A reverse myosin moves with a kinesin-like mechanism

Myosins act as molecular motors in the cell, by transforming chemical energy generated by the hydrolysis of ATP into mechanical energy in order to move along actin filaments [1]. Myosin VI moves in the opposite direction to all previously characterized myosins [2] (Figure 1). A recent study has highlighted how the mechanism of this reverse myosin differs from that of plus-end myosins in order to promote successive steps in the opposite direction. Analysis of different structures was used to model the movement of myosin VI on actin, suggesting that this movement is closer to that of kinesins than conventional myosins.

REFERENCES:
[1] H. L. Sweeney HL & A. Houdusse, Annu Rev Biophys. 2010 39, 539
[2] H. L. Sweeney HL & A. Houdusse, Cell 2010 141, 573

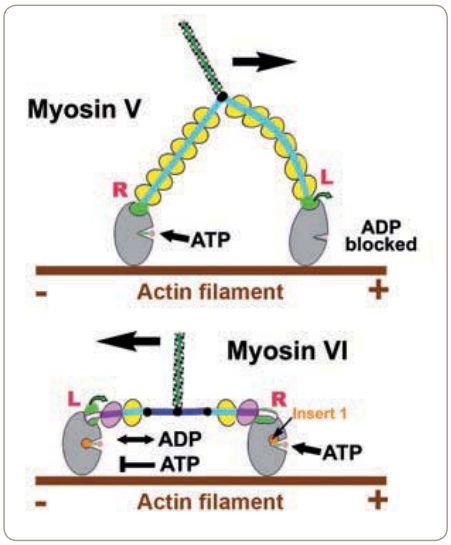

Figure 1: Diagram comparing, in the case of myosin V and VI, the mechanism involving two myosin heads of a dimer in motion. As myosin V and myosin VI move in opposite directions, the connection mechanism for communicating between the two heads of these motors is different. Myosin motor domains are shown in gray, with the converter subdomain in green. While the lever arm of myosin V is composed of a central rod (blue) bound to six calcium-binding proteins (yellow), that of myosin VI is different. In myosin V, the "lead" head (L) cannot release ADP while the "rear" head (R) has not released ADP, bound to ATP and detached itself from the actin. In myosin VI the lead head can release ADP and rebind ADP, but ATP binding is blocked until the rear head has detached itself. For more details, see Ref.1.

Myosin VI has very diverse roles in the cell. It is involved in endocytosis, secretory functions, the maintenance of stereocilia in inner ear hair cells, cytokinesis, cell adhesion, nuclear transcription, tumorigenesis and in the localization of neuronal growth cones. In these roles, it can act either as a transporter of cellular partners or as an anchor by which partners are securely attached to the actin cytoskeleton when opposite forces are applied.

Determination of several structural states of this reverse motor has allowed the authors to identify the specificities of myosin VI necessary for reverse directionality (Figure 1). These specificities include repositioning of the lever arm, rearrangements in the structure of the so-called converter region that can amplify the swing of the lever arm during the powerstroke, the mechanism by which the two myosin heads communicate to stay out of phase to facilitate their sequential movement, and a novel lever arm which includes a three helix bundle that unfolds upon dimerization of the motor [2].

In this *Molecular Cell* article, a comparison of several motor domain structures was used to model the processive movement of the motor and explain the origin of the variability of the steps achieved (Figure 2). An internal flexibility of the lever arm was demonstrated that allows a decoupling between the internal movements of the motor and the lever arm. Thus, the lead head can bind strongly to the actin filament on its new site (by releasing hydrolysis products), while maintaining a lever

PROXIMA1 & SWING BEAMLINES

Processive steps in the reverse direction require uncoupling of the lead head lever arm of Myosin VI

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arm in the rear position. The proposed model involves a rotation of the converter without rearrangements in its structure, thus keeping the orientation of the lever arm in place thanks to its internal flexibility in this state. Strong binding of the lead head indeed requires rearrangements of the motor domain that lead to a converter rotation. But unlike plus end motors, decoupling within the lever arm prevents the light chain binding region to directly follow this rotation. When the rear head is detached, the lever arm of the lead head is no longer constrained. Rearrangements can then occur in the converter leading to a 180° swing of the lever arm, followed by its recoupling. This promotes the transition for this head towards the rigor state, thus favoring the repositioning of the detached head towards the minus end of the actin filament, promoting movement in this direction. Therefore, to move in the opposite direction to other myosins, myosin VI has not changed the fundamental rearrangements that direct the converter when binding to actin. Instead, it has evolved into a mechanism similar to that of other motors such as kinesins, where these rearrangements are not coupled directly to the amplifying system of the lever arm movement.

To arrive at these conclusions, the "Structural Motility" research group at the Curie Institute in Paris crystallized several motor domain constructs with or without the myosin VI lever arm. These crystals were analyzed by X-ray diffraction on the PROXIMA1 beamline. SAXS measurements recorded on SWING made it possible to refute the suggestions of some molecular dynamics experts, who attributed the variation in step length to the internal dynamics of the converter in the lead head, while it re-attaches to the actin filament.

Figure 2: Superposition of the 3D model of myosin VI (MD-Ins2GFP) in the prepowerstroke state (blue, the state where the motor domain can hydrolyze ATP, which is in its active site) to the SAXS envelope (gray). In red, the position of myosin VI's lever arm if the converter fold would rearrange toward a conformation found in the rigor-like state (state where the motor domain has no nucleotide in the active site). These results indicate that the lead head of the motor adopts a well-defined orientation for its lever arm and that variability of the step sizes of myosin VI cannot thus come from the lead head exploring multiple conformations, as previously proposed.