Walking to work: roles for class V myosins as cargo transporters

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Abstract | Cells use molecular motors, such as myosins, to move, position and segregate their organelles. Class V myosins possess biochemical and structural properties that should make them ideal actin-based cargo transporters. Indeed, studies show that class V myosins function as cargo transporters in yeast, moving a range of organelles, such as the vacuole, peroxisomes and secretory vesicles. There is also increasing evidence in vertebrate cells that class V myosins not only tether organelles to actin but also can serve as short-range, point-to-point organelle transporters, usually following long-range, microtubule-dependent organelle transport.

Power stroke
The distance that the lever arm of myosin moves in a single event.

Brownian diffusion
The random, thermally driven motion of small objects in a fluid or gas.

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All cells, in their efforts to move and position their organelles, use molecular motors. Three classes of molecular motors — dyneins, kinesins and myosins — have evolved to transport organelles and other cargoes inside cells. The myosin superfamily members, which are separated into at least 35 classes[1] and which move on actin filaments, participate in a plethora of functions in cells, including cytokinesis, cell adhesion, endocytosis, exocytosis, movement of mRNA, movement of pigment granules, and cell motility. This diversity of function requires the various myosins to have different kinetic properties and structural adaptations. For example, some myosins are adapted to transport cargo, whereas others may function as load-bearing tension sensors.

In general, myosins are composed of three domains[2] (FIG. 1). The first is the motor domain or head, which is usually located at the amino terminus and which binds ATP and actin. The motor domain is mechanically linked to a second domain consisting of an extended α-helical 'neck' containing a variable number of IQ motifs, which bind light chains of calmodulin or members of the calmodulin family. The neck domain is sometimes referred to as the lever arm because it moves as a rigid body to generate the power stroke in response to small ATP-dependent changes in the conformation of the motor domain[3–6]. The tail domain contains the most class-specific variations and can include coiled-coil motifs for dimerization, as well as domains that accommodate the different functions of distinct myosin classes, including binding to membranes or to cargo receptors mediating specific interactions with vesicular or non-vesicular cargo[7].

Class V myosins have been implicated in both organelle transport and dynamic organelle tethering in several cellular systems. These myosins are found in almost all eukaryotic genomes sequenced to date[1]. Humans have 38 myosin genes, including three class V myosin genes (encoding myosin Va, myosin Vb and myosin Vc)[8], whereas the yeast *Saccharomyces cerevisiae* has only five myosin genes, two of which encode class V myosins (*MYO2* and *MYO4*)[8]. The class XI myosins found in plants are almost certainly derived from an ancient class V myosin-like molecule[1], implying that organisms which predate the division of plants and animals possessed this type of myosin.

In this Review, we describe the design features of class V myosins that should allow them to serve as cargo transporters *in vivo*. We then examine the evidence that these myosins actually transport organelles inside cells and define what we believe is the 'acid test' to prove unequivocally that any particular class V myosin might indeed drive organelle translocation *in vivo*.

Myosin Va properties and adaptations

The transportation of cargo within cells is a demanding process. Actin filament tracks exist as single filaments, branched networks or bundles of different geometries. In many cases, actin filaments are in close proximity with the two other cytoskeletal filaments, microtubules and intermediate filaments, forming meshes with small pore sizes. In the turbulent world of the cytoplasm, where the motor is constantly bombarded by water molecules and macromolecules, instantaneous detachment of the myosin from actin would be associated with rapid Brownian diffusion away from the track and an end to directed movement.

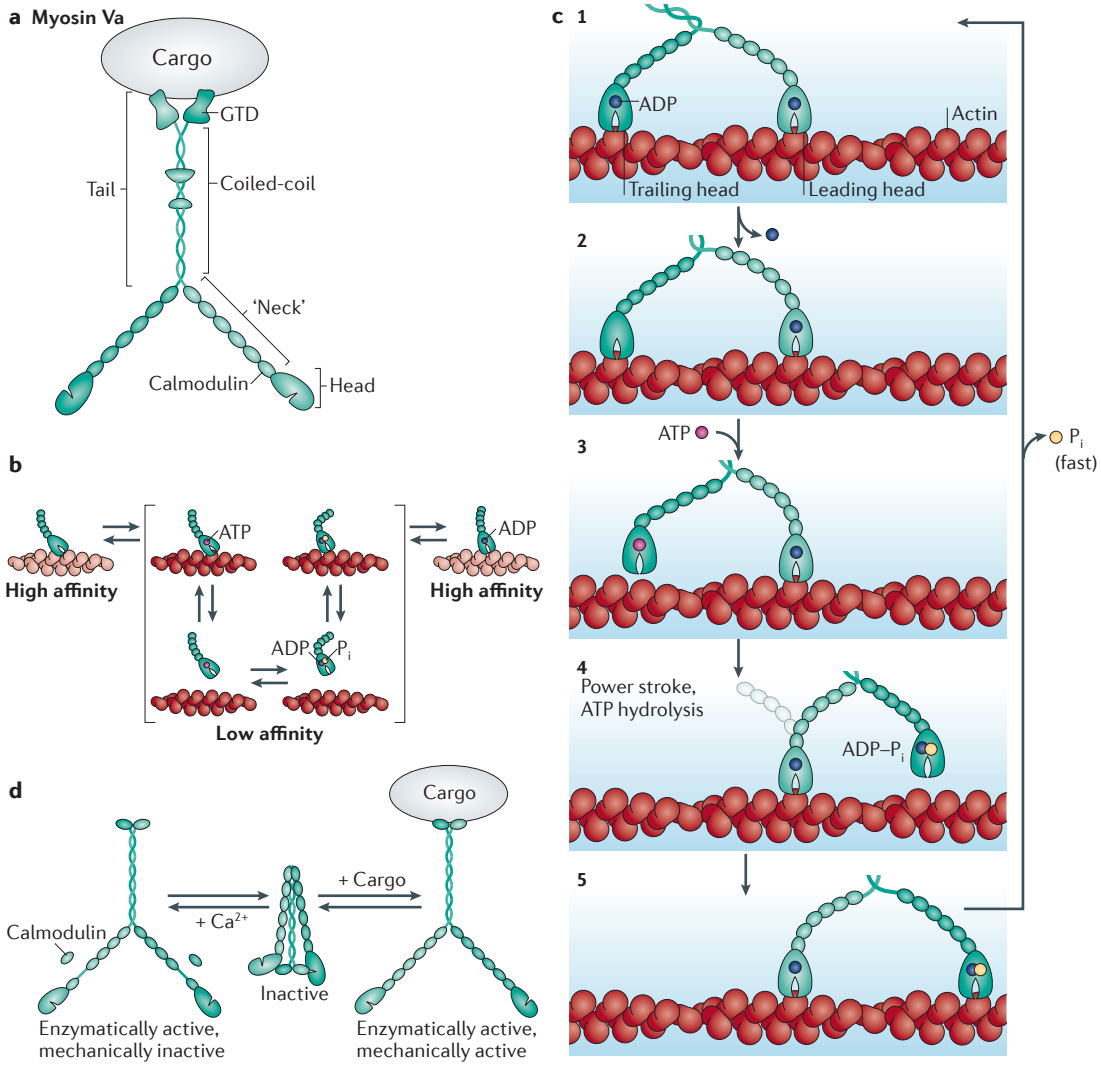


Figure 1 | **Properties of myosin Va. a** | Domain structure of myosin Va. The head domain, often called the motor domain, contains the nucleotide-binding and actin-binding sites. The 'neck' domain consists of an α-helical segment of the heavy chain containing six tandem IQ motifs, which each bind a calmodulin molecule. The tail domain consists of a coiled-coil-forming domain with periodic interruptions, which dimerizes the heavy chains, and two globular tail domains (GTDs), which bind cargo. **b** | Kinetic cycle for myosin Va. The cycle alternates between states with a high affinity for actin (myosin Va with no nucleotide bound and myosin Va with ADP bound) and states with a low affinity for actin (myosin Va bound to ATP or to ADP and inorganic phosphate (P_i)). ADP release from actomyosin–ADP is rate limiting[31]. **c** | Pathway of the processive movement of myosin Va along actin. Myosin Va probably dwells in a state with ADP bound to both heads (step 1)[36] and the two heads exert intramolecular strain[32–36] on each other, so that ADP is first released from the trailing head (step 2). This head subsequently binds ATP and rapidly dissociates from actin (step 3). The attached head undergoes a power stroke, positioning the new leading head (bound to ADP and P_i) to find a forward binding site via a thermally driven search[15] (step 4). Upon binding to actin (step 5), the leading head rapidly releases P_i and establishes a strong binding conformation[31], which brings myosin Va to the same state as in step 1 but translated forward by 36 nm. **d** | Regulation of myosin Va. In the absence of Ca^{2+} and cargo, myosin Va adopts a bent conformation that is enzymatically and mechanically inactive (middle), in which the motor domains interact with the cargo binding domains[37–39,138,139]. In the presence of Ca^{2+} (left), myosin Va unfolds into a conformation that has high ATPase activity but cannot move on actin because the neck is mechanically weakened by dissociation of calmodulins[41]. On cargo binding (right), myosin Va becomes extended and is both mechanically and enzymatically active. This active state has not been observed by electron microscopy but has been inferred from enzymatic studies and *in vitro* motility studies[44,45].

Vertebrate myosin Va was the first myosin shown *in vitro* to be capable of moving along actin filaments as a single molecule without detaching. This behaviour, which is known as processive movement, is likely to be a requirement in many cases for efficient cargo transport *in vivo*[9,10]. As it has been extremely well characterized *in vitro*, we describe here the array of evolutionary adaptations in both its structure and kinetics that should make myosin Va an efficient cargo transporter *in vivo*.

Structural adaptations allow processive movement. Myosin Va has coiled-coil forming sequences in its tail domain, which dimerize to form a two-headed motor[11], allowing the molecule to 'walk' along actin filaments by alternating the positions of the leading and trailing heads[12,13] (FIG. 1). The two heads must be able to simultaneously bind to two actin monomers within a filament, each of which has a helical pitch with crossover points every 36 nm[14]. If the two heads of the motor can span this distance, then the myosin can effectively transport its cargo linearly along an actin filament, rather than following the actin's helical pitch. Following the pitch would result in the myosin spiralling around the filament, potentially causing steric clashes between the cargo and the cell membrane (FIG. 1c). Myosin Va accomplishes this feat by having an elongated neck region containing six IQ motifs, each of which binds a calmodulin or a calmodulin family member (FIG. 1a). The 36 nm step size has been measured *in vitro* in single-molecule experiments involving optical trapping[9,15,16] and in super-resolution light microscopic observations of moving myosin Va molecules using total internal reflection fluorescence (TIRF) microscopy[17,18]. In addition, electron microscopic observation of myosin Va molecules associated with actin in the presence of ATP show that the two heads are bound to actin monomers spaced 36 nm apart[14]. Moreover, these features were recently confirmed using a novel, high-speed atomic force microscope (AFM) to image myosin Va moving along an actin filament[19].

The tail domain of myosin Va is significantly more elastic than that of the conventional skeletal muscle myosin (myosin II), probably owing to the multiple interruptions of its coiled-coil domain by loops of various sizes[20]. This property is interesting, as modelling predicts that an elastic tail would allow the motor to continue moving forward taking regularly spaced steps, albeit more slowly, while the cargo is being pulled through the viscous cytoplasm[21].

Myosins must have a means of binding their cargo; in the case of myosin Va, this is mediated by a pair of globular tail domains (GTDs) located at the carboxyl terminus, following the coiled-coil region. However, other parts of the molecule, such as flexible loops within the coiled-coil domain, also have roles in cargo binding[22]. Often, cargo binding is mediated by the interaction of the GTD with an adaptor protein, which in turn is bound to an organelle-specific RAB-family GTPase, thereby linking the myosin to the membranous cargo[22–26]. Furthermore, in cases when a given class V myosin moves multiple cargoes, there has to be means to select the correct cargo. In the case of myosin Va, different alternatively spliced isoforms found in melanocytes and brain cells bind different cargo[22,27,28].

Finally, myosin Va not only must be capable of navigating the complex array of actin filaments within cells but also must cooperate with microtubule motors, as most cargoes, at least in animal cells, are transported on both microtubules and actin filaments. Efficient switching between actin filament tracks has been shown *in vitro*[29,30]. This was found to be mediated by the long neck of myosin Va, which allows it to move from one actin filament to another when the filaments cross or when the actin filament contains a side branch generated by the actin nucleator actin-related protein 2/3 (Arp2/3)[30]. Furthermore, *in vitro* experiments, in which beads coated with both dynein and myosin V were applied to surfaces containing meshes of actin filaments and microtubules, indicated that track switching can occur efficiently[29].

Kinetic adaptations allow processive movement. The kinetic cycle of ATP hydrolysis by myosin Va, like that of most myosins, alternates between states with a high affinity for actin (myosin Va alone and myosin Va–ADP) and states with a low affinity (myosin Va–ATP and myosin Va–ADP–inorganic phosphate (P_i)) (FIG. 1b). In this cycle, the rate-limiting step is the dissociation of ADP from an actomyosin–ADP complex, whereas ATP hydrolysis and P_i release are fast[31]. This means that myosin Va spends most (~70%) of its kinetic cycle bound strongly to actin (that is, it has a high duty ratio, which is required for processive movement). Furthermore, the kinetic cycles of the two heads of myosin Va are strain-dependent, such that ADP release from the attached leading head is strongly suppressed as long as the trailing head is still attached to actin[32–36] (FIG. 1c). The leading head and its neck also cannot easily enter the post-power-stroke state as long as the trailing head remains attached[14]. These events ensure that the trailing head loses its ADP, binds a new ATP and detaches from actin, allowing the leading head to undergo its power stroke. This propels the former trailing head forwards to search for a new binding position on actin, whereas the former leading head remains attached.

Regulation of myosin Va. Given that myosin Va is capable of fast processive movement as a single molecule *in vitro*, it is imperative that its activity is tightly regulated in cells when it is not engaged in binding to cargo. Under ionic conditions that are similar to those found in most cells (that is, with a submicromolar concentration of free Ca^{2+}), myosin Va adopts a specific triangular, folded conformation, in which the GTDs bind to the motor (head) domains[37,38] (FIG. 1d). This folded conformation sediments at 14S in the analytical ultracentrifuge (and is thus frequently referred to as 14S), as opposed to 11S for the extended molecule[39]. The ATPase activity of the folded molecule is suppressed and the molecule does not bind strongly to actin[40,41].

Ca^{2+} ions shift the equilibrium to the extended, 11S conformation and activate the actin-activated ATPase activity of myosin Va *in vitro*. However, Ca^{2+} binding also results in the dissociation of one or more calmodulins from the neck region, resulting in a very flexible neck that cannot undergo mechanical work. Molecules under these conditions do not move on actin filaments unless very high concentrations (~10 μM) of calmodulin are present[39,42,43]. Thus, Ca^{2+} may not be the physiological activator of myosin Va. Instead, it has been shown that binding to melanophilin, a cargo receptor, activates myosin Va's ATPase activity, presumably by trapping the molecule in its extended, active conformation, and

Optical trapping
A technique that uses focused laser light in a light microscope to capture and manipulate objects with dielectric constants different from water. Sophisticated traps can be used to measure the mechanical properties of single molecules of motor proteins, such as the class V myosins.

Super-resolution light microscopy
Techniques that use Gaussian fits to the point spread function of light emitted from a single fluorophore to determine its position to within a few nanometres, which is significantly smaller than the diffraction limit.

Total internal reflection fluorescence
(TIRF). TIRF microscopy provides improved signal to noise ratios at the interface between media with differing refractive indices (such as coverslip–water), where fluorescence is excited by an evanescent field of light created when the incident light is totally internally reflected. It is ideal for visualizing the movement of single motor proteins.

High-speed atomic force microscope
(High-speed AFM). A modified AFM that can acquire images at a high rate from samples in aqueous solution, allowing the dynamic imaging of single molecules as they perform their tasks.

Myosin II
Also called conventional muscle myosin. The first myosin type to be discovered and the most conspicuous of the myosin superfamily. It is responsible for skeletal muscle contraction in muscle cells.

Duty ratio
The fractional time that a myosin spends in a state of high affinity for actin during an ATP hydrolysis cycle.

Melanosome
The pigment-producing organelle found in pigment-producing cells, such as melanocytes.

restores the myosin's ability to move along actin filaments *in vitro* in the absence of Ca^{2+} (REFS 44,45). Melanophilin, in turn, binds in a GTP-dependent manner to RAB27A, which is attached to the limiting membrane of the melanosome. This form of cargo-dependent control of myosin Va's mechanochemistry serves to avoid energy wastage, prevent cargo-free myosin Va molecules from piling up at actin filament plus ends and promote the recycling of the myosin by diffusion.

Properties of myosin V paralogues

The structural and kinetic properties of mammalian myosin Vb are similar to those of myosin Va, and, based on the high duty ratio of myosin Vb, it is predicted to be a processive motor at the single-molecule level[46]. By contrast, although mammalian myosin Vc and *Drosophila melanogaster* myosin V are similar in structure to myosin Va, kinetic studies demonstrate that ADP dissociation is not rate limiting for these myosins and that they have a lower duty ratio, which would result in non-processive interactions with actin at the single-molecule level[47–49]. However, as is seen with myosin Va, the kinetics of these motors may be affected by intramolecular strain[15,32–35], so there may be conditions in which they can move processively as single molecules. If not, it is possible that clusters of these myosins might transport cargo efficiently. Moreover, some class V myosins may be processive at the single-molecule level only under specific ionic conditions[50].

The situation is more complicated in *S. cerevisiae*. Although the structure of Myo2 is similar to that of mammalian class V myosin, it is only weakly processive as a single molecule[51], although this can be enhanced using artificial cargo (such as beads) that contain both Myo2 and Smy1, a kinesin family protein that interacts weakly and electrostatically with actin[52]. By contrast, Myo4 is different in structure from mammalian class V myosin[51]: the tail of one Myo4 molecule forms a heterodimeric coiled-coil with another yeast protein, Swi5-dependent HO expression 3 (She3), to form a single-headed motor[53]. The monomeric Myo4 motor does not move processively along actin *in vitro* as a single molecule, but if it is artificially dimerized or aggregated, or if more than one molecule is bound to a polymer bead, processive movements are possible[51,53].

Given that class V myosins in most organisms seem well-suited to supporting organelle transport, how does one go about proving that their favourite class V myosin does indeed serve as a point-to-point organelle transporter *in vivo*? BOX 1 and Supplementary information S1 (table) lists the types of evidence that can be used to support a claim of class V myosin-dependent organelle translocation, as well as the evidence currently available for various class V myosin–organelle pairs. The types of evidence include demonstrating colocalization between the myosin and the organelle using class V myosin-specific antibodies and green fluorescent protein (GFP)-tagged class V myosin chimaeras, as well as demonstrating defects in the movement and/or localization of the organelle when the function of the myosin is abrogated by gene deletion, RNA interference (RNAi)-mediated knockdown, or overexpression of a dominant-negative class V myosin construct. The acid test of class V myosin-dependent organelle transport is the rescue of mutant cells with mechanochemically compromised versions of the class V myosin in question (such as step-size mutants or enzymatically impaired mutants) and the demonstration of corresponding reductions in the velocity of its cognate organelle *in vivo*. In our opinion, this crucial test is the state-of-the-art approach to verifying motor protein function; however, it has so far been done in only a few instances. In the following sections, we describe these and other examples of apparent class V myosin-dependent organelle transport in more detail.

Cargo transport in yeast

The manner in which cells accomplish motor-dependent organelle movement and positioning is constrained by the organization of the two types of tracks that they can use: microtubules and filamentous actin (F-actin). The principal elements of this constraint involve the three-dimensional arrangement of these polymers inside the cell, and the fact that individual motors can move (at least, robustly) only in one direction on the polymer (for class V myosins, towards the barbed end of the actin filament). The budding yeast *S. cerevisiae* has solved this overarching design issue by building actin structures that are highly polarized (all barbed ends point in the same direction) and spatially arranged to drive organelle transport[54]. Specifically, this yeast supports most, if not all, organelle transport using actin cables that are generated by the nucleator formin, that run from the mother cell to the bud tip and that are oriented uniformly with their barbed ends pointing towards the bud tip. In this way, the yeast's two class V myosins (Myo2 and Myo4) can support, in an efficient way and in the complete absence of microtubule-dependent organelle transport, the host of membrane movements that drive bud growth and organelle inheritance[55,56] (BOX 1; FIGS 2,3; see Supplementary information S1 (table)). Below, we highlight some examples of cargo transport in yeast that support this general conclusion.

Myo2 and secretory vesicle transport. The transport of Golgi-derived, Sec4-positive secretory vesicles drives the vectorial growth of the bud. In what was the first unequivocal demonstration that a class V myosin actually moves an organelle *in vivo*, Schott and colleagues[57] showed that complementing the yeast Myo2-null mutant with versions of Myo2 possessing progressively shorter lever arms (which should result in the myosin taking progressively smaller steps) resulted in a progressive reduction in the velocity of secretory vesicle transport into the bud (from ~2.5 μm s^{-1} to ~0.2 μm s^{-1}). Somewhat surprisingly, the receptor for Myo2 on these vesicles has until recently evaded identification, despite being the only organelle cargo for this myosin that is required for viability. However, several recent papers[58–62] have provided important new insights into the organization and regulation of this receptor (FIG. 2). Overall, Myo2 is recruited to these vesicles through its sequential interaction with the functional RAB GTPase pair Ypt31–Ypt32

Box 1 | **Evidence for class V myosin-dependent organelle transport *in vivo***

To demonstrate that a class V myosin transports an organelle *in vivo*, one should first show colocalization of the endogenous class V myosin with its cognate organelle using specific antibodies, followed by colocalization using a green fluorescent protein (GFP)-tagged version of the myosin and markers for its cognate organelle in another colour to confirm their interaction and follow their dynamics. Second, one should identify abnormalities in the movement and/or distribution of the organelle in cells lacking the function of this class V myosin, which can be generated by gene knockout, RNA interference (RNAi) or the overexpression of a dominant-negative class V myosin construct. Such functional studies require extensive quantification and, in some cases, the application of stress to the mutant cell to demonstrate more clearly the requirement for the myosin. Third, one should complement the defect in mutant cells by reintroducing the myosin gene (or expressing an RNAi-immune version) to prove that the defect in organelle motility and/or distribution is due to the abrogation of the function of this myosin. Complementation also opens the door to defining what domains and/or properties of the myosin are required for organelle targeting and transport. Finally, the 'acid test' of class V myosin-dependent organelle motility is the rescue of mutant cells with mechanochemically compromised versions of the myosin in question (for example, step-size mutants or catalytic mutants) and the demonstration of corresponding reductions in the velocity of its cognate organelle *in vivo*. Other lines of evidence include the identification of the organelle receptor for the myosin, the identification of mechanisms that regulate the myosin–receptor interaction, and the reconstitution of the myosin-dependent organelle motility *in vitro*.

The table in this box is available in full Online (see Supplementary information S1 (table)). We discuss only those myosin–organelle and myosin–cargo pairs for which we think there is sufficient evidence to conclude that the myosin actually moves the organelle or non-vesicular cargo *in vivo*. Note that the wide variations in the speeds reported for yeast class V myosin Myo2-dependent organelle transport could be due, in part, to variations in the loads presented by the different organelles, together with variations in the numbers of Myo2 molecules recruited. Also note that the results of the acid test could be ambiguous for those class V myosin-dependent organelle movements that are extremely local or 'diffusive' in nature.

Organelle–class V myosin	Proposed function	Regulatory mechanisms for myosin–receptor complex identified
Secretory vesicles–yeast Myo2	Vectorial transport of the secretory vesicles that drives the growth of the bud	PtdIns4P levels, GEF for Sec4
Late Golgi–yeast Myo2	Transport of late-Golgi elements into the bud to facilitate inheritance of this compartment	No
Vacuole–yeast Myo2	Transport of portions of the vacuole into the bud to facilitate inheritance of this compartment	Cell cycle-regulated synthesis, degradation and phosphorylation of Vac17
Peroxisomes–yeast Myo2	Transport of peroxisomes into the bud to facilitate inheritance of this compartment	Cell cycle-regulated synthesis and degradation and phosphorylation of Inp2, degradation of Inp2 controlled by positional cues
mRNAs–yeast Myo4	Transport of mRNAs to the bud tip	No
Pigment granules–*D. melanogaster* myosin V	Transport of pigment granules within photoreceptor cells in response to light to create a functional pupil (sensory adaptation)	Elevated Ca^{2+}
Secretory vesicles–*D. melanogaster* myosin V	Transport of secretory vesicles that support the growth of the sensory membrane at the rhabdomere	No
Melanosome–mouse myosin Va	Capture and local movement of melanosomes in the cell periphery	GEF and GAP for RAB27A, cargo-dependent unfolding and activation of myosin Va
Melanosome–frog myosin V	Capture and local movement of melanosomes in the central cytoplasm	Phosphorylation of myosin V's GTD
CV membranes–*D. discoideum* MyoJ	Cortical capture of CV membranes and translocation of CV tubules following water discharge	No
Recycling endosome–mouse myosin Vb	Activity-dependent transport of recycling endosomes into the dendritic spines of hippocampal neurons to support functional and structural spine plasticity	Ca^{2+}-dependent unfolding and activation of myosin Vb, GEF and GAP for RAB11
SER–mouse myosin Va	Transport of SER tubules into the dendritic spines of cerebellar Purkinje neurons to support synaptic plasticity	No

CV, contractile vacuole; *D. discoideum*, *Dictyostelium discoideum*; *D. melanogaster*, *Drosophila melanogaster*; GAP, GTPase-activating protein; GEF, guanine nucleotide exchange factor; GTD, globular tail domain; Inp2, inheritance of peroxisomes 2; PtdIns4P, phosphatidylinositol-4-phosphate; SER, smooth endoplasmic reticulum; Vac17, vacuole-related 17.

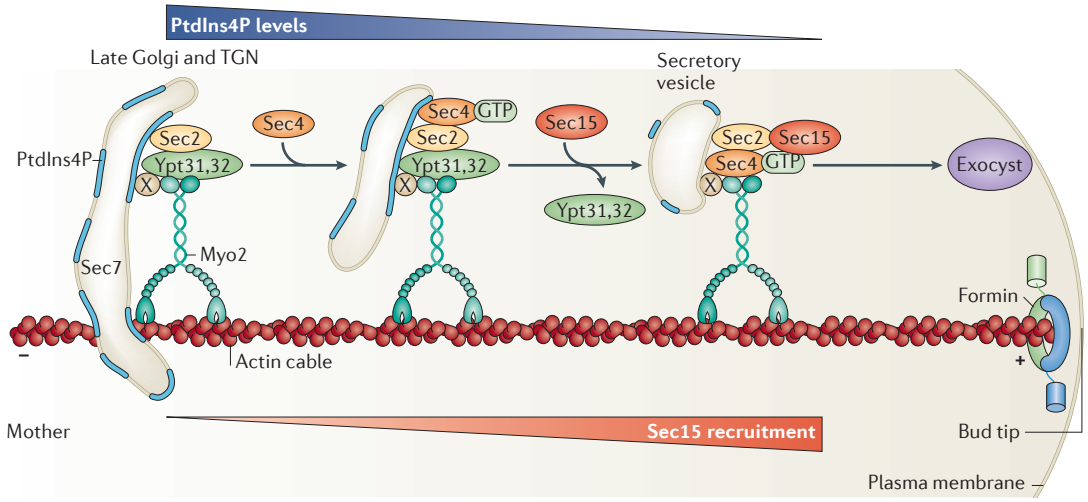


Figure 2 | **The recruitment of the yeast class V myosin Myo2 to secretory vesicles involves two RAB GTPases.** As in all eukaryotic cells, secretory vesicles in yeast form by budding at the *trans* face of the Golgi apparatus (the *trans*-Golgi network (TGN)). Budding and subsequent Myo2-dependent vesicle transport involves the sequential interaction of Myo2 with the functional RAB GTPase pair Ypt31–Ypt32 and Sec4, in a process that is coordinated by the Golgi membrane-enriched lipid phosphatidylinositol-4-phosphate (PtdIns4P)[54,58–60,140,141]. Myo2 is initially recruited to forming secretory vesicles by its combined interactions with Ypt31–Ypt32·GTP and PtdIns4P (the latter via an unidentified bridging protein, X). Sec2, the guanine nucleotide exchange factor (GEF) for Sec4, is also recruited to the TGN through interactions with PtdIns4P and Ypt31–YPT32·GTP. This creates a RAB GEF cascade in which the recruitment of Sec2 serves to activate Sec4, which in turn is required for the terminal transport of vesicles to sites of bud growth. The late-acting Sec4 effector protein Sec15, which is required for the final steps in vesicle docking and fusion at the exocyst, also interacts with Sec2, and its interaction competes with that of Ypt31–Ypt32. Importantly, the binding of PtdIns4P to Sec2 inhibits the Sec2–Sec15 interaction, thereby favouring the recruitment of Sec2 by Ypt31–Ypt32 as secretory vesicles initially bud from the TGN. Later, as secretory vesicles mature and approach the site of secretion, PtdIns4P levels in the vesicle membrane decline. This change in membrane lipid composition allows Sec15 to competitively replace Ypt31–Ypt32 on Sec2. This exchange, together with the presence within Myo2's globular tail domain (GTD) of a binding site for Sec4–GTP that is distinct from its binding site for Ypt31–Ypt32, results over time in the replacement of Ypt31–Ypt32 by Sec4 as the Myo2 receptor. The increased vesicle levels of active Sec4 and Sec15 that result from this PtdIns4P-orchestrated handoff serve to drive the final stage of vectorial vesicle transport and to prepare the vesicles for exocyst-dependent tethering, docking and fusion at the bud tip.

and Sec4. Myo2 also interacts with the Golgi-enriched lipid phosphatidylinositol-4-phosphate (PtdIns4P), and the decrease in the content of this lipid as the vesicle matures regulates both the RAB switch and the recruitment of Sec15, a late-acting Sec4 effector protein that is important for the final steps in vesicle docking and fusion at the exocyst.

In addition to moving post-Golgi secretory vesicles, Myo2 supports the inheritance of the Golgi apparatus by transporting late-Golgi cisternae into the bud at a rate of ~0.22 μm s^{-1} (REF. 63). Recently, the RAB GTPase Ypt11, which was known from previous work to interact with Myo2's GTD, was also shown to bind Ret2, a subunit of the Golgi-associated coatomer complex that is involved in generating cytosolic coatomer complex I (COPI)-coated vesicles for retrograde transport from the Golgi to the endoplasmic reticulum (ER)[64]. These interactions should allow Ypt11 to link Myo2 to late-Golgi elements for their Myo2-dependent, vectorial transport into the bud.

Myo2 and vacuole inheritance. The highly polarized mechanism of cell division in *S. cerevisiae*, with its large mother cell and tiny emerging bud (as opposed to the two evenly sized daughter cells created by median fission in most animal cells), places a high demand on the machinery driving organelle partitioning and inheritance. Myo2 and Myo4 have important roles in the delivery of most, if not all, organelles into the bud. Importantly, although these myosins support organelle inheritance, as evidence by slower growth when the Myo2- or Myo4-dependent transport of a particular organelle or non-vesicular cargo is specifically abrogated, these roles are not absolutely required for viability.

Vacuole inheritance in *S. cerevisiae* is supported by the Myo2-dependent transport of portions of the vacuole into the emerging bud[65]. During transport, Myo2 accumulates at the leading edge of the segregation structure, a finger-like projection of vacuolar membranes destined for the daughter cell that Myo2 pulls into the bud along the actin cable at a speed of ~0.15 μm s^{-1}. The vacuole-specific receptor for Myo2 is composed of the vacuole-specific adaptor protein vacuole-related 17 (Vac17) and the myristoylated and palmitoylated vacuole membrane protein Vac8 (REFS 66,67) (FIG. 3). Vac17 bridges the indirect interaction between Myo2 and Vac8 by binding simultaneously and directly to the GTD of Myo2 and Vac8.

RAB switch
The sequential interaction of an effector protein with two different RAB GTPases.

Exocyst
An eight-member protein complex that is required for vesicle docking and polarized exocytosis and is conserved from yeast to mammals.

Myristoylation
A protein post-translational modification in which the 14-carbon saturated fatty acid myristic acid is covalently attached to an amino-terminal Gly residue.

Palmitoylation
A protein post-translational modification in which the 16-carbon saturated fatty acid palmitic acid is covalently attached to a Cys residue.

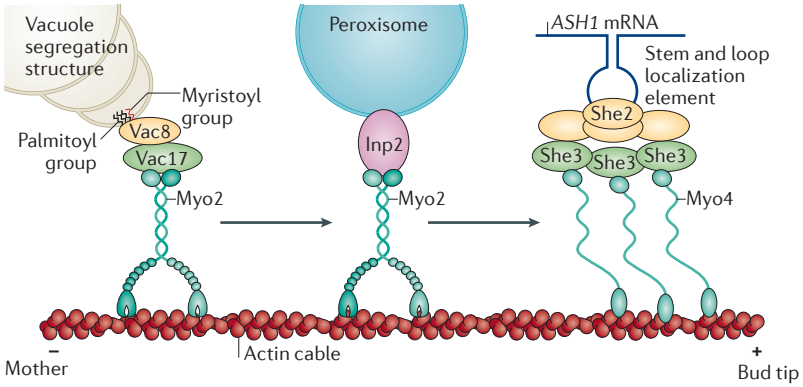


Figure 3 | **A plethora of receptors for the yeast class V myosins.** The two class V myosins in yeast, Myo2 and Myo4, are the real 'workhorses' for organelle movement, positioning and inheritance, in that they move at least six organelles (secretory vesicles, the late Golgi, the vacuole, the endoplasmic reticulum, peroxisomes and mitochondria), as well as mRNAs (for example, asymmetric synthesis of HO 1 (*ASH1*) mRNA)[55,56,77,102]. Binding of Myo2 and Myo4 to their diverse cargoes is mediated by cargo-specific receptors, one of which is shown in FIG. 2 and three of which are shown here. Multitasking by the yeast class V myosins is coordinated both temporally and spatially, primarily at the level of these receptors, through several mechanisms, including cell cycle-controlled receptor synthesis and proteolysis, and receptor phosphorylation. Myo2 binds multiple receptors through largely distinct regions on the surface of its globular tail domain (GTD). The determination of the structure of Myo2's GTD[142] (which can also be used to model the structure of the GTD in vertebrate class V myosins) has given valuable insights into the molecular details of class V myosin–cargo interactions. The vacuole-specific receptor for Myo2 comprises the vacuole-specific adaptor protein vacuole-related 17 (Vac17) and the myristoylated and palmitoylated vacuole membrane protein Vac8. During vacuole transport, Myo2 accumulates at the leading tip of the segregation structure, a finger-like projection of vacuolar membranes destined for the daughter cell, which Myo2 pulls into the bud along the actin cables[55]. Myo2 attaches to peroxisomes by a direct interaction with inheritance of peroxisomes 2 (Inp2), an integral peroxisomal membrane protein[56]. The cargo adaptor Swi5-dependent HO expression 2 (She2) not only links the Myo4–She3 complex to mRNAs but also serves to cluster Myo4–She3 complexes together in close proximity by virtue of its ability to self-associate. Although individual Myo4–She3 complexes are not processive, their She2-driven aggregation allows processive mRNA transport by increasing the probability, at any one moment during transport, of at least one complex being attached to an actin filament[52,53,77].

Vac17 also serves to coordinate vacuole inheritance with the cell cycle in two ways[68]. First, its synthesis and accumulation early in the cell cycle probably facilitates the initiation of vacuole movement. Second, it is phosphorylated by cyclin-dependent kinase 1 (Cdk1). This modification strengthens the interaction of Vac17 with Myo2 and promotes vacuole inheritance, thereby serving to coordinate vacuole movement with the cell cycle. Indeed, this may be a common cell cycle control mechanism in yeast, as the peroxisome protein inheritance of peroxisomes 2 (Inp2; see below) also contains Cdk1 phosphorylation sites.

Finally, Vac17 seems to control the ultimate position of the vacuole in the bud through its cell cycle-regulated degradation[67]. Specifically, when the vacuole reaches the bud, Vac17 is specifically degraded by cleavage at its PEST site, thereby releasing Myo2 from the vacuole. This serves to terminate further vacuole movement, thereby depositing the vacuole in the correct location in the bud, and to release Myo2, allowing it to carry out other functions.

PEST site
A sequence within proteins that serves as a site for cleavage by the Ca^{2+}-dependent protease calpain.

Myo2 and peroxisome inheritance. Myo2 supports the inheritance of peroxisomes by transporting them on actin cables into the bud at ~0.45 μm s^{-1} (REF. 69). Myo2 attaches to peroxisomes by a direct interaction with Inp2, an integral peroxisomal membrane protein[69]. Although Inp2 does not have homologues in other species, the peroxisome protein peroxisomal biogenesis 3 (Pex3), which is widely expressed in eukaryotes, plays a part in peroxisome inheritance in the fungus *Yarrowia lipolytica*[70]. This observation suggests that class V myosins may be involved in peroxisome inheritance in vertebrate cells as well.

In *S. cerevisiae*, the regulation of peroxisome inheritance seems to be focused largely on Inp2, which is subject to both cell cycle and positional cues[71]. First, like those of Vac17, Inp2 protein levels rise and fall during the cell cycle, being highest during the period when most peroxisomes are transported into the bud. This suggests that peroxisome inheritance is regulated, at least in part, by the availability of Inp2 for Myo2. Interestingly, the presence of Inp2 in the peroxisome membrane is only readily apparent on peroxisomes that have been transported into the bud. The underlying mechanism of this position-dependent asymmetry in receptor distribution is thought to be a negative feedback mechanism, in which peroxisomes that have been delivered to the bud relay a signal back to the mother cell that triggers the degradation of Inp2 in the mother cell[71,72].

Myo4 and mRNA transport. Yeast Myo4 is responsible for the transport of asymmetric synthesis of HO 1 (*ASH1*) mRNA[73,74], as well as a host of other mRNAs[75], to the bud tip. *In vivo* imaging of fluorescently tagged *ASH1* mRNA shows that Myo4 supports its continuous transport from mother to bud at a rate of ~0.3 μm s^{-1} (REF. 76). The fact that the single-headed Myo4–She3 molecule does not exhibit processive motility raises the question of how this motor generates processive movement of mRNA *in vivo*[51]. The answer to this conundrum is in the numbers[77]. Specifically, through the tetramerization of the molecule She2, which bridges the indirect interaction between Myo4 and the mRNA by simultaneously binding to a localization element in the mRNA and to She3 (REFS 78,79), multiple copies of Myo4–She3 are recruited to each localization element (FIG. 3). The inference, then, is that by recruiting multiple, non-processive Myo4 motors in such close proximity, the major hurdle to processive movement that is inherent in being a monomeric motor can be overcome to allow for the continuous movement of the mRNA that is observed *in vivo*. The fact that mouse myosin Va facilitates the localization of an mRNA-binding protein and its bound mRNA to the dendritic spines of hippocampal neurons[80], and that *D. melanogaster* myosin V contributes to the posterior accumulation of *Oskar* mRNA in embryos[81], suggests a more general connection between class V myosins and mRNA transport and/or localization.

Myo4 and ER inheritance. Myo4 seems to have a major role in ER inheritance into the bud. Indeed, Myo4-null cells exhibit a marked decrease in the delivery of cortical ER tubules (which normally move at a rate

of ~0.013 μm s^{-1}) into the bud[82]. However, the defect in ER inheritance in Myo4-null cells, although significant, is not complete. Indeed, a secondary, less efficient mechanism of ER inheritance seems to exist, in which ER tubules attach to the pre-bud site and are passively pulled into the growing bud[83].

Efficient ER inheritance also requires the Myo4-interacting protein She3, which is also involved in the Myo4-dependent movement of mRNA. A large fraction of She3 co-fractionates with ER membranes, suggesting that it may play a part in the attachment of Myo4 to the ER. Different domains of She3 are involved in ER inheritance and mRNA transport, and these two Myo4-dependent processes are independent of each other. Interestingly, there is evidence that messenger ribonucleoprotein (RNP) particles migrate with ER tubules into the emerging bud[84], and that this requires Myo4 and She3.

Cargo transport in *D. discoideum*

The contractile vacuole complex in *Dictyostelium discoideum* is a tubulovesicular osmoregulatory organelle that exhibits extensive motility along the actin-rich cortex. What is most striking is the conversion, following water discharge, of collapsed bladder membranes into radiating cortical tubules, which move along the cortex at a rate of ~0.5 μm s^{-1}. It has now been shown that the *D. discoideum* class V myosin MyoJ powers these movements[85]. Strong support for this conclusion was provided by the complementation of MyoJ-null cells with MyoJ containing a shorter neck, which resulted in a significant reduction in the speed of the radiating tubules. Moreover, as with class V myosins in vertebrate cells (see below), MyoJ was shown to cooperate with bidirectional, microtubule-dependent contractile vacuole membrane transport to properly distribute the contractile vacuole complex.

Cargo transport in *D. melanogaster*

When the fly eye is exposed to bright light, pigment granules located deep in the photoreceptor cell cytoplasm move rapidly to the cytoplasmic face of the photosensitive membrane organelle, the rhabdomere, thereby creating a functional pupil. Granule migration occurs along the rhabdomere terminal web (RTW), a polarized array of actin filaments that emanate from the base of the rhabdomere, extend deep into the photoreceptor cell cytoplasm and are oriented uniformly, with their barbed ends pointing towards the rhabdomere. The fact that myosin V localizes to these pigment granules, and that granule translocation is abrogated in a strong myosin V loss-of-function mutant, suggests that myosin V is responsible for moving the granules[86].

Pigment granule migration is triggered by the entry of Ca^{2+} into the cytoplasm downstream from light-activated Transient receptor potential (TRP) Ca^{2+} channels, and the increase in intracellular Ca^{2+} seems to directly activate myosin V to drive granule migration. Myosin V appears to function, therefore, as a sensory adaptation motor to drive the light-induced movement of pigment granules. Interestingly, a RAB-like protein known as Lightoid, which also associates with pigment granules and is required for granule movement, physically interacts with myosin V and is required for the association of myosin V with the granules, indicating that it serves as an essential component of the granule receptor for myosin V (REF. 86).

A second important part played by myosin V in photoreceptor cells involves the transport of post-Golgi secretory vesicles containing rhodopsin and other cargoes that fuel the rapid expansion of the sensory membrane[87]. Specifically, the movement of these vesicles out the RTW to the developing rhabdomere membrane requires myosin V, RAB11 and the RAB11 effector protein, RAB11-interacting protein (RIP11). Myosin V and RIP11 bind independently to RAB11 to create a ternary complex, which drives secretory vesicle movement. Given that *D. melanogaster* myosin V is not processive[48], pigment granules and secretory vesicles presumably recruit multiple myosin V molecules to allow processive movement of the organelles.

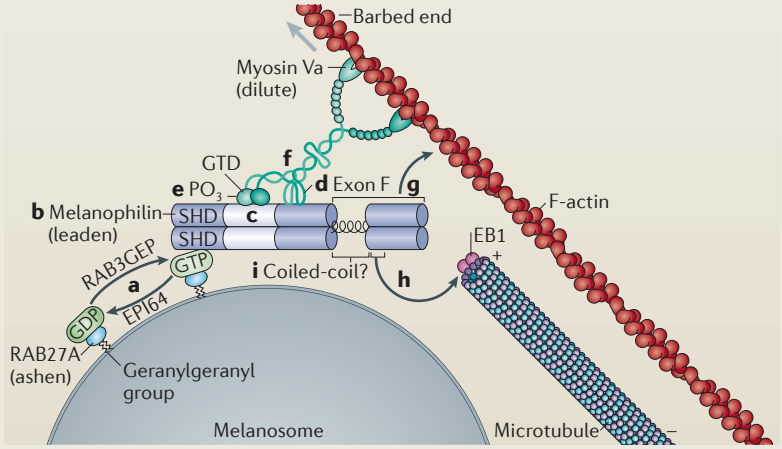
Evidence of cargo transport in vertebrates

Fly photoreceptor cells, with their RTW, and actin-centric organisms, like budding yeast and plants cells, use actin tracks of uniform polarity to support long-range, class V myosin-dependent organelle transport. By contrast, most cells, including vertebrate cells of all types (from minimally polarized-like liver hepatocytes to highly polarized-like neurons), seem to use microtubules and microtubule-based motors to drive the bulk of long-range organelle transport. Importantly, the organization of the microtubule cytoskeleton in these cells, in which most microtubule minus ends are anchored at the centrosome in the cell centre and most plus ends are found at the cell periphery, provides uniformly polarized, relatively straight, uninterrupted tracks that are ideal for driving organelle movements over much of the cell's three-dimensional space. Actin-based motors, such as the class V myosins, come into play at the end of the line to support track switching and the capture and/or local movement of organelles in the actin-rich periphery[88,89]. Below, we highlight three examples, one in melanocytes and two in neurons, in which a class V myosin cooperates with microtubule-dependent motors to drive point-to-point organelle transport in this second step.

Class V myosin and the transport of melanosomes. As with myosin V in fly photoreceptor cells, class V myosins have essential roles in the positioning of melanosomes within pigment-producing cells in mammals, fish and amphibians[90]. Myosin Va is recruited to the surface of melanosomes in mouse melanocytes by a receptor complex made up of the RAB GTPase RAB27A and one of its effector proteins, melanophilin[22,24,25,91] (BOX 2). Together, they form a tripartite complex, which connects the melanosome to the cortical actin cytoskeleton following the organelle's long-range, microtubule-dependent transport to the distal end of the melanocyte's dendrites. Numerous sites at which this receptor complex is regulated have been identified (BOX 2).

The study of mouse melanocytes lacking myosin Va (known as dilute melanocytes; FIG. 4a) led to the development of the cooperative-capture model of intracellular

Box 2 | **The melansome receptor for myosin Va**



The organization of the melanosome receptor for myosin Va[22,25,128]. RAB27A, when in its GTP-bound, active state and attached to the melanosome membrane via geranylgeranyl groups, binds its effector protein melanophilin, which then recruits myosin Va (see the figure). The labelled sites in the figure highlight the following: (**a**) the regulation of the nucleotide state of RAB27A by a RAB27A-specific guanine nucleotide exchange factor (RAB3-specific GDP–GTP exchange protein (RAB3GEP))[129] and GTPase-activating protein (EBP50-PDX interactor of 64 kDa (EPI64))[130]; (**b**) melanophilin's conserved helix–zinc-finger–helix SLP homology domain (SHD), which confers a specific interaction with RAB27A[131]; (**c**) melanophilin's low-affinity binding site for the globular tail domain (GTD) of myosin Va[132]; (**d**) exon F, an alternatively spliced, 27-residue exon that is present in the melanocyte-spliced isoform of myosin Va and binds melanophilin with high affinity[22]; (**e**) a conserved site within the GTD of frog class V myosin that is phosphorylated during mitosis by Ca^{2+}- and calmodulin-dependent kinase II (CaMKII), uncoupling the myosin from pigment granules[133]; (**f**) the ability of melanophilin to drive the unfolding of myosin Va from its closed, quiescent, 14S conformation to its extended, active 11S conformation[44,45]; (**g**) a binding site for filamentous actin (F-actin) within the carboxy-terminal domain of melanophilin which may increase the processivity of myosin Va when it is bound to melanophilin[134]; (**h**) two SKIP motifs that mediate melanophilin's interaction with the core microtubule plus end-tracking protein end-binding 1 (EB1), through which melanophilin associates transiently with the plus end of growing microtubules[135,136]; and (**i**) a short stretch of putative coiled-coil in melanophilin that is required for its function[137].

melanosome transport and distribution[92] (FIG. 4b). This model stressed a role for myosin Va in tethering melanosomes in the actin-rich periphery following their transport there by long-range, microtubule-dependent transport. This was based on evidence showing that myosin Va- dependent melanosome capture in the periphery seemed to be sufficient to drive melanosome positioning in cooperation with bidirectional, microtubule-based melanosome transport[92]. This was in contrast to the widely held and incorrect belief at the time that myosin Va drives the long-range transport of melanosomes. Moreover, crucial evidence for myosin Va- dependent melanosome movement was lacking. However, a class of melanosome movements (with a speed of ~0.15 μm s^{-1}) that were myosin Va- dependent (present in wild-type but not dilute melanocytes) and microtubule-independent, and that exhibited little directional persistence, were identified[92]. These movements could be due either to myosin Va moving melanosomes on actin or to myosin holding the organelles on actin and the actin moving (see also REF. 93).

Significantly stronger evidence that class V myosins move melanosomes has been obtained from studies of fish and frog pigment cells (known as melanophores; FIG. 4c). Specifically, the ~0.04 μm s^{-1} melanosome movements attributed to class V myosin within these cells can, over time, drive the long-range movement of the organelle, can often exhibit significant directional persistence and can show a significant decrease in run length when actin dynamics are suppressed by drugs that stabilize F-actin[94–97]. Moreover, high-resolution tracking of melanosome movements *in vivo* identified translocation events that occur in 35-nm-long steps, a size which corresponds to the step size of class V myosin[98]. Perhaps the 'nail in the coffin' would be to complement class V myosin-null melanocytes or melanophores with versions of the myosin that walk more slowly and show a corresponding decrease in the speed of melanosome movement *in vivo*.

Myosin Vb transports endosomes in hippocampal neurons. Previous studies have shown that vertebrate myosin Vb binds independently to RAB11A, a resident RAB GTPase in the recycling endosome, and to one of its effector proteins, RAB11 family-interacting protein 2 (FIP2), through its GTD[99,100]. Although the exact transport event supported by myosin Vb in this pathway remains unclear, its importance has been demonstrated for many cargoes by the overexpression of a dominant-negative myosin Vb tail construct, which markedly inhibits recycling[101–103]. Defining the precise role of myosin Vb should be facilitated by the recent demonstration that microvillus inclusion disease, a rare human disease characterized by the lack of apical microvilli on intestinal epithelial cells, is caused by loss-of-function mutations in myosin Vb[104].

Recycling endosomes also exist at the base of dendritic spines in hippocampal neurons, and these vesicles move into the spine when the neuron is stimulated strongly, that is, with a stimulus that can increase intraspinal Ca^{2+} levels and induce long-term potentiation (LTP). Importantly, exocytosis from these relocated recycling endosomes, which occurs locally within the spine and involves an unidentified transport carrier, appears to serve two critical functions[105]. First, it seems to serve as a major source of AMPA receptors for insertion into the postsynaptic membrane to create LTP. Second, it appears to provide the membrane lipids and other molecules needed to drive the activity-dependent growth in spine size and other structural remodelling that accompany LTP induction[105].

A recent study has now made a strong case for the idea that the stimulus-dependent translocation of recycling endosome membranes into spines is driven by myosin Vb[106] (FIG. 5a). Moreover, evidence was presented that the spike in intraspinal Ca^{2+} levels which occurs following the strong stimulation of spine NMDA (*N*-methyl-D-aspartate) receptors serves to activate myosin Vb by pushing it from its folded, quiescent 14S conformation to its extended, active 11S conformation. This Ca^{2+}-dependent unfolding of myosin Vb exposes the RAB11A- and FIP2-binding sites in its cargo-binding GTD, allowing the myosin to attach to recycling endosome membranes and transport them into the spine along actin tracks. Myosin Vb appears to act, therefore, as a Ca^{2+}

Recycling endosome
A generic, centrally located membrane compartment that receives endocytosed membrane receptors and recycles them back to the plasma membrane.

Long-term potentiation
(LTP). A form of synaptic plasticity that is thought to underlie memory formation, in which synapse use leads to long-term strengthening of the synapse.

AMPA receptors
The major excitatory ionotropic Glu receptors found in neurons. Their name comes from their ability to be activated by the artificial Glu analogue AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid).

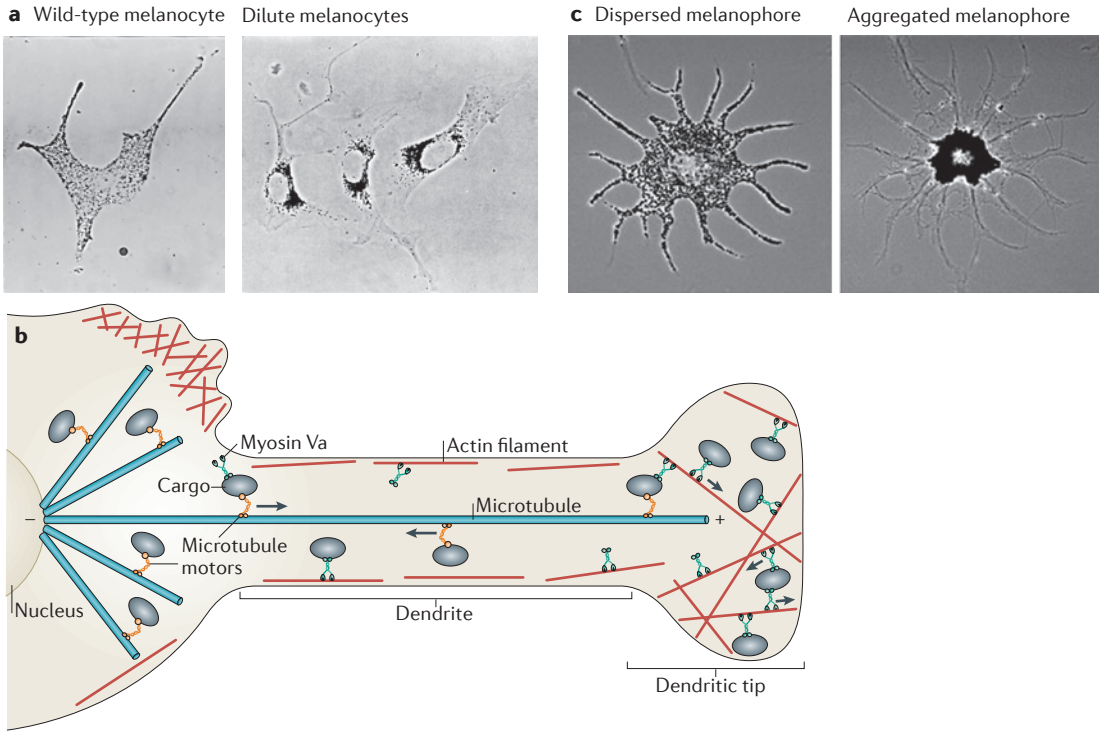


Figure 4 | **Myosin Va and its pervasive connection to melanosomes. a** | Melanosome distribution in melanocytes from wild-type and dilute mutant (myosin Va-null) mice. In the absence of the myosin Va-dependent capture of melanosomes in the periphery, melanosomes distribute according to microtubule density, leading to their accumulation in the cell centre. **b** | The cooperative-capture mechanism of melanosome transport and distribution in mouse melanocytes[92]. Long-range, bidirectional, microtubule-dependent transport carries melanosomes out along the cell's dendrites, where myosin Va functions to tether them (and possibly move them locally) in the actin cortex at the dendritic tip. **c** | Melanosome distribution in frog melanophores stimulated to disperse or aggregate the organelles. Frog class V myosin cooperates with the plus end-directed microtubule motor during dispersion to drive the even cytoplasmic distribution of melanosomes required to darken the animal[90,143]. Images in part **c** are reproduced, with permission from REF. 95 © (1998) Elsevier.

sensor to translate increases in spine Ca^{2+} into postsynaptic membrane transport. However, it is unclear how this Ca^{2+}-dependent activation step can avoid the dissociation of neck calmodulins and the concomitant decrease in mechanochemical integrity of the myosin that is seen *in vitro*[107].

Of note, a second study, which used RNAi experiments and the expression of a dominant-negative tail construct (which may not be isoform specific), argued that it is in fact myosin Va that mediates the movement of recycling endosomes into spines, although its attachment to recycling endosome membranes seems to be driven by a direct interaction with the C terminus of the AMPA receptor Glu receptor 1 (GLUR1) and with RAB11 (REF. 108). These findings are surprising, as previous studies have only identified a role for myosin Vb in recycling endosome dynamics, and hippocampal neurons from myosin Va-null mice exhibit normal postsynaptic GLUR distribution, excitatory synaptic transmission and both short-term and long-term potentiation[109,110].

Myosin Va transports the ER in Purkinje neurons. The extension of the ER into the dendritic spines of cerebellar Purkinje neurons, which is required for synaptic plasticity, does not occur when myosin Va is missing[111,112], indicating a requirement for myosin Va in this process. Indeed, a recent study[113] has now provided clear evidence that myosin Va acts as a point-to-point organelle transporter to pull ER tubules into these spines at a maximum velocity of ~0.45 μm s^{-1} (FIG. 5b). Myosin Va was shown to concentrate at the tip of the ER tubule as it moves into the spine, and rescue of dilute mutant Purkinje neurons with slow-walking versions of myosin Va results in corresponding decreases in the velocity of ER movement into spines. In keeping with the paradigm discussed above, this short-range, myosin Va- dependent movement of ER into spines occurs downstream of long-range, microtubule-dependent transport of ER out neuronal dendrites. The myosin Va- mediated transport of ER into spines was also shown to be required for the rise in spinal Ca^{2+} levels downstream of metabotrophic GLUR1 (mGLUR1) activation, which leads to long-term depression[113] (LTD), the major form of synaptic plasticity exhibited by Purkinje neurons (FIG. 5b). Interestingly, a role for class V myosins in ER transport may be evolutionarily ancient, given the role of yeast Myo4 in ER movement[82] and of class XI myosins, the plant versions of myosin V, in powering the rapid (60 μm s^{-1}) transport of ER networks that drives cytoplasmic streaming via viscous drag[114,115].

Long-term depression (LTD). A form of synaptic plasticity that is thought to underlie memory formation, in which synapse use leads to long-term weakening of the synapse.

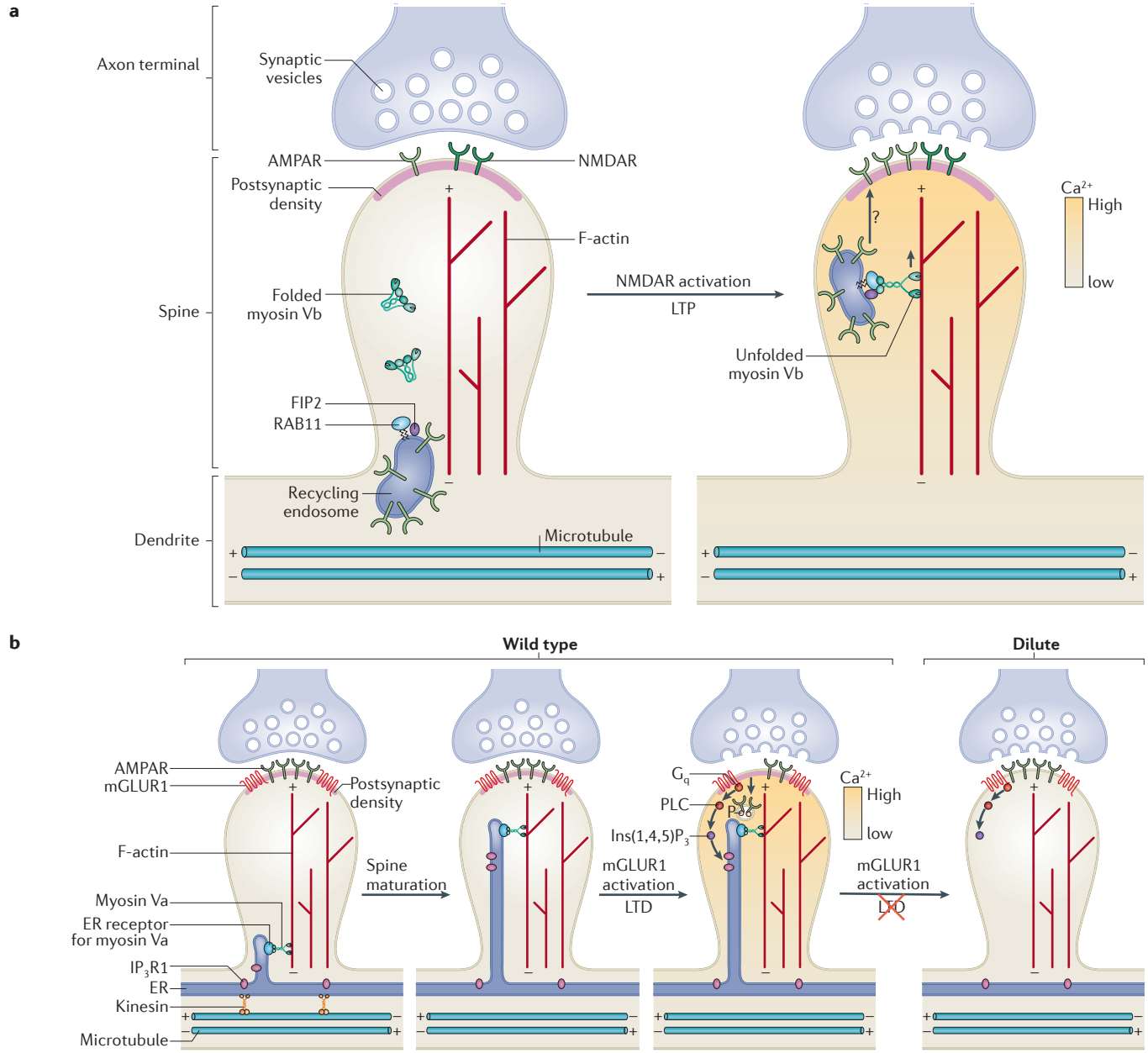


Figure 5 | **Type V myosins transport membrane compartments into dendritic spines to support synaptic plasticity.** **a** | Myosin Vb transports recycling endosomes into the dendritic spines of hippocampal neurons following stimulation[106]. These recycling endosomes serve as a major source of AMPA receptors (AMPARs) for insertion into the postsynaptic membrane to support long-term potentiation (LTP). Importantly, the rise in spinal Ca^{2+} levels downstream of strong NMDA (*N*-methyl-D-aspartate) receptor (NMDAR) activation is argued to open up the myosin, allowing it to bind RAB11 and RAB11 family-interacting protein 2 (FIP2) and thereby attach to recycling endosomes. **b** | Myosin Va translocates endoplasmic reticulum (ER) tubules into the dendritic spines of cerebellar Purkinje neurons[113]. In this system, the myosin appears to be unfolded and activated by interaction with its membrane cargo rather than by raised levels of cytosolic Ca^{2+}. Note that the receptor in the ER membrane for myosin Va has not been identified. Importantly, the spike in spinal Ca^{2+} levels downstream of strong signals that activate metabotrophic Glu receptor 1 (mGLUR1) requires the myosin Va-dependent transport of ER into the spine, as this Ca^{2+} comes from that stored in spine ER. The inositol-1,4,5-trisphosphate ($Ins(1,4,5)P_3$) generated downstream of mGLUR1 activation through the G protein G_q and phospholipase C (PLC) normally binds to the $Ins(1,4,5)P_3$ receptor IP_3R1 in the spine ER membrane, causing Ca^{2+} to flow out of the ER and into the spine head. In the absence of myosin Va and spine ER (as in the dilute mutant), the spike in spinal Ca^{2+} levels, and the subsequent protein kinase C (PKC)-driven phosphorylation of AMPARs that stimulates their endocytosis, resulting in a reduction in their levels at the postsynaptic membrane (the basis for LTD), does not occur[112,113]. For both of the class V myosin-dependent membrane transport steps depicted in this figure to occur with reasonable efficiency, the organization of filamentous actin (F-actin) in spines must be such that it can support outward movement (that is, with the majority of filaments being oriented with their barbed ends pointing towards the postsynaptic density)[144–146]. Please see REF. 107 for an in-depth discussion of the Ca^{2+}-dependent regulation of class V myosins *in vitro* and *in vivo*.

Anisotropic
A term referring to a complete lack of uniformity in orientation.

Filopodia
Thin, dynamic, cellular extensions containing actin filaments aligned in parallel with their barbed ends pointing towards the tip. They are often found in growth cones and at the leading edge of migrating cells.

Challenges in vertebrate cells. The key to obtaining clear examples of class V myosin-dependent organelle movement in animal cells, and presumably the reason why such movements are more often than not very hard to discern, almost certainly revolves around the organization of F-actin in the cortex. In those cases in which class V myosin-dependent organelle transport has been identified in a definitive way, such as the movements of ER and recycling endosomes into dendritic spines, the actin tracks must be sufficiently aligned and polarized over the relatively short distances travelled to support the efficient, directed transport that is seen. Typically, however, the organization of F-actin in the periphery of cells is highly anisotropic, although as it approaches the membrane the actin should become increasingly oriented with its barbed end facing 'out', owing to the nature of the machinery that drives actin assembly at the plasma membrane[116] (note that filopodia, the only place in animal cells where highly polarized, barbed end-out actin filaments exist, rarely contain organelles). Anisotropic actin organization does not allow organelles to move persistently in any one direction, making it difficult to demonstrate class V myosin-dependent organelle movement. Examples of this situation may include the class V myosin-dependent capture of melanosomes[92] and secretory vesicles[117,118] in the cortex.

However, class V myosins can readily navigate the Arp2/3-generated actin side branches that probably permeate much of the cortex, and they can also hop from one actin filament onto a crossing filament in anisotropic actin networks[30]. Moreover, such *in vitro* behaviours have now been witnessed in living cells using quantum dot-labelled myosin Va molecules[119,120]. Although the paths of individual myosin Va molecules look random in most cases, and their mean-squared displacements on timescales longer than 1 second resemble random diffusion, on short timescales (<0.2 seconds), clear evidence of directed transport is seen. These movements, which occur with the characteristic step size (36 nm) and speed (~0.5 μm s^{-1}) of myosin Va, almost certainly represent very short periods of processive movement within the highly anisotropic F-actin network in the cell cortex[119]. This suggests that future studies of class V myosin-dependent organelle movement should focus on observing very short periods of persistent movement *in vivo*.

Why does the cell not just build cortical actin tracks that are better suited to allow class V myosins to move organelles to the plasma membrane? Perhaps the cost of doing this, in terms of the other major functions supported by cortical actin (such as cell locomotion, cortical integrity and endocytosis), is too high. Moreover, driving relatively random walks on anisotropic actin networks that exhibit even modest barbed-end-out bias should allow class V myosins to eventually deliver vesicles to the membrane. Alternatively, the organization of cortical actin in cells within tissues may be organized for more effective class V myosin-dependent vesicle delivery — that is, with filaments more highly aligned and polarized towards the plasma membrane[121].

Conclusions

Class V myosins clearly drive organelle translocation in plants and yeast. However, neither of the yeast class V myosins is processive (Myo4 is not even dimeric), so this organism uses 'tricks' like the cargo-driven clustering of Myo4 to allow robust transport. Conversely, although vertebrate class V myosins are highly processive, concrete evidence that they move membranes over appreciable distances inside cells has been slower to appear. Indeed, debate continues regarding the extent to which class V myosins in animal cells serve as point-to-point organelle transporters or dynamic organelle tethers[88,122–127], and several reviews have commented on the relative paucity of data supporting the transport role[88,123,125]. As discussed above, this may be due in large part to the fact that, in animal cells, the actin tracks for class V myosins are not usually organized in a way that supports persistent movement in any one direction. Therefore, efforts to prove the existence of class V myosin-dependent organelle transport in vertebrate cells should focus on identifying short-range movements, such as those identified recently in the cell cortex using quantum dot-labelled myosin Va[119] and in the dendritic spines of neurons[106,113].

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Competing interests statement
The authors declare no competing financial interests.

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