

RIPK3 phosphorylates MLKL

Chan, FK., Gillespie, ME., Murphy, JM., Shamovsky, V.

European Bioinformatics Institute, New York University Langone Medical Center, Ontario Institute for Cancer Research, Oregon Health and Science University.

The contents of this document may be freely copied and distributed in any media, provided the authors, plus the institutions, are credited, as stated under the terms of [Creative Commons Attribution 4.0 International \(CC BY 4.0\) License](https://creativecommons.org/licenses/by/4.0/). For more information see our [license](https://reactome.org/licenses/).

06/10/2024

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. [↗](#)

Reactome database release: 90

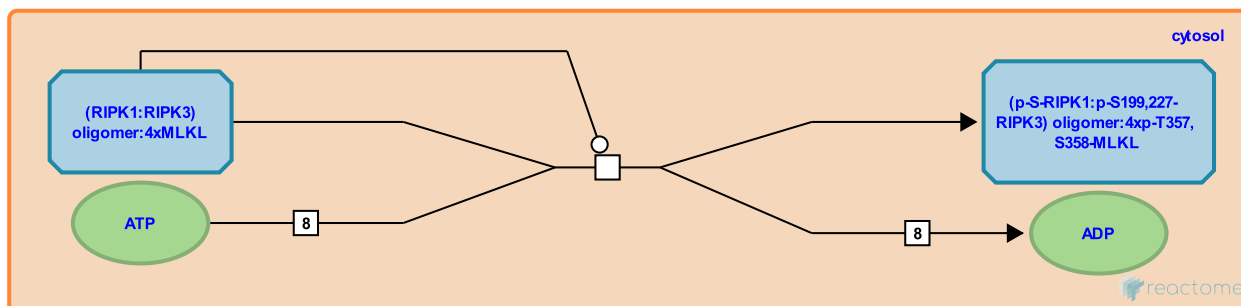
This document contains 1 reaction ([see Table of Contents](#))

RIPK3 phosphorylates MLKL ↗

Stable identifier: R-HSA-5218906

Type: transition

Compartments: cytosol



Receptor-interacting serine/threonine-protein kinase 3 (RIPK3) was shown to activate mixed lineage kinase domain-like protein (MLKL) by phosphorylation of the threonine 357 (T357) and serine 358 (S358) residues within the kinase-like domain in human MLKL and S345 in mouse MLKL (Sun L et al. 2012; Wang H et al. 2014; Murphy JM et al. 2013; Tanzer MC et al. 2015; Rodriguez DA et al. 2016). The precise mechanism of MLKL activation by RIPK3 is incompletely understood and may vary across species (Davies KA et al. 2020; reviewed by Murphy JM 2020). The pseudokinase domain (psKD) of MLKL is known to engage the kinase domain (KD) of RIPK3, stably in the case of the human system (Sun L et al. 2012; Davies KA et al. 2018; Petrie EJ et al. 2018, 2019a), but transiently in the mouse system (Tanzer MC et al. 2015; Rodriguez DA et al. 2016; Petrie EJ et al. 2019b). The kinase-dead RIPK3 mutants were unable to bind MLKL or mediate TNF-induced necroptosis in human and mouse cells (Sun L et al. 2012; Zhao J et al. 2012; Murphy JM et al. 2013; Chen W et al. 2013). Studies involving knockout of endogenous MLKL in human histiocytic lymphoma U937 and adenocarcinoma HT-29 cells support the idea that activation of MLKL relies on the RIPK3-mediated phosphorylation of T357 and S358 in human MLKL (Petrie EJ et al. 2018). While wild-type human MLKL could reconstitute the necroptotic signaling, both the T357E/S358E phosphomimic and the T357A/S358A phospho-ablating human MLKL constructs blocked necroptosis in MLKL^{-/-} U937 and HT-29 cell lines in the presence of necroptosis stimuli (Petrie EJ et al. 2018). Furthermore, introduction of constructs harboring mutations within the human MLKL pseudoactive site, such as those observed in colon, lung, and endometrial carcinomas and melanoma specimens, into MLKL^{-/-} U937 cells did not promote MLKL's killing activity, but rather delayed the kinetics of cell death following treatment with a necroptosis stimulus (Petrie EJ et al. 2018). Biophysical data suggest that defective MLKL variants are locked in a monomeric conformation, which hampers assembly into higher order oligomers that are responsible for cell death (Petrie EJ et al. 2018). Although wild-type human MLKL robustly bound human RIPK3 kinase domain, no binding was detected for the human MLKL T357E/S358E constructs (Petrie et al. 2018). These data support the idea that human MLKL activation relies on recruitment to human RIPK3 in cells as a precursor to its activation (Petrie EJ et al. 2019). RIPK3-mediated phosphorylation of human MLKL is thought to trigger a conformational change within the pseudokinase of MLKL that promotes the N-terminal four-helix bundle (4HB) domain exposure, enabling MLKL to form higher order MLKL assemblies which are trafficked to the plasma membrane (Sun L et al. 2012; Wang H et al. 2014; Petrie EJ et al. 2017, 2018, 2019; 2020; Samson AL et al. 2020). The phosphorylation of MLKL may induce disengagement of MLKL from RIPK3 followed by translocation to the plasma membrane where cell permeabilization occurs (Davies KA et al. 2020; Murphy JM 2020). Important to note that the assembly of MLKL into higher order species and the translocation of MLKL oligomers to the plasma membrane are hallmarks of necroptosis (Davies KA et al. 2020; Petrie EJ et al. 2020; Samson AL et al. 2020). This Reactome event shows that 4 molecules of MLKL are bound to RIPK1:RIPK3 oligomer, however the exact stoichiometry of MLKL binding remains unclear (Chen X et al. 2014; Cai Z et al. 2014; Davies KA et al. 2018; Petrie EJ et al. 2018; reviewed by Petrie EJ 2017). Single-cell imaging approaches revealed that endogenous human MLKL assembles into higher order species that are heterogeneous in MLKL stoichiometry (Samson AL et al. 2020). The mechanisms of necroptosis regulation and execution downstream of MLKL remain elusive.

Literature references

- Wang, X., Liu, L., Su, L., Wang, H., Wang, L.F., Sun, L. et al. (2014). Mixed lineage kinase domain-like protein MLKL causes necrotic membrane disruption upon phosphorylation by RIP3. *Mol. Cell*, 54, 133-46. ↗
- Wang, X., Yan, J., Liao, D., Wang, Z., Liu, W., Wang, L. et al. (2012). Mixed lineage kinase domain-like protein mediates necrosis signaling downstream of RIP3 kinase. *Cell*, 148, 213-27. ↗

Editions

2013-12-20	Authored	Shamovsky, V.
2014-10-31	Reviewed	Gillespie, ME.
2015-02-10	Edited	Shamovsky, V.
2015-02-15	Reviewed	Chan, FK.
2020-08-28	Reviewed	Murphy, JM.