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# Cargo Recognition and Cargo-Mediated Regulation of Unconventional Myosins

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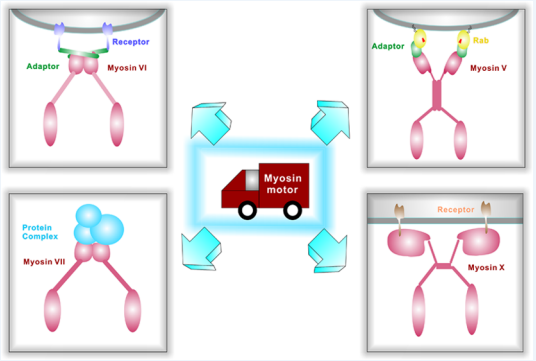
**CONSPECTUS:** Organized motions are hallmarks of living organisms. Such motions range from collective cell movements during development and muscle contractions at the macroscopic scale all the way down to cellular cargo (e.g., various biomolecules and organelles) transportation and mechanoforce sensing at more microscopic scales. Energy required for these biological motions is almost invariably provided by cellular chemical fuels in the form of nucleotide triphosphate. Biological systems have designed a group of nanoscale engines, known as molecular motors, to convert cellular chemical fuels into mechanical energy. Molecular motors come in various forms including cytoskeleton motors (myosin, kinesin, and dynein), nucleic-acid-based motors, cellular membrane-based rotary motors, and so on. The main focus of this Account is one subfamily of actin filament-based motors called unconventional myosins (other than muscle myosin II, the remaining myosins are collectively referred to as unconventional myosins). In general, myosins can use ATP to fuel two types of mechanomotions: dynamic tethering actin filaments with various cellular compartments or structures and actin filament-based intracellular transport.




In contrast to rich knowledge accumulated over many decades on ATP hydrolyzing motor heads and their interactions with actin filaments, how various myosins recognize their specific cargoes and whether and how cargoes can in return regulate functions of motors are less understood. Nonetheless, a series of biochemical and structural investigations in the past few years, including works from our own laboratory, begin to shed lights on these latter questions.

Some myosins (e.g., myosin-VI) can function both as cellular transporters and as mechanical tethers. To function as a processive transporter, myosins need to form dimers or multimers. To be a mechanical tether, a monomeric myosin is sufficient. It has been shown for myosin-VI that its cellular cargo proteins can play critical roles in determining the motor properties. Dab2, an adaptor protein linking endocytic vesicles with actin-filament-bound myosin-VI, can induce the motor to form a transport competent dimer. Such a cargo-mediated dimerization mechanism has also been observed in other myosins including myosin-V and myosin-VIIa.

The tail domains of myosins are very diverse both in their lengths and protein domain compositions and thus enable motors to engage a broad range of different cellular cargoes. Remarkably, the cargo binding tail of one myosin alone often can bind to multiple distinct target proteins. A series of atomic structures of myosin-V/cargo complexes solved recently reveals that the globular cargo binding tail of the motor contains a number of nonoverlapping target recognition sites for binding to its cargoes including melanophilin, vesicle adaptors RILPL2, and vesicle-bound GTPase Rab11. The structures of the MyTH4−FERM tandems from myosin-VIIa and myosin-X in complex with their respective targets reveal that MyTH4 and FERM domains extensively interact with each other forming structural and functional supramodules in both motors and demonstrate that the structurally similar MyTH4−FERM tandems of the two motors display totally different target binding modes. These structural studies have also shed light on why numerous mutations found in these myosins can cause devastating human diseases such as deafness and blindness, intellectual disabilities, immune disorders, and diabetes.

## 1. INTRODUCTION OF UNCONVENTIONAL MYOSINS

Living cells have evolved a variety of specific myosins to fulfill their distinct functions. Based on sequence analysis, the human genome contains about 40 myosins, which can be classified into 12 subfamilies (Figure 1). In addition to the class II myosins (also called conventional myosins), the myosin superfamily contains a large number of unconventional myosins with distinct amino acid sequences and lengths in their tails. In the human genome, 24 myosins belong to unconventional myosins, and 4 out of a total of 5 myosins are unconventional myosins in *Saccharomyces cerevisiae*, and 11 of 13 myosins belong to the unconventional class in *Drosophila*.[1−3]

Each myosin typically contains three major parts: an N-terminal motor head for binding to actin filaments and for ATP hydrolysis-induced energy production, a neck region for

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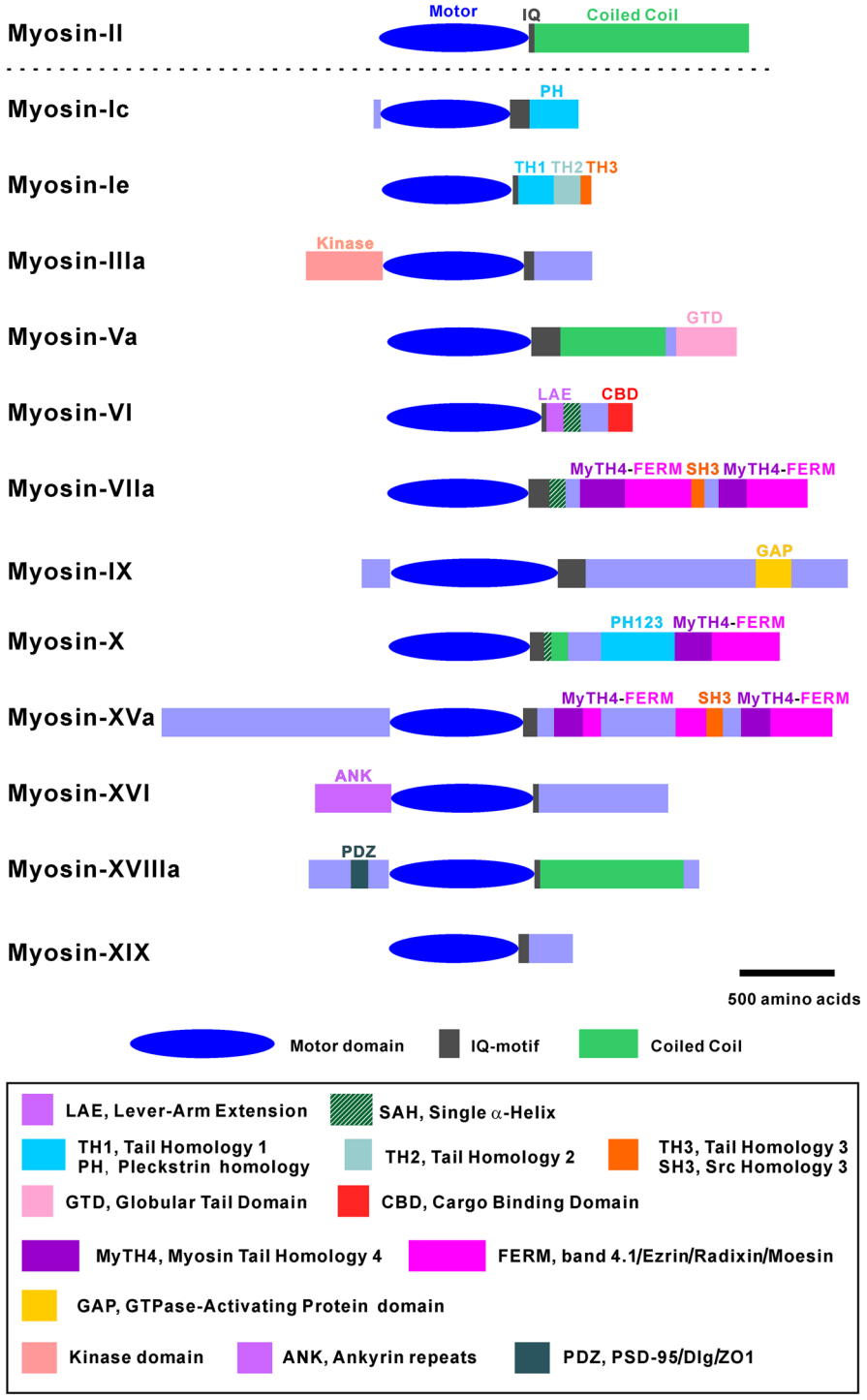


**Figure 1.** Schematic diagrams showing the domain organizations of human myosins. Different myosins have conserved motor heads and distinct tails with versatile functional domains. The definitions of protein domains are annotated in the text box.

mechanical force amplification, and a variable C-terminal tail for engaging with their specific targets. All myosins share a general chemomechanocycle of transforming chemical fuels into mechanical energy. In the ADP-bound and nucleotide-free states, myosin motor binds strongly to actin, but ATP or ADP-$P_i$ bound myosin has lower affinities for actin. Detailed descriptions of the chemomechanical coupling of the actin−myosin ATPase cycles can be found in several excellent reviews of the topic.[4−7] The large variations in the ATPase kinetic cycle properties of motors underlie their motile features and consequently determine their physiological functions (e.g., functioning as a tether or a transporter in living cells).[8,9] A myosin's cellular activity is governed by several key parameters including directionality on actin filaments, stepping or power stroke, processivity, and duty ratio (the fraction of strongly bound state with actin filaments per ATPase cycle). Several excellent reviews have provided comprehensive coverage on these points.[8−10] We will only very briefly touch these points here. In general, a myosin motor needs to have high duty ratio to achieve directional and long distance transport. The class I myosins and most myosin-II forms exhibit


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very low duty ratio. To support rapid muscle contraction, skeletal and smooth muscle myosin IIs assemble with actin filaments forming fibers. Polymerized myosin-II, each with a duty ratio of only 0.04−0.05, cross-links with actin filaments, sustaining the continuous actin filament sliding following a fashion like the walking of a millipede with many legs.[10] The more interesting case is class I myosins, of which duty ratios could be regulated by tensions applied to their motor heads. For example, the duty ratio of myo1c decreases from >0.9 to <0.2 when tension or load is released,[11] suggesting that it may function as a tension sensitive molecular tether instead of a cellular transporter. On the other hand, high duty ratio motors, represented by myosin-Va, which has a duty ratio of 0.7−0.8 (ref 8), ensure their processive movements and sequential steps along actin filaments in a dimerized manner.

The neck region of each myosin, which immediately follows the motor head, contains various numbers of continuous IQ-motifs for binding to its light chains (calmodulin (CaM) or CaM-like proteins). CaM binding to each IQ motif physically rigidifies its $\alpha$-helical conformation,[12] and the number of IQ-motifs determines the length of the lever arm of each myosin motor.[13] Several myosins (e.g., myosin-VI,[14,15] myosin-X,[16] and myosin-VIIa (our unpublished data)) contain a highly rigid single $\alpha$-helix (SAH) with different lengths, which can further extend the lengths of the lever arms of these motors.

Some myosins contain predicted coiled coils with various lengths after their neck regions. The predicted coiled coils of muscle myosin-II and unconventional myosin-V each form a parallel coiled-coil dimer, mediating dimerization and juxtaposition of two motor heads to a linear actin filament of these two motors.[17−19] Interestingly, a recent structural study showed that the predicted coiled coil of myosin-X forms an antiparallel dimer (anti-CC), which facilitates the motor to take straddled walking steps in addition to the classical hand-over-hand steps on bundled actin filaments.[20]

Fitting with their diverse cellular functions, variations of the C-terminal cargo recognition tails of unconventional myosins are much greater than their corresponding motor heads and neck regions (Figure 1). Many well-defined protein or lipid membrane-binding domains are present in the unconventional myosin tails, and these include SH3 domains in myosins-I, -VII, and -XV, PH domains in myosins-I and -X, and MyTH4−FERM tandems in myosins-VII, -X, and -XV. There are also class I myosin-specific domains such as TH1, TH2, and TH3 domains. Although less frequent, a few myosins contain additional defined protein domains N-terminal to their motor domains (e.g., a protein kinase domain in myosin-III, ankyrin repeats in myosin-XVI, and a PDZ domain in myosin-XVIII; see Figure 1 for details and definitions of the abbreviations). Given their widely known scaffolding capabilities, the ankyrin repeats and the PDZ domain situated at the N-terminal ends of myosins-XVI and -XVIII, respectively, may also be responsible for target recognition, like their C-terminal counterparts. The kinase domain of myosin-III has been implicated in regulating the ATPase activity of the motor domain via direct phosphorylation.[21]

The two major activities of unconventional myosins (i.e., cellular transportation and dynamic tethering) require myosins to be targeted to specific subcellular compartments, which is also facilitated by their tail domains via binding to specific target proteins or lipid membranes.[2,22−24] Myosins-I, -V, -VI, -VII, and -X have long been reported to participate in vesicle endocytosis or exocytosis and cellular organelle delivery within the actin-rich regions. Determined by the motors' distinct directionality and the overall directionality of actin filaments with plus ends pointing toward plasma membranes, myosin-V is responsible for exocytosis to transport vesicles toward plasma membranes.[25] Myosin-VI, the only reported motor directed to the minus end of actin filaments, often participates in endocytosis and moves vesicles toward the cell interior.[26] Myosins-VII, -X and -XV are often concentrated at the bundled actin-based protrusions such as stereocilia and filopodia and are known to promote stereocilia and filopodia formation and stability.[27−29] For the class I myosins, they are known as short-range tethers for linking membrane vesicles to cortical actin filaments during endocytosis, exocytosis, and trans-Golgi network (TGN) trafficking.[30]

How myosins recognize their cargoes via their distinct tail regions and how motor/cargo interactions are regulated are rapidly growing areas in the myosin field. In particular, structural studies at the atomic level in the past few years have provided valuable insights into the mechanistic bases governing how several representative unconventional myosins bind to their specific cargoes. In the following, we attempt to summarize these discoveries.

## 2. MYOSIN-VI CAN SWITCH BETWEEN TETHER AND TRANSPORTER

Being the only minus-ended motor, myosin-VI plays vital roles at early stages of endocytosis for clathrin-coated vesicles, at the late stage of endocytosis for uncoated vesicles, and in biosynthetic exocytosis at the Golgi network.[26,31] Myosin-VI is also known to function as a molecular tether by physically anchoring cytoskeletons to membranes to maintain highly organized cellular structures, such as stereocilia of hair cells in inner ears, leading edges of migrating cells, and apical−basal polarity of epithelia.[26] To function as a transporter, myosin-VI needs to dimerize in order to walk processively on actin filaments. On the other hand, a monomeric myosin-VI exhibits load-dependent anchoring and thus suffices to function as mechanical tether.[32] Given that myosin-VI does not contain a predicted coiled coil dimerization domain in its neck region, its tail cargo binding domain is believed to contain necessary element(s) that can modulate the monomer−dimer conversion of the motor.[26]

The atomic structures of the myosin-VI globular cargo binding domain (CBD) alone and in complex with a transporting cargo adaptor Dab2 reveal that myosin-VI undergoes a cargo binding-induced dimerization.[33] In the absence of Dab2, myosin-VI CBD folds into a stable monomer (Figure 2A). Before binding to myosin-VI CBD, the myosin-VI interaction region (MIR) of Dab2 exists as an unfolded monomer. Binding of Dab2 converts myosin-VI CBD into a stable dimer by forming a 2:2 myosin-VI CBD/Dab2MIR complex (Figure 2B). The structure of myosin-VI CBD changes very little and Dab2MIR folds into two $\alpha$-helices upon the complex formation. It is noted that two molecules of myosin-VI CBD do not directly contact each other, meaning that the dimerization is strictly mediated by Dab2MIR (Figure 2B). The binding between myosin-VI CBD and Dab2 is very strong ($K_d \approx 50$ nM).[33] Such strong binding is expected to convert myosin-VI efficiently into a processive dimeric transporter as soon as myosin-VI meets its Dab2-containing membrane vesicles (Figure 2D). The cargo binding-mediated dimerization mode has also been implicated for myosin-VII. Myosin-VIIa alone exists as a monomer in vitro, but its transporting function can be activated by its cargo Myrip.[34]

Several other targets including LMTK2, GIPC, and optineurin[26] have also been reported to bind to myosin-VI CBD, albeit these cargoes are not known to convert myosin-VI into dimers.


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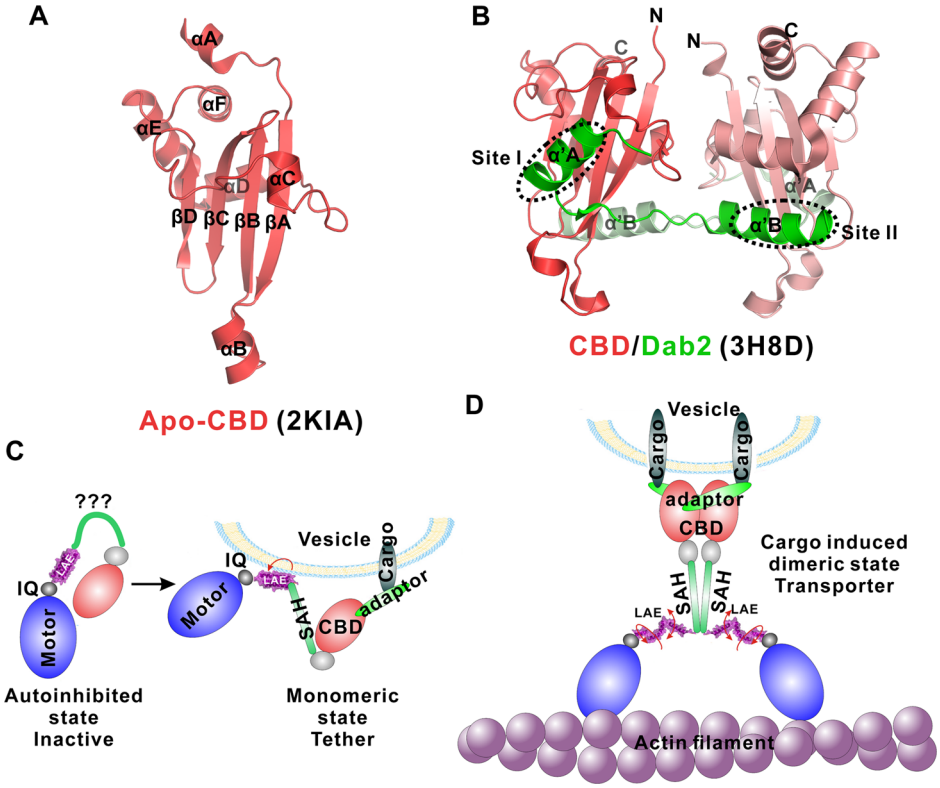


**Figure 2.** Cargo-induced dimerization of myosin-VI. (A) The structure of the monomeric, apo-form myosin-VI CBD. (B) The binding of cargo adaptor Dab2 induces CBD to form a 2:2 complex. (C) The tail of myosin-VI folds back to bind to the motor head, keeping the motor in an autoinhibited conformation (left). Cargo vesicles can open the autoinhibited conformation (right). (D) A walking model of myosin-VI with the dimerized tails induced by cargo adaptor binding and membrane-induced LAE expansion.

An ~90-residue subdomain immediately preceding myosin-VI CBD is responsible for binding to GIPC and optineurin, and the binding of these two cargoes do not induce dimer formation of the motor.[26,35] Myosin-VI is likely to play tethering roles in these monomeric myosin-VI/cargo interactions (Figure 2C).

A conserved ~80 amino acid region after the IQ motifs of myosin-VI, known as the lever arm extension (LAE), also attracted attention in recent years. It has been shown that the SAH following the LAE extends the lever arm length required for the large step size (~30−36 nm) of the motor,[15] although the IQ-motifs and SAH together are still not long enough. Interestingly, myosin LAE forms a compact three helical bundle structure with a length of ~3 nm before cargo binding.[36,37] Upon cargo vesicle binding, the LAE expands into a semirigid extended helical structure with a length of ~6−9 nm (Figure 2D), although the detailed mechanism governing the LAE expansion is a topic of debate.[15,36] The cargo binding-induced expansion of LAE, together with the IQ-motifs and SAH, has been proposed to allow the myosin-VI dimer to take "wiggly" walking steps on actin filaments with large step sizes of ~30−36 nm[15,36,38] (Figure 2C,D). Importantly, dissociation of cargo vesicles from myosin-VI tail leads to spontaneous conversion of the expanded LAE back to compact three helix bundle, which presumably coincides with the formation of the apo- and autoinhibited conformation of the motor (Figure 2C).

## 3. CLASS V MYOSINS RECOGNIZE DIVERSE CARGOES WITH DISTINCT MECHANISMS

The class V myosins are perhaps the best studied cargo transporting myosins. They are widely expressed in most eukaryotes, with yeast containing two paralogs (Myo2p and Myo4p) and human containing three (myosin-Va, -Vb, and -Vc). The class V myosins are capable of recognizing a diverse range of cargoes (e.g., membrane vesicles, organelles, protein complexes, and mRNAs) and therefore play very broad physiological functions.[25,39] The globular tail domain (GTD) at the C-terminus of myosin-V is responsible for cargo recognition.[25] Several recent structural studies have shed light on how the single GTD might be able to recognize such diverse cellular cargoes by myosin-V. The overall structures of the cargo-free GTD from yeast (Myo2p[40] and Myo4p[41]) and from mammals (myosin-Va, myosin-Vb, and myosin-Vc)[42,43] are highly similar (Figure 3A,B), despite their very low sequence similarities between the class V myosins from mammals and yeast (~20% sequence identity) and nonoverlapping cellular cargoes. Although sharing overall folding similarities, GTDs of yeast and mammalian myosin-V contain important structural differences. The most obvious difference is in subdomain I, where the two termini of mammalian myosin-V-GTD fold into short $\beta$-strands ($\beta$1 and $\beta$2) and form a small antiparallel $\beta$-sheet (Figure 3A,C) and the two termini of yeast myosin-V-GTD form $\alpha$-helical structures ($\alpha$1 and $\alpha$16) and interact with each other in an antiparallel manner (Figure 3B,C). Since subdomain I is extensively involved in target binding (Figure 3D, also see below), such structural differences explain at least in part the distinct cargo binding properties of the class V myosins between yeast and mammalian and among mammalian paralogs.

In vertebrates, myosin-Va can be detected in brain and other tissues such as melanocytes.[44] Mutations of human myosin-Va are known to cause Griscelli syndrome, a genetic disorder characterized by hypopigmentation with immune and intellectual deficiencies.[45] Myosin-Va defective patients have defects in the trafficking of melanosomes, vesicles containing the pigment


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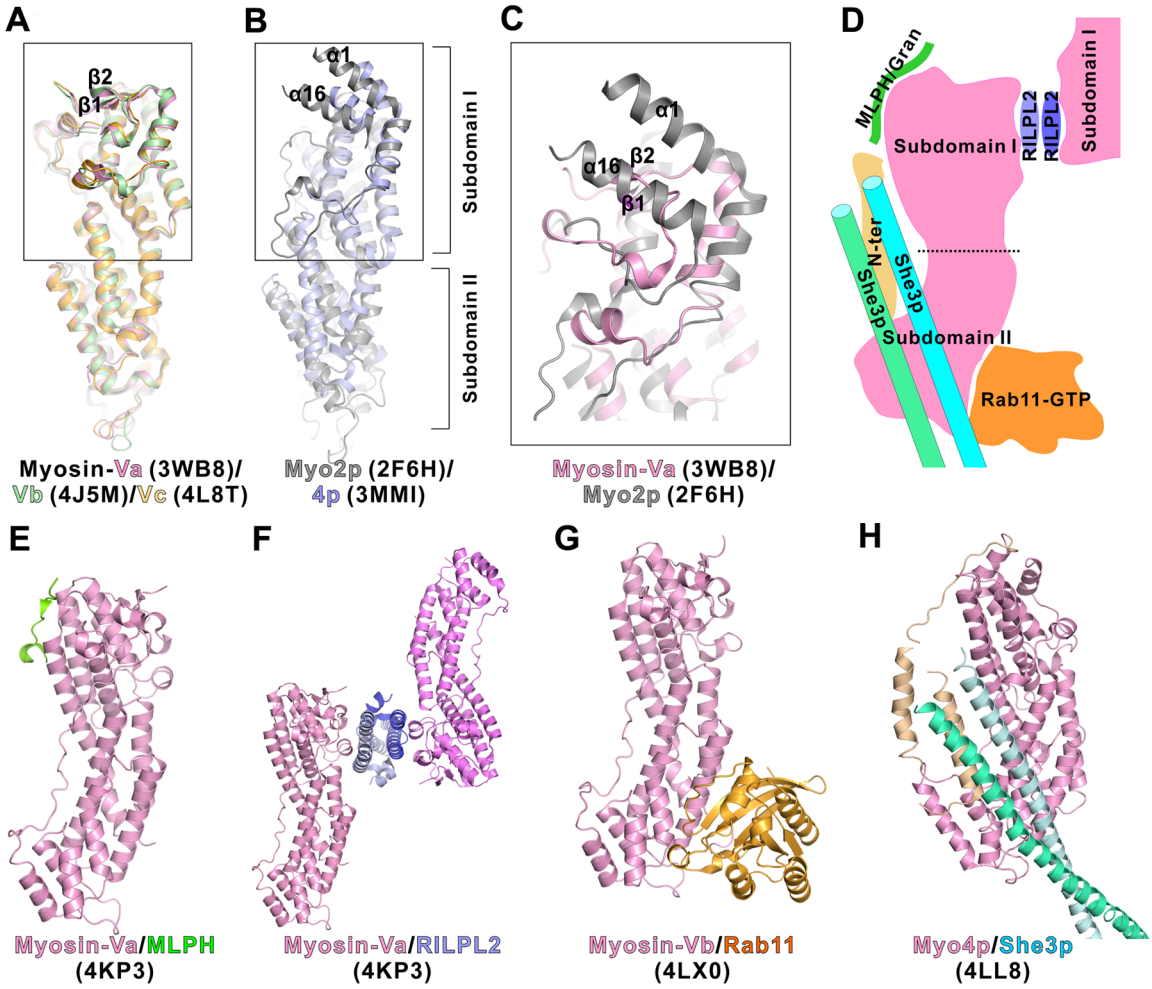


**Figure 3.** Different cargo recognition mechanisms of the class V myosins. (A) Superposition of the structures of mammalian myosin-V-GTDs. (B) Superposition of the two yeast myosin-V-GTD structures. (C) Enlarged view of the upper part of subdomain I showing the structural difference between yeast Myo2p and mammalian myosin-Va. (D) Schematic model shows different binding sites on myosin-V-GTD. (E) MLPH interacts with subdomain I of myosin-Va-GTD. (F) RILPL2 dimer interacts with two molecules of myosin-Va-GTD at the opposite site of subdomain I and forms a dimer of dimers complex. (G) Crystal structure shows the binding of Rab11 to subdomain II of myosin-Vb-GTD. (H) She3p binds to Myo4p at subdomain II with the site partially overlapping with that of Rab11.

melanin.[44] Melanophilin (MLPH), an effector of melanosomes adaptor Rab27a, can directly interact with GTD of myosin-Va.[46] The crystal structures of the myosin-Va/MLPH complex[47,48] show that a short stretch of sequence in MLPH specifically interacts with a conserved pocket in the upper part of subdomain I in GTD (Figure 3E). Interestingly, through sequence and structural analysis of different paralogs/orthologs of this class, only myosin-Va in vertebrates share the same binding pockets. This is consistent with the specificity of MLPH binding shown by biochemical experiments.[47] MLPH can specifically discriminate very similar GTDs among the three isoforms of myosin-V (i.e., only binding to myosin-Va, but not -Vb and -Vc). Similarly, granuphilin (Gran) was reported as an adaptor for secretory granules.[49] Biochemical data indicate that Gran shares the same binding pocket with MLPH on myosin-Va.[47] Therefore, Gran may also be able to differentiate myosin-V paralogs and specifically interact with myosin-Va. One might envision that myosin-Va (and like other two paralogs of myosin-V), via binding to different cargoes using the same binding site, can execute distinct cellular roles.

Myosin-Va GTD contains other target binding sites in addition to the MLPH/Gran binding pocket. For example, another myosin-Va cargo, Rab interacting lysosomal protein-like 2 (RILPL2), has been shown to bind to a site opposite to the MLPH binding site in subdomain I of GTD with a very different mechanism[47] (Figure 3D,F). RILPL2 forms a stable dimer, and the resulting myosin-Va GTD/RILPL2 complex adopts a dimer of dimers assembly (Figure 3F). The dimerization of RILPL2 is required for its binding to myosin-Va.[47] Again, the binding site for RILPL2 in GTD is only conserved in myosin-Va; therefore RILPL2 only binds to myosin-Va but not to the other two paralogs. Both RILPL2 and MLPH bind to subdomain I of myosin-Va-GTD, leaving subdomain II completely free to interact with additional targets (Figure 3D, and see below).[48] It should be noted that tissue-specific alternative splicing of myosin-V's tail regions N-terminal to GTD also directly regulates cargo bindings.[25] Since these splicing sites are all outside the GTD, myosin-V's cargo binding capacity is further expanded by its alternative splicing.

In addition to binding to cargo vesicles through vesicle adaptor proteins, myosin-V can directly bind to Rab proteins.[50] For example, Rab11−myosin-Vb interaction is required for transferrin receptor-containing vesicle trafficking.[50] A recent structural study showed that myosin-Vb-GTD can bind to the GTP- and GDP-bound forms


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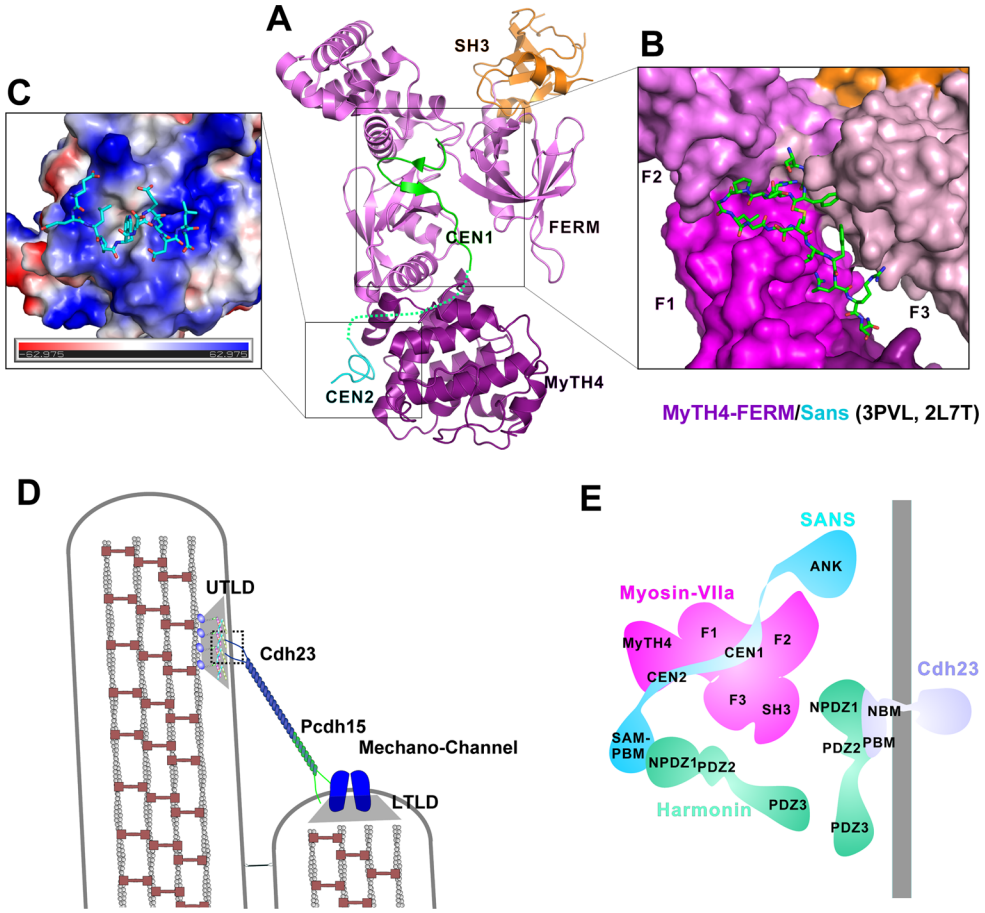


**Figure 4.** The myosin-VIIa/sans complex structure. (A) The CEN region of sans interacts with the N-terminal MyTH4−FERM supramodule of myosin-VIIa. (B) In the myosin-VIIa/sans complex, CEN1 (green) binds to the center of the "cloverleaf" of myosin-VIIa FERM. (C) CEN2 (cyan) binds to the positively charged pocket of myosin-VIIa MyTH4. (D) Diagram of stereocilia showing the location of the myosin-VIIa−sans−harmonin complex at UTLD. (E) Schematic model showing the assembly of myosin-VIIa−sans−harmonin complex at UTLD.

of Rab11, although GTP-Rab11 binds to myosin-Vb-GTD with a higher affinity.[48] Instead of binding to subdomain I like MLPH and RILPL2 do, Rab11 binds to the lower part of subdomain II of myosin-Vb-GTD (Figure 3G). Sequence analysis shows that residues involved in Rab11 binding are largely identical between myosin-Va and myosin-Vb, but obviously different in myosin-Vc, indicating that Rab11 can also bind to myosin-Va but not to myosin-Vc. Besides the direct binding to Rab11, myosin-Vb can also interact with the Rab11 adaptor protein called Rab11-FIP2.[25] It is possible that the simultaneous interactions of myosin-Vb with Rab11 and Rab11-FIP2 can increase both the binding affinity and specificity between myosin-Vb and the Rab11-containing cargo vesicles. The direct interactions between Rab GTPases and myosin-V provide a mechanistic link for broad ranges of actin-based vesicle trafficking events driven by the class V myosins.

Myo4p, one of the two members of yeast myosin-V, has been shown to transport cargoes such as messenger ribonucleoprotein particles (MRPs) and tubular endoplasmic reticulum.[25,51] However, no direct interactions between these cargoes and the motor have been observed. A recent crystal structure revealed that Myo4p-GTD interacts with She3p, a coiled coil protein that can link MRPs with Myo4p.[52] Similar to RILPL2, She3p formed a dimer via its coiled coil domain, and this dimer interacts with Myo4p-GTD at subdomain II (Figure 3H). The She3p binding site on Myo4p and the Rab11 binding site on myosin-Vb partially overlap with each other (Figure 3D,G,H), indicating that subdomain II of myosin-V can also bind to multiple cargoes via distinct surfaces. Interestingly, Myo4p lacks the long coiled coil dimerization region found in mammalian myosin-V; thus cargo-free Myo4p is a monomer. The formation of She3p dimer raises a possibility that Myo4p may represent another example of a myosin that can undergo cargo-mediated dimerization.[52,53]

## 4. MYOSIN-VIIa/SANS/HARMONIN TRIPARTITE COMPLEX

The class VII myosins are MyTH4−FERM containing myosins. Whether myosin-VII can act as a transporter is being debated because it lacks a defined coiled coil dimerization domain.[14] However, it has been suggested that cargo binding can induce dimerization in myosin-VIIa.[34]

The best studied member of the class VII myosins is myosin-VIIa (aka USH1B), mutations of which can cause the most severe type of the Usher syndrome characterized by early onset hearing loss and visual impairment.[28] Myosin-VIIa is important for the development of stereocilia, the actin based protrusions localized at the apical surface of cochlear and vestibular hair cells of ears responsible for sound perceptions and balance sensing. Mutations of myosin-VIIa cause morphological defects of stereocilia and mislocalization of its binding proteins including multi-PDZ domain scaffold protein harmonin (aka USH1C) and a SAM domain containing scaffold protein sans (aka USH1G), two of the five Usher I proteins.[28,54] It has been shown that myosin-VIIa,


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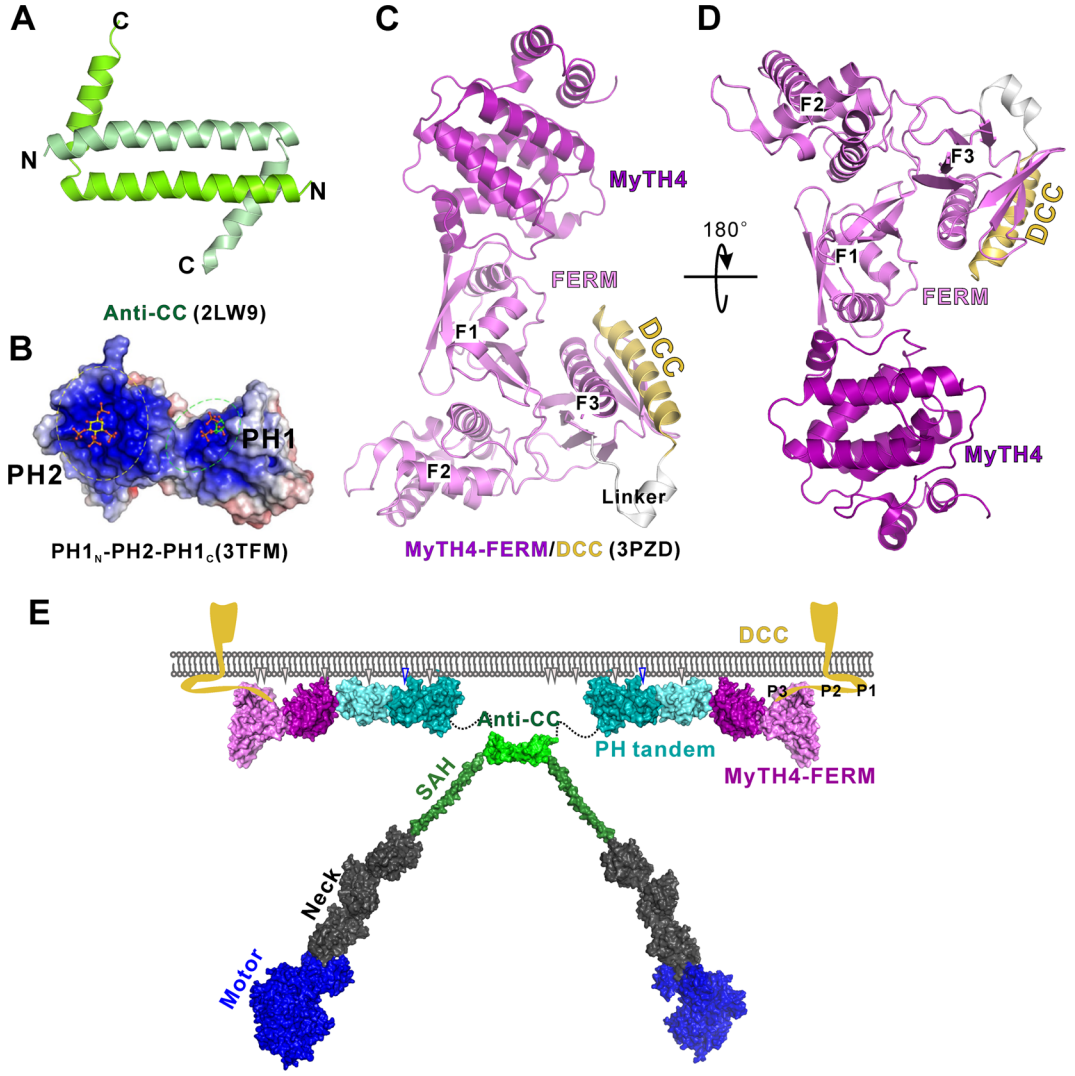


**Figure 5.** Multidomain cooperation of the myosin-X tail. (A) The predicted coiled-coil region of myosin-X forms an antiparallel coiled coil dimer. (B) Two distinct positively charged lipid binding pockets of the $PH1_N$–PH2–$PH1_C$ tandem renders the PH tandem as a high affinity, cooperative PI(3,4,5)P3 sensor. (C and D) Ribbon representation of the MyTH4–FERM/DCC_P3 complex structure showing the specific DCC binding by myosin-X. The ribbon diagram at right is at the same orientation as myosin-VIIa MyTH4–FERM in Figure 4A. (E) Structural model of the full-length, dimeric myosin-X simultaneously bound to DCC and PI(3,4,5)P3-containing membranes via its tail domains.

through sans, forms a very stable tripartite complex with harmonin.[54,55] The central conserved region of sans (called "CEN") can interact with the first MyTH4–FERM tandem of myosin-VIIa with a high affinity ($K_d \approx 50$ nM). The N-terminal half of CEN (CEN1) binds to the center of the "cloverleaf" of the FERM domain (Figure 4A,B) and the C-terminal half of CEN (CEN2) binds to a positively charged surface of the MyTH4 domain (Figure 4A,C), and thus the entire CEN binds very tightly to the MyTH4–FERM supramodule of myosin-VIIa. The stable myosin-VIIa/sans/harmonin tripartite complex is likely to play critical roles to maintain the tip-link structure and thus to stabilize stereocilia by firmly anchoring the protein complex at the upper tip link density (UTLD) at the bases of the link[56] (Figure 4D,E). It is noted that the canonical binding sites of the myosin-VIIa FERM domain (i.e., the outer surfaces of the three cloverleaves) are unoccupied (Figure 4A). Additional targets may bind to this MyTH4–FERM supramodule. Additionally, myosin-VIIa contains another MyTH4–FERM tandem at the C-terminus (Figure 1), which is likely to bind to another host of cargo proteins. A very recent study reported that myosin-VIIb can link with harmonin in brush borders of mammalian intestines,[57] which are also actin based protrusions similar to stereocilia of hair cells. All these studies indicate that, together with their cargo proteins like harmonin and sans, the class VII myosins play critical roles in the formation and maintenance of the actin-rich protrusions such as stereocilia in hair cells, microvilli in brush borders and photoreceptor cells.

## 5. MULTIDOMAIN COOPERATION OF THE MYOSIN-X TAIL

Myosin-X is another MyTH4–FERM tandem-containing myosin that is critical for the formation of filopodial structures in various tissues in mammals. As such, myosin-X is essential for numerous developmental functions such as neuronal network formation and angiogenesis.[27] Myosin-X contains a motor head, a coiled coil neck, and a tail region composed of three PH domains and a MyTH4–FERM tandem (Figure 1). Unexpectedly, the predicted coiled coil domain in the neck region of myosin-X forms an antiparallel dimer instead of commonly expected parallel dimer[20] (Figure 5A). On bundled actin


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filaments, the anti-CC-mediated myosin-X dimer may take straddled, duck-like walking steps in addition to the conventional hand-over-hand walking mode, a unique property that may provide a mechanistic explanation to the long processivity and actin bundling capacity of the motor.[58,59]

In the myosin-X tail, three consecutive PH domains and the MyTH4−FERM tandem bind to their targets each in a unique manner. The split $PH1_N$−PH2−$PH1_C$ tandem of myosin-X forms an integral structural supramodule, allowing myosin-X to bind to $PI(3,4,5)P_3$ containing membrane with high specificity and cooperativity. As such, the PH tandem has a capacity of functioning as an acute PI3-kinase activity sensor[60] (Figure 5B). The MyTH4−FERM tandem of myosin-X forms another structural supramodule, which via its F3 lobe of the FERM domain binds to the cytoplasmic tail of an axon guidance receptor called DCC[61,62] (Figure 5C and D). Although the structure of the MyTH4−FERM tandems from myosin-X and myosin-VIIa are highly similar, their cargo recognition modes are totally different (Figures 4A and 5D).

In general, the binding between myosin tails and their targets are strong, with $K_d$ values ranging from ∼50 to 300 nM.[33,47,54] In contrast, myosin-X MyTH4−FERM/DCC complex has a relative low affinity with $K_d \approx 2000$ nM. However, multivalent weak interactions between two or more domains in the single myosin-X tail with membrane-anchored ligands can greatly enhance their binding affinities (Figure 5E). The cooperative interactions of its PH123 and MyTH4−FERM tandems with PI(3,4,5)P3 and DCC, respectively, ensure that myosin-X binds to its cargoes with high affinity and exquisite specificity. The anti-CC-mediated dimerization further enhances the binding between myosin-X and its membrane cargoes (Figure 5B). As such, myosin-X is often found to be highly enriched in specialized actin-rich membrane regions such as tips of filopodia.[27] A potential advantage of such coincidence detection is that the interaction can be differentially tuned by any one or combinations of these elements. For example, PI3-kinase activation signals can affect PH123's membrane association, netrin signal can potentiate the MyTH4−FERM/DCC interaction, and degree of bundled actin can further influence the concentration of myosin-X at the growing tips of filopodia. Therefore, myosin-X is ideally suited to interpret and integrate various cellular signals at filopodia tips. Since tails of many myosins contain multiple well-recognized protein domains (Figure 1), we anticipate that some of these myosins may also adopt multivalent targeting recognition modes as myosin-X does. Finally, it is reasonable to anticipate that the highly diverse tail domains are critical for extremely broad functions but each with very specific and often highly regulated physiological functions of unconventional myosins.

## 6. CONCLUSIONS AND PERSPECTIVES

Structural elucidations of a number of unconventional myosin tails in complex with their respective cargoes in the past few years have provided important insights into the cargo recognition mechanisms of several different classes of unconventional myosins. These studies have also indicated that the functional properties of myosin motors are often regulated by their specific cargoes. We believe that cellular functions of myosin motors often coevolve with their cellular cargoes. We anticipate that more atomic level structures of myosin motors, with and without their cargoes bound, will become available in the coming years. Together with detailed knowledge obtained from single molecule level biophysical, ultrahigh resolution imaging, and cellular and genetic studies, our understandings of myosins in various aspects of cellular motions will reach an unprecedented level.

Considering the highly organized cellular environment, myosins must be effectively regulated to perform their specific functions at precise location and time. Such spatial and temporal regulations also include cargo loading and unloading by myosins, although much less is understood in this aspect. It has been indicated that many different mechanisms including but certainly not limited to protein phosphorylation, calcium-dependent cargo loading and unloading, lipid signaling, and small GTPase-mediated cargo recognition can regulate motor/cargo interactions.[22] It is also possible that degradation of motor or cargo adaptor can regulate cargo unloading.[63] An important direction of future research is to elucidate regulatory mechanisms governing reversible cargo loading and unloading of unconventional myosins.

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