## Phylogeny

Intestinal Cell Kinase (ICK), also known as Ciliogenesis-associated kinase 1 (Cilk1), is a serine/threonine protein kinase classified within the CMGC (CDK, MAPK, GSK3, CLK) group of the human kinome (wu2012intestinalcellkinase pages 1-2, chowdhury2023cmgckinasesin pages 10-12, howard2014ancestralresurrectionreveals pages 2-3). ICK belongs to the RCK (Regulatory ciliary kinase) family, a paralogous superfamily that also includes Male Germ cell-Associated Kinase (MAK) and MAK-related Kinase (MOK) (chowdhury2023cmgckinasesin pages 10-12, wu2012intestinalcellkinase pages 1-2, howard2014ancestralresurrectionreveals pages 2-3). This classification is consistent with the kinome analysis by Manning et al., 2002 (chowdhury2023cmgckinasesin pages 10-12, howard2014ancestralresurrectionreveals pages 2-3, chaya2024ccrkmakickkinasesignaling pages 42-45). ICK and MAK are considered paralogs (moon2014intestinalcellkinase pages 3-4). The RCK family structurally resembles both MAPKs and CDKs (chowdhury2023cmgckinasesin pages 10-12).

Orthologs of ICK are conserved across species, including *Chlamydomonas* LF4, *Tetrahymena* LF4A, *Leishmania mexicana* LmxMPK9, and *Caenorhabditis elegans* DYF-5 (chaya2024ccrkmakickkinasesignaling pages 1-5, moon2014intestinalcellkinase pages 3-4). A homolog also exists in *Saccharomyces cerevisiae* that is regulated by the yeast CAK kinase (sturgill2010thepromoterfor pages 9-10).

## Reaction Catalyzed

The reaction catalyzed is the transfer of a γ-phosphate group from an ATP molecule to a serine or threonine residue on a protein substrate (wu2012intestinalcellkinase pages 1-2, chaya2024ccrkmakickkinasesignaling pages 1-5).

ATP + a protein → ADP + a phosphoprotein

## Cofactor Requirements

The catalytic activity of ICK requires a divalent cation, specifically Mg²⁺, as a cofactor (moon2014intestinalcellkinase pages 1-2, chaya2024ccrkmakickkinasesignaling pages 42-45, wang2022modulationofprimary pages 8-9).

## Substrate Specificity

ICK phosphorylates substrates containing a consensus sequence characterized by a strong preference for Arginine (R) at the -3 position and Proline (P) at the -2 position relative to the phosphoacceptor site (howard2014ancestralresurrectionreveals pages 3-5, tong2018modulationofgsk3β pages 5-6). A general motif has been described as [R-P-X-S/T-P/A/T/S] (wu2012intestinalcellkinase pages 1-2). Mammalian RCK kinases, including ICK, show a preference for Proline at the +1 position; however, this specificity is plastic and depends on the phosphoacceptor residue (howard2014ancestralresurrectionreveals pages 3-5). A threonine phosphoacceptor favors a +1 proline, whereas a serine phosphoacceptor can also accommodate a +1 arginine (howard2014ancestralresurrectionreveals pages 6-9). A comprehensive atlas of substrate specificities has documented ICK’s preferred motifs, but these are not detailed in the provided context (chaya2024ccrkmakickkinasesignaling pages 42-45, moon2014intestinalcellkinase pages 1-2).

## Structure

ICK consists of an N-terminal catalytic kinase domain and a C-terminal non-catalytic domain (wu2012intestinalcellkinase pages 1-2, moon2014intestinalcellkinase pages 1-2). The kinase domain shares structural similarity with MAPKs and contains a conserved TDY motif (Thr-157, Asp-158, Tyr-159) within its activation loop, which is essential for catalytic activity (sturgill2010thepromoterfor pages 1-2, moon2014intestinalcellkinase pages 1-2). The C-terminal non-catalytic domain is required for the regulation of ciliogenesis (chaya2024ccrkmakickkinasesignaling pages 42-45, wang2022modulationofprimary pages 8-9). While no experimentally determined 3D structures are described, AlphaFold models are available (moon2014intestinalcellkinase pages 1-2).

## Regulation

The primary regulatory mechanism for ICK is dual phosphorylation of the TDY motif within its activation loop (wu2012intestinalcellkinase pages 1-2). Full activation requires phosphorylation of Thr-157 by the upstream kinase CCRK (Cell Cycle-Related Kinase/CDK20), while Tyr-159 is phosphorylated via autophosphorylation (sturgill2010thepromoterfor pages 1-2). ICK is inactivated by dephosphorylation, which can be mediated by phosphoprotein phosphatase 5 (PP5) under conditions of oxidative stress (wu2012intestinalcellkinase pages 1-2, noguchi2021ccrkcdk20regulatesciliary pages 15-17). The dynamic cycle of phosphorylation and dephosphorylation is essential for its function in regulating intraflagellar transport (noguchi2021ccrkcdk20regulatesciliary pages 11-14). Additionally, ICK activity is negatively regulated by fibroblast growth factor (FGF) receptors, and inhibition of these receptors leads to increased ICK activity (chaya2024ccrkmakickkinasesignaling pages 1-5).

## Function

ICK is widely expressed, with high levels observed in proliferative tissues such as the intestinal crypt epithelium, retinal photoreceptors, bone, cartilage, and embryonic myocardium (sturgill2010thepromoterfor pages 1-2, moon2014intestinalcellkinase pages 1-2, ding2017intestinalcellkinase pages 6-10, chaya2024ccrkmakickkinasesignaling pages 42-45). It functions downstream of the upstream kinase CCRK (moon2014intestinalcellkinase pages 4-5).

Known substrates of ICK include the kinesin motor protein KIF3A, the mTORC1 component Raptor at residue Thr-908, and BAT3/Scythe at T1080 (chaya2024ccrkmakickkinasesignaling pages 1-5, wu2012intestinalcellkinase pages 1-2, sturgill2010thepromoterfor pages 9-10). ICK interacts with the IFT-B complex, phosphatase PP5, and the F-box protein FBX9 (wu2012intestinalcellkinase pages 1-2, sturgill2010thepromoterfor pages 9-10, noguchi2021ccrkcdk20regulatesciliary pages 11-14).

ICK is a key regulator of ciliogenesis, controlling primary cilia length by modulating the turnaround of intraflagellar transport (IFT) at the ciliary tip (chaya2024ccrkmakickkinasesignaling pages 1-5, moon2014intestinalcellkinase pages 1-2). This function is critical for the Sonic Hedgehog (SHH) signaling pathway (moon2014intestinalcellkinase pages 1-2, chaya2024ccrkmakickkinasesignaling pages 42-45). Through phosphorylation of Raptor, ICK promotes mTORC1 activation, thereby regulating cell proliferation and growth (wu2012intestinalcellkinase pages 1-2). It is also required for normal chondrocyte proliferation and maturation during skeletal development (ding2017intestinalcellkinase pages 6-10). In the context of protein malnutrition, ICK expression is transiently increased, where it supports intestinal epithelial cell proliferation and survival by activating the Wnt/β-catenin pathway and suppressing caspase-dependent apoptosis (bolick2014intestinalcellkinase pages 6-7).

## Inhibitors

The flavonoid kinase inhibitor alvocidib (flavopiridol) modulates ICK activity (wang2022modulationofprimary pages 8-9). Indirect modulation can be achieved through pharmacological inhibition of FGF receptors (chaya2024ccrkmakickkinasesignaling pages 1-5).

## Other Comments

Mutations in the *ICK* gene cause severe, neonatally lethal ciliopathies, including endocrine-cerebro-osteodysplasia (ECO) syndrome and short rib-polydactyly syndrome (SRPS) (chaya2024ccrkmakickkinasesignaling pages 1-5). Pathogenic variants are also associated with juvenile myoclonic epilepsy (wang2022modulationofprimary pages 8-9).

The autosomal recessive R272Q mutation, which causes ECO syndrome, impairs kinase activation and proper protein localization (moon2014intestinalcellkinase pages 1-2, sturgill2010thepromoterfor pages 9-10). This loss-of-function mutation disrupts ciliogenesis and SHH signaling, leading to severe developmental defects, including short limbs, polydactyly, bowed long bones, and reduced bone mineralization (ding2017intestinalcellkinase pages 6-10, moon2014intestinalcellkinase pages 1-2).

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