is thought to trigger conformational changes and stimulate the kinase domain of ALPK1 to phosphorylate and further activate TIFA, which eventually trigger activation of the downstream NF-κB pathway (Zhou P et al. 2018). Many Gram-negative bacteria induce host cytokine expression in a type III secretion system (T3SS)-dependent manner. T3SS of Y. pseudotuberculosis was required for ADP-heptose induced activation of NF-κB signaling, indicating that a bacterial injection system mediated ADP-heptose translocation to induce activation of ALPK1 upon Y. pseudotuberculosis infection (Zhou P et al. 2018). However, sensing of ADP-heptose by ALPK1 is not just limited to bacteria with T3SS. Burkholderia cenocepacia, enterotoxigenic E. coli, and diffuse-adhering E. coli, also stimulated NF-κB activation through the ALPK1-TIFA axis in an hldE-dependent manner in HEK293T cells (Zhou P et al. 2018). Further, TIFAsome formation and NF-kB activation could be triggered by H. pylori's component secreted in a type IV secretion system (T4SS)-dependent fashion (Zimmermann S et al. 2017; Stein SC et al. 2017). Animal studies indicate that injection of ADP-heptose induced massive neutrophil recruitment with increased production of several NF-κB-dependent cytokines and chemokines in wild type (WT), but not in Alpk1-/- mice. On infection with B. cenocepacia, which triggers lung inflammation in WT, but not Alpk1-/- mice showed increased expression of NF-κB-dependent cytokines and chemokines in the lungs, which led to higher bacterial load in the lungs of Alpk-/-, but not WT, mice (Zhou P et al. 2018). Thus, in vitro and in vivo studies identified ADP-heptose as a bona fide bacterial immunomodulator which is sensed by cytosolic innate immune receptor ALPK1.

Followed by: ALPK1:ADP-heptose binds TIFA

Literature references

Zhou, P., She, Y., Dong, N., Li, P., He, H., Borio, A. et al. (2018). Alpha-kinase 1 is a cytosolic innate immune receptor for bacterial ADP-heptose. *Nature, 561*, 122-126. ↗

García-Weber, D., Dangeard, AS., Cornil, J., Thai, L., Rytter, H., Zamyatina, A. et al. (2018). ADP-heptose is a newly identified pathogen-associated molecular pattern of Shigella flexneri. *EMBO Rep., 19*. ↗

Editions

2019-05-17	Authored	Shamovsky, V.
2019-06-03	Reviewed	Gillespie, ME.
2019-07-08	Reviewed	Shao, F.
2019-08-09	Edited	Shamovsky, V.

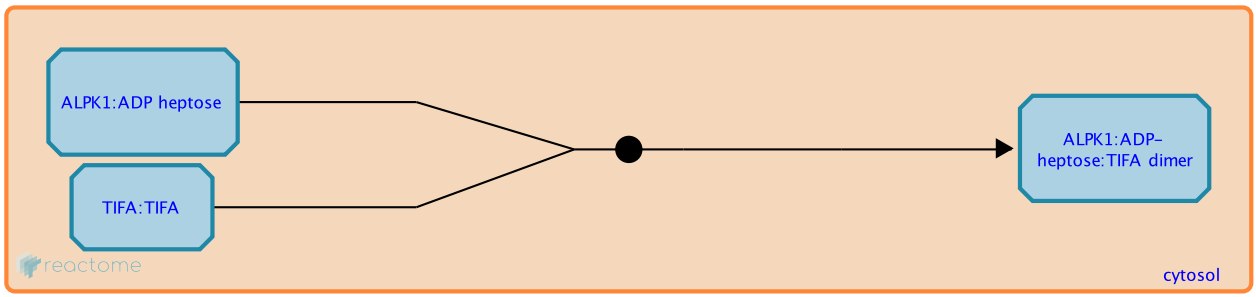
ALPK1:ADP-heptose binds TIFA ↗

Location: Alpha-protein kinase 1 signaling pathway

Stable identifier: R-HSA-9645524

Type: binding

Compartments: cytosol



A fluorescence-activated cell sorting (FACS)-based, genome-wide CRISPR-Cas9 screen on a HEK293T NF-κB reporter cell line identified alpha protein kinase 1 (ALPK1), tumor necrosis factor (TNF-α) receptor–associated factor (TRAF)–interacting protein with the forkhead-associated domain (TIFA) and TRAF6 as mediators of NF-kB activation induced by bacterial ADP L-glycero-β-d-manno-heptose (ADP-heptose) or by Yersinia pseudotuberculosis (Zhou P et al. 2018). ADP-heptose is metabolic intermediate in the lipopolysaccharide (LPS) biosynthesis, which is present in all Gram-negative and some Gram-positive bacteria (Tang W et al. 2018). ADP-heptose stimulated coimmunoprecipitation of TIFA with ALPK1 and TRAF6 (Zhou P et al. 2018). Deletion of ALPK1 or TIFA abolished ADP-heptose-induced NF-κB activation and cytokine expression in HEK293T cells. Defective NF-κB activation in ADP-heptose-treated TIFA-/- HEK293T cells was restored by wild-type TIFA but not by a T9A mutant (Zhou P et al. 2018). Further, ALPK1 kinase activity was required for ADP-heptose-induced phosphorylation of TIFA in HEK293T cells, thus indicating that ALPK1 acts upstream of TIFA (Zhou P et al. 2018). Similarly, ADP-heptose sensing and TIFA oligomerization was ALPK1-dependent during Shigella flexneri infection (Garcia-Weber D et al. 2018). These findings are supported by studies showing that d-glycero-β-d-manno-heptose 1,7-bisphosphate (HBP), another intermediate of the LPS biosynthesis pathway, induced activation of ALPK1-TIFA-dependent NF-κB signaling in host cells upon Neisseria meningitidis, Shigella flexneri, Salmonella enterica serovar Typhimurium or Helicobacter pylori (H. pylori) infections (Zimmermann S et al. 2017; Milivojevic M et al. 2017; Gaudet RG et al. 2017). It is important to note that HBP is converted by host-derived adenylyltransferases, such as nicotinamide nucleotide adenylyltransferase, to ADP-heptose 7-P, a substrate which can then activate ALPK1 and the downstream NF-κB response (Zhou P et al. 2018).

Preceded by: ALPK1 binds ADP-heptose

Followed by: ALPK1 phosphorylates TIFA

Literature references

Zhou, P., She, Y., Dong, N., Li, P., He, H., Borio, A. et al. (2018). Alpha-kinase 1 is a cytosolic innate immune receptor for bacterial ADP-heptose. *Nature, 561*, 122-126. ↗

Editions

2019-05-17	Authored	Shamovsky, V.
2019-06-03	Reviewed	Gillespie, ME.
2019-07-08	Reviewed	Shao, F.
2019-08-09	Edited	Shamovsky, V.

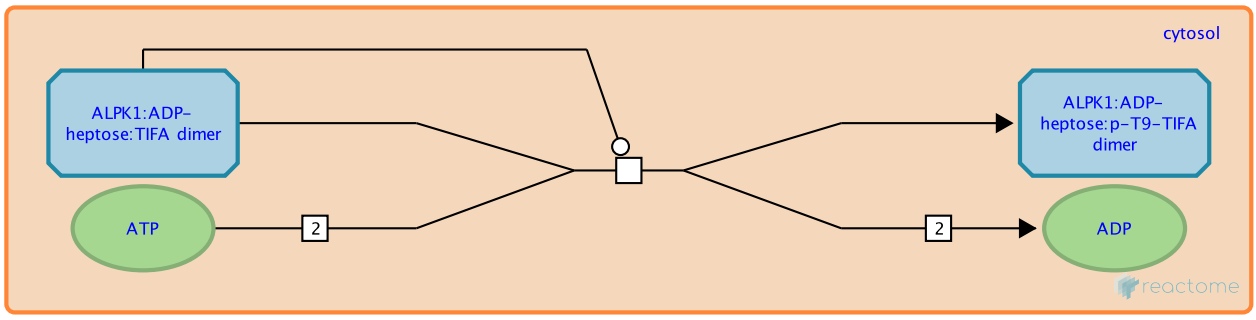
ALPK1 phosphorylates TIFA ↗

Location: Alpha-protein kinase 1 signaling pathway

Stable identifier: R-HSA-9645535

Type: transition

Compartments: cytosol



TRAF-interacting protein with a forkhead-associated (FHA) domain (TIFA) was reported to trigger NF-kappa B mediated inflammatory responses to Helicobacter pylori, Shigella flexneri, Yersinia pseudotuberculosis infections (Gall A et al. 2017; Milivojevic M et al. 2017; Gaudet RG et al. 2017; García-Weber D et al. 2018; Zhou P et al. 2018). During infection, ADP-heptose-activated alpha protein kinase 1 (ALPK1) binds and phosphorylates TIFA at threonine 9 (T9) (Zhou P et al. 2018). Defective NF-κB activation in TIFA -/- human embryonic kidney 293T (HEK293T) cells was restored by wild-type TIFA but not by the non-phosphorylatable T9A TIFA variant upon H. pylori, S. flexneri, Y. pseudotuberculosis infections (Zimmermann S et al. 2017; Gaudet RG et al. 2017; Zhou P et al. 2018). Moreover, T9A TIFA variant was unable to oligomerize preventing the S. flexneri- induced formation of the TIFA:TRAF6:TAK1-Ub complex in TIFA -/- HEK293T cells (Gaudet RG et al. 2015, 2017). Unphosphorylated TIFA is thought to exist as an intrinsic dimer in solution (Huang CC et al. 2012). When T9 is phosphorylated, this is recognized by the FHA domain of other TIFA dimers leading to its oligomerization (Huang CC et al. 2012). Oligomerized TIFA promotes activation of an innate immune response by inducing the oligomerization and polyubiquitination of TRAF6, which leads to the activation of TAK1 (MAP3K7) and IKK (Ea CK et al. 2004).

Preceded by: ALPK1:ADP-heptose binds TIFA

Followed by: TIFA oligomerization

Literature references

Zhou, P., She, Y., Dong, N., Li, P., He, H., Borio, A. et al. (2018). Alpha-kinase 1 is a cytosolic innate immune receptor for bacterial ADP-heptose. *Nature, 561*, 122-126. ↗

Editions

2019-05-17	Authored	Shamovsky, V.
2019-06-03	Reviewed	Gillespie, ME.
2019-07-08	Reviewed	Shao, F.
2019-08-09	Edited	Shamovsky, V.

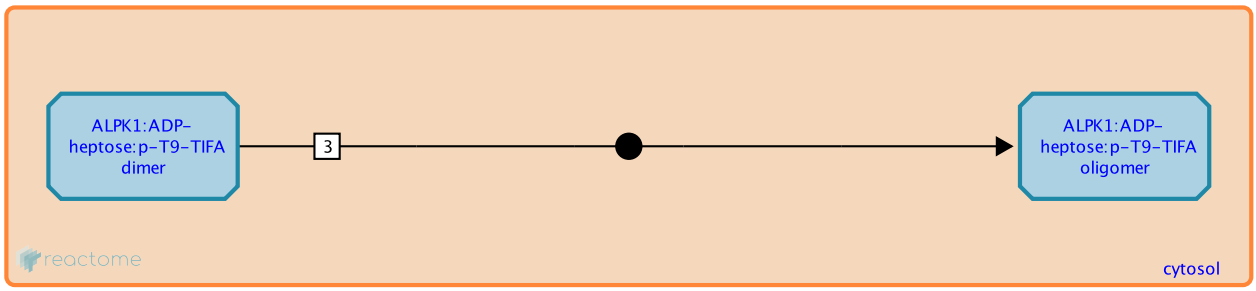
TIFA oligomerization ↗

Location: Alpha-protein kinase 1 signaling pathway

Stable identifier: R-HSA-9645481

Type: binding

Compartments: cytosol



TRAF-interacting protein with a forkhead-associated (FHA) domain (TIFA) was reported to induce NF-kappa B mediated inflammatory responses to Helicobacter pylori, Shigella flexneri, Yersinia pseudotuberculosis and other infections in various human cells (Gall A et al. 2017; Milivojevic M et al. 2017; Gaudet RG et al. 2017; García-Weber D et al. 2018; Zhou P et al. 2018). During infection, alpha protein kinase 1 (ALPK1) phosphorylates TIFA at threonine 9 (T9) (Zimmerman S et al. 2017; Milivojevic M et al. 2017; Zhou P et al. 2018). Unphosphorylated TIFA is thought to exist as an intrinsic dimer in solution (Huang CC et al. 2012). When T9 is phosphorylated, this is recognized by the FHA domain of other TIFA dimers leading to TIFA self-oligomerization (Huang CC et al. 2012). FHA domain is the only signaling domain that recognizes phosphothreonine (pT) specifically (Hammet A et al. 2003). T9A TIFA variant was unable to oligomerize preventing the S. flexneri- induced formation of the TIFA:TRAF6:TAK1-Ub complex in TIFA -/- HEK293T cells (Gaudet RG et al. 2015, 2017). Structural studies of the truncated TIFA in complex with its T9 phosphorylated N-terminal peptide (1-15) confirmed that the pT9–FHA domain interaction can occur only between different sets of dimers rather than between the two protomers within a dimer (Weng JH et al. 2015). Glycerol-gradient ultracentrifugation analysis revealed that a very small amount of TIFA formed oligomers and only these oligomeric forms of TIFA were able to activate IKK in vitro (Ea CK et al. 2004). Mutations in the FHA domain caused a shift in the distribution of the molecular sizes of TIFA toward the middle of the gradient, but none of the fractions containing TIFA-FHA mutant was able to activate IKK (Ea CK et al. 2004). Homo-oligomerization of TIFA was also observed when glutathione S-transferase (GST)- tagged TIFA or its mutants were co-expressed with FLAG-TIFA in human embryonic kidney 293T (HEK293T) cells followed by GST-pull down assay (Takatsuna H et al. 2003). TIFA aggregation was measured by clear-native PAGE (CN-PAGE) in 1XFLAG-TIFA expressing HEK293T cells infected with Salmonella for 6 hr (Gaudet RG et al. 2017). ALPK1 depletion completely prevented the formation of TIFA oligomers after Shigella flexneri infection in HeLa cells (Milivojevic M et al. 2017). TIFA oligomerization was restored by overexpressing a siRNA-resistant full length ALPK1 construct. In contrast, overexpressing a construct deleted of the kinase domain of ALPK1 failed to do so, showing that the kinase domain of ALPK1 is essential for the regulation of TIFA oligomerization (Milivojevic M et al. 2017).

Preceded by: ALPK1 phosphorylates TIFA

Followed by: ALPK1:ADP-heptose:TIFA oligomer recruits TRAF6

Literature references

Weng, JH., Hsieh, YC., Huang, CC., Wei, TY., Lim, LH., Chen, YH. et al. (2015). Uncovering the Mechanism of Forkhead-Associated Domain-Mediated TIFA Oligomerization That Plays a Central Role in Immune Responses. *Biochemistry, 54*, 6219-29. ↗

Editions

2019-05-17	Authored	Shamovsky, V.
2019-06-03	Reviewed	Gillespie, ME.
2019-07-08	Reviewed	Shao, F.
2019-08-09	Edited	Shamovsky, V.

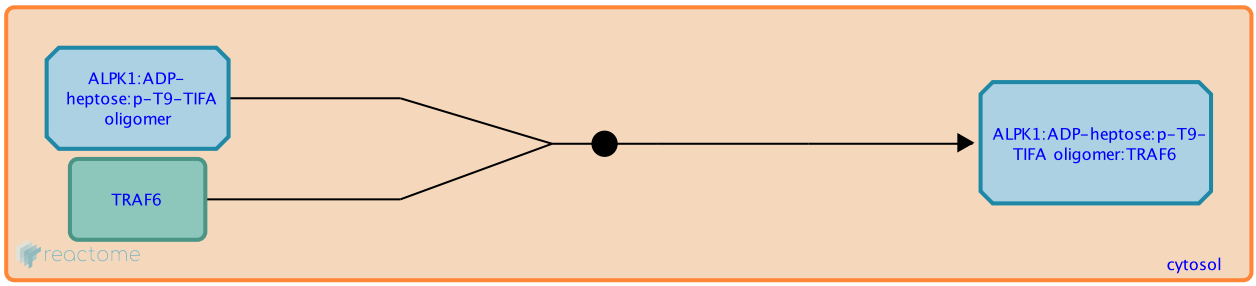
ALPK1:ADP-heptose:TIFA oligomer recruits TRAF6 ↗

Location: Alpha-protein kinase 1 signaling pathway

Stable identifier: R-HSA-9645520

Type: binding

Compartments: cytosol



A fluorescence-activated cell sorting (FACS)-based, genome-wide CRISPR-Cas9 screen on a HEK293T NF-κB reporter cell line identified alpha protein kinase 1 (ALPK1), tumor necrosis factor (TNF-α) receptor-associated factor (TRAF)-interacting protein with the forkhead-associated domain (TIFA) and TRAF6 as mediators of NF-kB activation induced by bacterial ADP L-glycero-β-d-manno-heptose (ADP-heptose) or by Yersinia pseudotuberculosis (Y. pseudotuberculosis) (Zhou P et al. 2018). ADP-heptose is metabolic intermediate in the lipopolysaccharide (LPS) biosynthesis, which is present in all Gram-negative and some Gram-positive bacteria (Tang W et a. 2018). ADP-heptose stimulated coimmunoprecipitation of TIFA with ALPK1 and TRAF6 in HEK293T cells (Zhou P et al. 2018). The co-localization of both proteins was visualized in Shigella flexneri (S. flexneri)-infected HeLa cells co-transfected with TIFA-myc and TRAF6-Flag cDNA constructs (Milivojevic M et al. 2017). The same result was obtained upon infection of human epithelial colorectal adenocarcinoma Caco-2 cells. The interaction between TIFA and TRAF6 was further confirmed by co-immunoprecipitation assay in HeLa cells co-transfected with TIFA-myc and TRAF6-Flag cDNA constructs (Milivojevic M et al. 2017). The E178A TIFA mutant was unable to bind TRAF6 suggesting that TRAF6 activation was dependent on the TRAF6 binding motif of TIFA (Milivojevic M et al. 2017). Finally, structural studies further support the interaction between TIFA and TRAF6 (Huang WC et al. 2019). Small interfering RNA (siRNA) oligonucleotides targeting Ubc13, TRAF6, or TRAF2 strongly inhibited TIFA-mediated NF-κB activation upon the expression of these genes in HEK293 cells transfected with TIFA expression vector and a luciferase reporter gene thus suggesting that Ubc13, TRAF2, and TRAF6 are required for TIFA-mediated NF-κB activation in living cells (Ea CK et al. 2004). Further, analysis of the molecular sizes by glycerol-gradient ultracentrifugation showed that only the high-molecular-weight forms of TIFA co-sedimented with TRAF6, suggesting that oligomerization of TIFA greatly enhances its ability to bind to TRAF6 (Ea CK et al. 2004). The TIFA mutant that did not bind to TRAF6 was also unable to induce TRAF6 oligomerization (Ea CK et al. 2004). In vitro ubiquitination assay in the presence of E1, Ubc13-Uev1A, purified endogenous TRAF6, Ub, and ATP showed that TIFA enhanced the Ub ligase activity of TRAF6 (Ea CK et al. 2004). ALPK1 kinase activity was found to control TIFA oligomerization and TRAF6 activation in response to the invasive bacteria Y. pseudotuberculosis, S. flexneri and Salmonella typhimurium as well as to the extracellular pathogen Neisseria meningitidis (Milivojevic M et al. 2017; Zhou P et al. 2018). Thus, ALPK1 induces TIFA oligomerization upon bacterial infection (Zhou P et al. 2018; Milivojevic M et al. 2017). The oligomerized forms of TIFA bind to TRAF6 and promote TRAF6 oligomerization (Ea CK et al. 2004). As a result, the TRAF6 Ub ligase is activated to catalyze K63-linked polyubiquitination in conjunction with the Ubc13-Uev1A E2 complex (Ea CK et al. 2004). Activated TRAF6 promotes polyubiquitin-mediated activation of the protein kinase TAK1 (MAP3K7) complex (Ea CK et al. 2004). TAK1 is then phosphorylates IkB kinase (IKK) at key serine residues within the activation loop,

thereby activating IKK complex (Israël A 2010).

Preceded by: TIFA oligomerization

Followed by: TRAF6 oligomerizes within the ALPK1:ADP-heptose:TIFA oligomer complex

Literature references

Zhou, P., She, Y., Dong, N., Li, P., He, H., Borio, A. et al. (2018). Alpha-kinase 1 is a cytosolic innate immune receptor for bacterial ADP-heptose. *Nature, 561*, 122-126. ↗

Ea, CK., Sun, L., Inoue, J., Chen, ZJ. (2004). TIFA activates IkappaB kinase (IKK) by promoting oligomerization and ubiquitination of TRAF6. *Proc. Natl. Acad. Sci. U.S.A., 101*, 15318-23. ↗

Editions

2019-05-17	Authored	Shamovsky, V.
2019-06-03	Reviewed	Gillespie, ME.
2019-07-08	Reviewed	Shao, F.
2019-08-09	Edited	Shamovsky, V.

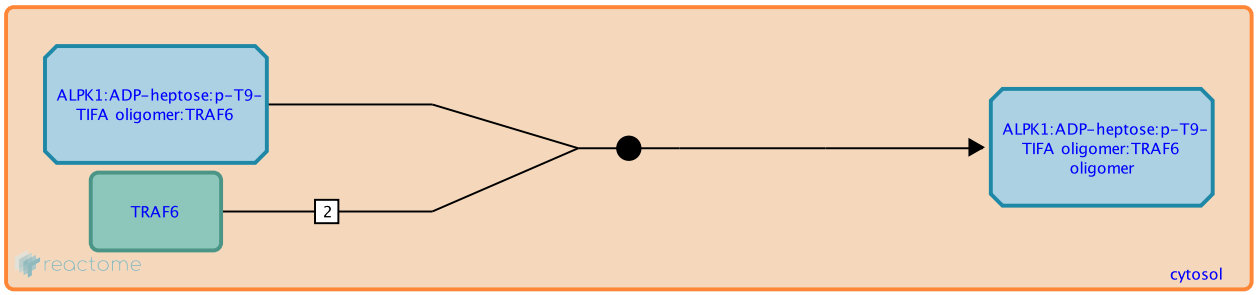
TRAF6 oligomerizes within the ALPK1:ADP-heptose:TIFA oligomer complex ↗

Location: Alpha-protein kinase 1 signaling pathway

Stable identifier: R-HSA-9645501

Type: binding

Compartments: cytosol



Tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6) is a RING domain Ub ligase (E3), which, in conjunction with Ubc13-Uev1A, catalyzes K63-linked polyubiquitination that is required for activation of transforming growth factor β-activated kinase (TAK1 or MAP3K7) and IκB kinase (IKK). TRAF6 is activated downstream of alpha-protein kinase 1 (ALPK1) by binding to TRAF-interacting protein with FHA domain (TIFA) (Milivojevic M et al. 2017; Zhou P et al. 2018). TIFA recruits TRAF6 via a consensus TRAF6-binding motif of TIFA. Glycerol-gradient ultracentrifugation showed that only the high-molecular-weight forms of TIFA co-sedimented with TRAF6, suggesting that oligomerization of TIFA greatly enhanced its ability to bind to TRAF6 (Ea CK et al. 2004). In vitro ubiquitination assay in the presence of E1, Ubc13-Uev1A, purified endogenous TRAF6, Ub, and ATP showed that TIFA enhanced the Ub ligase activity of TRAF6 (Ea CK et al. 2004). Incubation of TRAF6 with wild-type or mutant TIFA proteins followed by gel-filtration chromatography showed that TIFA protein led to oligomerization of TRAF6, which eluted from the gel filtration column in the void volume (>700 kDa) (Ea CK et al. 2004). Importantly, these high-molecular-weight forms of TRAF6 displayed greatly increased Ub ligase activity as compared with TRAF6 of lower-molecular-weight species, despite a similar amount of TRAF6 protein in these fractions. Moreover, only the fraction with significantly higher Ub ligase activity of TRAF6 was able to activate IKK (Ea CK et al. 2004). The TIFA mutant that did not bind to TRAF6 was also unable to induce TRAF6 oligomerization (Ea CK et al. 2004). Cell fractionation and immunoblot analysis also suggest that TRAF6 forms oligomers in a TIFA-dependent manner in HEK293T cells in response to Shigella flexneri infection (Gaudet RG et al. 2017). Thus, upon bacterial infection such as Yersinia pseudotuberculosis, Shigella flexneri, Salmonella typhimurium or Neisseria meningitidis ALPK1 kinase activity induces TIFA oligomerization (Milivojevic M et al. 2017; Zhou P et al. 2018). The oligomerized forms of TIFA bind to TRAF6 and promote TRAF6 oligomerization (Ea CK et al. 2004). As a result, the TRAF6 Ub-ligase is activated to catalyze K63-linked polyubiquitination in conjunction with the Ubc13-Uev1A E2 complex (Ea CK et al. 2004). Activated TRAF6 promotes polyubiquitin-mediated activation the protein kinase TAK1 (MAP3K7) complex (Ea CK et al. 2004). Activated TAK1 (MAP3K7) in turn phosphorylates IkB kinase (IKK) at key serine residues within the activation loop, thereby activating IKK complex (Israël A 2010).

Preceded by: ALPK1:ADP-heptose:TIFA oligomer recruits TRAF6

Followed by: Auto ubiquitination of TRAF6 bound to ALPK1:ADP-heptose:TIFA oligomer

Literature references

Weng, JH., Hsieh, YC., Huang, CC., Wei, TY., Lim, LH., Chen, YH. et al. (2015). Uncovering the Mechanism of Forkhead-Associated Domain-Mediated TIFA Oligomerization That Plays a Central Role in Immune Responses. *Biochemistry, 54*, 6219-29. ↗

Editions

2019-05-17	Authored	Shamovsky, V.
2019-06-03	Reviewed	Gillespie, ME.
2019-07-08	Reviewed	Shao, F.
2019-08-09	Edited	Shamovsky, V.

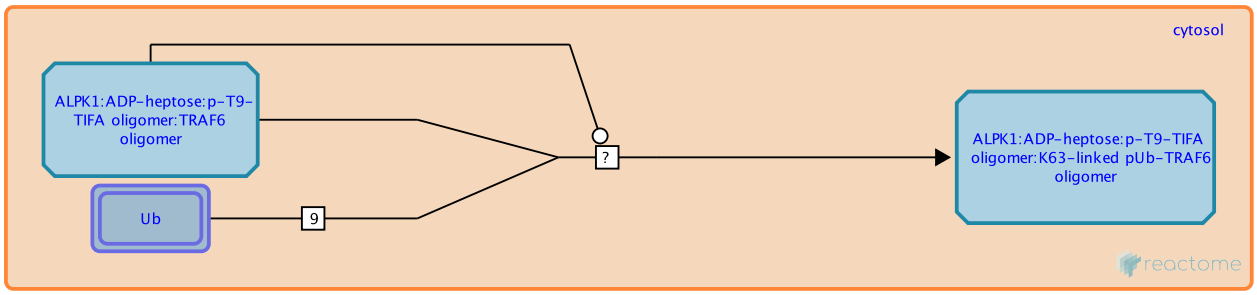
Auto ubiquitination of TRAF6 bound to ALPK1:ADP-heptose:TIFA oligomer ↗

Location: Alpha-protein kinase 1 signaling pathway

Stable identifier: R-HSA-9645414

Type: uncertain

Compartments: cytosol



TNF Receptor Associated Factor 6 (TRAF6) possesses ubiquitin ligase activity and undergoes K-63-linked auto-ubiquitination after its oligomerization. In the first step, ubiquitin is activated by an E1 ubiquitin activating enzyme. The activated ubiquitin is transferred to a E2 conjugating enzyme (a heterodimer of proteins Ubc13 and Uev1A) forming the E2-Ub thioester. Finally, in the presence of ubiquitin-protein ligase E3 (TRAF6, a RING-domain E3), ubiquitin is attached to the target protein (TRAF6 on residue Lysine 124) through an isopeptide bond between the C-terminus of ubiquitin and the epsilon-amino group of a lysine residue in the target protein. In contrast to K-48-linked ubiquitination that leads to the proteosomal degradation of the target protein, K-63-linked polyubiquitin chains act as a scaffold to assemble protein kinase complexes and mediate their activation through proteosome-independent mechanisms. This K63 polyubiquitinated TRAF6 activates the TAK1 kinase complex.

Preceded by: TRAF6 oligomerizes within the ALPK1:ADP-heptose:TIFA oligomer complex

Followed by: Activated TRAF6 synthesizes unanchored polyubiquitin chains upon ALPK1:ADP-heptose stimulation

Literature references

Lamothe, B., Besse, A., Campos, AD., Webster, WK., Wu, H., Darnay, BG. (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. ↗

Editions

2019-05-17	Authored	Shamovsky, V.
2019-06-03	Reviewed	Gillespie, ME.
2019-07-08	Reviewed	Shao, F.
2019-08-09	Edited	Shamovsky, V.

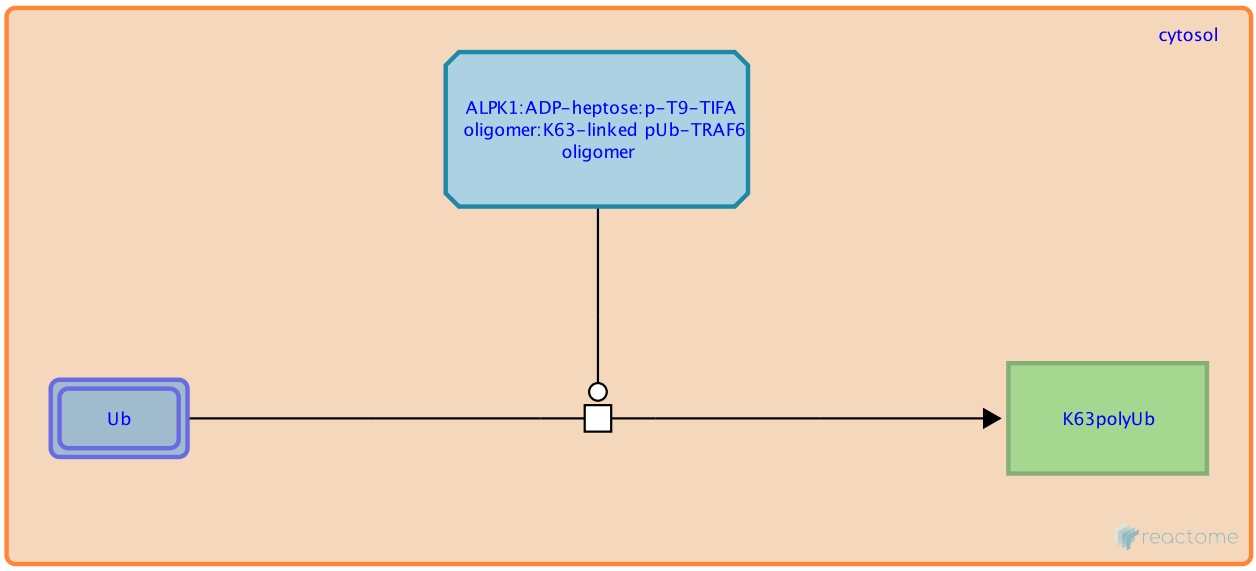
Activated TRAF6 synthesizes unanchored polyubiquitin chains upon ALPK1:ADP-heptose stimulation ↗

Location: Alpha-protein kinase 1 signaling pathway

Stable identifier: R-HSA-9645394

Type: transition

Compartments: cytosol



E3 ubiquitin ligase TRAF6 generates free K63 -linked polyubiquitin chains that non-covalently associate with ubiquitin receptors of TAB2/TAB3 regulatory proteins of the TAK1 complex, leading to the activation of the TAK1 kinase.

Preceded by: Auto ubiquitination of TRAF6 bound to ALPK1:ADP-heptose:TIFA oligomer

Followed by: ALPK1:ADP-heptose:p-T9-TIFA oligomer:K63pUb-TRAF6 oligomer recruits MAP3K7 (TAK1)

Literature references

Xia, ZP., Sun, L., Chen, X., Pineda, G., Jiang, X., Adhikari, A. et al. (2009). Direct activation of protein kinases by unanchored polyubiquitin chains. *Nature*. ↗

Editions

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2019-06-03	Reviewed	Gillespie, ME.
2019-07-08	Reviewed	Shao, F.
2019-08-09	Edited	Shamovsky, V.

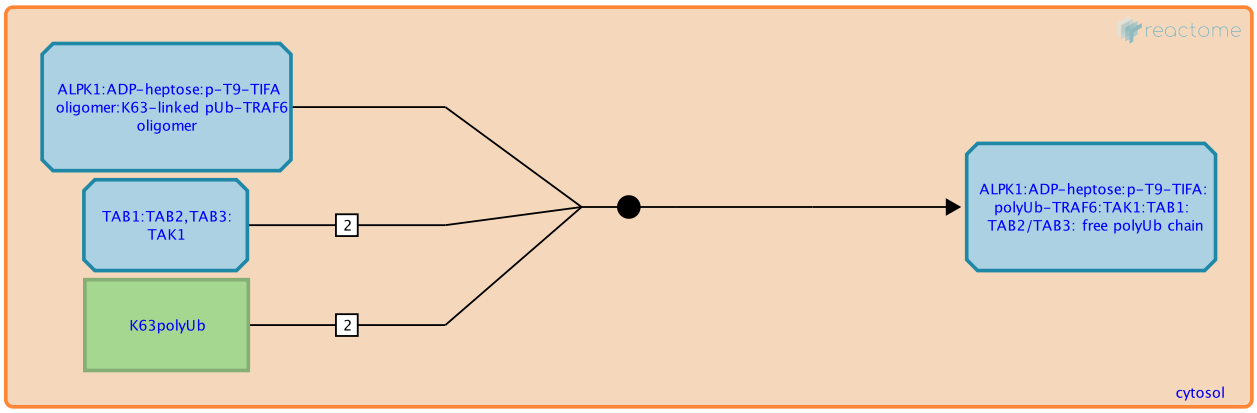
ALPK1:ADP-heptose:p-T9-TIFA oligomer:K63pUb-TRAF6 oligomer recruits MAP3K7 (TAK1)

Location: Alpha-protein kinase 1 signaling pathway

Stable identifier: R-HSA-9645406

Type: binding

Compartments: cytosol



TAK1-binding protein 2 (TAB2) and/or TAB3, as part of a complex that also contains TAK1 (MAP3K7) and TAB1, binds polyubiquitinated TRAF6. The TAB2 and TAB3 regulatory subunits of the TAK1 complex contain C-terminal Npl4 zinc finger (NZF) motifs that recognize Lys63-pUb chains (Kanayama et al. 2004). The recognition mechanism is specific for Lys63-linked ubiquitin chains (Kulathu Y et al 2009). TAK1 can be activated by unattached Lys63-polyubiquitinated chains when TRAF6 has no detectable polyubiquitination (Xia et al. 2009) and thus the synthesis of these chains by TRAF6 may be the signal transduction mechanism. This binding leads to autophosphorylation and activation of TAK1.

Preceded by: Activated TRAF6 synthesizes unanchored polyubiquitin chains upon ALPK1:ADP-heptose stimulation

Followed by: Auto phosphorylation of TAK1 within the ALPK1:ADP-heptose:p-T9-TIFA:pUb-TRAF6: free K63 pUb:TAB1:TAB2/TAB3 :MAP3K7 complex

Literature references

Xia, ZP., Sun, L., Chen, X., Pineda, G., Jiang, X., Adhikari, A. et al. (2009). Direct activation of protein kinases by unanchored polyubiquitin chains. *Nature*.

Kanayama, A., Seth, RB., Sun, L., Ea, CK., Hong, M., Shaito, A. et al. (2004). TAB2 and TAB3 activate the NF-kappaB pathway through binding to polyubiquitin chains. *Mol Cell, 15*, 535-48.

Adhikari, A., Xu, M., Chen, ZJ. (2007). Ubiquitin-mediated activation of TAK1 and IKK. *Oncogene, 26*, 3214-26.

Editions

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2019-06-03	Reviewed	Gillespie, ME.
2019-07-08	Reviewed	Shao, F.
2019-08-09	Edited	Shamovsky, V.

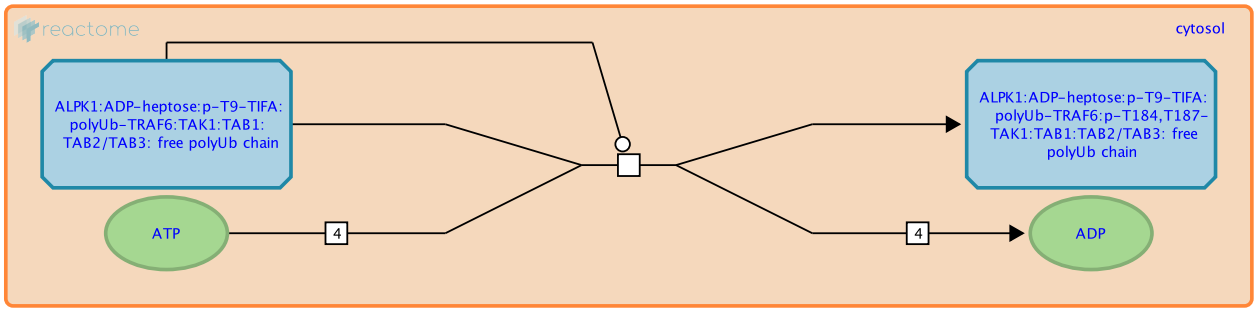
Auto phosphorylation of TAK1 within the ALPK1:ADP-heptose:p-T9-TIFA:pUb-TRAF6: free K63 pUb:TAB1:TAB2/TAB3 :MAP3K7 complex ↗

Location: Alpha-protein kinase 1 signaling pathway

Stable identifier: R-HSA-9645442

Type: transition

Compartments: cytosol



The TAK1 complex consists of transforming growth factor-beta (TGFB)-activated kinase (TAK1 or MAP3K7), TAK1-binding protein 1 (TAB1), TAB2 and TAB3. TAK1 requires TAB1 for its kinase activity (Shibuya et al. 1996, Sakurai et al. 2000). TAB1 promotes TAK1 autophosphorylation at the kinase activation lobe, probably through an allosteric mechanism (Brown et al. 2005, Ono et al. 2001). The TAK1 complex is regulated by polyubiquitination. Binding of TAB2 and TAB3 to Lys63-linked polyubiquitin chains leads to the activation of TAK1 by an uncertain mechanism. Binding of multiple TAK1 complexes to the same polyubiquitin chain may promote oligomerization of TAK1, facilitating TAK1 autophosphorylation and subsequent activation of its kinase activity (Kishimoto et al. 2000). The binding of TAB2/3 to polyubiquitinated TRAF6 may facilitate polyubiquitination of TAB2/3 by TRAF6 (Ishitani et al. 2003), which might result in conformational changes within the TAK1 complex that lead to TAK1 activation. Another possibility is that TAB2/3 may recruit the IKK complex by binding to ubiquitinated NEMO; polyubiquitin chains may function as a scaffold for higher-order signaling complexes that allow interaction between TAK1 and IKK (Kanayama et al. 2004). TAB1 promotes TAK1 autophosphorylation at the kinase activation lobe, probably through an allosteric mechanism (Brown et al. 2005, Ono et al. 2001).

Preceded by: ALPK1:ADP-heptose:p-T9-TIFA oligomer:K63pUb-TRAF6 oligomer recruits MAP3K7 (TAK1)

Literature references

Kishimoto, K., Matsumoto, K., Ninomiya-Tsuji, J. (2000). TAK1 mitogen-activated protein kinase kinase kinase is activated by autophosphorylation within its activation loop. *J Biol Chem, 275*, 7359-64. ↗

Sato, S., Sanjo, H., Takeda, K., Ninomiya-Tsuji, J., Yamamoto, M., Kawai, T. et al. (2005). Essential function for the kinase TAK1 in innate and adaptive immune responses. *Nat Immunol, 6*, 1087-95. ↗

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Brown, K., Vial, SC., Dedi, N., Long, JM., Dunster, NJ., Cheetham, GM. (2005). Structural basis for the interaction of TAK1 kinase with its activating protein TAB1. *J. Mol. Biol., 354*, 1013-20. ↗

Editions

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2019-06-03	Reviewed	Gillespie, ME.
2019-07-08	Reviewed	Shao, F.
2019-08-09	Edited	Shamovsky, V.

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