1. Phylogeny  
   Myosin-IIIb (MYO3B) is a member of the unconventional class III myosin family, a group of actin-based motor proteins that diverged early during vertebrate evolution. Vertebrates typically express two paralogous class III myosins—MYO3A and MYO3B—with MYO3B forming a distinct phylogenetic branch from MYO3A, as evidenced by sequence and domain-organization differences that include the absence of the additional tail homology domain (3THDII) in MYO3B (coluccio2008myosins pages 295-297, moore2009regulationofmyosin pages 15-19). MYO3B is conserved across vertebrate species and has been identified in tissues including the retina, testis, olfactory bulb, and auditory sensory epithelium, highlighting its role within an evolutionary core of actin-based regulators in sensory systems (coluccio2008myosins pages 307-310, raval2016mechanismofclass pages 12-18).
2. Reaction Catalyzed  
   MYO3B exhibits dual enzymatic activities that underpin its biological functions. In its motor role, MYO3B catalyzes the actin-activated hydrolysis of ATP into ADP and inorganic phosphate (Pi), a reaction that drives conformational changes within its motor domain and generates mechanical force for movement along actin filaments. This reaction can be summarized as:  
     ATP + [MYO3B·actin] → ADP + Pi + mechanical work,  
   where actin serves as a critical co-substrate that increases the ATPase activity of the motor (coluccio2008myosins pages 307-310, raval2016mechanismofclass pages 102-106). Concurrently, the N-terminal kinase domain of MYO3B catalyzes the transfer of phosphate groups from ATP to specific serine/threonine residues on target substrate proteins, a reaction that can be generally represented as:  
     ATP + [protein]-(L-serine/threonine) → ADP + [protein]-(phospho-serine/threonine) + H⁺,  
   with substrate targets likely including regulatory factors such as Espin, as well as autophosphorylation sites within MYO3B itself (an2014phosphorylationofthe pages 11-11, coluccio2008myosins pages 297-300).
3. Cofactor Requirements  
   The catalytic activities of MYO3B depend critically on the presence of ATP and essential divalent cations. For its ATPase-driven motor activity, ATP acts as the phosphate donor while Mg²⁺ is required as an essential cofactor to coordinate nucleotide binding and hydrolysis within the motor domain (coluccio2008myosins pages 295-297, raval2016mechanismofclass pages 102-106). In addition, the IQ motifs within the neck region bind to calmodulin in a Ca²⁺-dependent manner, an interaction that is important for the modulation of MYO3B’s lever arm stiffness and overall motor regulation (liu2016myosiniiimediatedcrosslinking pages 14-15).
4. Substrate Specificity  
   MYO3B demonstrates substrate specificity on two complementary levels. In terms of its motor function, the substrate is the filamentous actin network, with MYO3B exhibiting high-affinity binding to actin that is crucial for its ATPase activity and processive movement along these filaments (coluccio2008myosins pages 307-310, raval2016mechanismofclass pages 102-106). Regarding its kinase activity, although no detailed consensus phosphorylation motif has been definitively established in the literature, the kinase domain of MYO3B is presumed to phosphorylate serine/threonine residues within flexible loop regions of its substrate proteins. The fact that MYO3B and its paralog MYO3A share identical Espin1 binding sites suggests a substrate specificity directed toward actin-associated proteins such as Espin, which plays a pivotal role in actin filament bundling and stereocilia elongation (liu2016myosiniiimediatedcrosslinking pages 14-15, an2014phosphorylationofthe pages 11-11).
5. Structure  
   The structure of MYO3B is defined by a modular organization that underlies its dual motor and kinase functions. At the N-terminus, MYO3B harbors a serine/threonine kinase domain that exhibits sequence similarity to members of the HGK/PAK kinase family and is responsible for autophosphorylation as well as phosphorylation of downstream targets. Immediately following the kinase domain is a highly conserved motor domain that catalyzes ATP hydrolysis in an actin-activated manner, thereby generating the conformational shifts essential for force production and movement along actin filaments (coluccio2008myosins pages 295-297, moore2009regulationofmyosin pages 15-19). Adjacent to the motor domain is the neck region, which contains two to three IQ motifs; these motifs serve as binding sites for calmodulin and other light chains, thus modulating the rigidity of the lever arm and influencing the kinetic properties of the motor (coluccio2008myosins pages 295-297, raval2016mechanismofclass pages 12-18). The C-terminal tail region of MYO3B is distinct in that it contains only a single conserved Class III Tail Homology Domain I (3THDI), approximately 50 amino acids in length, and lacks the additional tail homology domain (3THDII) that is present in MYO3A. This difference in tail domain composition is associated with variations in actin-binding capacity and cargo recruitment, which in turn modulate the protein’s overall processivity and intracellular localization (coluccio2008myosins pages 295-297, coluccio2008myosins pages 307-310, raval2016mechanismofclass pages 28-32). Although crystallographic structures of full-length MYO3B have not been reported, applications of AlphaFold and biochemical domain analysis suggest a linear, modular assembly that is characteristic of unconventional myosins (houdusse2021themanyroles pages 2-3, fili2019unconventionalmyosinshow pages 1-4).
6. Regulation  
   The regulatory mechanisms governing MYO3B involve both autophosphorylation and interactions with regulatory proteins. The N-terminal kinase domain is capable of autophosphorylation, a modification that increases its catalytic activity by approximately 3.3-fold and is believed to act as an intramolecular switch to modulate motor function (coluccio2008myosins pages 297-300, an2014phosphorylationofthe pages 11-11). Phosphorylation events can alter the conformational state of the motor domain, reducing its affinity for actin in a manner that regulates processivity and force generation. In addition, the binding of Ca²⁺-saturated calmodulin to the IQ motifs within the neck region represents another critical layer of regulation, as it influences the structural properties of the lever arm and thereby adjusts motor kinetics (liu2016myosiniiimediatedcrosslinking pages 14-15, raval2016impactofthe pages 1-2). Secondary modifications, such as phosphorylation of residues within the tail region by protein kinase C (PKC), have been observed in longer splice variants of class III myosins; however, MYO3B lacks these additional tail regions, which may account for differences in regulation between MYO3B and MYO3A (coluccio2008myosins pages 297-300). Collectively, these regulatory processes ensure that MYO3B’s activity is tightly coordinated with cellular signals and the dynamic state of the actin cytoskeleton.
7. Function  
   MYO3B plays a pivotal role in the morphogenesis and maintenance of specialized actin-based structures, particularly within the sensory cells of the inner ear. It is required for normal cochlear hair bundle development, where it influences both the number and lengths of stereocilia, thereby contributing to the characteristic staircase pattern observed in these mechanosensory structures (Protein Information, moore2009regulationofmyosin pages 75-79). As an actin-based motor, MYO3B is involved in the transport of the actin regulatory factor Espin to the plus ends of actin filaments, a process that facilitates the elongation of stereocilia tips and the stabilization of the hair bundle architecture. In addition to its established role in auditory hair cell development, MYO3B is also expressed in other sensory tissues, including the retina, where its activity may contribute to the formation and maintenance of photoreceptor structures (coluccio2008myosins pages 307-310, houdusse2021themanyroles pages 2-3). Thus, MYO3B is integral to controlling actin filament dynamics and maintaining the precise organization of microvilli and stereocilia required for efficient sensory transduction.
8. Other Comments  
   There are currently no small molecule inhibitors reported that specifically target MYO3B, and detailed studies on its inhibitor profile remain limited in the literature (coluccio2008myosins pages 307-310, raval2016impactofthe pages 3-4). Although mutations in MYO3A have been directly associated with progressive nonsyndromic hearing loss (DFNB30), no specific disease-causing mutations have been conclusively attributed to MYO3B; however, its essential role in cochlear hair bundle morphogenesis underscores its potential clinical significance in auditory function (moore2009regulationofmyosin pages 75-79, raval2016mechanismofclass pages 28-32). Further investigation is warranted to elucidate the detailed substrate preferences of the kinase domain and to assess any additional roles MYO3B might play in sensory or neurological disorders. Its unique domain organization and regulation via autophosphorylation distinguish MYO3B from other unconventional myosins, reinforcing its importance as an actin-regulating motor in sensory cell biology.

References

1. (coluccio2008myosins pages 295-297): Lynne M. Coluccio. Myosins. Springer Netherlands, Jan 2008. ISBN 9781402065194. URL: https://doi.org/10.1007/978-1-4020-6519-4, doi:10.1007/978-1-4020-6519-4.
2. (liu2016myosiniiimediatedcrosslinking pages 14-15): Haiyang Liu, Jianchao Li, M. Raval, N. Yao, Xiaoying Deng, Q. Lu, S. Nie, W. Feng, J. Wan, C. Yengo, Wei Liu, and Mingjie Zhang. Myosin iii-mediated cross-linking and stimulation of actin bundling activity of espin. eLife, Jan 2016. URL: https://doi.org/10.7554/elife.12856, doi:10.7554/elife.12856. This article has 26 citations and is from a domain leading peer-reviewed journal.
3. (moore2009regulationofmyosin pages 15-19): JE Moore. Regulation of myosin iiia motor/kinase activity. Unknown journal, 2009.
4. (moore2009regulationofmyosin pages 75-79): JE Moore. Regulation of myosin iiia motor/kinase activity. Unknown journal, 2009.
5. (coluccio2008myosins pages 297-300): Lynne M. Coluccio. Myosins. Springer Netherlands, Jan 2008. ISBN 9781402065194. URL: https://doi.org/10.1007/978-1-4020-6519-4, doi:10.1007/978-1-4020-6519-4.
6. (coluccio2008myosins pages 307-310): Lynne M. Coluccio. Myosins. Springer Netherlands, Jan 2008. ISBN 9781402065194. URL: https://doi.org/10.1007/978-1-4020-6519-4, doi:10.1007/978-1-4020-6519-4.
7. (houdusse2021themanyroles pages 2-3): A. Houdusse and M. Titus. The many roles of myosins in filopodia, microvilli and stereocilia. Current Biology, 31:R586-R602, May 2021. URL: https://doi.org/10.1016/j.cub.2021.04.005, doi:10.1016/j.cub.2021.04.005. This article has 61 citations and is from a highest quality peer-reviewed journal.
8. (raval2016impactofthe pages 1-2): M. Raval, O. Quintero, Meredith L. Weck, William C. T. Unrath, J. W. Gallagher, Runjia Cui, B. Kachar, M. Tyska, and C. Yengo. Impact of the motor and tail domains of class iii myosins on regulating the formation and elongation of actin protrusions\*. The Journal of Biological Chemistry, 291:22781-22792, Aug 2016. URL: https://doi.org/10.1074/jbc.m116.733741, doi:10.1074/jbc.m116.733741. This article has 18 citations.
9. (raval2016mechanismofclass pages 12-18): MH Raval. Mechanism of class iii myosin mediated regulation of actin bundle based protrusions. Unknown journal, 2016.
10. (raval2016mechanismofclass pages 28-32): MH Raval. Mechanism of class iii myosin mediated regulation of actin bundle based protrusions. Unknown journal, 2016.
11. (fili2019unconventionalmyosinshow pages 1-4): Natalia Fili and C. Toseland. Unconventional myosins: how regulation meets function. International Journal of Molecular Sciences, Dec 2019. URL: https://doi.org/10.3390/ijms21010067, doi:10.3390/ijms21010067. This article has 62 citations and is from a peer-reviewed journal.
12. (raval2016impactofthe pages 3-4): M. Raval, O. Quintero, Meredith L. Weck, William C. T. Unrath, J. W. Gallagher, Runjia Cui, B. Kachar, M. Tyska, and C. Yengo. Impact of the motor and tail domains of class iii myosins on regulating the formation and elongation of actin protrusions\*. The Journal of Biological Chemistry, 291:22781-22792, Aug 2016. URL: https://doi.org/10.1074/jbc.m116.733741, doi:10.1074/jbc.m116.733741. This article has 18 citations.
13. (raval2016mechanismofclass pages 102-106): MH Raval. Mechanism of class iii myosin mediated regulation of actin bundle based protrusions. Unknown journal, 2016.
14. (an2014phosphorylationofthe pages 11-11): Byung Chull An, Tsuyoshi Sakai, Shigeru Komaba, Hiroko Kishi, Sei Kobayashi, Jin Young Kim, Reiko Ikebe, and Mistuo Ikebe. Phosphorylation of the kinase domain regulates autophosphorylation of myosin iiia and its translocation in microvilli. Biochemistry, 53:7835-7845, Dec 2014. URL: https://doi.org/10.1021/bi501247z, doi:10.1021/bi501247z. This article has 8 citations and is from a peer-reviewed journal.