Molecular Structures and High Resolution TEM

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Structural analysis of the processive motor domain of myosin IX bound to actin filaments

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Myosins are molecular motors with the ability to convert chemical energy into mechanical force. The hydrolysis of ATP enables myosins to move along actin filament tracks. Myosins have diverse cellular functions and are important for cell morphology and migration. Class IX myosins belong to the unconventional myosins and show several unique motor properties compared to other myosin classes. Remarkably, they are able to take multiple successive steps along actin filaments without dissociating although they are single-headed motors. The successive hand-over-hand mechanism of double-headed myosins where always one of the two heads stays attached to the actin filament cannot be operating in myosin IX. The mechanism of the single-headed processive movement of myosin IX still remains to be determined. It is supposed that a long basic insertion of approximately 120 amino acid residues within loop 2 in the head domain acts as actin tether [1,2]. This would ensure a persistent binding to F-actin during the whole ATPase cycle and allow for a single-headed processive movement. In previous studies we have shown that deletion of the basic insertion in the head domain of myosin IXb from rat (Myo9b) is accompanied with a decreased actin affinity [3]. Furthermore, a calmodulin-binding site (CaM-BS) could be identified within the basic insertion [4]. CaM is sensitive to changes in the Ca^{2+} concentration and reacts with a conformational change. Therefor Ca^{2+}/CaM may regulate the Myo9 motor properties. The contribution of CaM to the processive behavior of myosin IX is still unknown. To elucidate the mechanism of single-headed processive movement, the binding behavior of the *C. elegans* Myo9 motor domain (Figure 1) to actin filaments and the structure of decorated actin filaments were analyzed by electron microscopy using negative staining. Phalloidin stabilized actin filaments were decorated with Myo 9 motor domain on grids with a 10-15 nm thin carbon film. For negative staining 1% uranyl acetate solution was used. Filaments were imaged using a Zeiss Libra 120 transmission electron microscope at an acceleration voltage of 120 kV. First experiments have shown that actin binding of Myo 9 motor domain seems to be regulated by a still unknown mechanism that differs from other myosin classes (Figure 2). Recombinant Myo9 protein fragments were created that contain solely the insertion of the motor domain with and without the CaM-binding site (Figure 1). These constructs will aid in the analysis of the binding behavior of the insertion and the putative regulatory function of the additional CaM-BS and contribute to a better understanding of how processive movement with a single motor domain is achieved.

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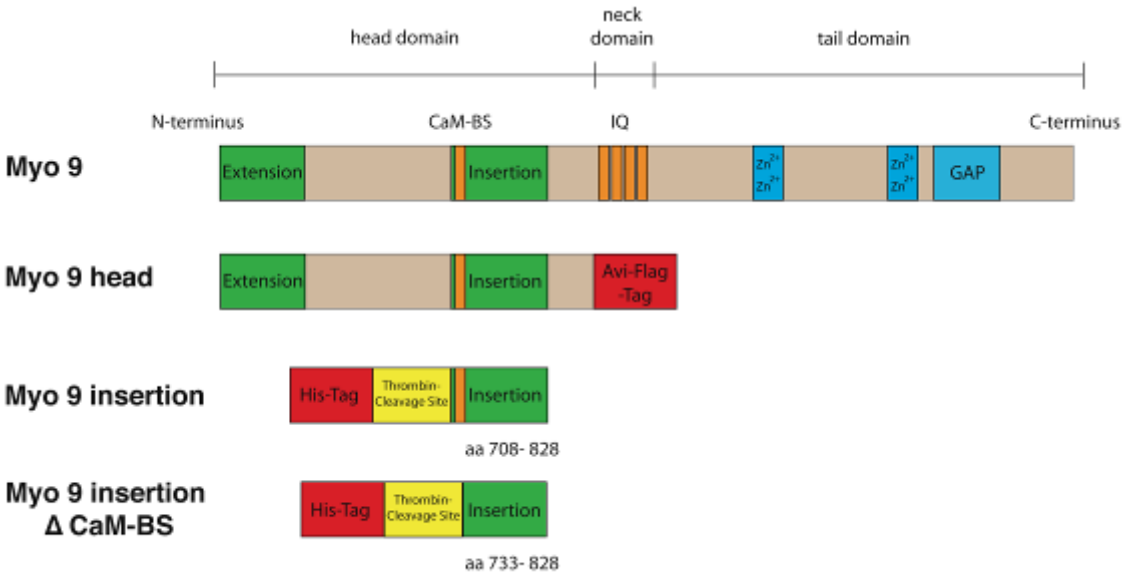

Figure 1. Schematic drawing of myosin 9 and used constructs. Myo 9 head contains a large basic insertion with a CaM-BS. Myo 9 insertion consists of solely the insertion with the CaM-BS. Myo 9 insertion Δ CaM-BS consists of a truncated insertion without the CaM-BS.

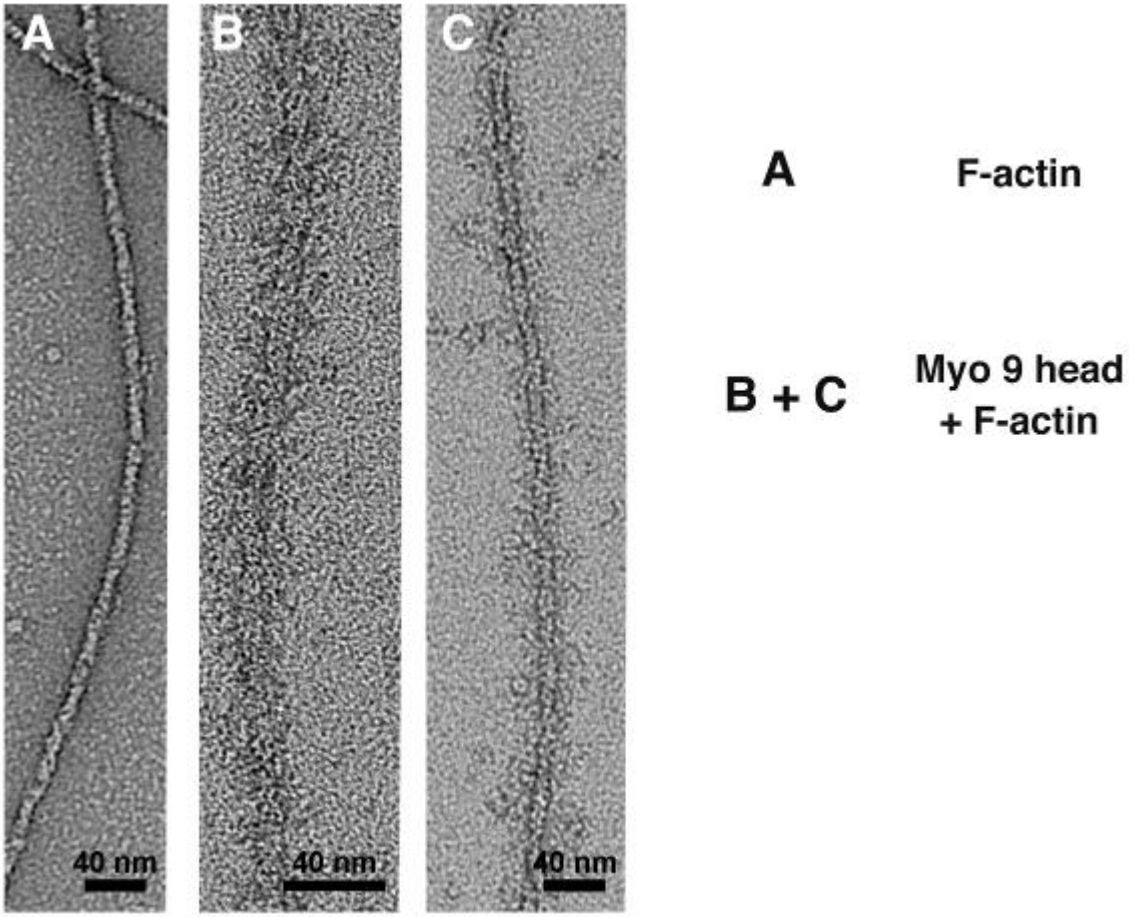

Figure 2. Actin filaments partially decorated with Myo 9 head. A) Phalloidin stabilized actin filament without Myo 9 head. B+C Phalloidin stabilized actin filaments partially decorated with Myo 9 head. Staining with 1% uranyl acetate. Scale bar 40 nm.