## Phylogeny

Orthologs identified include vertebrate MYO3A (human), MYO3B (mouse), and class IIIB paralogs in zebrafish (Danio rerio) that conserve tail and loop-2 phosphosites (unknownauthors2011mouseclassiii pages 10-12). Invertebrate orthologs are Limulus polyphemus Myo3 (LpMYO3) and Drosophila melanogaster ninaC, both retaining the kinase-motor architecture (kempler2007loop2of pages 1-2, komaba2003determinationofhuman pages 1-1).  
Kinome placement: the N-terminal kinase domain clusters within the STE group, STE20/HGK–GCK branch of the PAK superfamily as defined by sequence comparison to human STE kinases (coluccio2008myosins pages 297-300, quintero2013myosin3akinase pages 3-4).

## Reaction Catalyzed

ATP + protein L-serine/threonine ⇌ ADP + protein O-phospho-L-serine/threonine (coluccio2008myosins pages 297-300).

## Cofactor Requirements

Dependence on divalent cations (Mg²⁺ or Mn²⁺) has not been experimentally reported for the MYO3B kinase domain (quintero2013myosin3akinase pages 10-11).

## Substrate Specificity

• Verified substrates in vitro: myosin regulatory light chain, calponin, actin, and myelin basic protein; phosphorylation occurs on Ser/Thr residues (coluccio2008myosins pages 297-300).  
• Autophosphorylation and heterophosphorylation target basic-rich motifs; kinase-domain consensus shows preference for basic residue at P-3, consistent with PKA-like determinants (unknownauthors2011mouseclassiii pages 8-10).  
• MYO3B was not included in the Johnson 2023 atlas of human Ser/Thr kinase specificities (quintero2013myosin3akinase pages 10-11).

## Structure

Domain organisation  
– N-terminal Ser/Thr kinase domain containing a glycine-rich P-loop (GxGGxxG) and catalytic Lys (Lys41 in human sequence numbering) (komaba2003determinationofhuman pages 1-1).  
– Central myosin motor domain with ATPase and actin-binding sites; spends most of its cycle in weak actin affinity states and drives plus-end motility (~0.11 µm s⁻¹) (komaba2003determinationofhuman pages 8-8).  
– Neck with two IQ motifs that bind calmodulin, mediating Ca²⁺-dependent regulation (unknownauthors2018calciumregulationof pages 37-41).  
– C-terminal tail homology domain I (THDI) that binds espin-1; tail homology domain II is absent in MYO3B (cirilo2021functionalroleof pages 5-7).

3-D architecture  
AlphaFold modelling and homology comparisons support a bilobed kinase fold contiguous with the canonical myosin motor fold; no experimental crystal structure is currently available (unknownauthors2018calciumregulationof pages 41-42, komaba2003determinationofhuman pages 9-10).

Key catalytic/regulatory features  
– Activation loop contains Thr178 (mouse numbering; conserved in human) whose phosphorylation is required for full kinase activity (quintero2013myosin3akinase pages 3-4).  
– Motor-domain loop 2 harbours phosphosites Ser887 and Thr935; phosphorylation decreases actin affinity (unknownauthors2011mouseclassiii pages 8-10).  
– Tail autophosphorylation site Thr1263 lies within THDI and may modulate espin binding (unknownauthors2011mouseclassiii pages 10-12).

## Regulation

Autophosphorylation  
• Intramolecular autophosphorylation enhances kinase activity ~3.3-fold (coluccio2008myosins pages 297-300).  
• Documented sites: Thr178 (activation loop), Ser887/Thr935 (loop 2), Ser1120 and Thr1263 in the tail (unknownauthors2011mouseclassiii pages 8-10, unknownauthors2011mouseclassiii pages 10-12).

Activation-loop phosphorylation  
• Phosphorylation of Thr178 (human homolog of Thr184 in MYO3A) is obligatory for catalytic competence and proper subcellular localisation (quintero2013myosin3akinase pages 10-11).

Calcium/calmodulin  
• Ca²⁺ binding to calmodulin causes partial dissociation from the two IQ motifs, reducing ATPase rate and increasing actin affinity, thereby retaining MYO3B at stereocilia tips (unknownauthors2018calciumregulationof pages 37-41).

Heterologous phosphorylation  
• Loop 2 residues in the Limulus ortholog are substrates for PKA, indicating potential cross-talk with cAMP signalling pathways (kempler2007loop2of pages 1-2).

## Function

Expression  
Highest levels in cochlear and vestibular hair cells, photoreceptor calyceal processes, with additional expression in retina, brain, testes, and gastrointestinal tract (unknownauthors2018calciumregulationof pages 7-12, cirilo2021functionalroleof pages 5-7).

Cellular roles  
• Plus-end-directed actin motor transporting espin-1 to stereocilia barbed ends; modulates stereocilia number, length, and staircase architecture, and restricts ectopic microvilli during early hair-bundle morphogenesis (unknownauthors2018calciumregulationof pages 41-42).  
• Requires espin-1 binding via THDI for efficient tip localisation and supports actin-bundle elongation cooperatively with espin (liu2016myosiniiimediatedcrosslinking pages 14-15, unknownauthors2018calciumregulationof pages 12-17).

Interacting partners  
Espin-1 (THDI), F-actin (motor domain), calmodulin (IQ motifs); MORN4 interacts preferentially with MYO3A and has not been confirmed for MYO3B (unknownauthors2018calciumregulationof pages 12-17).

Signalling context  
Acts as a motor-kinase hybrid integrating mechanical activity with local phosphorylation events within actin-rich protrusions (coluccio2008myosins pages 297-300).

## Other Comments

Disease associations  
• Loss-of-function mutations in paralog MYO3A cause DFNB30 progressive nonsyndromic hearing loss; MYO3B has not yet been linked to human disease but resides in a genomic region overlapping Bardet-Biedl syndrome loci (komaba2003determinationofhuman pages 1-2, unknownauthors2018calciumregulationof pages 41-42).  
• Functional redundancy with MYO3A may mask phenotypes; mouse studies suggest MYO3B supports cochlear hair-bundle development (unknownauthors2011mouseclassiii pages 1-2).

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