## Phylogeny

TRPM6 belongs to the melastatin-related (TRPM) subfamily of the transient receptor potential (TRP) ion channel family (azim2019exomesequencingidentifies pages 6-6, chubanov2014trpm6 pages 1-3). According to the kinome analysis by Manning et al., TRPM6 and its close homolog TRPM7 are classified within the atypical alpha-kinase group, a unique subset of kinases characterized by the fusion of an ion channel to a kinase domain (chubanov2004disruptionoftrpm6trpm7 pages 4-6, schlingmann2007trpm6andtrpm7—gatekeepers pages 1-7, runnels2011trpm6andtrpm7 pages 1-2). The human TRPM6 gene has orthologs in other vertebrates, including mouse, rat, and zebrafish, indicating conserved function across species (chubanov2014trpm6 pages 1-3, schmitz2005thechannelkinases pages 1-2). TRPM6 is a paralog of TRPM7, with which it shares approximately 77% sequence identity in the kinase domain, but the two proteins are functionally nonredundant (chubanov2014trpm6 pages 3-6, schmitz2005thechannelkinases pages 1-2).

## Reaction Catalyzed

The C-terminal alpha-kinase domain of TRPM6 functions as an atypical serine/threonine kinase that catalyzes the phosphorylation of substrate proteins via the transfer of a phosphate group from ATP (chubanov2004disruptionoftrpm6trpm7 pages 4-6, chubanov2014trpm6 pages 1-3). The kinase is specific for ATP and cannot utilize GTP as a phosphate donor (runnels2011trpm6andtrpm7 pages 1-2). The chemical reaction is: ATP + protein → ADP + phosphoprotein (runnels2011trpm6andtrpm7 pages 9-10, schmitz2005thechannelkinases pages 1-2, schlingmann2007trpm6andtrpm7—gatekeepers pages 27-31).

## Cofactor Requirements

The catalytic activity of the TRPM6 kinase domain requires divalent cations as cofactors (azim2019exomesequencingidentifies pages 6-6, chubanov2014trpm6 pages 1-3). Specifically, it requires Mg2+ or Mn2+ for its activity, with Mg2+ being particularly critical (chubanov2004disruptionoftrpm6trpm7 pages 4-6, runnels2011trpm6andtrpm7 pages 1-2). The presence of Mn2+ has been shown to greatly enhance kinase activity (runnels2011trpm6andtrpm7 pages 1-2).

## Substrate Specificity

A consensus phosphorylation motif for the TRPM6 kinase has not been defined in the provided literature, and sources including Johnson et al. (2023, Nature) do not report one (runnels2011trpm6andtrpm7 pages 9-10, schmitz2005thechannelkinases pages 1-2). The kinase phosphorylates serine and threonine residues that are typically located within alpha-helical regions of substrate proteins (chubanov2014trpm6 pages 6-9, runnels2011trpm6andtrpm7 pages 2-3). Substrates phosphorylated by the related TRPM7, and in some cases TRPM6, include myosin II heavy chains (IIA, IIB, IIC), annexin A1, and elongation factor 2 kinase (chubanov2014trpm6 pages 6-9, runnels2011trpm6andtrpm7 pages 2-3).

## Structure

TRPM6 is a bifunctional protein with a modular structure, comprising a large N-terminal domain with ankyrin-like repeats, a central ion channel domain with six transmembrane helices (S1-S6), and a C-terminal alpha-kinase domain (chubanov2014trpm6 pages 1-3, chubanov2014trpm6 pages 3-6). The ion channel pore is formed by a loop between helices S5 and S6 containing a conserved EVY motif critical for ion selectivity (chubanov2014trpm6 pages 3-6). The kinase domain has a characteristic alpha-kinase fold with separate N- and C-lobes that form the catalytic site, and includes a zinc-binding module crucial for domain stability (chubanov2014trpm6 pages 1-3, runnels2011trpm6andtrpm7 pages 1-2). Structural insights, including the conformation of the activation loop and C-helix which are crucial for catalytic activity, are derived from homology modeling using the crystal structure of mouse TRPM7 kinase (PDB: 1IA9) as well as from cryo-EM studies and AlphaFold models (chubanov2014trpm6 pages 1-3, runnels2011trpm6andtrpm7 pages 9-10, schlingmann2007trpm6andtrpm7—gatekeepers pages 27-31).

## Regulation

TRPM6 activity is regulated by autophosphorylation, allosteric effectors, and protein-protein interactions. The kinase undergoes extensive autophosphorylation in a Ser/Thr-rich region, which enhances substrate recognition (chubanov2014trpm6 pages 1-3, runnels2011trpm6andtrpm7 pages 2-3). Phosphorylation occurs on both serine and threonine residues, with phosphoserine predominating (schmitz2005thechannelkinases pages 8-9). Autophosphorylation at residue Thr1851 is crucial for coupling kinase activity to channel function (runnels2011trpm6andtrpm7 pages 5-6). Mg-ATP acts as an allosteric regulator that enhances channel activity, while free intracellular Mg2+ can be inhibitory (azim2019exomesequencingidentifies pages 6-6, chubanov2014trpm6 pages 6-9). A non-reciprocal regulatory relationship exists with TRPM7, where TRPM6 can cross-phosphorylate TRPM7, but not vice versa (schmitz2005thechannelkinases pages 8-9). The scaffold protein RACK1 interacts with the kinase domain (residues 1857-1885) to mediate inhibition and regulation (runnels2011trpm6andtrpm7 pages 5-6). TRPM6 expression is regulated by epidermal growth factor (EGF) (chubanov2014trpm6 pages 3-6).

## Function

TRPM6 is a bifunctional protein that functions as both a Mg2+-permeable ion channel and a serine/threonine protein kinase (azim2019exomesequencingidentifies pages 6-6). It is essential for systemic magnesium homeostasis, mediating active transcellular Mg2+ absorption in the intestine and reabsorption in the distal convoluted tubule (DCT) of the kidney (azim2019exomesequencingidentifies pages 6-6, voets2004trpm6formsthe pages 1-1). Its expression is predominantly restricted to these epithelial tissues (chubanov2014trpm6 pages 1-3, chubanov2014trpm6 pages 3-6). TRPM6 forms functional heterotetrameric complexes with the ubiquitously expressed TRPM7; this interaction is essential for the efficient trafficking of TRPM6 to the plasma membrane and for its function (chubanov2004disruptionoftrpm6trpm7 pages 4-6, schmitz2005thechannelkinases pages 7-8). Other known interacting partners include RACK1, REA (repressor for estrogen receptor activity), and MsrB1 (chubanov2014trpm6 pages 6-9).

## Inhibitors

The available information on specific kinase inhibitors for TRPM6 is contradictory. Most sources state that specific kinase inhibitors are not well-characterized or are not mentioned (azim2019exomesequencingidentifies pages 6-6, chubanov2004disruptionoftrpm6trpm7 pages 4-6, runnels2011trpm6andtrpm7 pages 1-2). One source indicates that TRPM6 is inhibited by specific kinase inhibitors, but notes that few highly selective small molecules have been reported (schaffers2018theriseand pages 3-3). The channel activity is blocked by ruthenium red in a voltage-dependent manner and is activated by 2-APB, which contrasts with its inhibitory effect on TRPM7 (schlingmann2007trpm6andtrpm7—gatekeepers pages 11-15, voets2004trpm6formsthe pages 1-1).

## Other Comments

Loss-of-function mutations in the *TRPM6* gene cause hypomagnesemia with secondary hypocalcemia (HSH), a rare autosomal recessive disorder characterized by defective intestinal Mg2+ absorption and renal Mg2+ wasting (azim2019exomesequencingidentifies pages 6-6, chubanov2014trpm6 pages 1-3). Documented mutations include missense (e.g., S141L), frameshift, splice-site, and nonsense variants that result in truncated or nonfunctional proteins, or disrupt the TRPM6/TRPM7 complex formation (azim2019exomesequencingidentifies pages 6-6, chubanov2004disruptionoftrpm6trpm7 pages 4-6, schlingmann2007trpm6andtrpm7—gatekeepers pages 11-15). Homozygous deletion of *Trpm6* in mice leads to embryonic lethality and neural tube defects, underscoring its essential role in prenatal development (chubanov2014trpm6 pages 1-3, chubanov2016epithelialmagnesiumtransport pages 32-32).

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