ALPK1 binds ADP-heptose

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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.

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Literature references

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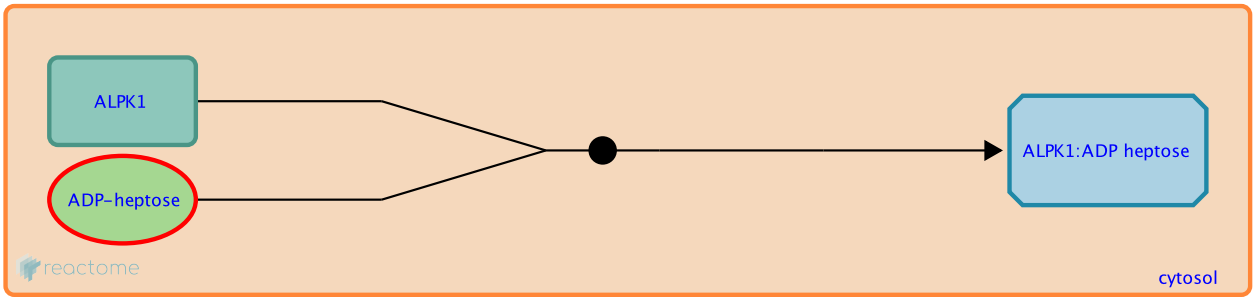
Stable identifier: R-HSA-9645428

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 phosphorylation 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.

Literature references

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Editions

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