A plant calmodulin-binding motor is part kinesin and part myosin

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Kinesins and myosins are molecular motors that move on microtubules and actin filaments, respectively. These motor proteins are involved in a variety of cellular processes such as intracellular transport, spindle formation and chromosome segregation in eukaryotes (Langford, 1995; Hirokawa, 1998). Although kinesins and myosins hydrolyze ATP to generate force for their movement on cytoskeletal filaments and have similar structural organization (head/motor, stalk and tail) there is virtually no amino acid (aa) sequence identity between the microtubule- and actin-based motors (Kull et al., 1996).

We report here that a novel calmodulin-binding protein from plants has domains that are present in microtubule- and actin-based motors, suggesting that this class of plant motors is unique in having kinesin and myosin features. In a protein–protein interaction based screening with calmodulin we recently isolated a cDNA from Arabidopsis encoding a novel calmodulin (CaM)-binding protein, KCBP, with a kinesin-like motor domain at the C-terminus of the protein (Reddy et al., 1996). Motility studies with the C-terminal kinesin-like motor domain alone have shown that it is a minus-end directed microtubule motor (Song et al., 1997). KCBP is unique among all known kinesins and kinesin-like proteins in having a CaM-binding domain (CBD). The homolog of KCBP has been cloned from three other plant systems, suggesting that it is ubiquitous in flowering plants (Narasimhulu and Reddy, 1998). However, a KCBP homolog has not been found in yeast and Caenorhabditis elegans, whose genomes have been completely sequenced, or in any other non-plant systems.

In our ongoing analysis of KCBP function, we analyzed KCBP sequence using BLASTP and SMART programs (http://coot.embl-heidelberg.de/SMART) and identified, in addition to kinesin-like motor domain at the C-terminus, a region that is conserved in the tails of some members of myosins (Figure 1). The C-terminal region (amino acids 860–1217) of KCBP showed similarities with the motor region of a large number of microtubule-based motors of which KIFC3, a C-terminal motor, showed the highest sequence similarity (43% identity and 56% similarity, Figure 1A). The tail of myosin VIIa has two long repeats (about ~460 aa per repeat), each containing a MyTH4 (myosin tail homology 4) domain (~110 aa) and a talin-like (~350 aa) domain. Although the MyTH4 domain and the talin-like regions exist singly in some myosins (IV and XII) and in the band 4.1 superfamily (examples include talin and ERM proteins), respectively, the presence of these two regions together (MyTH4+talin-like) occurs only in myosin VIIa (as two repeats) and myosin X (one repeat) (Chen et al., 1996). The N-terminal tail (aa 121–612) of KCBP showed significant similarities to the MyTH4 and talin-like (band 4.1) regions present in myosins VIIa and X. The MyTH4 and the talin-like domains of KCBP showed the highest similarity with the myosin VIIa tail (29% identity and 45% similarity in the MyTH4 domain and 23% identity and 37% similarity in the talin-like region) (Figure 1B). The MyTH4 domain and talin-like region have not been found in any other known members of the kinesin superfamily. Therefore, we conclude that the KCBP is a molecular hybrid consisting of a motor domain from microtubule-based motors and a tail region of actin-based motors (Figure 1C).

The significance of MyTH4 and talin-like domains in KCBP and myosins is not known at this time, but the existence of talin-like region together with MyTH4 domain in KCBP, myosin VIIa and X is interesting and points to some functional significance. Based on what is known about KCBP and myosin VIIa, we propose that these domains may be involved in one or more of the following functions. One possibility is that the KCBP and myosin VIIa-like proteins could interact with some unknown common protein(s) through their tail homology regions to either cross-bridge microtubule and actin filaments or facilitate cargo exchange between these two types of molecular motors. An elegant study by Lille and Brown (1992) showed that a lethal mutation resulting from a defect in a myosin motor could be complemented by a kinesin motor protein which also suggests that actin- and microtubule-motors are likely to interact with some common set of proteins. Furthermore,
Fig. 1. Comparison of Arabidopsis KCBP sequence (AtKCBP, L40358) with human KIFC3 head (HsKIFC3, AF004426) and myosin VIIa tail (HsMyoVIIa, U55208) regions. The star and plus symbols indicate identical and similar amino acids (aa), respectively, between KCBP and other motors. The dashes indicate the gaps in alignment. (A) Alignment of head domains of KCBP (aa 860–1217) and KIFC3 (aa 279–636). (B) Alignment of KCBP tail region (aa 121–612) with the two repeats of MyoVIIa tail region (aa 1116–1453; 1713–2175). (C) Schematic diagram of KCBP, MyoVIIa and KIFC3 proteins. The blue color represents conserved tail regions between KCBP and MyoVIIa and the red color represents conserved head domains between KCBP and KIFC3 proteins. MyTH4, myosin tail homology 4; talin-like, talin-like domain; CC, coiled-coil region; IQs, calmodulin-binding IQ motifs; CBD, calmodulin-binding domain.
in a recent study, Huang et al. (1999) have shown a direct interaction between the tail regions of a microtubule-based kinesin and an actin-based myosin. This kind of interaction may facilitate the transfer of cargo from kinesin tail to myosin tail. On the other hand, the talin-like region in these motors may be involved in anchoring the microtubules and actin filaments to plasma membrane as the corresponding region in talin has been shown to bind membranes (Girault et al., 1998). Biochemical studies with the myosin VIIa full-length gene product showed its tight association with membranes probably through its talin domain (Girault et al., 1998). Functional studies with KCBP have shown its involvement in cell division, especially in some plant-specific processes and in trichome morphogenesis. Recently in a genetic screen Krishnakumar and Oppenheimer (1999) have isolated three extragenic suppressors of a mutation in the tail region of KCBP, suggesting that there are at least three gene products that are likely to interact with the tail region of KCBP. The identity of these suppressor gene products is currently unknown. These genetic studies coupled with the yeast-two hybrid system should help identify the proteins that interact with the KCBP tail.

Another interesting feature shared by the KCBP and myosins is the presence of a CBD. However, the CBDs are found at different locations in these motors (see Figure 1C) and the amino acid sequence of CBDs is not conserved between KCBP and myosins. A calcium-dependent CBD is mapped to the C-terminus (1217–1240) of KCBP (Reddy et al., 1996) and the binding of calmodulin to motor inhibits the interaction of KCBP with microtubules (Song et al., 1997). The IQ motifs in myosins bind CaM in the absence of Ca$^{2+}$ and Ca$^{2+}$ induces the dissociation of CaM light chains from myosins, resulting in inhibition of motility (Hasson and Moseker, 1995). Therefore, calcium stops the motility of KCBP and myosins by two very different mechanisms.

The presence of kinesin and myosin features in KCBP raises an interesting question about the evolutionary relationship between these molecular motors. Did the members of the kinesin and myosin superfamilies evolve from a common ancestor through divergent evolution or from separate ancestral proteins through independent pathways (convergent evolution)? To understand the evolutionary relationship between proteins showing no sequence identity (for example, motor regions of myosins, kinesins and their related G proteins), Kull et al. (1998) formulated several criteria to evaluate evolutionary relationships among such proteins. Using these criteria it was concluded that the motors of kinesins and myosins have evolved from an ancestral proto-motor by divergent evolution (Kull et al., 1998). The sequence similarities between the tails of a kinesin and myosins support the hypothesis that the molecular motors may have evolved from a common ancestral protein.

In summary, KCBP is the only known motor protein with a kinesin head and myosin tail. In addition, KCBP is the only known kinesin-like protein with a calmodulin-binding domain. These features make KCBP unique among members of the kinesin superfamily in eukaryotes. Based on sequence analyses and functional characterization of different regions of KCBP, we conclude that the KCBP is part kinesin and part myosin.

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References


