CMLS, Cell. Mol. Life Sci. 56 (1999) 233–242
1420-682X/99/040233-10 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Myosin-V: head to tail

D. W. Provance Jr. and J. A. Mercer*

McLaughlin Research Institute, 1520 23rd Street South, Great Falls (Montana 59405, USA),
Fax +1 406 454 6019, e-mail: umbjm@montana.edu

Abstract. The myosin-V family is the most extensively studied of the unconventional myosin families. Most organisms examined have at least one member of the myosin-V family; many have multiple members. The wide range of species in which myosin-V has been identified suggests that myosin-V is a fundamental component of organelle transport in all higher eukaryotes. Possible cargoes for myosin-V range from melanosomes and synaptic vesicles in mammals to vacuoles and messenger RNA in yeast. In this review, we discuss the current state of research on the cellular function of myosin-V as described by the actions of the head, neck and tail domains.

Key words. Myosin-V; vesicle transport; *dilute*; p190; IQ motif; motility.

History of the myosin-V family

The myosin-V family is a class that differs structurally from other myosins by an extended neck domain and a tail domain that allows dimerization, but not the formation of filaments due to the presence of a globular carboxy-terminus (fig. 1). Members of the myosin-V family have been identified in humans, mice, chickens, flies, worms, yeast and plants (table 1). The complete sequence of the genome of *Saccharomyces cerevisiae* shows that eukaryotes only need two myosin-Vs, along with two myosin-Is and one myosin-II.

An evolutionary tree (fig. 2) suggests that the myosin-V family diverged from other myosins prior to the divergence of yeast and mammals [1]. Phylogenetic analyses [1–3] have identified likely orthologs and homologs. Members that are definite orthologs are *Myo5a* (murine) and *MYO5a* (human). The p190 myosin from chicken is a likely ortholog of *Myo5a* and *MYO5a* as shown graphically in figure 2. The relationships between members need to be considered when extrapolating the results with one family member to the other members. The relevance of experiments on one member to the other members depends on their relatedness.

Investigators in the field have been reorganizing the nomenclature to provide a consensus on the names for the loci, the alleles and the gene products. In the following section, the historical names are presented for continuity from isolation to the individual studies on myosin-V function. The modern nomenclature has been used throughout the rest of the text.

The classical coat-color locus *dilute* (denoted originally by the single letter *d*, now *Myo5a*) was first identified and propagated by mouse fanciers at the turn of the century [4, 5] and encodes a member of the myosin-V family on chromosome 9. The chromosomal alteration in the mutant allele in common strains ($Myo5a^{d}$) was found to be the ectopic insertion of a murine leukemia provirus, *Emv-3*. The presence of the provirus allowed the cloning of flanking genomic regions [6], and subsequently *Myo5a* complementary DNAs (cDNAs) [7]. The mutations in a number of alleles of *Myo5a* have been molecularly characterized to regions in both the head and tail domains [8, 9].

The second mammalian member of the myosin-V family, myr 6 (myosin from rat, clone #6, now *Myo5b*), was identified by degenerate polymerase chain reaction (PCR) from rat brain stem cDNA [10]. Comparison of the *Myo5b* sequence with that of *Myo5a* shows 77% similarity in the head domain and 78% similarity overall. Further demonstration that *Myo5b* is distinct from *Myo5a* comes from genetic mapping data placing *Myo5b* on chromosome 18 at Mouse Genome Database position 48 [10].

The cloning of *Myo5b* has shed light on a troubling sequence in the data base for a putative murine glu-

* Corresponding author.

tamic acid decarboxylase (GAD) [11, 12]. Initial database searches with *Myo5a* and chicken myosin-V sequences identified this GAD as having similarities with the tail domains of these myosins. Characterization of the mouse homolog of *Myo5b*, using PCR, demonstrated that the putative GAD was a fusion of a short 5′ sequence and the tail of *Myo5b* [10]. No loss in GAD activity in *Myo5a*$^{d\text{-}l}$ null mutants has been detected [J. A. Mercer, unpublished results], and no GAD activity copurified with chicken myosin-V [13].

The human homolog of *Myo5a*, *MYO5a* (originally myoxin or *MYH12*), was cloned serendipitously during the screening of a human brain cDNA expression library with an anti-Ras antibody [14, 15]. *MYO5a* was mapped to human chromosome 15 using a somatic cell hybrid panel. Since this region of human chromosome 15 is syntenic with the central region of mouse chromosome 9, which contains the *Myo5a* locus, MYO5a and *Myo5a* are almost certainly homologs (also see fig. 1). Mutations in the MYO5a locus have been observed in cases of Griscelli disease [16].

A large number of temperature-sensitive mutants have been made in *S. cerevisiae* that are deficient in cellular processes. One cell-cycle mutant selected by size fractionation, *cdc66-1*, was found to remain biosynthetically active at the restrictive temperature, but could not proliferate and displayed an unbudded morphology [17]. Complementation experiments showed that *cdc66-1* encoded a myosin named *MYO2*, the second myosin found in yeast [18]. *MYO2* is an essential gene and the original mutant allele, renamed *myo2-66*, only supplies partial function probably because the mutation (Glu511Lys) lies in the actin-binding region of the motor domain [19].

Table 1. Members of the myosin-V family.

Locus	Species	GenBank accession no.	a.k.a.*
Myo5a	murine	X57377 [7]	*d*, *dilute*
MYO5a	human	Y07759 [14]	myoxin, MYH12
p190	*P. gallus*	Z11718† [23], X67251 [93]	myosin-Va
Myo5b	murine	U60416 [10]	myr 6
MYO2	*S. cerevisiae*	M35532 [18]	
MYO4	*S. cerevisiae*	M90057 [21]	
Dros-V	*D. melanogaster*	AF003826† [30], Y08160 [29]	*didum*
hum-2	*C. elegans*	U52516 [31]	
MYA1	*A. thaliana*	Z28389[32]	
MYA2	*A. thaliana*	Z34293 [33]	

The locus name for each known member of the myosin-V family is presented, with the species of origin and its GenBank accession number.

* Some members were known before the myosin-V classification and the historical names are presented.

† The myosin-V in both chicken and fly were cloned by two independent laboratories, and the sequence used for the phylogenetic analysis is indicated.

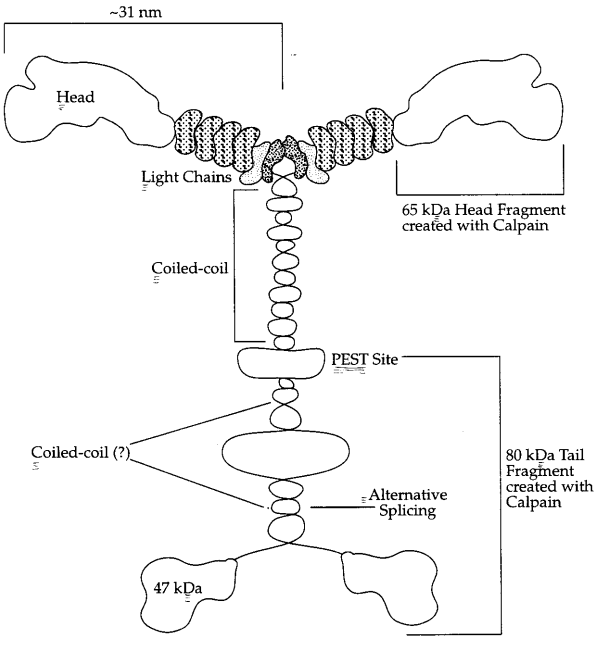

Figure 1. Model for the structure of brain myosin-V, based on the model originally presented by Cheney et al. [34], with the addition of the fragments generated through calpain digest [36]. The neck shows three different light chains to reflect the ability of IQ motifs to bind calmodulin and the 17- and 23-kDa essential light chain of nonmuscle and smooth muscle myosin-II [34, 35]. The second and third coiled-coil motifs are predicted to allow dimerization, but the likelihood of the third is significantly reduced by alternative splicing, at least for myosin-Va in melanocytes (see text, [80]).

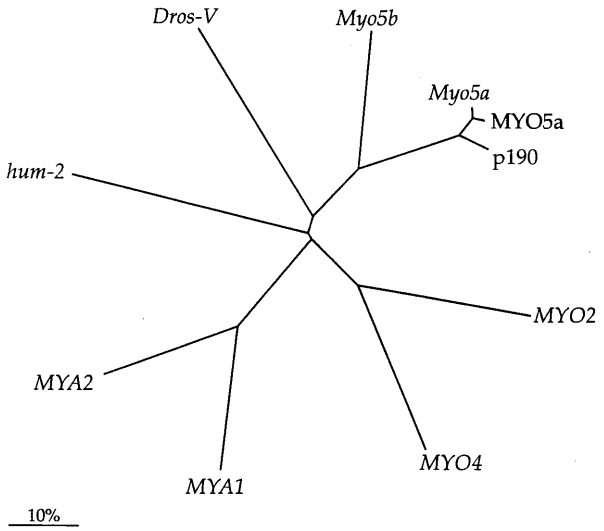

Figure 2. Phylogenetic analysis of the myosin-V family, performed with Clustal W on full-length sequences [94].

Haarer et al. used a myosin probe [20] to identify other myosin cDNA clones, including *MYO4*, the fourth myosin and the second member of the myosin-V family found in yeast [21]. The tail domains of Myo2p and Myo4p are shorter than those of the mammalian members of the family. In addition, there are fewer regions predicted to form coiled-coil domains in their myosin-V tail regions.

Myosin-V was identified in chicken brain as a 190-kDa protein (p190) that specifically precipitated with filamentous actin in an ATP-dependent manner [22]. Biochemical characterization suggested p190 was a myosin [13]. The cloning of the p190 gene [23] was interesting in several respects. First, following the cloning, was the initial phylogenetic analysis of the available myosin sequences, which showed that *Myo5a*, *MYO2*, *MYO4* and chicken myosin-V constituted a separate class of myosins. Second, it coined the term 'IQ motif' to describe the imperfect tandem repeats found in the necks of all myosins, described by Mercer et al. [7]. Third, they showed that calmodulin, which copurified with chicken myosin-V, can bind to chimeric proteins containing IQ motifs. There is a chicken mutant in chicken, *lavender*, that has a similar melanocyte morphology to that seen for *dilute* melanocytes ($Myo5a^d/Myo5a^d$), with a perinuclear melanosome accumulation [24]. No genetic mapping data are available for *lavender* in chicken, but *lavender* could be a mutation in the locus encoding chicken myosin-V.

The identification of myosin-V in squid began with cell biology. The movement of organelles in squid axoplasm was observed to exhibit actin-dependent component [25]. This group applied the technique for purification of myosin-V from chicken to squid optic lobes and isolated a 196-kDa protein [26]. The complete sequence of p196 is not currently available, but the sequences of proteolytic fragments align well with other members of the myosin-V family [27]. Squid myosin-V has been shown to be associated with endoplasmic reticulum-derived tubulovesicular organelles, and tail-specific antibody inhibition studies suggest that myosin-V can directly support vesicle transport [28].

The most recent member of the myosin-V family has been found in *Drosophila melanogaster* by two independent laboratories [29, 30]. Bownes's group used an enhancer-trap strategy to study oogenesis, and isolated a myosin-V (*didum*, *dilute*-like *Drosophila* unconventional myosin) cDNA [29]. A more focused search for myosins in the fly using degenerate PCR produced *Drosophila* myosin-V (*Dros*-V), predicted to encode a 207-kDa heavy chain [30]. Sequence analysis revealed that the third IQ motif began with LS, which is apparently unique to *D. melanogaster*. Regulation of the motor domain could require species-specific light chains unique to each family member.

A myosin-V, *hum-2* (heavy chain of an unconventional myosin), has been identified in *Caenorhabditis elegans* using a PCR-based strategy [31]. The percent similarity of *hum-2* in table 1 shows it to be nearly equally divergent from all of the other members. It will be of interest to learn how researchers will apply the cell fate map of *C. elegans* to study the function of this member of the myosin-V family, particularly if hum-2 functions in the movement of vesicles within the less complex nervous system of *C. elegans*.

Finally, MYA1 and MYA2 are two members of the myosin-V family identified from *Arabidopsis thaliana* [32, 33]. MYA1 and MYA2 are expected to function as vesicle motors in plants.

Myosin-V domain structure

The model of a chicken myosin-V multimer, based on the rotary-shadowed electron micrographs of Cheney et al. and primary structure analysis [34], provides a framework for our discussion of myosin-V function (fig. 1). The criteria used to classify a protein as a myosin are limited to the amino-terminus, or the head domain, where the mechano-enzymatic reaction takes place through cooperation between the ATPase domain and the actin-binding domain. The neck domain is depicted with light chains bound. The tail is shown with the proximal coiled-coil domain followed by a less definitive model for the arrangement of the heavy chain to the globular carboxy-terminus, the cargo binding domain. A region known to exhibit alternative splicing in murine myosin-Va is identified. Not shown are the dynein light chains predicted to be dimerized and associated with the tail domain [35]. The functions of the head, neck and tail domains will be considered below in the order in which they are translated.

Head

The force required for the movement of cargoes is generated in the head domain. The ATPase activities and actin-binding affinities vary widely among the myosins studied. All of the characteristics for the myosin-V family are based on biochemical studies of chicken myosin-V [13, 34, 36], and these studies describe some properties that apparently are unique to the myosin-V family.

The ATPase activity of a myosin is routinely described by its K^+-EDTA-ATPase and Mg^{++}-ATPase activity, including the influence of F-actin and Ca^{++} in modulating these activities. The K^+-EDTA-ATPase activity of chicken myosin-V was reported to be 1.8 ATP/s/head [36], which is significantly lower than that of skeletal muscle myosin II ($\sim$10 ATP/s/head, [37]) and

Acanthamoeba myosin I (12–22 ATP/s/head, [38]). The basal Mg^{++}-ATPase activity of chicken myosin-V also is quite low, 0–0.3 ATP/s/head, and does not appear to be activated by F-actin in the absence of Ca^{++}. The addition of Ca^{++}, with F-actin, stimulates the myosin-V Mg^{++}-ATPase substantially to an activity that is among the highest of the known myosins, with a V_{max} of 27 ATP/s. This regulation of Mg^{++}-ATPase by Ca^{++} occurs over a narrow range in vitro, between 1 and 3 μM [36].

In the sliding filament motility assay, chicken myosin-V performs as a barbed end-directed motor that can translocate F-actin at ~300 nm/s [34]. The rate of motility by chicken myosin-V also is influenced by the levels of Ca^{++}, but not in the manner anticipated from the in vitro biochemical assays. Increasing the concentration of Ca^{++} to 10 μM caused an immediate decrease in filament velocity, followed by a gradual decline. Spiking the reaction with calmodulin prior to the addition of Ca^{++} prevented the gradual decline, but the initial decrease was still observed [34].

An assay that more closely mimics the proposed role of chicken myosin-V in vivo utilized beads of 800 and 300 nm in diameter, immunoadsorbed with chicken myosin-V using an antibody against the tail domain of chicken myosin-V [39]. Motility was again more robust in the absence of Ca^{++}, with chicken myosin-V absorbed from crude chicken brain extracts performing as well as purified chicken myosin-V. The 800-nm beads were transported at rates of 450 nm/s, and 300-nm beads were transported at 600 nm/s. The addition of antibodies directed against the head domain inhibited motility [39].

Myosin-V has demonstrated a robust in vitro Mg^{++}-ATPase activity in the presence of Ca^{++} and F-actin, but its activity is virtually zero in the absence of Ca^{++} [36]. Examining the conversion of ATP into mechanical energy through motility assays results in seemingly contradictory data, since rates were greatest in the absence of Ca^{++}. A plausible scenario, assuming in vitro ATPase rates are relevant to motility assays, is that the presence of Ca^{++} shifts myosin-V into an extremely low gear. This in turn should provide greater force to successfully transport large cargoes. Greater insight into regulation by Ca^{++} will require understanding the influences of the neck domain and bound light chains on modulating motor activity.

Neck

Predictably, the neck domain in myosins follows the head domain and consists of an α-helix that contains sequences for light-chain binding. In conventional myosins, light chains apparently contribute to the structural stability and rigidity of the α-helical region [40, 41]. Known myosin-V light chains are all members of the EF-hand family of Ca^{++}-binding proteins [41a]. Quick-freeze, deep-etch electron micrographs of chicken myosin-V show this region to be a long, thickened segment, consistent with it being an extended α-helix with bound light chains [34].

The peptide sequence in the neck region conforms to the imperfect repeat of 'IQxxxRGxxxR', termed the IQ motif [2, 42, 43]. Members of the myosin-V family contain six IQ motifs; with the more carboxy-terminal repeats showing the greatest divergence [7, 23]. Multiple lines of evidence demonstrate the direct interaction between calmodulin and myosin-V, mediated through the IQ motifs.

From the beginning, calmodulin was copurified with chicken myosin-V at a ratio of 4 to 1 [34]. Immunofluorescent localization of Myo2p in yeast showed colocalization with calmodulin [44]. In gel overlay experiments, the IQ motifs from both chicken myosin-V and Myo2p, expressed as bacterial fusion proteins, are sufficient to bind calmodulin [23, 44]. In addition, immunoprecipitation of Myo2p coprecipitates calmodulin in either the presence or absence of Ca^{++} [44].

Along with calmodulin, two other proteins with relative masses of 17 and 23 kDa were also found to copurify with chicken myosin-V [34]. Sequencing demonstrated these light chains to be identical to the 17- and 23-kDa essential light chains of nonmuscle and smooth muscle myosin II [45]. In yeast, a Myo2p light chain (Mlc1p) was unmasked by the Yeast Genome Sequencing Project, based on sequence similarity with other myosin light chains [46]. Binding of Mlc1p to Myo2p has been shown through genetic interactions and gel overlay assays [46].

The stability of Myo2p appears to depend on the availability of light chains. The overexpression of Myo2p has toxic effects in yeast, but increasing the expression of Mlc1p prevents this toxicity [1]. The haploinsufficiency of *MLC1* also suggests that a constant ratio of light chains to heavy chains is essential for Myo2p stability. In addition, mutations outside the Ca^{++} binding sites of calmodulin cause synthetic lethality with *myo2-66*. Presumably the mutations in calmodulin would affect binding to Myo2p [2].

The same investigators generated a mutant Myo2p without the six IQ motifs (MYO2-Δ6IQ). This neckless Myo2p was able to rescue yeast without full-length Myo2p [46]. Bud formation and cell size were slightly different than wild type, but the localization of Myo2p-Δ6IQ appeared normal. Calmodulin, on the other hand, was delocalized, solidifying the role of IQ motifs in binding calmodulin and localizing it to the bud site.

One speculation on the function of the neck domain is that the neck acts as a lever for augmenting the power

stroke of the myosin head [40]. Consistent with this hypothesis, myosin-II constructs containing variable numbers of light-chain binding domains have different motility rates [47, 48]. The long length of the myosin-V neck, in addition to possibly providing leverage, also suggests that myosin-V may span a relatively large space (~ 27 nm) so that it can interact with two antiparallel actin filaments [34]. This may be important in light of the isometric actin networks found in squid axoplasm and nerve growth cones, two environments in which myosin-V has been found [23, 49].

Tail

The tail domain uniquely describes a particular myosin within a family and is generally believed to interact with a specific cellular structure, its cargo. A major research focus has been to identify the cargoes of the individual members of the myosin-V family. The identity of the cargo should define where a myosin applies the force generated in the head domain, essential to understanding that myosin's function. Possible myosin-V cargoes have been described in neurons, pigment cells and yeast.

All mutations at the *Myo5a* locus cause a dilution in coat color; most alleles also have a neurological phenotype characterized by ataxia, opisthotonos and death at about 3 weeks of age [50]. This coincides with an extremely active developmental period in the central nervous system of the mouse. The temporal and spatial patterns of *Myo5a* messenger RNA (mRNA) expression were not very informative [7]. Transcripts were present at high levels in every organ but liver in adult mice; they were also present in virtually every neuron in the brain. High levels of *Myo5a* expression were also observed in the central nervous system of embryos, 3 weeks before the onset of any phenotype in the $Myo5a^{d\text{-}l}$ null mutant. The delay is likely to be caused by overlapping functions between members of the same family, as hypothesized for the myosin-I family [51].

Recently, the *MYO5a* locus was shown to be mutated in human cases of Griscelli disease, characterized by autosomal recessive inheritance; hypopigmentation with silvery hair color; and immunosuppression, especially low natural killer cell activity [16]. The condition is fatal in childhood or adolescence. In the first report of Griscelli disease, no evidence of a neurological phenotype was noted [52]. However, in the recent paper identifying mutations, one patient of four had a neurological syndrome characterized by hypotonia. This patient had a nonsense mutation in the head region of the myosin that would almost certainly result in a nonfunctional protein; the other mutation detected was a missense mutation in the tail, two patients who had no neurological phenotype. These phenotypes are remarkably consistent with the $Myo5a^{d\text{-}l}$ and $Myo5a^{d}$ phenotypes seen in mice, respectively.

In addition to Griscelli disease, two other rare hypopigmentation syndromes in humans are characterized by symptoms similar to those of $Myo5a^{d}$-mutant mice. Cross-syndrome patients are described as having a metallic sheen to their hair and are unresponsive, with hypotonic characteristics [53]. Elejalde syndrome patients have silver hair and exhibit hypotonia, and in one case, grand mal seizures [54, 55]. It is very likely that these syndromes are allelic with Griscelli disease, considering the phenotypic variation in alleles of *Myo5a*, suggesting that studies in mice will be germane to determining the function of human myosin-V.

Myosin-V function in neurons

The inactivation of myosin-V by chromophore-assisted laser inactivation (CALI) in growth cones of chicken dorsal root ganglion neurons caused retraction of filopodia [56]. In contrast, explants of cultured superior cervical ganglia from $Myo5a^{d\text{-}l}$ mice appear normal in terms of morphology, outgrowth and the cytoskeletal organization of growth cones [57]. This is consistent with the absence of gross abnormalities in the central nervous systems of $Myo5a^{d\text{-}l}$ mice prior to the onset of the neurological phenotype, arguing for a physiological role for myosin-V and against a developmental function.

Purkinje cells stained for myosin-V show punctate staining in the cell body and the dendrites [23]. In the cell body, colocalization of myosin-V was observed with wheat-germ agglutinin. While this suggested Golgi-derived vesicles as a cargo, the nonspecific staining could have labeled other organelles, including lysosomes and endosomes. Purkinje cells from both the mouse and rat null mutants at the *Myo5a* locus do not extend their smooth endoplasmic reticulum into the dendritic spines to the same extent as wild-type controls [58, 59]. It must be emphasized that these analyses were performed after the onset of the neurological phenotype, however, and therefore cannot exclude the possibility of secondary or tertiary effects of the opisthotonic neurological condition, including dehydration and malnutrition.

Punctate staining for myosin-V was observed in retinas labeled in areas of rod photoreceptor synapses [60]. Consistent with this localization, brain myosin-V has recently been found associated with synaptic vesicles immunoprecipitated with antibodies against synaptobrevin II and rab3 [61]. The association appeared to be disrupted by Ca^{++} and Mg^{++}, but not necessarily due to actions on the neck.

During the purification of myosin-V, an early step generates a brain vesicle fraction [62]. These vesicles exhibited characteristics of synaptic vesicles and were motile in vitro; antibodies directed against the head domain of myosin-V could inhibit the in vitro motility [63]. The vesicle fraction consisted of 60- and 90-nm vesicles; myosin-V was found associated with a subset of 90-nm vesicles, but not with copurified 60-nm vesicles. This observation could account for the punctate subcellular localization of myosin-V in growth cones [23, 57]. Evans et al. described these vesicles, viewed at the level of electron microscopy, as 50–100 nm in size and associated with both actin filaments and microtubules [57].

In squid axoplasm, both actin- and microtubule-based movement has been observed for individual tubular vesicular organelles [25]. A dual filament model of transport has been proposed to explain this observation; it stipulates that organellar movement can consist of long, rapid motility along microtubules and short, stochastic motility along actin filaments [64, 65]. For some organelle movements in squid giant axon, myosin-V appears to be the actin-dependent motor. Isolation of endoplasmic reticulum-derived vesicles partially purifies squid myosin-V. These vesicles exhibit actin-based motility in vitro [28]. This motility was inhibited with antibodies against the head domain and by antibodies against a unique peptide in the tail of squid myosin-V. Immunoelectron microscopy demonstrated that some of the vesicles to which squid myosin-V localized also were labeled for kinesin [28]. Recently, two-hybrid experiments and immunofluorescent results demonstrated a direct interaction between the tail of myosin-Va and ubiquitous kinesin [66], consistent with the dual filament model of transport.

Myosin-V and melanosomes

Normally, pigment is synthesized in melanosomes by melanocytes and transported through dendritic processes toward the hair bulb [67]. Early observations of *dilute* melanocytes in situ described the absence of dendrites, and it was originally hypothesized that myosin-Va functioned in the formation of dendritic processes [68]. This has been shown not to be the function of myosin-Va, and myosin-Va does not play a role in melanosome pigmentation itself [69–72].

The absence of myosin-Va causes a striking difference in the distribution of melanosomes in primary murine melanocytes. Melanosomes are concentrated in the perinuclear region with clumps of melanosomes found sporadically in dendrites, rather than the uniform distribution observed in wild-type melanocytes [70–72]. This suggested that myosin-Va function is required during melanosome transport, either directly as the transporter or in cooperation with another motor, possibly as a tether or dock after transport [71]. These are not mutually exclusive hypotheses.

Consistent with these hypotheses, disruption of actin filaments influences the distribution of mammalian melanosomes [73], and immunofluorescent localization of myosin-Va in wild-type melanocytes revealed a punctate distribution in the periphery of the cell [71]. Later immunofluorescent data from B16 melanoma cells showed more colocalization of myosin-Va staining with melanosomes [72, 74]. Immunoelectron labeling showed myosin-Va labeling concentrated in a small region on the cytoplasmic face of melanosomes [74].

Based on their velocity and persistence to move in a single direction, the movement of melanosomes within wild-type melanocytes has been described as exhibiting both microtubule- and actin-based motility [75]. In *dilute* melanocytes, melanosomes were described to only exhibit microtubule-based movement. Wild-type melanocytes accumulated melanosomes in the perinuclear region in response to the expression of a headless myosin-V construct. The authors concluded from these observations that the majority of melanosome transport occurs along microtubules and that myosin-V 'captures' melanosomes in the periphery [75]. This disagrees with the dual filament model of transport by suggesting that the actin-based motor (in this case myosin-Va) does not contribute significantly to the distance traveled by the cargo, but merely acts as a tether or dock.

Melanosome movement in both amphibian and teleost melanophores has a well-established dependence on microtubules and microtubule-dependent motor function [76, 77]. Recently, an actin-dependent component to melanosome motility has been described in melanophores. In teleost melanophores, melanosome movement persisted in the absence of microtubules, and the disassembly of actin prevented the normal dispersion of pigment induced by caffeine treatment [78]. In another study, melanosomes purified from *Xenopus* melanophores were capable of moving along *Nitella* actin filaments. Myosin-V, but not myosin-I, was demonstrated to be associated with purified melanosomes, suggesting that myosin-V is the actin-based motor [79].

The results in melanocytes and neurons suggest that myosin-V has cell type-specific cargoes. Transcripts from the *Myo5a* locus have a tissue-specific splicing pattern in a region immediately 5′ of the globular carboxy-terminus [80, 81]. This creates multiple isoforms of myosin-Va. Melanocytes express one predominate isoform that differs from the brain-expressed isoforms by the inclusion of a 240-bp exon and the absence of a 9-bp exon [80]. The insertion point of this exon coincides with a predicted coiled-coil motif depicted in the model of brain myosin-Va structure (fig. 2). These splic-

ing patterns could significantly alter the conformation of the globular carboxy-terminus and provide different cargo specificities between melanocytes and neurons. Different cargoes for myosin-Va in melanocytes and neurons could explain how the single mutant allele at the *dilute suppressor* (*dsu*) locus can semidominantly suppress the coat-color phenotype, but not the neurological phenotypes of $Myo5a^{d\text{-}l}$ [82].

Myosin-V in *S. cerevisiae*

Studies into the function of members of the myosin-V family have been facilitated by the power of yeast genetics. Initial studies on a temperature-sensitive *Myo2* mutant (*myo2-66*) at the restrictive temperature showed an accumulation of vesicles, abnormal F-actin structures, disruption of directed growth but not secretion, and delocalization of chitin, a marker for the bud site [18]. Double-mutant analysis placed myo2p function at a post-Golgi stage of secretion [83]. Examination of vacuole partitioning in *myo2-66* mutants during budding demonstrated a disproportionate number of cells that do not transfer vacuoles to the daughter cells. Further analysis revealed colocalization between Myo2p and a vacuole-specific marker (60-kDa vacuolar ATPase subunit) [84]. Recently, a point mutation was identified in the tail of Myo2p (Gly1248Asp, *myo2-2*) that disrupts vacuole inheritance and causes the mislocalization of the mutant gene product compared with wild-type Myo2p [85]. These data strongly support a role for *MYO2* in the transport of vacuole-derived vesicles to the bud site in yeast and that the tail domain is essential for cargo interactions.

The second member of the myosin-V family in yeast, Myo4p, has been shown to be involved in mating type switching [86, 87]. Conversion of the mating type locus involves the cleavage of DNA at the *MAT* locus by the *HO* endonuclease and is restricted to the mother cell [88]. Daughter cells are prevented from switching through the repression of HO transcription by Ash1p, which is preferentially accumulated in the nuclei of daughter cells and not mother cells [86, 87, 89]. This asymmetric distribution of Ash1p has been attributed to the accumulation of *ASH1* mRNA in daughter cell nuclei through a process that depends on both actin and Myo4p function [90]. This would suggest that the cargo of Myo4p is *ASH1* mRNA.

The presence of two myosin-V family members in yeast provided an opportunity to test for possible functional redundancy between family members [21]. Myo4p does not appear to influence vacuole inheritance, the hypothesized role of myo2p [84]. Furthermore, the overexpression of Myo4p cannot rescue the loss of Myo2p function, suggesting that family members have distinct functions and do not overlap, at least in yeast [21].

The loss of Myo2p function can be rescued by *SMY1* (suppressor of myosin), which is a member of the kinesin superfamily [91]. Deletion of *SMY1* indicates that it is not an essential gene, but the loss of SMY1p in conjunction with the reduced activity of *myo2-66* is lethal. The distribution of SMY1p has been suggested to be similar to myo2p by extrapolating the localization of each with actin [19]. The same publication also reported the discovery of Smy2 (GenBank accession no. M90654), but no other data have been published. It is interesting that a member of the kinesin family can suppress the loss of Myo2p function in yeast considering the proposed cooperation between myosin-V and microtubule-based motors in higher eukaryotes.

Conclusion and future

While the tail domain appears to mediate the binding to cargo, sequences known to interact directly with phospholipids are not present in the tail domains of members of the myosin-V family [92]. This suggests that the tail of a myosin-V interacts with its cargo through an intermediary protein (or protein complex) present on the cytoplasmic face of the cargo. Future studies will be aimed at identifying the mechanisms involved in cargo binding.

The initiation of cargo binding most likely occurs to begin transporting a vesicle from where it is synthesized to where it will ultimately be used and probably involves simple affinity. The events that take place after the cargo is delivered to its final destination are less intuitive. The modular protein AF-6 [3] shares a homologous domain with the tail of myosin-V [4], and could provide a mechanism for cargo release near the plasma membrane by competing for cargo binding. AF-6 includes a PDZ domain known to localize proteins under the plasma membrane [5] and has been shown to be involved in clustering of receptors [6]. Considering that synaptic vesicles are a possible cargo and need to be targeted to the cell membrane, it may be important to consider AF-6 function. The simple null *AF-6* mutant is not informative, since homozygotes die during mid-gestation [92a].

In summary, significant progress has been made on the elucidation of functions for several members of the myosin-V family through identification of potential cargoes. This does not imply that these cargoes rely exclusively on myosin-V function for transport; in fact, some of the possible cargoes also have exhibited microtubule-dependent motility. The concept of cooperation with microtubule-based molecular motors, as described by the dual filament model of transport, will be essential for a complete understanding of myosin-V function.

1 Cheney R. E., Riley M. A. and Mooseker M. S. (1993) Phylogenetic analysis of the myosin superfamily. Cell Motil. Cytoskeleton **24:** 215–223
2 Mooseker M. S. and Cheney R. E. (1995) Unconventional myosins. Annu. Rev. Cell Dev. Biol. **11:** 633–675
3 Sellers J. R., Goodson H. V. and Wang F. (1996) A myosin family reunion. J. Muscle Res. Cell Motil. **17:** 7–22
4 Silvers W. K. (1979) The Coat Colors of Mice, Springer, New York
5 Green M. C. (1989) Catalog of mutant genes and polymorphic loci. In: Genetic Variants and Strains of the Laboratory Mouse, 2nd ed., pp. 82–83, Lyon M. F. and Searle A. G. (eds), Oxford University Press, Oxford
6 Strobel M. C., Seperack P. K., Copeland N. G. and Jenkins N. A. (1990) Molecular analysis of two mouse *dilute* locus deletion mutations: spontaneous *dilute-lethal-20J* and radiation-induced *dilute* prenatal lethal Aa2 alleles. Mol. Cell Biol. **10:** 501–509
7 Mercer J. A., Seperack P. K., Strobel M. C., Copeland N. G. and Jenkins N. A. (1991) Novel myosin heavy chain encoded by murine *dilute* coat colour locus. Nature **349:** 709–713
8 Huang J., Cope M., Mermall V., Strobel M., Kendrick-Jones J., Russell L. et al. (1998) Molecular genetic dissection of mouse unconventional myosin-Va head region mutations. Genetics **148:** 1951–1961
9 Huang J., Mermall V., Strobel M., Russell L., Mooseker M., Copeland N. et al. (1998) Molecular genetic dissection of mouse unconventional myosin-Va tail region mutations. Genetics **148:** 1963–1972
10 Zhao L. P., Koslovsky J. S., Reinhard J., Bahler M., Witt A. E., Provance D. W. et al. (1996) Cloning and characterization of myr 6, an unconventional myosin dilute/myosin-V family. Proc. Natl. Acad. Sci. USA **93:** 10826–10831
11 Huang W. M., Reed-Fourquet L., Wu E. and Wu J. Y. (1990) Molecular cloning and amino acid sequence of brain L-glutamate decarboxylase. Proc. Natl. Acad. Sci. USA **87:** 8491–8495
12 Wu J., Huang W., Reed-Fourquet L., Bao J., Nathan B., Wu E. et al. (1991) Structure and function of L-glutamate decarboxylase. Neurochem. Res. **16:** 227–233
13 Espindola F. S., Espreafico E. M., Coelho M. V., Martins A. R., Costa F. R., Mooseker M. S. et al. (1992) Biochemical and immunological characterization of p190-calmodulin complex from vertebrate brain: a novel calmodulin-binding myosin. J. Cell Biol. **118:** 359–368
14 Engle L. J. and Kennett R. H. (1994) Cloning, analysis and chromosomal localization of myoxin (*MYH12*), the human homologue to the mouse *dilute* gene. Genomics **19:** 407–416
15 Moore K. J., Testa J. R., Francke U., Milatovich A., Copeland N. G. and Jenkins N. A. (1995) Cloning and regional assignment of the human myosin heavy chain 12 (MYH12) gene to chromosomal band 15q21. Cytogenet. Cell Genet. **69:** 53–58
16 Pastural E., Barrat F. J., Dufourcq-Lagelouse R., Certain S., Sanal O., Jabado N. et al. (1997) Griscelli disease maps to chromosome 15q21 and is associated with mutations in the Myosin-Va gene. Nature Genet. **16:** 289–292
17 Prendergast J. A., Murray L. E., Rowley A., Carruthers D. R., Singer R. A. and Johnston G. C. (1990) Size selection identifies new genes that regulate *Saccharomyces cerevisiae* cell proliferation. Genetics **124:** 81–90
18 Johnston G. C., Prendergast J. A. and Singer R. A. (1991) The *Saccharomyces cerevisiae MYO2* gene encodes an essential myosin for vectorial transport of vesicles. J. Cell Biol. **113:** 539–551
19 Lillie S. H. and Brown S. S. (1994) Immunofluorescence localization of the unconventional myosin, myo 2p, and the putative kinesin-related protein, smy1p, to the same regions of polarized growth in *Saccaromyces cerevisiae*. J. Cell Biol. **125:** 825–842
20 Pascolo S., Ghazvini M., Boyer J., Colleaux L., Thierry A. and Dujon B. (1992) The sequence of a 9.3-kb segment located on the left arm of the yeast chromosome-XI reveals five open reading frames including the CCE1 gene and putative products related to MYO2 and to the ribosomal protein L10. Yeast **8:** 987–995
21 Haarer B. K., Petzold A., Lillie S. H. and Brown S. S. (1994) Identification of *MYO4*, a second class V myosin gene in yeast. J. Cell Sci. **107:** 1055–1064
22 Larson R. E., Pitta D. E. and Ferro J. A. (1988) A novel 190-kDa calmodulin-binding protein associated with brain actomyosin. Braz. J. Med. Res. **21:** 213–217
23 Espreafico E. M., Cheney R. E., Matteoli M., Nascimento A. A., De Camilli P. V., Larson R. E. et al. (1992) Primary structure and cellular localization of chicken brain myosin-V (p190), an unconventional myosin with calmodulin light chains. J. Cell Biol. **119:** 1541–1557
24 Mayerson P. L. and Brumbaugh J. A. (1981) Lavender, a chick melanocyte mutation with defective melanosome translocation: a possible role for 10-nm filaments and microfilaments but not microtubules. J. Cell Sci. **51:** 25–51
25 Kuznetsov S., Langford G. and Weiss D. (1992) Actin-dependent organelle movement in squid axoplasm. Nature **356:** 722–725
26 Cohen D., Kuznetsov S. and Langford G. (1994) Myosin V in squid axoplasm and optic lobes. Mol. Biol. Cell **5:** 278a
27 Molyneaux B. and Langford G. (1997) Squid myosin V transports organelles in nerve cells. Mol. Biol. Cell **8:** 371a
28 Tabb J., Molyneaux B., Cohen D., Kuznetsov S. and Langford G. (1998) Transport of ER vesicles on actin filaments in neurons by myosin V. J. Cell Sci. **111:** 3221–3234
29 MacIver B., McCormack A., Slee R. and Bownes M. (1998) Identification of an essential gene encoding a class-V myosin in *Drosphila melanogaster*. Eur. J. Biochem. **257:** 529–537
30 Bonafé N. and Sellers J. R. (1998) Molecular characterization of myosin V from *Drosophila melanogaster*. J. Muscle Res. Cell. Motil. **19:** 129–141
31 Baker J. and Titus M. (1995) A family of unconventional myosins from the nematode *Caenorhabditis elegans*. J. Mol. Biol. **272:** 523–535
32 Kinkema M. and Schiefelbein J. (1994) A myosin from a higher plant has structural similarities to class V myosins. J. Mol. Biol. **239:** 591–597
33 Kinkema M., Wang H. and Schiefelbein J. (1994) Molecular analysis of the myosin gene family in *Arabidopsis thaliana*. Plant Mol. Biol. **26:** 1139–1153
34 Cheney R. E., O'Shea M. K., Heuser J. E., Coelho M. V., Wolenski J. S., Espreafico E. M. et al. (1993) Brain myosin-V is a two-headed unconventional myosin with motor activity. Cell **75:** 13–23
35 Benashski S., Harrison A., Patel-King R. and King S. (1997) Dimerization of the highly conserved light chain shared by dynein and myosin V. J. Biol. Chem. **272:** 20929–20935
36 Nascimento A. A. C., Cheney R. E., Tauhata S. B. F. and Larson R. E. (1996) Enzymatic characterization and functional domain mapping ofbrain myosin-V. J. Biol. Chem. **271:** 17561–17569
37 Margossian S. S. and Lowey S. (1982) Preparation of myosin and its subfragments from rabbit skeletal muscle. Methods Enzymol. **85:** 55–71
38 Pollard T. D., Doberstein S. K. and Zot H. G. (1991) Myosin-I. Ann. Rev. Physiol. **53:** 653–681
39 Wolenski J. S., Cheney R. E., Mooseker M. S. and Forscher P. (1995) In vitro motility of immunoadsorbed brain myosin-V using a Limulus acrosomal process and optical tweezer-based assay. J. Cell Sci. **108:** 1489–1496
40 Rayment I., Holden H. M., Whittaker M., Yohn C. B., Lorenz M., Holmes K. C. et al. (1993) Structure of the actin-myosin complex and its implications for muscle contraction. Science **261:** 58–65
41 Spudich J. A. (1994) How molecular motors work. Nature **372:** 515–518
41a Persechini A., Moncrief N. D. and Kretsinger R. H. (1989) The EF-hand family of calcium-modulated proteins. Trends Neurosci. **12:** 462–467

42 Wolenski J. S. (1995) Regulation of calmodulin-binding myosins. Trends Cell Biol. **5:** 310–316
43 Larson R. E. (1996) Myosin-V: a class of unconventional molecular motors. Braz. J. Med. Biol. Res. **29:** 309–318
44 Brockerhoff S. E., Stevens R. C. and Davis T. N. (1994) The unconventional myosin Myo2p is a calmodulin target at sites of cell growth in *S. cerevisiae*. J. Cell Biol. **124:** 315–323
45 Espindola F., Cheney R., King S., Suter D. and Mooseker M. (1996) Mol. Biol. Cell **7:** 327a
46 Stevens R. and Davis T. (1998) Mlc1p is a light chain for the unconventional myosin Myo2p in *Saccharomyces cerevisiae*. J. Cell Biol. **142:** 711–722
47 Uyeda T. Q., Warrick H. M., Kron S. J. and Spudich J. A. (1991) Quantized velocities at low myosin densities in an in vitro motility assay. Nature **352:** 307–311
48 Anson M., Geeves M. A., Kurzawa S. E. and Manstein D. J. (1996) Myosin motors with artificial lever arms. EMBO J. **15:** 6069–6074
49 Tabb J. S., Harmon K. O., DePina A. S. and Langford G. M. (1996) Localization of myosin on tubulovesicular organelles in the squid giant axon by immuno-EM. Biol. Bull. **191:** 274–275
50 Searle A. G. (1952) A lethal allele of *dilute* in the house mouse. Heredity **6:** 395–401
51 Ostap E. M. and Pollard T. D. (1996) Overlapping functions of myosin-I isoforms? J. Cell Biol. **133:** 221–224
52 Griscelli C., Durandy A., Guy G. D., Daguillard F., Herzog C. and Prunieras M. (1978) A syndrome associating partial albinism and immunodeficiency. Am. J. Med. **65:** 691–702
53 Cross H. E. M., McKusick V. A. and Breen W. (1967) A new oculocerebral syndrome with hypopigmentation. J. Pediat. **70:** 398–406
54 Elejalde B. R., Valencia A., Gilbert E. F., Marin G., Molina J. and Holguin J. (1977) Neuro-ectodermal melanolysosomal disease: an autosomal recessive pigment mutation in man. Am. J. Hum. Genet. **29:** 39A
55 Elejalde B. R., Holguin J., Valencia A., Gilbert E. F., Molina J., Marin G. et al. (1979) Mutations affecting pigmentation in man: I. Neuroectodermal melanolysosomal disease. Am. J. Med. Genet. **3:** 65–80
56 Wang F. S., Wolenski J. S., Cheney R. E., Mooseker M. S. and Jay D. G. (1995) Perturbation of myosin-V by CALI causes immediate filopodial retraction in neuronal growth cones. Mol. Biol. Cell **6**(Suppl.)**:** 145a
57 Evans L. L., Hammer J. and Bridgman P. C. (1997) Subcellular localization of myosin V in nerve growth cones and outgrowth from dilute-lethal neurons. J. Cell Sci. **110:** 439–449
58 Dekkerohno K. (1996) Endoplasmic reticulum is missing in dendritic spines of Purkinje cells of the ataxic mutant rat. Brain Res. **714:** 226–230
59 Takagishi Y., Oda S., Hayasaka S., Dekkerohno K., Shikata T., Inouye M. et al. (1996) The dilute-lethal (d(l)) gene attacks a Ca^{2+} store in the dendritic spine of Purkinje cells in mice. Neurosci. Lett. **215:** 169–172
60 Schlamp C. L. and Williams D. S. (1996) Myosin V in the retina: localization in the rod photoreceptor. Exp. Eye Res. **63:** 613–619
61 Prekeris R. and Terrian D. M. (1997) Brain myosin-V is a synaptic vesicle-associated motor protein: evidence for a Ca^{++}-dependent interaction with the synaptobrevin-synaptophysin complex. J. Cell Biol. **137:** 1589–1601
62 Cheney R. (1998) Purification and assay of myosin V. Methods Enzymol. **298:** 2–18
63 Evans L. L., Lee A. J., Bridgman P. C. and Mooseker M. S. (1998) Vesicle-associated brain myosin-V can be activated to catalyze actin-based transport. J. Cell. Sci. **111:** 2055–2066
64 Langford G. (1995) Actin- and microtubule-dependent organelle motors: interrelationship between the two motility systems. Curr. Opin. Cell Biol. **7:** 82–88
65 Langford G. and Molyneaux B. (1998) Myosin V in the brain: mutations lead to neurological defects. Brain Res. Rev. **28:** 1–8
66 Huang J., Brady S., Richards B., Stenoien D., Resau J., Copeland N. et al. (1998) Direct interaction of microtubule- and actin-based transport motors. Nature **397:** 267–270
67 Hirobe T. (1995) Structure and function of melanocytes: Microscopic morphology and cell biology of mouse melanocytes in the epidermis and hair follicle. Histol. Histopathol. **10:** 223–237
68 Markert C. L. and Silvers W. K. (1956) The effects of genotype and cell environment on melanoblast differentiation in the house mouse. Genetics **41:** 429–450
69 Hearing V. J., Phillips P. and Lutzner M. A. (1973) The fine structure of melanogenesis in coat color mutants of the mouse. J. Ultrastruct. Res. **43:** 88–106
70 Koyama Y. and Takeuchi T. (1981) Ultrastructural observations on melanosome aggregation in genetically defective melanocytes of the mouse. Anat. Rec. **4:** 599–611
71 Provance D. W., Wei M., Ipe V. and Mercer J. A. (1996) Cultured melanocytes from *dilute* mutant mice exhibit dendritic morphology and altered melanosome distribution. Proc. Natl. Acad. Sci. USA **93:** 14554–14558
72 Wu X., Bowers B., Wei Q., Kocher B. and Hammer J. A. III (1997) Myosin V associates with melanosomes in mouse melanocytes: evidence that myosin V is an organelle motor. J. Cell Sci. **110:** 847–859
73 Koyama Y. and Takeuchi T. (1980) Differential effect of cytochalasin B on the aggregation of melanosomes in cultured mouse melanoma cells. Anat. Rec. **4:** 449–459
74 Nascimento A. A., Amaral R. G., Bizario J. C., Larson R. E. and Espreafico E. M. (1997) Subcellular localization of myosin-V in the B16 melanoma cells, a wild-type cell line for the dilute gene. Mol. Biol. Cell **8:** 1971–1988
75 Wu X., Bowers B., Rao K., Wei Q. and Hammer J. (1998) Visualization of melanosome dynamics within wild-type and *dilute* melanocytes suggests a paradigm for myosin V function in vivo. J. Cell Biol. **143:** 1899–1918
76 Rodionov V., Gyoeva F. and Gelfand V. (1991) Kinesin is responsible for centrifugal movement of pigment granules in melanophores. Proc. Natl. Acad. Sci. USA **88:** 4956–4960
77 Tuma M., Zill A., Le Bot N., Vernow I. and Gelfand V. (1998) Heterotrimerick kinesin II is the microtubule motor protein responsible for pigment dispersion in *Xenopus* melanophores. J. Cell Biol. **143:** 1547–1558
78 Rodionov V., Hope A., Svitkina T. and Borisy G. (1998) Functional coordination of microtubule-based and actin-based motility in melanophores. Curr. Biol. **8:** 165–168
79 Rogers S. and Gelfand V. (1998) Myosin cooperates with microtubule motors during organelle transport in melanophores. Curr. Biol. **8:** 161–164
80 Seperack P. K., Mercer J. A., Strobel M. C., Copeland N. G. and Jenkins N. A. (1995) Retroviral sequences located within an intron of the *dilute* gene alter *dilute* expression in a tissue-specific manner. EMBO J. **14:** 2326–2332
81 Lambert J., Naeyaert J., Callens T., De Paepe A. and Messiaen L. (1998) Human myosin V gene produces different transcripts in a cell type-dependent manner. Biochem. Biophys. Res. Commun. **252:** 329–333
82 Moore K. J., Swing D. A., Rinchik E. M., Mucenski M. L., Buchberg A. M., Copeland N. G. et al. (1988) The murine dilute suppressor gene *dsu* suppresses the coat-color phenotype of three pigment mutations that alter melanocyte morphology, *d*, *ash* and *ln*. Genetics **119:** 933–941
83 Govindan B., Bowser R. and Novick P. (1995) The role of Myo2, a yeast class V myosin, in vesicular transport. J. Cell Biol. **128:** 1055–1068
84 Hill K. L., Catlett N. L. and Weisman L. S. (1996) Actin and myosin function in directed vacuole movement during cell division in *Saccharomyces cerevisiae*. J. Cell Biol. **135:** 1535–1549
85 Catlett N. and Weisman L. (1998) The terminal tail region of a yeast myosin-V mediates its attachment to vacuole membranes and sites of polarized growth. Proc. Natl. Acad. Sci. USA **95:** 14799–14804

86 Jansen R.-P., Dowzer C., Michaelis C., Galova M. and Nasmyth K. (1996) Mother cell-specific HO expression in budding yeast depends on the unconventional myosin Myo4p and other cytoplasmic proteins. Cell **84:** 687–697

87 Bobola N., Jansen R.-P., Shin T. and Nasmyth K. (1996) Asymmetric accumulation of Ash1p in postanaphase nuclei depends on a myosin and restricts yeast mating-type switching to mother cells. Cell **84:** 699–709

88 Nasmyth K. (1993) Regulating the *HO* endonuclease in yeast. Curr. Opin. Genet. Dev. **3:** 286–294

89 Sil A. and Herskowitz I. (1996) Identification of an asymmetrically localized determinant, Ash1p, required for lineage-specific transcription of the yeast *HO* gene. Cell **84:** 711–722

90 Long R. M., Singer R. H., Meng X., Gonsalez I., Nasmyth K. and Jansen R.-P. (1997) Mating type switching in yeast controlled by asymmetic localization of ASH1 mRNA. Science **277:** 383–387

91 Lillie S. H. and Brown S. S. (1992) Suppression of a myosin defect by a kinesin-related gene. Nature **356:** 358–361

92 Li D., Miller M. and Chantler P. D. (1994) Association of a cellular myosin II with anionic phospholipids and neuronal plasma membrane. Proc. Natl. Acad. Sci. USA **91:** 853–857

92a Zhadanov A. B., Provance D. W. Jr., Speer C. A., Goss D., Blixt J. A., Coffin J. D. et al. (1999) Absence of the tight junctional protein AF-6 disrupts epithelial cell-cell junctions and cell polarity during mouse development. Curr. Biol. **9:** 880–888

93 Sanders G., Lichte B., Meyer H. E. and Kilimann M. W. (1992) cDNA encoding the chicken ortholog of the mouse dilute gene product. Sequence comparison reveals a myosin I subfamily with conserved C-terminal domains. FEBS Lett. **311:** 295–298

94 Thompson J., Higgins D. and Gibson T. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22:** 4673–4680