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Regulation of the function of mammalian myosin and its conformational change

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Abstract

It has been known that the phosphorylation of the regulatory light chain, residing at the head/rod junction of the molecule activates the motor activity of smooth muscle and non-muscle conventional myosin (myosin II), and triggers a large conformational change of the molecule from the inhibited folded conformation to the active extended conformation. Recent structural analysis has revealed the structural basis of the inhibition of the motor function of the two heads in the inhibited conformation. On the other hand, recent studies have revealed that a processive unconventional myosin, myosin V, also shows a large change in the conformation from the folded to an extended form and this explains the activation mechanism of myosin V motor activity. These findings suggest the presence of a common scenario for the regulation of motor protein functions.

Conformational change and the regulation of myosin II

The regulation of actomyosin was first studied with skeletal muscle. Ebashi and Ebashi [1,2] found that a fraction extracted from muscle at very low ionic strength has a strong inhibitory activity against the superprecipitation, which was regarded as an in vitro model of contraction. This regulatory component was named native tropomyosin and subsequently found to be a complex of tropomyosin and troponin. A number of subsequent studies have established that the troponin–tropomyosin complex, the thin filament-linked regulatory components, is responsible for the Ca^{2+}-dependent regulation of striated muscle actomyosin [3]. On the other hand, a myosin-linked regulation mechanism was first observed in Molluscan muscle actomyosin [4]. It was found that a class of light chain, called regulatory light chain (RLC), is dissociated from myosin by EDTA, and this abolishes the Ca^{2+}-dependent regulation of actomyosin, thus making actomyosin constitutively active. Since RLC contains EF-hand Ca^{2+}-binding motifs, it was thought that the Ca^{2+}-binding to RLC is critical for the regulation. Subsequent 3D structural analysis revealed that the Ca^{2+}-binding site is actually in the essential light chain (ELC) and RLC stabilizes Ca^{2+}-binding to ELC [5].

The regulation of vertebrate smooth muscle actomyosin is also myosin-linked, but the mode of regulation is different from that of Molluscan actomyosin. It was found that smooth muscle myosin is phosphorylated at high, but not at low concentration of Ca^{2+}, and the phosphorylation is required for the activation of actomyosin [6–8]. Once myosin is phosphorylated, Ca^{2+} is no longer required for the activation. The Ca^{2+} sensor was identified to be a ubiquitous Ca^{2+}-binding protein, calmodulin. A calmodulin-dependent specific protein kinase, called myosin light chain kinase (MLCK), phosphorylates the RLC at Ser19 and this induces the activation of the mechanoenzymatic activity of smooth muscle myosin. While MLCK is the major protein kinase responsible for phosphorylating myosin II in smooth muscle, recent studies have suggested that Ca^{2+}-independent kinases play a role in non-muscle cells

Abbreviations: 3D, three dimensional; PKC, protein kinase C; PDGF, platelet derived growth factor; S1, myosin subfragment 1; HMM, heavy meromyosin.

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[9–13]. In migrating cells, ZIP (Zipper-interacting protein) kinase is critical for myosin II phosphorylation [14]. On the other hand, several protein kinases may be involved in the phosphorylation of RLC during mitosis [15–17].

While the phosphorylation of RLC at Ser19 is responsible for the activation of the motor activity of myosin II, RLC can be phosphorylated at other sites, i.e., Thr18, Thr9, and Ser1/Ser2. The phosphorylation at Thr18 was originally attributed to MLCK [18,19]. However, the rate of phosphorylation at Thr18 by MLCK is more than 1000-fold slower than that of Ser19, and it is not likely that the activation of MLCK induces Thr18 phosphorylation in cells. In contrast, it was found that ZIP kinase phosphorylates Ser19 and Thr18 with the same rate constant [12]. Therefore, it is anticipated that the protein kinases, which can phosphorylate Thr18 at a significant rate such as ZIP kinase are responsible for Thr18 phosphorylation in cells. It was also shown that ILK (integrin-linked kinase) can efficiently phosphorylate Thr18, and this kinase may also participate in Thr18 phosphorylation [20]. A critical issue is that each site has different effects on the myosin II function. While Thr18 resides right next to the Ser19 site, its effects on the myosin function may be different. It was reported that phosphorylation at Thr18 in addition to Ser19 further increases the actomyosin ATPase activity [18] and stabilizes filament formation [21]. Consistently, it was also found that the diphosphorylated myosin II is accumulated at the large stress-fibers and the cell cortical region while the singly phosphorylated myosin at Ser19 is present in the anterior region of the motile cells where dynamic actomyosin reorganization takes place [14]. These results suggest that Thr18 and Ser19, whose phosphorylation increases the ATPase activity and filament formation, are the activation sites of myosin. It was thought previously that mono-phosphorylated myosin at Thr18 does not exist since the rate of phosphorylation by MLCK at this site is much slower than at the Ser19 site. However, since Ca^{2+}-independent MLC kinases such as ZIP kinase phosphorylate the Thr18 site with the same potency as the Ser19 site, it is anticipated that mono-phosphorylated myosin II at Thr18 exists in cells. However, the functional nature of this myosin species is unknown and this is one of the important unanswered questions to be solved.

On the other hand, the phosphorylation of Ser1/Ser2 and Thr9 neither promotes the ATPase activity nor filament formation. In vitro studies have suggested that the phosphorylation of these sites decreases the affinity for actin thus inhibiting the actomyosin motor activity [22,23], therefore, these sites are referred to as the inhibitory sites. The protein kinases responsible for the phosphorylation at these sites are PKC [22–24] and cdc2 kinase [25]. While the rate of phosphorylation is greater for Thr9 than Ser1/Ser2 sites, the predominant phosphorylation sites in cells are Ser1/Ser2 [26] and this is thought to be due to the large difference in the dephosphorylation rates between these sites [27]. The importance of the phosphorylation at the inhibitory sites in cell contractility and motility has not been well studied. Quite recently, Komatsu and Ikebe [24] found that PDGF induced the phosphorylation of myosin IIA at the Ser1/Ser2 sites by activation of cPKC. This is accompanied by the loss of stress-fibers and cell shape change. It has been thought that myosin II in non-muscle cells does not form stable filament structure unlike in striated muscle cells. Since the dynamic reorganization of myosin filaments is critical for actin cytoskeletal reorganization during diverse motile events of the cells, it is likely that the phosphorylation of myosin II at the inhibitory sites plays a role in the dynamic reorganization of actomyosin II structure during various types of cell movements.

One of the unique features of the regulation of smooth muscle and non-muscle myosin II is that the filament formation in addition to the ATPase activity is regulated unlike that of striated myosin II. This is closely related to the phosphorylation-dependent change in the conformation of smooth muscle myosin II. Watanabe and his colleagues originally found that the smooth muscle myosin filament is disassembled upon the addition of ATP [28]. The 10S sedimentation velocity of disassembled myosin is significantly larger than that of the monomeric myosin in high ionic strength (6S). It was originally thought that the 10S component was the dimer of myosin [28], but electron microscopic observations showed that the tail of myosin bent back towards the head–neck junction in 10S myosin, thus showing high sedimentation velocity [29–32] (Fig. 1). Importantly, the phosphorylation of RLC destabilizes the folded 10S conformation [32–34]. In the folded conformation, one-third length from the tip of the tail associates with the head–neck junction of the molecule. Since RLC resides in the head–neck junction of myosin molecule, it is thought that the negative charge of the phosphate moiety destabilizes the ionic interaction between the RLC and the tail. Supporting this view, substitution of Ser19 of RLC by an acidic residue destabilized the folded conformation and induces filament formation in the presence of ATP. Since the folded conformation is not favorable for the intermolecular association at the C-terminal end of the tail domain that is responsible for filament formation, it is thought that the phosphorylation of RLC induces the elongated 6S myosin thus stabilizing the formation of myosin filaments [35].

On the other hand, the activation of the motor activity by phosphorylation may require the negative charge at the specific 3D location, since the substitution of Ser19 by acidic residues does not completely mimic the motor activity [21]. It has been suggested that the interaction between the two-heads is required for the regulation of the motor activity, since S1 having intact light chains of smooth muscle myosin is constitutively active [18]. Based upon the structural analysis of 2D crystals on a lipid monolayer, Wendt et al. [36,37] showed that the N-terminal portion of the motor domain of one head associates with the converter domain of the other head. According to their structural analysis of 10S myosin, one head is in the pre-

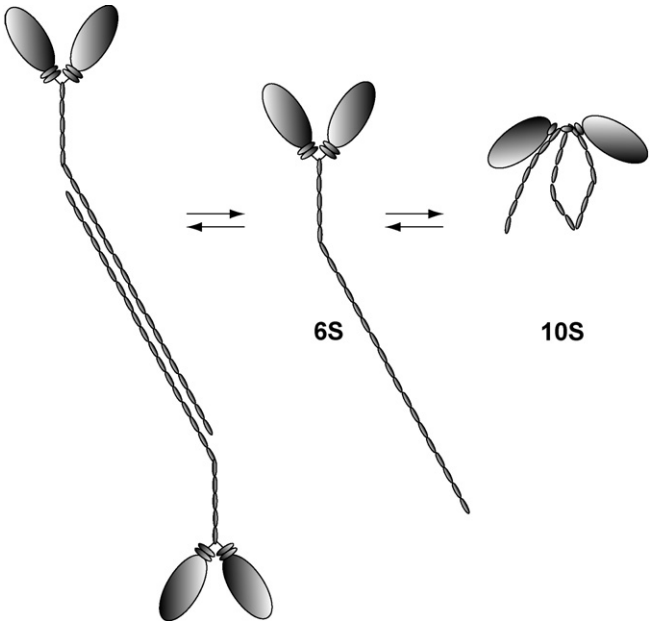

Fig. 1. Schematic drawing of the conformational transition of vertebrate smooth muscle and non-muscle myosin II. In the folded 10S conformation, one-third length from the tip of the tail associates with the head–neck junction of the molecule where the RLC resides. The negative charge of the phosphate moiety at Ser19/Thr18 of RLC destabilizes the ionic interaction between the RLC and the tail, thus destabilizing the folded conformation. Since the folded conformation is not favorable for filament formation, this induces the conversion to the elongated 6S myosin thus facilitating the formation of myosin filaments.

power stroke state while the other head is in the post-power stroke state. Based upon the analysis, it was proposed that one of the two-heads of this conformation cannot bind to actin, and the other head shows inhibited product release because of the hindered converter domain. A similar structure was also found in Tarantura myosin in the native thick filament using electron microscopic image reconstitution technique [38] but the functional regulation of this myosin is unknown. Quite recently, Burgess et al. [39], using negative staining electron microscopy and single particle image processing, reported that the two-heads of smooth muscle HMM in the inhibited state shows asymmetrical association resembling the blocked and free heads found by 2D crystal analysis. In addition, it was found that the tail of HMM lies between the heads contacting the blocked motor domain, unlike in the 2D crystal. The two-heads structure of myosin II is asymmetrical and it is anticipated that the asymmetrical structural nature can be reflected by functional asymmetry. To date, such functional asymmetry has not been clearly shown and further studies are required. On the other hand, it has been shown that the affinity of 10S myosin for actin is extremely low, suggesting that both heads may not be able to bind actin [18,40]. The result suggests that both heads may be in the pre-power stroke state. Recently, Salzameda et al. [41] proposed a model based upon chemical cross-linking of 10S myosin between RLC and the tail within a single molecule. Their model seems to be inconsistent with the structural model obtained from 2D crystal analysis. Sheng et al. [42] found by using cryo-atomic force microscopy that 50% of the molecules showed the two-heads separated in the inhibited state. The results did not support the structural model obtained by 2D crystal analysis. These findings are consistent with the functional data showing that the cross-talk between the two RLC lobes is important for the regulation of motor activity [43]. The structural basis of the inhibition of myosin II motor activity is controversial and further studies are required for clarifying the molecular mechanism of the regulation of myosin II motor function.

Conformational change and the regulation of myosin V

Myosin V is an unconventional two-headed myosin that processively moves along an actin filament [44–52]. This property is quite different from conventional myosin, which can strongly bind to actin within a short time during the cross-bridge cycling thus enabling many myosin heads in a thick filament to interact with an actin filament without interfering with each other. Myosin V strongly binds to actin within a large portion of the cycling time during the cross-bridge cycle, termed high duty ratio motor [53] and the two-heads concertedly interact with actin [46,48] thus walking on the actin filament for a long distance without dissociating from the actin track.

Each heavy chain of myosin V consists of a motor domain, an expanded neck domain that contains six IQ motifs that bind calmodulins or light chains, a series of coiled-coils separated by several flexible regions, and the C-terminal globular tail domain (GTD) [54,55]. Based upon the processive nature of its motor activity, it has been thought that myosin V functions as a cargo transporter and that the GTD is involved in the various cargos binding [56,57]. For example, melanophilin associated with the melanosome is a myosin V cargo molecule and plays the role as a linker protein to connect myosin Va and Rab27 in melanosomes [58–61]. Melanophilin binds to myosin V at the GTD in conjunction with the melanocyte myosin Va specific exon-F, which is present in the C-terminal short coiled-coil region [58]. One of the most important questions is how the transport of such cargos by myosin V is controlled. The regulation of the ATPase activities of myosin Va was first reported for chick myosin Va, and it was found that the actin-activated ATPase activity of myosin Va was significantly increased by micromolar concentrations of Ca^{2+} [55]. On the other hand, both the truncated two-headed myosin Va without the GTD domain (HMM-like construct) and the single-headed myosin Va (S1-like construct) showed no activation of the ATPase activity in Ca^{2+} [62,63]. Both truncated constructs have high ATPase activity in EGTA, which is similar to that of full-length myosin Va (M5aFull) in Ca^{2+}. The results suggest that the Ca^{2+}-dependent regulation observed for M5aFull is due to inhibition in EGTA rather than activation in Ca^{2+}. The over-all steady state ATPase activity in EGTA

is 1/10 of that in the presence of Ca^{2+}. Recent kinetic analysis revealed that the majority of the apparent steady state ATPase activity in EGTA is derived from the minor contamination of the unregulated (constitutively active) fraction of myosin Va, and that the ATPase activity in EGTA is less than 1/100 of that of the activated condition [64]. Quite interestingly, the Pi release rate is decreased more than 1000-fold in the inhibited state, which makes this step the rate limiting step for the actin-activated ATP hydrolysis cycle of M5aFull. Because of the large decrease in the Pi off rate, myosin Va spends a majority of the ATP hydrolysis cycling time in the weak actin-binding state. These findings suggest that myosin Va molecules at a low [Ca^{2+}] are inhibited as a cargo transporter not only due to the decrease in the cross-bridge cycling rate but also due to the decrease in the duty ratio thus being dissociated from actin.

A critical finding is that Ca^{2+} induces a significant conformational change of myosin Va under physiological conditions. Sedimentation velocity analysis revealed that the M5aFull undergoes a Ca^{2+}-induced change in the sedimentation coefficient from 14S to 11S, suggesting a large change in the conformation of the molecule [65–67]. In contrast, the truncated myosin Va without the GTD (HMM construct) or the S1 construct did not show the Ca^{2+}-dependent change in the sedimentation coefficient. These findings suggested that the full-length molecule forms a compact structure, in which the GTD bends back towards the motor/neck domain. The conformational change in the molecule was visualized by electron microscopy. At high ionic strength and at low Ca^{2+}, M5aFull showed an extended conformation that was similar to those images previously reported [55]. On the other hand, M5aFull showed a folded shape at low ionic strength in the presence of EGTA, in which the tail domain was folded back towards the head–neck region [65–67]. In the presence of micromolar Ca^{2+} concentration, an extended conformation was predominantly found even at low ionic strength. These results are consistent with centrifugation analysis and show that 14S M5aFull represents a folded conformation, while 11S M5aFull represents an extended conformation. The folded conformation has a triangular shape, suggesting that GTD directly contacts the motor domain [65]. Furthermore, the conformational transition is closely correlated with activation of the actin-activated ATPase activity of myosin Va [65–67]. These findings suggested that the conformational transition is closely correlated with activation of the actin-activated ATPase activity of myosin Va and that the inhibition of the ATPase activity is related to the interaction between the GTD and the motor domain. Supporting this view, it was found that the isolated GTD inhibits the ATPase activity of the tail-less myosin Va construct [68]. The result suggests that the GTD functions as an intra-molecular inhibitor of myosin Va. The GTD-induced inhibition of the actin-activated ATPase activity was studied with various tail-truncated myosin Va, and it was found that the C-terminal 57 residues of the first long coiled-coil domain are required for the GTD- induced inhibition [68]. The GTD-induced inhibition was correlated with the formation of the triangular folded shape of the complex of the tail-truncated myosin Va and the GTD. This finding further supported the idea that the inhibition of the ATPase activity is due to the formation of the triangular folded conformation of myosin Va. The result also suggests that the C-terminal end of the first long coiled-coil domain functions as the GTD anchoring site, thus stabilizing the folded conformation (Fig. 2). An important issue is how the GTD binding to the head domain inhibits the ATPase activity. According to the triangular conformation of the myosin Va, it was thought that the motor domain is flexible enough to interact with the GTD, which resides at the C-terminal end of the long coiled-coil domain. Supporting this view, it was shown that the deletion of any of two IQ domains at the neck abolishes the GTD-induced inhibition of the ATPase activity, while the additional deletion with comparable length to the two IQs in the coiled-coil restores the GTD-induced regulation [68]. The result suggested that the length of the neck and the coiled-coil is important for the head domain to interact with the GTD. Based upon the findings, a model for the formation of the inhibited state of myosin Va was proposed as follows. GTD binds to the C-terminal end of the first long coiled-coil. The neck–tail junction of myosin Va is flexible and the long neck enables the head domain to reach the GTD associated at the end of coiled-coil domain. Once the heads interact with the GTD, the triangular inhibited conformation is stabilized. The binding of the head domain to GTD prevents its conformational change during ATP turnover or interaction with actin, thus inhibiting the actin-activated ATPase activity of the motor domain.

The assignment of the GTD binding site in the motor domain is controversial. Taylor and colleagues proposed based upon 2D crystal analysis that the loop 1 which is near the entrance of the ATP-binding pocket in the motor domain is critical for the GTD binding [69]. They proposed that binding of the GTD to loop 1 decreases the rate of nucleotide exchange, thus inhibiting the ATPase activity of the motor domain. On the other hand, based on the averaged images of the inhibited myosin Va conformation obtained by electron microscopy and molecular modeling, Knight and colleagues proposed that the GTD binds to a lobe of the motor domain (P117–P137) that contains several conserved acidic residues [70]. Since the assigned GTD-binding site has no direct interaction with the ATP-binding site, they proposed that the GTD allosterically regulates motor activity. The crystal structures of the motor domain of chicken myosin Va [71] and the GTD of Myo2p, a yeast myosin V [72], were determined recently, and based upon these structures, it is unlikely that the GTD binds to both the lobe P117–P137 and loop-1 simultaneously. Quite recently, we found that the mutation of myosin Va at Asp136, a conserved acidic residue among the myosin V subfamily in the P117–P137 lobe completely abolishes the GTD-induced inhibition of the actin-activated ATPase

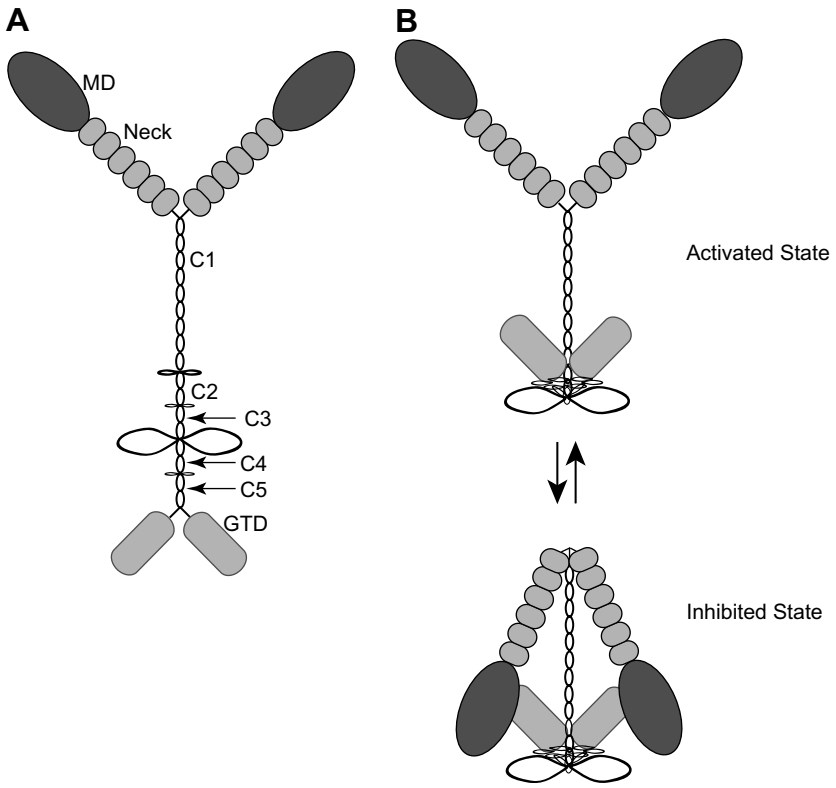

Fig. 2. Schematic drawing of the conformational transition of vertebrate myosin V. (A) Predicted structure of myosin Va. The motor domain (MD) is followed by a neck that consists of six bound light chains. The following 500 residues are predicted to form coiled-coils (C1–C5) separated by flexible regions. The C-terminal domain forms a globular tail. (B) The conformational model of the inhibited state and the activated state of myosin Va. In the activated state, myosin Va is in extended conformation, while the inhibited state of myosin Va shows a folded shape, in which the tail domain was folded back towards the head–neck region.

activity of myosin Va [73]. Furthermore, this mutation also disrupted the formation of the triangular conformation of myosin Va at low Ca^{2+}. The result is consistent with the structural model proposed by Knight and colleagues [70], and show that Asp136 is the GTD binding site in the motor domain critical for the inhibition of the motor function.

The important question is the mechanism by which the GTD inhibits the ATPase activity of the motor domain. Since Asp136 is away from the ATP-binding site and the actin-binding site, it is less likely that interaction of the residues in the GTD with Asp136 directly alters ATP-binding or actin-binding. It is known that ATP hydrolysis induces a small structural change in the motor domain that is connected to the converter and lever arm. In other word, the ATP hydrolysis cycle is closely coupled with the structural change of myosin. Therefore, it is likely that the ATP hydrolysis cycle rate is slowed if GTD binding to the motor domain inhibits the conformational change of the motor/converter domain. Based upon the 3D structure of the motor domain and GTD domain of myosin V, it is likely that the bound GTD at Asp136 is in close proximity to the converter and calmodulin bound to the first IQ. Therefore, it is likely that the bound GTD can interact with the converter/neck domain of myosin Va that prevents the movement of the converter/neck during the ATP hydrolysis cycle. The inhibition of the free conformational change may result in the inhibition of Pi release from the active site pocket. Supporting this view, it has been thought that Pi is released from the back door of the active site [74] and that a conformational change leading to the movement of the converter/neck occurs before Pi release [75].

As described above, a micromolar concentration of Ca^{2+} stimulates the actin-activated ATPase activity and induces an extended conformation of myosin Va. The activation by Ca^{2+} is presumably through calmodulin bound to the IQ motifs, since the bound GTD is in close proximity to the calmodulin bound to the first IQ. Therefore, it is plausible that the Ca^{2+}-induced conformational change of calmodulin associated with the first IQ disrupts the interaction between the motor domain and the GTD, thus abolishing the inhibition of the ATPase activity by the GTD. The mechanism of Ca^{2+}-induced activation of myosin Va motor activity requires further studies.

Since the cargo binding site is in the GTD domain, an important question is whether or not the binding of the cargo molecule activates the motor function of myosin V. Recently, it was reported that the actin-activated ATPase activity of myosin Va is enhanced by its cargo-binding protein, melanophilin [76]. Melanophilin activates the actin-activated ATPase activity of M5aFull in EGTA (inhibited state) to 20–30% of the maximum ATPase activity observed in Ca^{2+}. Considering that the activity in the

inhibited state is less than 1/100 of the maximum activity [64], malanophilin can activate the ATPase activity by at least 20- to 30-fold. Therefore, it is likely that the binding of melanophilin disrupts the interaction between the GTD and Asp136 in the motor domain, thus inhibiting the formation of the folded conformation. The above findings support the idea that the cargo binding to the tail domain of myosin Va in the inhibited state may interrupt the interaction between GTD and the head of myosin Va, thus shifting the conformational equilibrium toward the active state. It is known that myosin Va carries various cargo molecules and it is likely that the binding of these cargo molecules switches on the motor activity of myosin V. It is expected that such a mechanism is advantageous for the energy usage in cells. A question is whether the myosin V motor function in cells is regulated via a conformational transition found in in vitro studies. It has been shown that the majority of myosin Va shows diffuse localization in cells. Since the active myosin Va strongly binds to actin, it is anticipated that myosin Va is predominantly present in a low actin affinity form, i.e., the inhibited conformation, in cells. Since myosin Va is highly expressed in certain cell types [54,77], it is anticipated myosin Va would consumes a significant amount of energy if all myosin Va molecules in cells were in the active form. It would be advantageous for cells if myosin V becomes active only when its cargo transporting activity is required at certain location in cells. Further study is required to clarify whether or not the conformational change takes place in cells and is regulated by the binding of cargo molecules and/or change in the local Ca^{2+} concentration.

Is the tail inhibition mechanism common among the motor proteins?

The inhibited structure of myosin V resembles that of vertebrate smooth muscle and non-muscle myosin II, in which the tip of the one motor head binds to the converter region of the other. The inhibition of the converter/neck movement may be a common mechanism for the regulation of motor proteins. A similar tail inhibition model has been proposed for the regulation of kinesin [78,79]. The globular tail domain of kinesin binds to the neck domain to form a folded conformation and this is correlated with the inhibition of the motor activity [80]. It was found that full-length kinesin undergoes a 9S compact conformation to a 6S extended conformation, while the C-terminal domain of truncated kinesin shows extended conformation [78]. The ATPase activity of full-length kinesin is activated by cargo binding, while the C-terminal domain truncated kinesin is constitutively active. Furthermore, it was found that the expressed C-terminal globular domain inhibits the ATPase activity of the C-terminal domain of truncated kinesin [79]. These findings suggest that the regulatory function of the tail domain on the motor activity may be a common mechanism among the motor proteins. The regulation of the motor activity of other members of myosin superfamily is not well understood except for myosin I [81]. In the tail inhibition mechanism, it is thought that the tail domain of each heavy chain binds to the head of myosin. Therefore, the stability of the inhibited conformation is greatly facilitated by the binding between the tail and the head in each heavy chain. Other myosin family members such as myosin VI, VII, and X, are originally thought to be two-headed myosins like myosin V. However, recent studies have suggested that the predicted coiled-coil domains of these myosins are not stable enough to hold the two heavy chains together [82]. At present, it is unknown whether or not these myosins exist as dimers in cells, but it is likely that the regulation of these motor molecules is closely related to the monomer–dimer transition of these molecules in cells.

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