## Phylogeny

The Interleukin-1 Receptor-Associated Kinase (IRAK) family of serine/threonine kinases consists of IRAK1, IRAK2, IRAK3 (also known as IRAK-M), and IRAK4 (janssens2003functionaldiversityand pages 1-2, suzuki2005irakskeyregulatory pages 1-2). In the human kinome, the IRAK family is classified within the TKL (Tyrosine Kinase-Like) group (janssens2003functionaldiversityand pages 1-2, mahmoud2023modulationofirak pages 4-6, wang2006crystalstructuresof pages 7-8). The IRAK family is related to the Pelle kinase in *Drosophila* (suzuki2005irakskeyregulatory pages 1-2). An IRAK4-like kinase is considered the ancestral gene from which other IRAKs diverged (freihat2017doesthenovel pages 29-33). IRAK proteins are highly conserved across vertebrates (pereira2023regulationofinnate pages 1-2). While human IRAK3 is a class I pseudokinase, the rodent ortholog contains an asparagine in a key catalytic position, suggesting it may possess kinase activity (freihat2017doesthenovel pages 83-87, freihat2017doesthenovel pages 29-33).

## Reaction Catalyzed

In humans, IRAK3 is a pseudokinase that lacks phosphotransferase activity (lange2021dimericstructureof pages 1-3, freihat2017doesthenovel pages 29-33, mahmoud2023modulationofirak pages 4-6). Its catalytic inactivity stems from substitutions of key residues, including a serine (Ser293) replacing the aspartic acid in the canonical HRD motif and a DFA sequence replacing the DFG motif (freihat2017doesthenovel pages 83-87, lange2021dimericstructureof pages 3-4). However, IRAK3 contains an embedded guanylate cyclase (GC) center that catalyzes the conversion of GTP to cyclic GMP (cGMP) (turek2023mutationsinthe pages 1-2, freihat2017doesthenovel pages 29-33).

## Cofactor Requirements

Due to its pseudokinase nature, IRAK3 does not effectively bind cations like Mg²⁺ or Mn²⁺ required for kinase activity (freihat2017doesthenovel pages 83-87). Its guanylate cyclase activity is predicted to require metal ion cofactors, such as Mn²⁺, which bind to residues D377 and D385 (turek2023mutationsinthe pages 7-10).

## Substrate Specificity

As a catalytically inactive pseudokinase, human IRAK3 does not phosphorylate substrates, and thus no kinase substrate motif is known (freihat2017doesthenovel pages 83-87). High-throughput screening studies have confirmed that IRAK3 shows little to no kinase activity (suzuki2005irakskeyregulatory pages 1-2, mahmoud2023modulationofirak pages 4-6). The substrate for its guanylate cyclase activity is GTP (turek2023mutationsinthe pages 1-2, freihat2017doesthenovel pages 29-33).

## Structure

IRAK3 has a multi-domain organization consisting of an N-terminal death domain (DD), a proline-serine-threonine rich (PST) region, a central pseudokinase domain, and a C-terminal domain (lange2021dimericstructureof pages 1-3, flannery2010theinterleukin1receptorassociated pages 5-9). The DD is essential for interactions with MyD88 and other IRAK proteins (freihat2017doesthenovel pages 83-87).

The pseudokinase domain adopts a canonical kinase fold with N- and C-lobes but exists in a closed, pseudoactive conformation (lange2021dimericstructureof pages 1-3). The activation loop is in a pseudoactive state, with its DFA motif conformation classified in the BLAminus cluster (lange2021dimericstructureof pages 3-4). The conformation of the hydrophobic spine also corresponds to an inactive kinase state (lange2021dimericstructureof pages 3-4). A unique feature of IRAK3 is its formation of a head-to-head dimer through its pseudokinase domain, stabilized by a redox-sensitive disulfide bond between C291 (in the catalytic loop) of one protomer and C202 (in the αC helix) of the partner protomer (lange2021dimericstructureof pages 1-3, horne2021forwhomthe pages 2-2). Within the pseudokinase domain is a guanylate cyclase (GC) center (freihat2017doesthenovel pages 29-33).

## Regulation

Regulation of IRAK proteins can involve post-translational modifications such as ubiquitination by E3 ligases like TRAF6 and Pellino family members (pereira2023regulationofinnate pages 1-2). However, specific details on the ubiquitination of IRAK3 are not provided in the context (horne2021forwhomthe pages 2-2, patra2016recentprogressin pages 1-3, pereira2023regulationofinnate pages 6-7, singer2018inhibitionofinterleukin1 pages 2-6). Unlike active kinases IRAK1 and IRAK4, IRAK3 does not perform autophosphorylation (freihat2017doesthenovel pages 29-33). Its function is subject to allosteric and conformational regulation, potentially via its unique dimerization interface, which is sensitive to redox conditions (lange2021dimericstructureof pages 1-3, horne2021forwhomthe pages 2-2). This dimerization may serve as an allosteric mechanism for the negative regulation of IRAK4 activity (lange2021dimericstructureof pages 1-3, lange2021dimericstructureof pages 19-19).

## Function

IRAK3 is mainly expressed in monocytes, macrophages, and dendritic cells (freihat2017doesthenovel pages 33-38, mahmoud2023modulationofirak pages 4-6). Its expression is also found in airway epithelial cells, particularly in asthmatic patients (freihat2017doesthenovel pages 33-38).

IRAK3 is a negative regulator of TLR and IL-1R signaling pathways (freihat2017doesthenovel pages 33-38, turek2023mutationsinthe pages 1-2). As a component of the myddosome, it interacts with MyD88 and other IRAKs (turek2023mutationsinthe pages 1-2). One source states it interacts with IRAK2 but not IRAK1, while another states it forms heterodimers with IRAK1 and IRAK4 (freihat2017doesthenovel pages 33-38, freihat2017doesthenovel pages 83-87). By inhibiting the dissociation of signaling complexes, IRAK3 dampens downstream NF-κB and MAPK pathway activation, which reduces the production of proinflammatory cytokines like IL-6 and TNF-α (freihat2017doesthenovel pages 33-38, turek2023mutationsinthe pages 1-2). Its guanylate cyclase product, cGMP, also suppresses NFκB activity (turek2023mutationsinthe pages 1-2). At low TLR ligand concentrations, IRAK3 is preferentially recruited to activate inhibitory pathways, including the induction of molecules like SOCS1, SHIP1, and A20 (freihat2017doesthenovel pages 33-38).

## Inhibitors

Although IRAK3 is a pseudokinase, it retains an intact ATP-binding cleft and can bind ATP-competitive small-molecule inhibitors, potentially with low affinity (freihat2017doesthenovel pages 83-87, freihat2017doesthenovel pages 83-87). No specific experimental inhibitors for IRAK3 are detailed in the provided context (patra2016recentprogressin pages 1-3, pereira2023regulationofinnate pages 6-7, singer2018inhibitionofinterleukin1 pages 2-6).

## Other Comments

Dysregulation of IRAK3 is implicated in autoimmune and inflammatory diseases (turek2023mutationsinthe pages 1-2). Elevated expression is linked to immunosuppression and poorer outcomes in sepsis and acute lung injury from pneumococcal pneumonia (freihat2017doesthenovel pages 33-38). Asthma-associated mutations have been mapped to a conserved surface on IRAK3 that is likely involved in its interaction with IRAK4 (lange2021dimericstructureof pages 1-3). Specific mutations in the death domain (E71A, Q78G, W74A) disrupt IRAK4 binding and impair IRAK3’s regulatory function (freihat2017doesthenovel pages 83-87). Mutations near the guanylate cyclase center (e.g., affecting D377 and D385) alter subcellular localization and its immunomodulatory capacity (turek2023mutationsinthe pages 7-10).

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