Multifunctional myosin VI has a multitude of cargoes

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In humans, a vast array of cytoskeletal motor proteins (19 dyneins, 43 kinesins, and 39 myosins) (1) move along microtubule and actin filament tracks to generate an enormous range of cellular functions. The kinesin and dynein motor proteins drive long-distance transport along microtubules, whereas the myosins are responsible for short-range delivery along actin filaments (2). The myosin motor proteins are composed of three functional domains: a motor domain with catalytic ATPase and actin-binding sites, a neck (lever arm with 1–6 bound calmodulins), and a cargo-binding tail domain. The mechanochemical properties of the myosins have been studied in great detail, but far less is known about their exact cellular functions (3). Although the kinetic properties of the motor domains of the myosins are fine-tuned for their cellular roles, it is the identity of their cargo-binding proteins that determines their precise cellular functions. In PNAS, Finan et al. (4) use a biochemical interaction screen to identify the cargo-binding partners of myosin VI/Jaguar in *Drosophila* to further our understanding of the cellular roles of this motor protein.

Myosin VI is believed to have unique functions in the cell, because it is the only myosin shown to move toward the minus end of actin filaments, in the opposite direction of all other myosins (5). However, as in vertebrates, loss of myosin VI in *Drosophila* is not lethal, but causes a variety of well-documented phenotypes during embryonic development. For example, the absence of myosin VI in *Drosophila* leads to defects in actin-dependent pseudocleavage furrow formation in the syncytial blastoderm (6), and it affects border cell migration during oogenesis (7) as well as dorsal closure in late embryogenesis (8). Although these defects clearly demonstrate an important role for myosin VI during *Drosophila* development, compared with mammalian cells, far less molecular details are known about the exact cellular functions and pathways that require myosin VI.

In mammalian cells, myosin VI plays very specific roles in a number of cellular processes, such as clathrin-mediated endocytosis from the apical domain of polarized epithelial cells and the transport of endosomes and sorting of endocytic cargo (9, 10). Myosin VI is also required for the polarized delivery of cargo from the Golgi complex or the recycling endosome to specific sections of the plasma membrane, such as the cleavage furrow in dividing cells, the dendritic surface in neuronal cells, the basolateral domain in epithelial cells, and the leading edge in migratory cells (11). In addition to this role in polarized cargo transport, myosin VI may also anchor membranes to the cytoskeletal framework, thereby supporting the steady-state organization of a highly polarized cell, such as hair cells of the inner ear (12). Myosin VI is able to perform so many different functions in mammalian cells because it binds to a wide spectrum of interacting proteins (cargoes; Fig. 1). Loss of these cargo/adaptor proteins or mutations in the binding sites in the myosin VI cargo-binding tail domain abolishes the ability of this motor to target specific cellular compartments, to associate with its diverse cargoes, and to function in specific pathways (11).

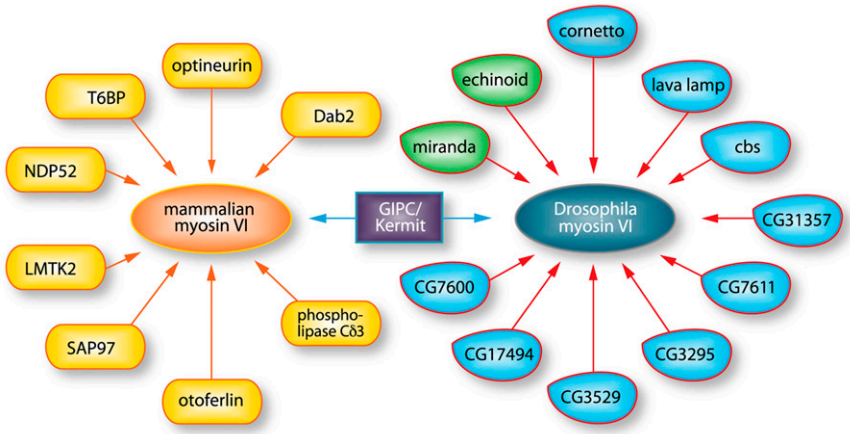

Fig. 1. Myosin VI-interacting proteins. A schematic overview of myosin VI binding partners identified in mammalian cells/tissues (yellow) and in *Drosophila* (blue). Previously known *Drosophila* myosin VI binding partners are highlighted in green, and the unique direct-binding proteins identified in Finan et al. (4) are in blue. Thus far, only GIPC/Kermit (purple) has been shown to bind to both mammalian and *Drosophila* myosin VI.

Although myosin VI is broadly expressed in *Drosophila* and, when knocked down, multiple phenotypes are generated, very few of its binding partners are known. The PNAS study by Finan et al. (4) now fills this gap in our knowledge and provides us with important information on the cargoes of *Drosophila* myosin VI. They used an affinity chromatography/mass spectrometry approach to identify the *Drosophila* myosin VI cargo-binding proteins. Previous studies in mammalian cells had used either two-hybrid screens or coimmunoprecipitation/mass spectrometry to identify myosin VI binding partners (11). Interestingly, in mammalian tissues and *Drosophila* cells, distinct (almost nonoverlapping) sets of myosin VI cargo/adaptor proteins were identified (Fig. 1). Given the weak binding affinity of the interactions detected, the different species/cells/tissues screened and the different methodologies used, it is not surprising that different interacting proteins were identified. Thus, a variety of complementary approaches will be required to screen a wide range of different species and cell types to yield the full complement of myosin VI binding partners.

In mammalian myosin VI, two regions in the C-terminal cargo-binding tail domain have been identified as cargo-binding sites: (*i*) a region containing an RRL sequence motif that binds GIPC, T6BP, NDP52, and optineurin, and (*ii*) a WWY motif that binds LMTK2 and Dab2 (11, 13). Between these cargo/adaptor-binding sites, a lipid-binding region has also been identified. The RRL motif is conserved between mammalian and *Drosophila* myosin VIs, whereas the WWY motif is changed to LWY in the *Drosophila* variant.

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Interestingly, the *Drosophila* homolog of GIPC called Kermit, which is likely to bind via the conserved RRL motif, is recognized as a myosin VI binding partner by Finan et al. (4). However, the *Drosophila* homolog of Dab2 was not identified (Fig. 1), which may be due to the W→L substitution in the conserved WWY motif in *Drosophila* myosin VI. It will be interesting to see if the other cargo/binding partners identified are functionally interchangeable in *Drosophila* and mammalian myosin VIs.

To confirm their initial biochemical/proteomic approach, Finan et al. (4) tested whether a number of the candidate proteins (major hits) would directly bind to the *Drosophila* myosin VI tail. Nearly half of the proteins tested bound directly and reproducibly to the myosin VI tail (Fig. 1), including Miranda (a protein already known to bind to myosin VI), Cornetto, Kermit (the ortholog of mammalian GIPC), and a number of presumed Golgi proteins and proteins similar to those in mammalian cells involved in vesicle trafficking. Focusing on one of the unique interacting proteins, Cornetto—a protein with no reported function—Finan et al. (4) show that it binds to *Drosophila* myosin VI with a relatively weak affinity (in the micromolar range). This weak affinity is to be expected, because it is now recognized that in the cell, multicomponent motor complexes are believed to initially exist as weak transient interactions that are assembled, reinforced, and processed for transport around the cell to their destinations, where they are induced to rapidly disassemble. Exploring the interaction in further detail, Finan et al. (4) demonstrate that myosin VI and Cornetto play a role in secretion of a specific protein (lipid-modified Hedgehog) in *Drosophila*. Although the adaptor proteins optineurin (in mammals; ref. 11) and Cornetto (in *Drosophila*; ref. 4) are unrelated proteins, they both mediate a role for myosin VI in the secretary pathway, suggesting that the cellular functions and pathways that require myosin VI appear to be conserved in mammals and *Drosophila*. Thus, further comparisons of the binding partners between the evolutionary distant myosin VI from mammals and *Drosophila* may provide important insights into the general mechanisms of cargo selection used in this class of myosin.

Finan et al. use a biochemical interaction screen to identify the cargo-binding partners of myosin VI/Jaguar in *Drosophila*.

The study by Finan et al. (4) convincingly demonstrates that myosin VI in *Drosophila*, like in mammalian cells, is able to associate with a large number of different adaptor proteins and cargoes, confirming the mammalian paradigm that one motor can execute multiple functions using an army of adaptor proteins. A future challenge will be to understand how myosin VI selects a specific cargo and how its attachment to adaptor proteins is regulated. Multiple binding partners compete for the two binding sites identified in the cargo-binding domain of mammalian myosin VI; therefore, the spatiotemporal control of adaptor protein concentration might determine which cargo binds. In the tail region, alternative splicing occurs both in mammalian and *Drosophila* myosin VI, giving rise in mammalian myosin VI to four different isoforms containing a small insert, a large insert, no inserts, or both inserts in the tail (14). How the presence or absence of these inserts influences the functions of myosin VI is not known; however, the different isoforms are expressed in a tissue-specific manner. It is thus possible that, depending on the isoform expressed, the cargo-binding region might undergo conformational changes to expose either one (RRL) or the other (WWY) binding site. Finally, the lipid composition of a specific membrane compartment may also assist myosin-cargo attachment and release, triggered by specific lipid signals and turnover.

It is now clear that cargo binding is not only crucial for the cellular functions of the myosins but also important for their regulation (14). Myosin VI can function both as a nonprocessive monomer and as a processive dimer. Binding of mammalian myosin VI to optineurin or Dab2 has been shown to induce dimerization, highlighting an important function of cargo attachment for regulating motor properties (15, 16). In addition, it has been suggested that all myosins may exist in an autoinhibitory compact state with the tail/cargo-binding region folded and interacting with the motor domain. Upon activation by specific signals such as phosphorylation or ligand binding, the cargo binds to its specific binding site, thus unfolding the tail, and the myosin adopts the extended active state ready for function—this is certainly the case in vitro for myosins II, V, and VII (17, 18), and it is also likely to occur in myosin VI.

A detailed characterization of the roles of myosin VI and its cargo/adaptor proteins in selected pathways in specific cells/tissues is needed to understand the precise cellular functions. In addition, further levels of complexity exist, because each motor does not function in isolation, but is part of a vast array of motor (both microtubule and actin-based) protein complexes, which under tight regulatory control act cooperatively to drive these cellular pathways.

1. Garret RH, Grisham CM (2010) *Biochemistry* (Brooks/-Cole, Boston), 4th Ed.
2. Brown SS (1999) Cooperation between microtubule- and actin-based motor proteins. *Annu Rev Cell Dev Biol* 15:63–80.
3. O'Connell CB, Tyska MJ, Mooseker MS (2007) Myosin at work: Motor adaptations for a variety of cellular functions. *Biochim Biophys Acta* 1773:615–630.
4. Finan D, Hartman MA, Spudich JA (2011) Proteomics approach to study the functions of *Drosophila* myosin VI through identification of multiple cargo-binding proteins. *Proc Natl Acad Sci USA* 108:5566–5571.
5. Wells AL, et al. (1999) Myosin VI is an actin-based motor that moves backwards. *Nature* 401:505–508.
6. Mermall V, Miller KG (1995) The 95F unconventional myosin is required for proper organization of the *Drosophila* syncytial blastoderm. *J Cell Biol* 129:1575–1588.
7. Geisbrecht ER, Montell DJ (2002) Myosin VI is required for E-cadherin-mediated border cell migration. *Nat Cell Biol* 4:616–620.
8. Lin HP, et al. (2007) Cell adhesion molecule Echinoid associates with unconventional myosin VI/Jaguar motor to regulate cell morphology during dorsal closure in *Drosophila*. *Dev Biol* 311:423–433.
9. Morris SM, et al. (2002) Myosin VI binds to and localises with Dab2, potentially linking receptor-mediated endocytosis and the actin cytoskeleton. *Traffic* 3:331–341.
10. Hasson T (2003) Myosin VI: Two distinct roles in endocytosis. *J Cell Sci* 116:3453–3461.
11. Buss F, Kendrick-Jones J (2007) Myosin VI: A multifunctional motor protein. *Myosins: A Superfamily of Molecular Motors*, ed Coluccio LM (Springer, New York), pp 325–352.
12. Sweeney HL, Houdusse A (2007) What can myosin VI do in cells? *Curr Opin Cell Biol* 19:57–66.
13. Spudich G, et al. (2007) Myosin VI targeting to clathrin-coated structures and dimerization is mediated by binding to Disabled-2 and PtdIns(4,5)P2. *Nat Cell Biol* 9:176–183.
14. Buss F, Kendrick-Jones J (2008) How are the cellular functions of myosin VI regulated within the cell? *Biochem Biophys Res Commun* 369:165–175.
15. Sweeney HL, Houdusse A (2010) Myosin VI rewrites the rules for myosin motors. *Cell* 141:573–582.
16. Yu C, et al. (2009) Myosin VI undergoes cargo-mediated dimerization. *Cell* 138:537–548.
17. Sellers JR, Knight PJ (2007) Folding and regulation in myosins II and V. *J Muscle Res Cell Motil* 28: 363–370.
18. Umeki N, et al. (2009) The tail binds to the head-neck domain, inhibiting ATPase activity of myosin VIIA. *Proc Natl Acad Sci USA* 106:8483–8488.