Myomics: myosin VI structural and functional plasticity

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Myosin VI is a minus end-directed actin motor protein that fulfils several roles in the cell. The interaction of myosin VI with its cellular cargoes is dictated by the presence of binding domains at the C-terminus of the protein. In this review, we describe how alternative splicing and structural and conformational changes modulate the plasticity of the myosin VI interactome. Recent findings highlight how the various partners can cooperate or compete for binding to allow a precise temporal and spatial regulation of myosin VI recruitment to different cellular compartments, where its motor or anchor function is needed.

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Introduction

Myosins are a superfamily of actin motor proteins that use ATP hydrolysis to move molecular cargoes along the actin filaments inside the cell. All characterized myosins move toward the plus-end of the filaments except for myosin VI, which moves in the opposite direction [1] due to a unique reverse gear [2]. This peculiarity accounts for the variety of the cellular roles that myosin VI can perform, ranging from vesicle transport and clathrin-mediated endocytosis to autophagy and cell migration [3,4]. The multifunctionality of myosin VI derives from its ability to interact with a plethora of cargoes, identified over the years with traditional as well as mass spectrometry approaches (reviewed in Ref. [5]).

In recent years, structural studies focused on the interplay between myosin VI and its interactors, revealing important myosin VI features that allow the fine modulation of its activity and functions. Here, we review major advances and remaining open questions in the understanding of myosin VI structural and functional plasticity, with a particular focus on its regulation.
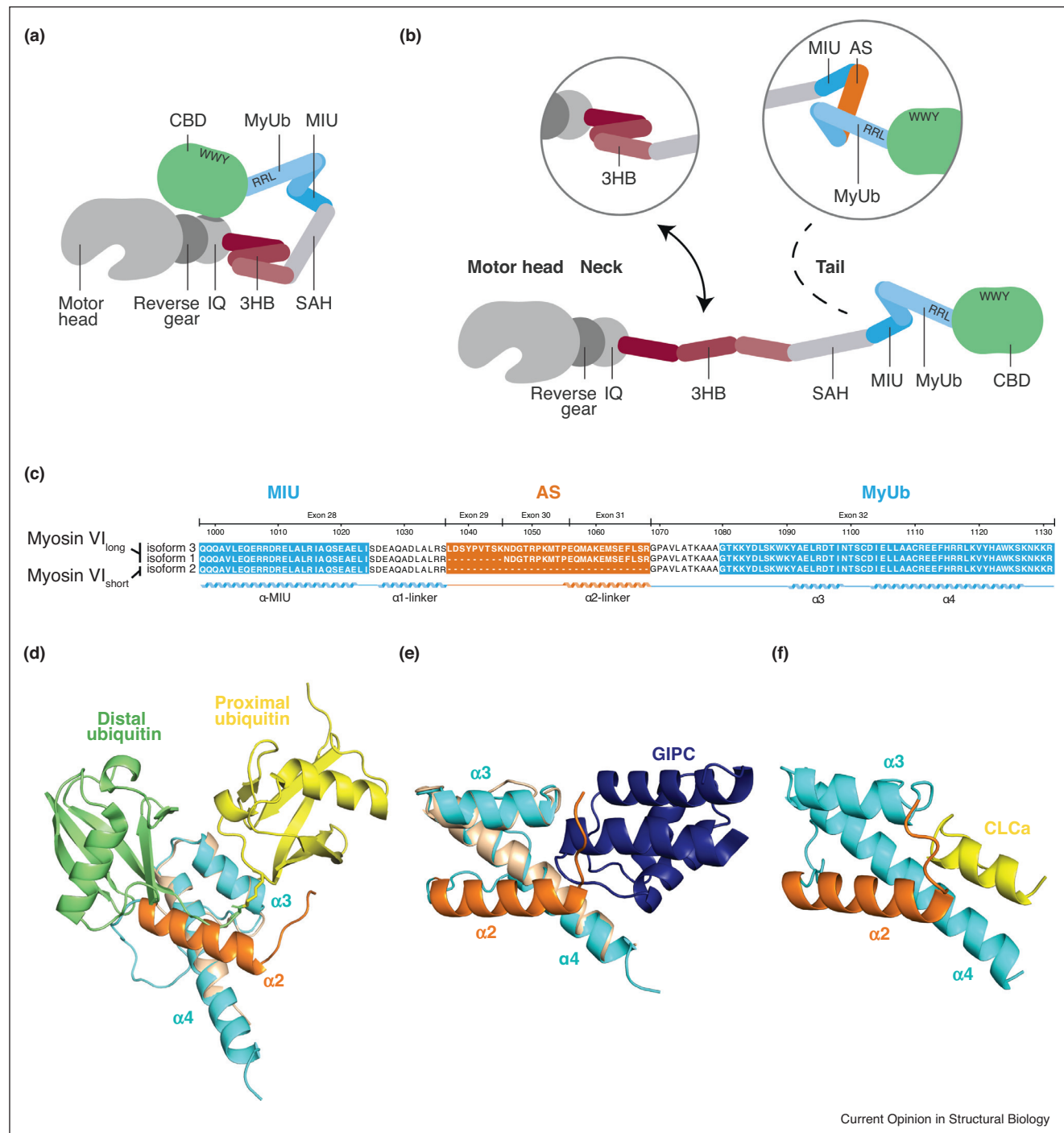
Myosin VI structural plasticity: how conformation dictates its interactome

As a member of the myosin superfamily, myosin VI is composed of a conserved structure that includes a catalytic motor head, which is responsible for binding to actin and hydrolysis of ATP; a neck region, which contains the reverse gear and an IQ domain that binds the calcium sensor calmodulin; and a tail domain, which binds cargoes and anchors the protein to specific membrane compartments (Figure 1a,b). This latter region is the critical determinant for functions unique to myosin VI and is composed of an N-terminal helical region and a C-terminal globular region called the cargo-binding domain, CBD.

Pioneering work from the Buss and Kendrick-Jones laboratories identified two short amino acid sequences in the tail region, the RRL [6] and WWY [7] motifs, as critical for interactor binding. In addition, a short domain in the helical region was identified as the motif interacting with ubiquitin, MIU [8], a feature suggesting a possible ubiquitin-mediated regulation of myosin VI binding. More recent structural studies confirmed the importance of these motifs, further defining and expanding these three binding surfaces as responsible for all of the identified myosin VI interactors.

The RRL$^{1116\text{-}1118}$ (positions refer to isoform 3, Q9UM54-3 uniprot) motif turned out to be part of a two-helix bundle domain, called M̲yosin VI U̲biquitin-binding (MyUb$^{1080\text{-}1131}$) domain [9]. This second ubiquitin-binding domain interacts preferentially with K63-linked polyubiquitin chains [9], a non-proteasomal signal that modulates various cellular pathways in which myosin VI has been shown to be involved, including autophagy, signaling and membrane trafficking events [4,10,11]. Ubiquitin binds the MyUb at the opposite side of the RRL motif, raising the intriguing hypothesis that myosin VI interaction with the RRL binders may be modulated by ubiquitin, a topic that will be further discussed in the next section. Amino acid substitution of the RRL$^{116\text{-}1118}$ motif with the alanine triplet AAA was initially reported to abolish myosin VI interaction with several interactors [12]. Subsequent studies found that a single substitution of the second arginine within the triplet, R1117, impairs the structural integrity of the entire region [9,13], challenging the contribution of these residues to the interaction with binders [13–15,16$^{\bullet\bullet}$]. Further dissection of the motif, using single-point mutants showed that, while

Figure 1

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Myosin VI structural plasticity. Scheme of myosin VI domain organization. Motor head and neck domains are in grey. Reverse gear and IQ domains are highlighted. In the tail domain, the extensible three α-helix bundle (3HB) is in dark red, the single α-helix (SAH) is in grey and the CBD is in green. MIU and the MyUb are in blue; AS is in orange. RRL and the WWY motifs are highlighted. **(a)** Myosin VI monomer is shown in the compact inactive conformation, where the tail back-folds onto the motor domain [46,49–51]. Calcium binding to calmodulin bound to the neck directs a major rearrangement of the motor from a dormant state to a primed state, cargo-binding state [49], shown in **(b)**. The 3HB can be found in a folded conformation (left circle) or in an extended conformation, that may function as a lever arm extension to allow processive movement [43]. The alternative structure of the tail region including the AS is shown in the right circle [13]. **(c)** Amino acid sequence alignment of the three myosin VI isoforms covering the region of alternative splicing between MIU and MyUb. Numbering above the sequence alignment refers to isoform 3. Constitutive and alternative exons are depicted above the sequence. Isoform 1 and isoform 3 are collectively defined as myosin VI$_{long}$ [13]; isoform 2 is myosin VI$_{short}$. The RRL motif is highlighted in red; secondary structure elements (PDB 2N12) are depicted below the sequence. **(d,e)**

R1116 and L1118 are critical for both GIPC and opti-
neurin binding [13,15], they appear to be dispensable for
the interaction with NDP52 and TAX1BP1 [13,14],
whose precise binding surface remains to be established
[17].

The second protein-protein interaction domain of myosin
VI is located in the globular domain that encompasses the
WWY[1201-1203] motif, initially identified as the determi-
nant for binding to the endocytic adaptor protein Dab-2
[7,18,19] and the ESCRT-0 component Tom1 [20,21].
Similarly, structural studies expanded the molecular
understanding by showing that the two prototypic
WWY interactors present distinct key interface residues
that interact with myosin VI. Dab-2 contacts two interac-
tion surfaces on myosin VI that are located on the oppo-
site sides of a central beta sheet within the globular CBD
domain [22]. Site I includes the WWY motif. Single point
mutations within either binding site are sufficient to
abrogate (site I) or reduce (site II) Dab-2 binding [22].
Tom1, instead, uses only site I to interact with myosin VI
[16••]. Previous results were thus confirmed as W1202
participates in the binding to both Dab-2 [22] and Tom1
[16••]. Considering the overlap of several interface resi-
dues at site I, it is tempting to speculate about a competi-
tion between Tom1 and Dab-2 that could account for the
formation of distinct complexes acting in different intra-
cellular processes [16••].

A third protein–protein interaction domain within myosin
VI is present only in the long isoform and is responsible
for the functional connection of myosin VI to clathrin-
mediated endocytosis [13,23••]. A well-known feature of
myosin VI is the presence of an alternatively spliced (AS)
region [24], which is positioned between the MIU and the
MyUb domains [9,13]. As shown in Figure 1c, this AS
region is encoded by an exon cassette composed of three
short exons. Partial or total inclusion of the exon cassette
generates myosin VI$_{long}$, whereas exon skipping gener-
ates myosin VI$_{short}$. Structural studies addressing the
differences between the isoforms have changed the view
of how myosin VI functions are regulated [13]. Indeed,
following inclusion, exon 31 codifies for an additional
α-helix, named α2-linker, that engages the two-helix
bundle of the MyUb domain, masking the RRL interac-
tion motif [13]. As a consequence, myosin VI$_{long}$ has a
severely reduced ability to bind K63-linked ubiquitin
chains and RRL-mediated interactors such as GIPC
[9,13,15] (Figure 1d,e). Intriguingly, while losing the
RRL interaction surface, myosin VI$_{long}$ acquires an addi-
tional binding surface for clathrin [13], which was recently
structurally defined [23••] and whose functional activities

are discussed below. Upon myosin VI interaction, resi-
dues 46-61 of the clathrin light chain (CLCa) fold into a
short α-helix that appears to complete the myosin VI$_{long}$
three-helix bundle domain (Figure 1f). Thus, the differ-
ent isoform structural architectures establish mutual
exclusivity of myosin VI-binding partners, clathrin versus
RRL interactors, and in turn, function. Although not
conserved in the primary sequence, an alternatively
spliced exon that codifies for a putative α-helix with
length and position similar to the α2-linker is evolution-
ary conserved in mammals [13]. It remains to be
addressed whether the structural conservation reflects a
general mechanism of regulation to yield two functionally
distinct proteins from the same gene.

The structural plasticity of myosin VI reflects its inter-
actome and the variety of functions that this motor
protein can play in the same cell and in different tissues.
Distinct adaptors that bind the myosin VI tail modulate
this motor/anchor protein temporally and spatially by
recruiting it to specific cell compartments. Moreover,
the presence of different and shared protein-protein
interaction surfaces suggests the existence of cooperative
and competitive mechanisms among partners that are just
starting to be elucidated [16••,25].

Ubiquitin and autophagy receptors: cooperation or competition?

The presence of different interaction surfaces on myosin
VI is exploited by the cell to coordinate different cargoes
when and where a concerted action is needed. A striking
example is the autophagy pathway, where myosin VI is
required for maturation of autophagosomes and, in par-
ticular, for their fusion with endosomes to generate the
amphisome [16••,21]. A recent structural study elucidated
the mechanistic basis underpinning the specific interac-
tion between myosin VI and Tom1 during the autophagic
process [16••]. Myosin VI appears to act as a bridge
between the two organelles based on its ability to interact
with the autophagy receptors optineurin, TAX1BP1 and
NDP52, and with endosomal Tom1 [16••] by using two
distinct surfaces in the helical and globular domains,
respectively (Figure 2a). In this context, ubiquitin is
predicted to play an important regulatory role. As men-
tioned above, the detailed interaction of the autophagy
receptors with the MyUb of myosin VI remains elusive.
The autophagy receptors bind myosin VI at low affinity
through their ubiquitin-binding domains, zinc-fingers for
TAX1BP1 and NDP52, and UBAN for optineurin
[17,26,27], suggesting a possible bipartite interaction with
ubiquitin. While initial studies seem to exclude a simul-
taneous interaction with myosin VI and ubiquitin for

(Figure 1 Legend Continued) Structure of the myosin VI$_{long}$ (PDB 2N12) superimposed on the structure of the myosin VI$_{short}$ in complex with K63-
linked di-ubiquitin (PDB 2N13, d) or with GIPC-GH2 (PDB 5V6E, e). The α2-helix severely clashes with the second half of the distal ubiquitin and
with the GH2 domain of GIPC. **(f)** Structure of the myosin VI$_{long}$ in complex with CLCa (PDB 6E5N).

Figure 2
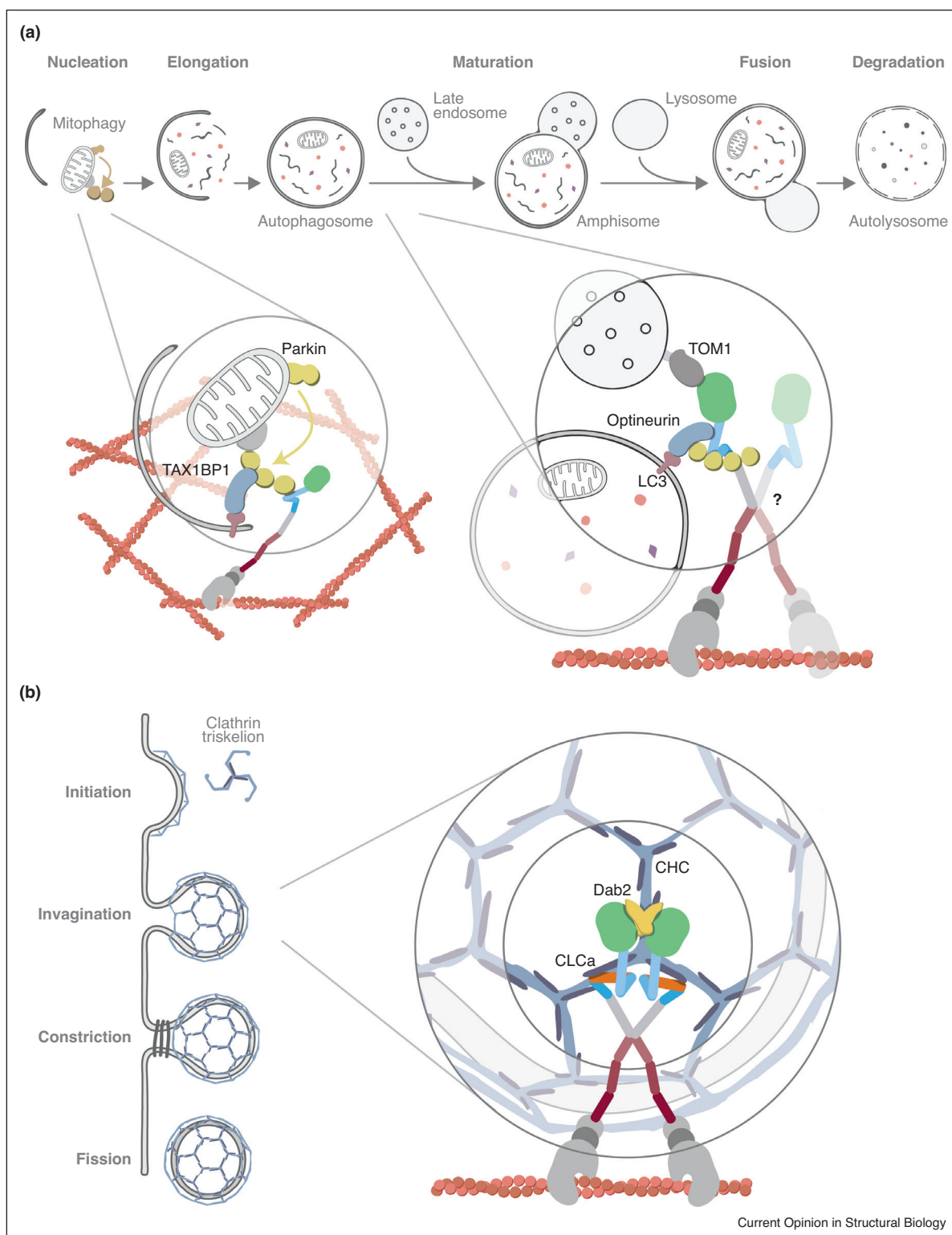
Myosin VI in autophagy and clathrin-mediated endocytosis. **(a)** Proposed models depicting the roles of myosin VI during mitophagy and autophagosome maturation. Left, myosin VI is recruited independently from the autophagic receptors (TAX1BP1 is shown as an example) by Parkin-mediated ubiquitination of the mitochondrial membrane proteins [31•]. Right, at later stages, ubiquitination of the autophagy receptors promotes the fusion of the autophagosomes with the endosome through the bridging ability of the ubiquitin receptor myosin VI [17] that simultaneously binds Tom1 and an autophagy receptor (optineurin is shown as an example). Coincident detection of the autophagy receptor

TAX1BP1 [17,26], gel filtration data show that K63-linked or M1-linked ubiquitin chains can induce the formation of a quaternary complex with myosin VI, the autophagy receptors and Tom1, even if the precise configuration of the multiple interactions remains undefined [16••]. The origin of the ubiquitin chains is unknown and it is tempting to speculate that ubiquitination of the autophagic receptors may provide the missing link. The coincident detection of the autophagy receptor and ubiquitin attached to it would allow the existence of a stable complex which, together with Tom1, is required at the later steps of the autophagosome maturation (Figure 2a). Up to now, ubiquitination has been demonstrated for optineurin [9,28] and was shown to increase the affinity of this autophagic receptor for myosin VI binding [9]. Moreover, coincident detection of optineurin and ubiquitin has been shown to contribute to cell migration [9,13,29], leading to the idea of precise temporal and spatial regulation of myosin VI recruitment by optineurin ubiquitination at the leading edge of migrating cells, a model that deserves experimental validation [13].

Whether a similar ubiquitin-modulated type of interaction may exist also for the WWY binders remains to be established. While Tom1 has been characterized as a myosin VI partner in autophagy [16••,21], its function in endosomal sorting is still unknown. It is tempting to speculate that, like its associated components of the ESCRT-0 complex Hrs and STAM1/2 [30], Tom1 may undergo ubiquitination at the endosome, leading to recruitment of the ubiquitin receptor to myosin VI.

Finally, Kruppa *et al.* recently demonstrated that myosin VI is recruited to damaged mitochondria where it promotes assembly of actin cages to isolate and prevent refusion of dysfunctional organelles [31•]. In this specific case, no cooperation was detected between the autophagy receptors and myosin VI, both of which are independently recruited to the outer mitochondrial membrane, when decorated with ubiquitin by the E3 ligase Parkin, through their ubiquitin-binding domains (Figure 2a) [31•].

Clathrin-mediated endocytosis: regulation in time and space
A further example of spatial and temporal regulation of myosin VI functions is represented by the induction of myosin VI$_{long}$ in epithelial tissues. During apico-basal polarization, epithelial cells switch the myosin VI isoform expression to include the AS region [24] that, together

**Box 1 Monomeric versus dimeric/oligomeric myosin VI: what is the role of the cargoes?**

Monomer to dimer conversion is considered to be required for the activation of the motor ability of myosin VI, but how dimerization occurs is still a matter of debate. The first half of the SAH domain appears to promote internal dimerization [43,44], but this capacity is weak at low local concentrations [45], and binding with cargoes is thought to promote/stabilize the dimeric status. By using dual interaction surfaces, two Dab2 monomers may mediate myosin VI dimerization by extending across the dimer to interact with distinct sites on each monomer [22]. The same effect could be induced by cargo homodimerization, thereby bringing together two myosin VI monomers, as in the case of optineurin and NDP52 [46,47]. GIPC, instead, can form oligomers that recruit several myosin VI molecules oriented in different directions [15,48]. Other myosin VI binding partners, like Tom1, seem to lack the features required to induce myosin VI dimerization [16••], which is expected if myosin VI functions as a tether between organelles (Figure 2a). Whether both of these functional states of myosin VI occur *in vivo* remains unknown and requires further investigation.

with the MyUb domain, specifically interacts with clathrin [13]. The isoform switch reflects a specific need of the cell; when epithelial cells become polarized, a dense actin network creates an obstacle for clathrin-mediated endocytosis (CME) from the apical surface. The molecular mechanism behind this specialized function has been recently dissected with the identification of the myosin VI direct interactor CLCa [23••]. The coordinated action of myosin VI$_{long}$ and CLCa provides the force necessary for the invagination and fission of the clathrin-coated pits beneath the apical surface of the cell [23••] (Figure 2b). The sequential recruitment of Huntingtin-interacting protein 1-related protein (Hip1R) and myosin VI plays an essential role in directing actin-mediated budding. This temporal regulation is coordinated by CLCa, which binds the two proteins in a mutually exclusive way [23••]. CLCa-mediated recruitment of myosin VI at the clathrin-coated pits displaces Hip1R, which is free to regulate actin polymerization at the invaginating neck while myosin VI, through interaction with Dab-2 [22], undergoes dimerization to become the processive motor required for vesicle fission [23••] (see Box 1).

Interestingly, myosin VI recruitment appears to be sensitive to the saddle-shaped curvature of the membranes [32••], which is typically present at the base of apical microvilli and at the endocytic sites where myosin VI$_{long}$ localizes. This shape-driven recruitment of myosin VI appears to be induced by lipid bilayer engagement, and to remodel membrane geometry [32••], possibly adding

(Figure 2 Legend Continued) through the RRL motif and the possible ubiquitin attached to it by the MyUb domain is shown. The monomeric or dimeric status of myosin VI in this context remains unknown (reported as a question mark). **(b)** Model of the multiple interactions occurring during CME. Myosin VI is recruited to clathrin-coated pits through interaction with CLCa [23••]. Myosin VI dimerization may then be stabilized by the binding of two Dab2 molecules [22], allowing processive movement on the actin filaments.

Figure 3
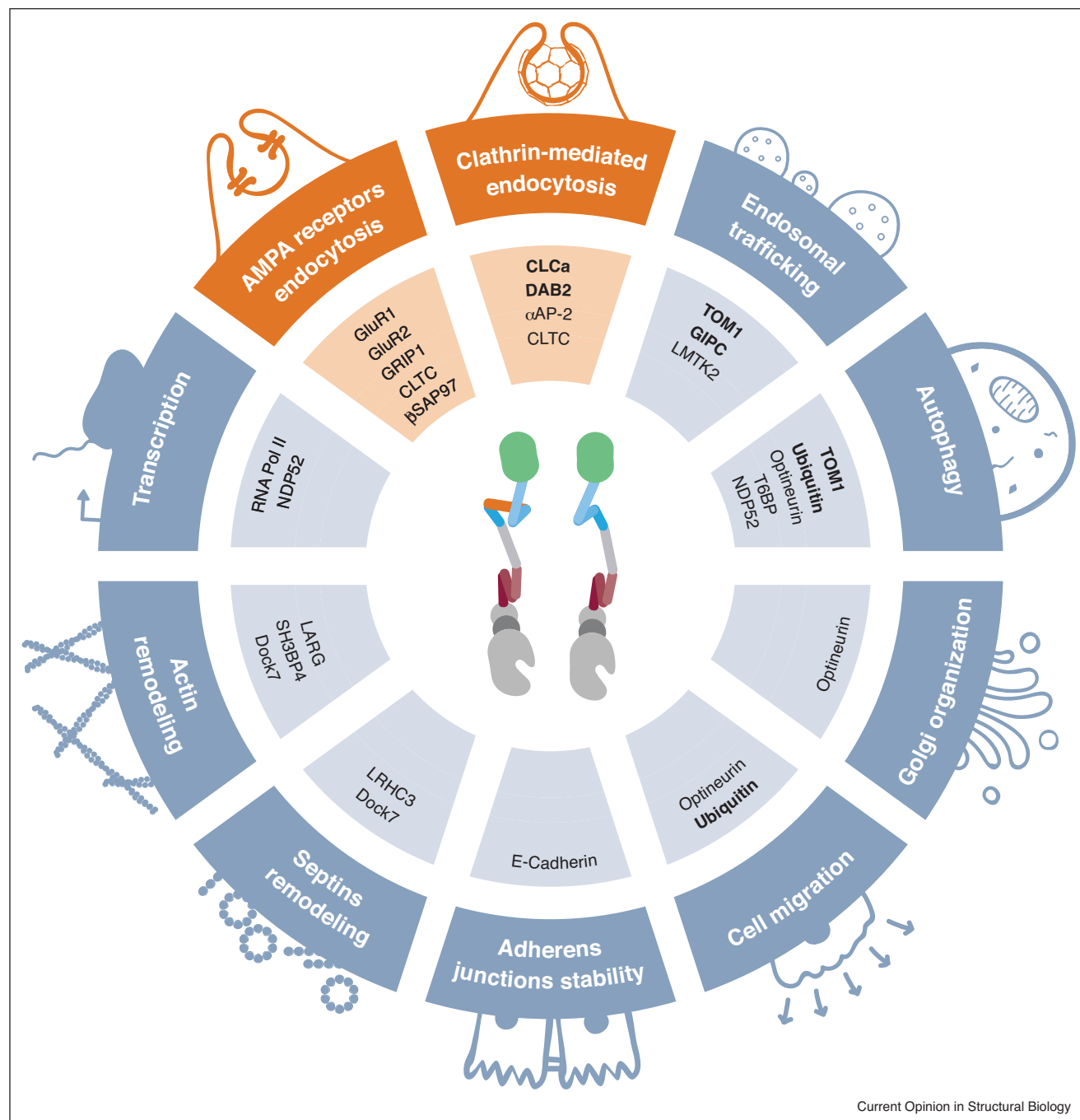
Map of the myosin VI physiological roles. A conceptual drawing of the different functions of myosin VI is displayed with the main interactors indicated; those with a solved structure in complex with myosin VI are marked in bold. The selective functions of myosin VI$_{long}$ are in orange.

another contribution of myosin VI to the detachment of the vesicles during CME.

In addition, myosin VI$_{long}$ appears to be needed to maintain synaptic plasticity [33•]. In neurons, it is well-known that the constitutive internalization of the AMPA receptor is clathrin-independent, while activity-induced endocytosis requires CME [34,35]. A recent study showed that the interaction of the AMPA receptor with the clathrin-mediated adaptors GRIP1 and βSAP97 drives the specific recruitment of myosin VI$_{long}$ at the Purkinje cell spine site where it mediates removal of surface AMPA receptors during long-term depression [33•].

Conclusions

Myosin VI is characterized by a powerful structural plasticity that has evolved in order to ensure that the appropriate myosin VI isoform is activated at the required site and time (Figure 3). Many of the currently proposed models of regulation are based on the structural definition of the binding between myosin VI and its interactors, but an emerging theme of study is the biophysical characterization of the myosin VI mechanosensory capacity. Indeed, myosin VI has an intrinsic force sensitivity and is able to respond to increasing loads [36,37]. Under low loads, myosin VI functions as a processive transporter, but it is converted to an anchor when a sufficient load is applied [38]. This conversion appears to be essential to preserve the structural integrity of the stereocilia [39] and the adherens junctions under tensile stress conditions [40••]. In this latter context, myosin VI interacts with E-cadherin, coupling it to the actin cytoskeleton and providing a feedback mechanism that, upon increased mechanical tension, leads to an increased strength of the junctions via activation of the GTPase RhoA [40••,41]. By regulating junction integrity, myosin VI could have a strong impact on tissue remodeling during physiological and pathological processes of collective cell migration, such as border cell migration in *Drosophila* [42] and cancer metastasis. Whether this role can be exerted by both myosin VI isoforms remains to be established.

Conflict of interest statement

Nothing declared.

Acknowledgements

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- •• of outstanding interest

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