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Sequence and Phylogenetic Analysis of Squid Myosin-V: A Vesicle Motor in Nerve Cells

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We have shown that vesicles in the axoplasm of the squid giant axon move on actin filaments and that movement is inhibited by myosin V-specific antibodies [Tabb et al., 1998]. In the study reported in this article, experiments were performed to clone and sequence the cDNA for squid brain myosin V. Five proteolytic fragments of purified squid brain myosin V were analyzed by direct protein sequencing [Tabb et al., 1998]. Based on this sequence information, degenerate primers were constructed and used to isolate cDNA clones by PCR. Five clones, representing overlapping segments of the gene, were sequenced. The sequence data and the previous biochemical characterization of the molecule support the classification of this vesicle-associated myosin as a member of the class V myosins. Motif analysis of the head, neck, and tail domains revealed that squid MyoV has consensus sequences for all the motifs found in vertebrate members of the myosin V family of motor proteins. A phylogenetic tree was constructed from a sequence alignment by the neighbor-joining method, using Megalign (DNAStar, Madison, WI); the resulting phylogenetic tree showed that squid MyoV is more closely related to vertebrate MyoV (mouse *dilute*, chicken *dilute*, rat *myr6*, and human *myo5a*) than *Drosophila* and yeast (*myo2*, and *myo4*) myosins V. These new data on the phylogenetic relationships of squid myosin V to vertebrate myosin V strengthens the argument that myosin V functions as a vesicle motor in vertebrate neurons. Cell Motil. Cytoskeleton 46:108–115, 2000.

Key words: Key words: axonal transport; actin filaments; vesicle/organelle movement; giant axon; molecular motor

INTRODUCTION

The movement of vesicles from the neuronal cell body to the axon and dendrites requires molecular motors. We have shown that vesicles in the giant axon of the squid, undergoing fast axonal transport, are transported on microtubules and on actin filaments. Using video microscopy, we observed individual vesicles switching from microtubules to actin filaments and vice versa [Kutnetsov et al., 1992, www.dartmouth.edu/langford/Movies.html]. The ability of vesicles to switch from microtubules to actin filaments provides a means for vesicle transport in actin-rich regions of the cell that do not contain microtubules, such as the axon terminal and dendritic spines. We have succeeded in identifying vesicles of the smooth endoplasmic reticulum (ER) as one specific class of organelle that moves on actin filaments and microtubules. An antibody to an ER-resident protein, protein disulfide isomerase (PDI), was used to identify the smooth ER [Tabb et al., 1998], and an antibody to purified squid brain myosin V was generated and used in

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immunogold EM studies to show that this myosin localizes to the isolated ER vesicles [Tabb et al., 1996]. Dual labeling with the squid MyoV antibody and a kinesin heavy chain antibody showed that these two motors colocalized on the same vesicle [Tabb et al., 1998]. Myosin V was also purified from squid brain and its identity confirmed by comparisons of amino acid sequences of tryptic peptides of this myosin with those of other known members of the myosin V family [Tabb et al., 1998]. RT-PCR was used to clone a 0.6-kb fragment in the head domain of the protein and a 0.5-kb fragment in the tail domain [Molyneaux and Langford, 1997]. The sequences of the cloned fragments showed homology to the other members of the myosin V family.

The present report presents the complete sequence of squid brain myosin V and a new phylogenetic tree for this family of myosins. A previous study showed that two different antibodies to squid brain myosin V inhibited movement of vesicles on actin filaments [Tabb et al., 1998]. Both antibodies inhibited actin-based motile activity (number of vesicles moving/field/min) in axoplasm by greater than 90%, as compared with motility assays with control antibodies [Tabb et al., 1998]. These studies provided direct evidence that vesicles in the giant axon of the squid are transported on actin filaments by myosin V [Langford, 1999]. These data confirm the role of actin filaments in vesicle movement and provide support for the dual filament model of vesicle transport [Langford and Molyneaux, 1998]. In support of these results are the observations that Purkinje cells in the brains of rats and mice carrying the *dilute* mutation, a myosin V defect, failed to localize ER to dendritic spines [Dekker-Ohno et al., 1996]. Collectively these recent studies strongly argue for the role of myosin V as an important organelle motor in neurons. A loss of its function could lead specifically to loss of calcium signaling in both the dendrites and axons.

MATERIALS AND METHODS

mRNA Purification and cDNA Synthesis

Molecular cloning methods were performed according to Sambrook et al. [1989]. To isolate mRNA for the amplification of the cDNA fragments, 1 g of squid optic lobe was homogenized in 10 ml Trizol (Gibco-BRL, Gaithersburg, MD). Aliquots were frozen in liquid nitrogen and stored at −80°C. Total RNA was isolated from the homogenate according to the manufacturer's instructions. mRNA was isolated with the PolyATtract mRNA Isolation System (Promega, Madison, WI) according to the manufacturer's instructions and used to synthesize double-stranded DNA with the Marathon cDNA Amplification Kit (Clontech, Palo Alto, CA).

Designing Primers for the Polymerase Chain Reaction (PCR)

Degenerate primers (k1−k5) were designed according to Koelle and Horvitz [1996] from short sequences of squid myosin V obtained through peptide sequencing [Tabb et al., 1998]. Based on the alignment of these peptide segments in homologous proteins, forward primer fk1 (AA(CT)-CCI-ATI-ATG-GA(AG)-(AT)(GC)I-TT(CT)-GG(TAGC)-AA (CT)-GC) and reverse primer rk2 (TT(ACGT)-GC(AGCT)-ACI-ATC-CA(AG)-TC(AG)-AA) was predicted to define a PCR fragment of approximately 0.6 kb in the head domain of squid myosin V. The fk4 (GA(AG)-CA(TC)-GA(GA)-GCI-ATI-GCI-GGI-(TC)T(ACGT)-AA(CT)-AA) and the rk3 (AT(TGCA)-GCI-ACI-GT(GA)-TC(AG)-TC(CT)-TC (AG)-TA) primers were predicted to define a region of 0.5 kb in the tail domain. The fk5 (GCI-GT(ACGT)-GA(TC)-CCI-GA(AG)-GT(ACGT)-AT(ACT)-AC) and rk3 primers were predicted to define a region of 120 amino acids that were nested within the fk4/rk3 fragment. For the 4-kb fragment, the specific primers fk6 (CAA-ACC-AAG-GAG-AAA-GTC-CGC-ACA-TCC-AG) and rk7 (AAC-CGC-ATG-ATT-TCT-CAG-CAC-ACG-CAG-AT) were designed from the sequence data obtained from the 0.5-kb and 0.6-kb fragments using the program OLIGO [Rychlik, 1992]. Primers were synthesized by Integrated DNA Technologies (Coralville, IA).

Polymerase Chain Reactions

PCR reactions were performed in a Perkin-Elmer GeneAmp 2400 in 0.2-ml thin-walled tubes (Perkin-Elmer, Foster City, CA) with Advantage cDNA polymerase (Clontech). The following "touchdown" PCR program was run: 94°C × 30 s; 5 cycles of 94°C × 5 s, 72°C × 4 min; 5 cycles of 94°C × 5 s, 70°C × 4 min; 25 cycles of 94°C × 5 s, 68°C × 4 min. The 4-min extension time was used for the 4-kb fragment; 2 min was used for the other fragments.

Cloning

cDNA fragments were electroeluted from agarose gels and directly ligated into the T/A overhang of the pGEM-T vector (Promega) according to manufacturer's instructions. The ligations were electroporated into TB1 *Escherichia coli* and plated on IPTG, X-Gal, and 100 μg/ml ampicillin-containing agar plates.

Sequencing

The minipreparations of plasmid DNA were performed according to manufacturer's (Promega, Madison, WI) instructions. Sequencing reactions were performed by the dideoxy method with ABI Prism Big Dye Terminator reactions (Perkin-Elmer) and sequenced by the Dartmouth Molecular Biology Core Facility with an ABI

Automated Sequencer (Perkin-Elmer). A minimum of three clones was sequenced in both the forward and reverse directions for each cDNA fragment and a primer walk strategy was used to sequence the 4-kb fragment. Sequence compilation and analysis were performed using Gene Inspector and Gene Construction Kit (TextCo, Lebanon, NH), the LaserGene Suite (DNAStar, Madison, WI) and Genetics Computer Group (GCG, Madison, WI) computer programs.

Phylogenetic Analysis

Sequence alignment of the myosin superfamily for the creation of the phylogenetic tree was performed using Megalign (DNAStar, Madison, WI) and the blosum62 amino acid table. Myosin V sequences were obtained from the NCBI database and aligned from N-terminus to C-terminus. Gene Inspector and Megalign were used to produce multiple sequence alignments using a progressive pairwise method. The clade was created using myosin V sequence data from arthropods (*Drosophila melanogaster*), vertebrates (*Homo sapiens*, *Gallus gallus*, *Mus musculus*), fungi (*Saccharomyces cerevisiae*), and mollusks (*Loligo peleai*). Human myosin VI was used as the outgroup.

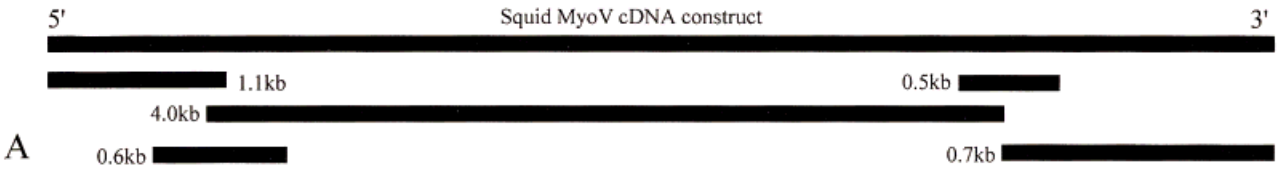
RESULTS

Five overlapping PCR fragments were cloned to obtain the gene sequence of squid brain myosin V (Fig. 1a). To obtain the first PCR fragment, squid optic lobe cDNA was amplified with the fk1/rk2 primer pair, using the polymerase chain reaction (PCR) at $MgCl_2$ concentrations ranging from 0 to 8.0 mM. A 0.6-kb fragment was obtained in the PCR reaction containing 1.5 mM $MgCl_2$. The reactions were re-amplified, run on a 1.0% agarose gel and the 0.6-kb band in each gel lane was excised. The DNA was eluted and used directly as the template for sequencing reactions in the forward and reverse directions. A second PCR fragment of 0.5 kb was obtained by PCR with squid optic lobe cDNA and the fk4/rk3 primers. Using the 0.5-kb product as a template, the nested primers, fk5 and rk3, yielded the expected 0.375-kb fragment. The 0.5-kb fragment was ligated into the pGEM-T vector and cloned into TB1 *E. coli* A third PCR fragment of 4 kb between the 0.6-kb and 0.5-kb fragments was amplified with specific primers designed from the sequences of the smaller fragments. A fourth PCR fragment of 0.7 kb at the 3′ end of the gene was amplified with a specific primer and 3′ RACE using an oligo dT primer. A fifth PCR fragment of 1.1 kb at the 5′-most end of the gene was amplified with a specific primer and 5′ RACE using the AP1 primer ligated to the 5′ end. A minimum of three clones for each fragment were sequenced in both directions, and the base sequence for the squid myosin V gene was determined. Both upstream and downstream UTRs were examined carefully to identify the start and stop codons. The deduced amino acid sequence is shown in Figure 1b. The highly conserved ATP-binding (dark green) and actin-binding (light green) motifs in the head domain and IQ motifs (red) in the neck domain are highlighted. The AF6/cno domain in the globular tail that binds kinesin [Huang et al., 1999] is highlighted in blue.

Figure 1c shows a schematic diagram of the primary structure of squid MyoV. Specific conserved functional sites are indicated, including a putative PEST site (brown), a region rich in proline, glutamate, serine, and threonine and the site where calpain cleaves the polypeptide into separate head and tail components. Calpain, a Ca^{2+}-activated protease, cleaves both chicken and squid MyoV immediately after the PEST site yielding a 130-kD head fragment and an 80-kD tail fragment [Nascimento et al., 1996; Molyneaux et al., 1997]. The AF6/cno domain in the tail that has been shown to bind kinesin [Huang et al., 1999] is indicated in blue. A 3D model of the MyoV homodimer with light chains bound to the neck region is illustrated in Figure 1d.

The deduced amino acid sequence of squid brain MyoV was aligned with other class V myosins to identify sites of conservation and divergence in each region of the molecule (Fig. 2). The most conserved sequences were within the head (Fig. 2a–f) and neck (Fig. 2g) regions at functional sites involved in ATP, actin, and calmodulin binding. The sequences in the tail region (Fig. 2h–l) were the most divergent part of the protein; however, a region within the AF6/cno region (Fig. 2l) of the tail is conserved among most species (Fig. 2, light blue). Huang and colleagues [1999] showed the binding site for kinesin to be a function of this site. A hydrophobicity plot (Fig. 3) of the squid MyoV tail showed that it contains an obvious hydrophilic region corresponding to the domain identified as the putative kinesin binding motif [Huang et al., 1999].

Motif analysis of the head, neck and tail domains revealed that squid brain myosin V has consensus sequences for all the major motifs found in other members of the myosin V family (Fig. 2). In the head domain (aa1–764), the ATP- and actin-binding sites occur at aa166–179 and aa505–533, respectively. The head domain contains three loops typical of myosin motors. Sequences corresponding to loops 1, 2, and 3 are indicated in Figure 2. Loop 1 is thought to be responsible for determining the velocity at which myosin moves along actin filaments and loop 2 has been suggested to control the rates of phosphate release and to set the V_{max} for ATPase activity [Rayment et al., 1993]. The neck domain (Fig. 2g) is characterized by 6 IQ motifs, each of which contains the consensus sequence IQxxxRGxxxRxxY.



A

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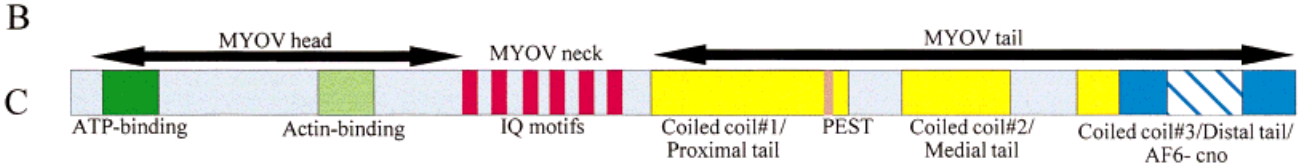
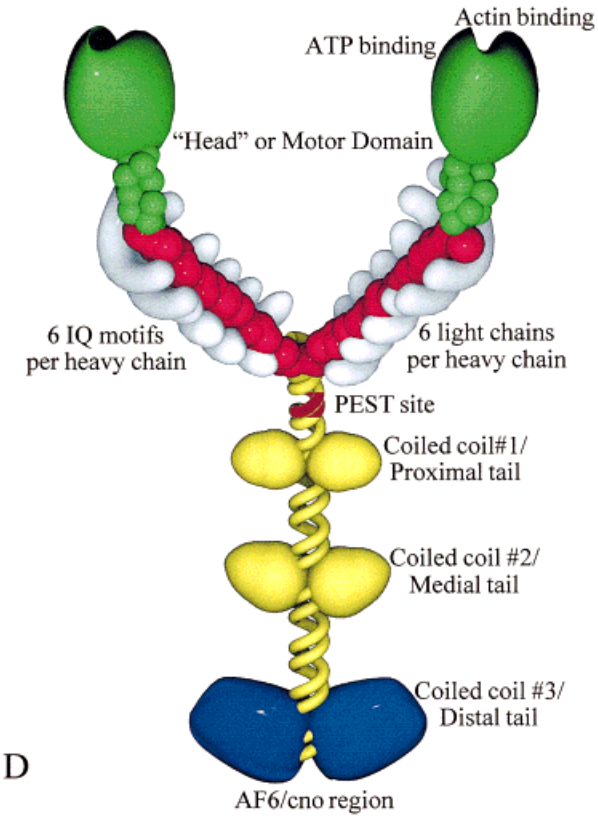
QAPNERNYHIFYQLCASSKCAELKSFQLTHQDKFLYTNQGESPHIQEIDDADLFIKTRSAFTELGISEDDQMKMFGIVSAILHLGNIAFESGEDESTCCVSKKNSHFDIVCDLLSLNKDEMQMWLCN
RQITSGSERIIKPLTAKEAGYGKDALAKHIYARVFDWIVAKINRNLLTHEDTQNFIGVLDIYGFETFRINSFEQFCINYANERLQLQFNTRVFTLEQQEYRKEGLEWHEISHYDNTPCIDLIESS QGIL
ASLDDECKMMSGNDANWCTSLFKKLSGTKCFEKPRTSQTSFTVCHFAEKVTYMCHGFMEKNMDTVSDLQIETLKGSTNPLVMELFQEKKLERKMSTMSEESYLNQPKKSKKHKQTVGISVQ
RILEQFDEDSLRHQPSLRRCIKPNDEKKPFRFNCKRVVEQLRACGVLETIRISAAGFPARETYENIFNMFQQLLDVIEVDKSDPKLSCEKLMQKYIEDPDKYRFGKTKIFFRAGQLALLDKLLSDRRI
RWSVMVQKQVRTFLCKRRFNKIRRSIFLLQVYGRGYLARLAFQERRRKFAAIRIQAYFRGYLCRKIYQQKKLAAIVVQRYARKALAQRLFRQLVEDHKATVIQTRVRAYLARRKFEKVR
RGMVLLQSHVRRRAAKKVFKELKRKAKDSDELKLSNRRLCNKIIELSNILKEKEGDLKKFRAIQSSTSQVQEQYEKLVSEHDNCRVLKVKIQEYEFMIERLEGDIKHKTEEFTVLMAEKNKMKT
SYDDDKQTMEKKNQELHQQLLDAQSLIKTNEETIANYVKKEEDHSALDNQKEREIEKAHHNKILHDYEVLKNHYDNLRHEMTVLKQTHRRTPSDNSTVSFESAEGEPVAAEPEG EEDEERQV
AHVVAQVVAEPDDKEDQGYGTGKRQERPIPAERKSRTVQRQATLDEPEKVDLALIMKLQNRIKELEKEIKRLNTERERREEEENKDKEHTVYNSLKMQELENDNDRMKREINNLMTAISKSPKY
EEGITDAGKAFKEKYDTMVEELERRRAEILYIKAIWLETGIEKKDDLHAKHGGEDVSEEEELKTALQFHQKLNRLLENQLQETEKKSKHTEQDLLTQIEELTKENEQKQVISQLNSIATNLSSKTG
DEISVTMGQDIIRITTENLELRETVSKQTEQIRKLKKTLKVYARKLKDGEGPFRRFQSLVSATSLSPTSAAEIAAELDRDEQESSGVMAAVKHQEREYLGMLEYNKVDESALIKNLVHDLQPYVAE
SMLPGLPAYIIFMCIRHTDHINDDEKVCALLTGVVNGIKRVVKKSNNDVERMTLWLANSCRLLHNLKQYSGEKRYQT SNTPKQNEHCLRNFDLSEYRPVFNDLCVYNYRQLIKVMKDNIEK
LIVPAILEHEAIAGLNKDDRRGRVPTNETEPDALDNLQKIMSQYLRVLRNHAVDPEVITLIVKQLFYDMSVKALNNLLLRRDMCNWHKGTQIRYNISHLEQWLREYHLQDAGAFSTM
EPLIQASQLLQARKTDADVDSVCQMCPKLKTAQIIKILNQYTPVRGYEDDTAISFIRKVQEKLSQTRETDMGTNLLMDTQYAFPVTFPFNPSNIALERIVPDKLHLGFIKRV

B



C



D

Fig. 1. Diagrammatic representation of the PCR fragments used to sequence squid brain myosin V and the deduced amino acid sequence. **A:** Locations of the 5-cDNA clones used to sequence squid MyoV. The sequence of the 4-kb fragment was used to generate primers for sequencing the 1.1-kb and 0.7-kb fragments (GeneBank accession number AF197336). **B:** Deduced amino acid sequence of squid MyoV highlighting the conserved ATP-binding site (green), actin-binding site (light green), IQ domains (red) and the region of the tail previously shown to bind kinesin (blue) [Huang et al., 1999]. **C:** Schematic diagram of squid brain myosinV showing the location of several notable motifs including the PEST site and the α-helical coils in the tail portion of the protein that allow the homodimer to form. **D:** 3D model of squid MyoV in its homodimeric form. Based on electron micrographs and the predicted secondary structure of chicken myosin V [Espreafico et al., 1992; Larson, 1996]. The regions are color-coded for each structural motif. The same set of colors is used in each panel, and in Fig. 2.

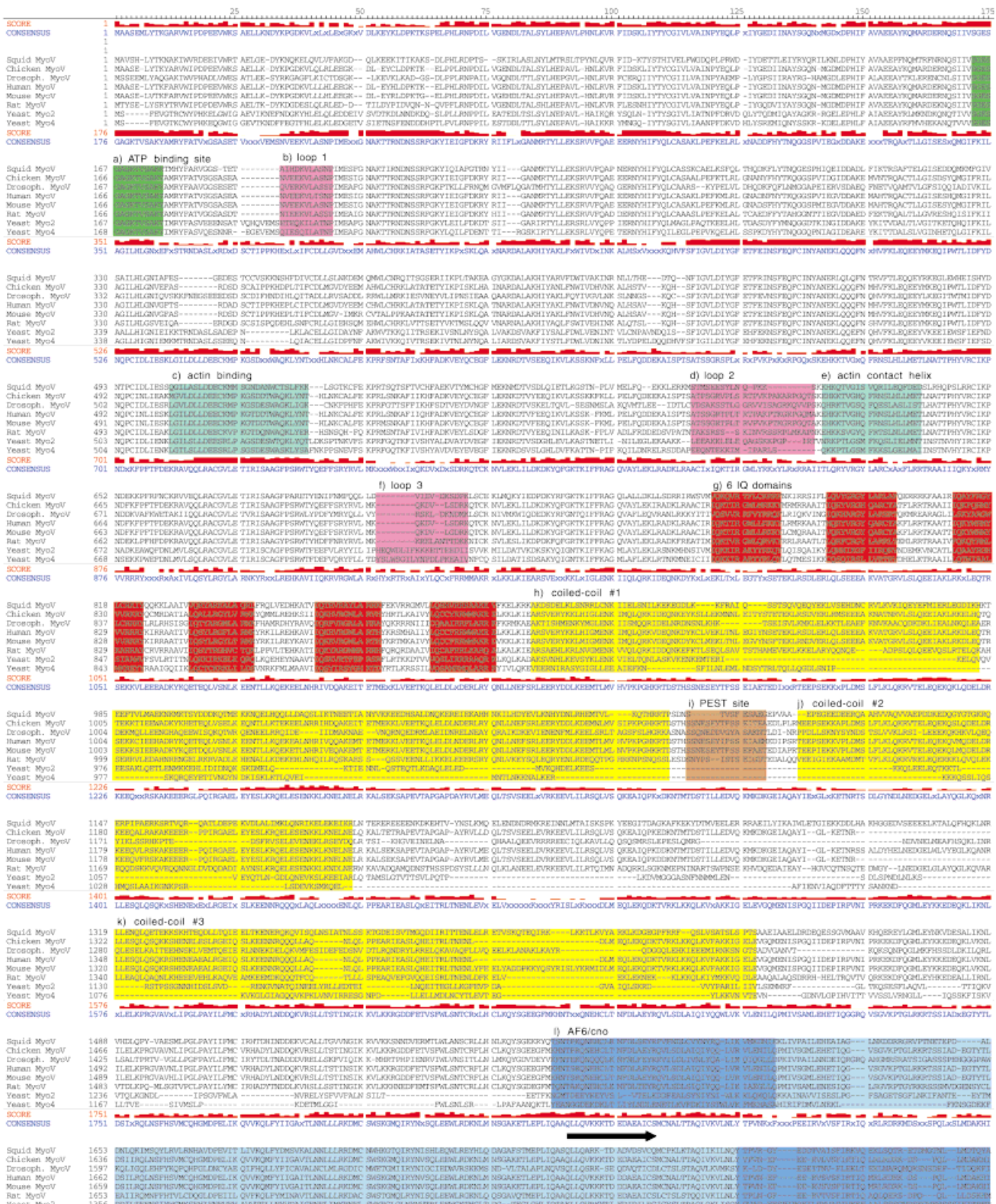

a) ATP binding site
b) loop 1
c) actin binding
d) loop 2
e) actin contact helix
f) loop 3
g) 6 IQ domains
h) coiled-coil #1
i) PEST site
j) coiled-coil #2
k) coiled-coil #3
l) AF6/cno

Figure 2.

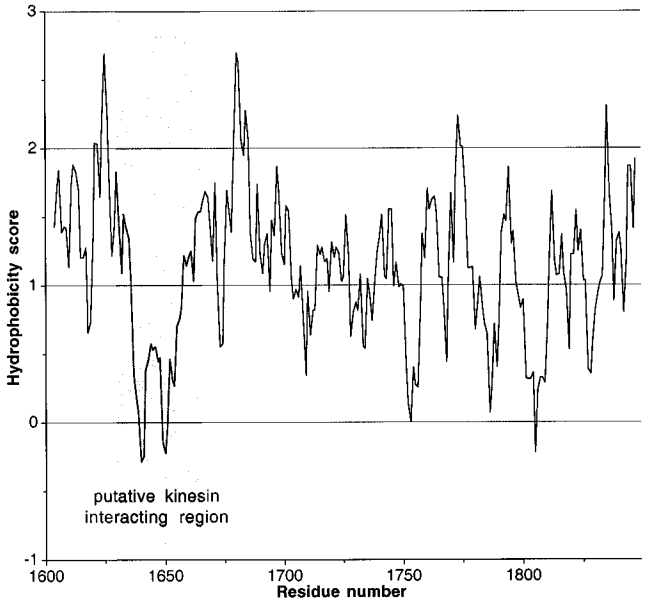


Fig. 3. Hydrophobicity plot of the AF6/cno region of squid MyoV with the putative kinesin binding region highlighted. Note that the region of MyoV shown to bind to mouse kinesin khcU is more hydrophilic than the surrounding regions.

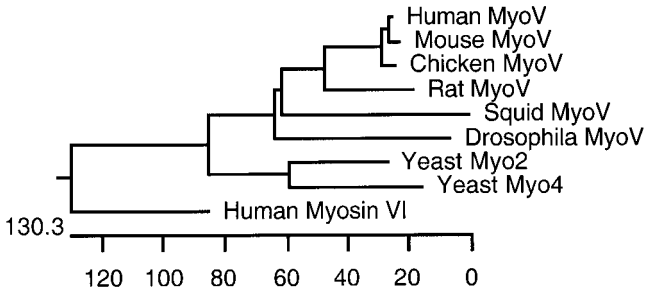


Fig. 4. Phylogenetic tree shows the relative evolutionary divergence of the class V Myosins (human MyoV splice variant, mouse *dilute*, chicken *dilute*, rat *myr6*, *Drosophila* MyoV, and yeasts *myo2* and *myo4*) with the evolutionary distance indicated on the × axis (GCG, Wisconsin Group). Human myosin VI was used as the outgroup. Mollusk MyoV is contained within a clade that includes vertebrate and avian MyoV. Invertebrate and fungal MyoV form a distinct and separate lineage removed from the mammal, avian, and mollusk MyoV proteins.

The proximal tail (Fig. 2h, aa906–1088) is a predicted α-helix, and the medial tail (Fig. 2j, aa1017–1203) is predicted to have a combination of globular and α-helical segments. The distal tail consists an α-helical segment (Fig. 2k, aa1323–1443) and a large globular tail domain at the C-terminal (Fig. 2l, aa1578–1846). The latter is a distinctive feature of class V myosins. This region also contains the AF6/cno and the putative kinesin binding domain (Fig. 2l light blue).

A dot matrix plot of the amino acid sequences of squid versus human brain myosin V revealed the level of similarity of the sequences of these two members of the myosin V family (Fig. 4). The similarity was highest in the head and globular tail domains of the protein and lowest in the proximal tail.

The program Megalign (LaserGene Software Suite, DNAStar, Madison, WI) was used to align the proteins and generate a relational cladogram using the neighbor joining method (Fig. 5). GCG (Wisconsin Package version 10.0, Genetics Computer Group, Madison, WI) was also used to confirm that the phylogenetic relationships were accurate and that both programs produced the same cladogram. The resulting phylogenetic tree revealed that squid myosin V is more closely related to vertebrate myosin V (*dilute* and *myr6*) than myosin V in *Drosophila* and yeast (Fig. 5).

Fig. 2. Alignment of squid myosin V (accession number: AF197336) with the class V myosins from mammals (human alt. spliced myosin V accession number: AF090428, mouse : 227523, rat: AAB38840), birds (chicken: Z11718), invertebrates (*Drosophila*: AAC99496), and fungi (yeast: CAA62184 and 6319290). The conserved protein motifs in the head (**a–f**), neck (**g**) and tail (h–l) regions are labeled and color coded as in Fig. 1. Alignments were performed using the blosum62 amino acid table using Gene Inspector 1.5 [TextCo, 1999]. Structural motifs for the Myosin V proteins were adapted from [Espreafico et al., 1992; Larson, 1996] as follows. **a:** ATP binding (aa166–179, green). **b:** loop1 20 kD:50-kD junction (aa184–194, magenta). **c:** Actin binding (aa505–533, light green). **d:** Loop 2 (aa605–627, magenta). **e:** Actin contact helix (aa629–647, light green). **f:** Loop 3 (aa609–617, magenta). **g:** 6 IQ domains (aa765, 787, 813, 836, 861, 884, red). **h:** Coiled coil 1 (aa906–1088, yellow). **i:** PEST site (aa1092–1106, brown). **j:** coiled coil 2 (aa1017–1203, yellow). **k:** Coiled coil 3 (aa1323–1443, yellow). **i:** The distal tail includes the AF6/cno domain (aa1578–1846, blue); lighter-colored area, minimum mouse MyoV sequence shown to bind mouse kinesin (aa763–856) [Huang et al., 1999]; black arrow, QLLQ (aa1740–1758) function-blocking antibody shown to inhibit actin-based motility of squid brain myosin V [Tabb et al, 1998].

DISCUSSION

Five overlapping cDNA fragments of squid myosin V were cloned to yield the entire 5547-bp squid brain myosin V coding sequence (accession number: AF197336). This is one of a small number of genes in squid that have been sequenced to date. When compared to other myosin V sequences, the deduced amino acid sequence of squid brain myosin V showed homology to chick brain MyoV, human MyoV(*myo5a*), mouse MyoV (*dilute*, the myo5a locus), rat MyoV (*myr6*), *Drosophila* MyoV, and the yeast class V myosins (*myo2*, *myo4*). As previously noted, squid brain MyoV, while highly similar in sequence to mammalian MyoV, displays specific

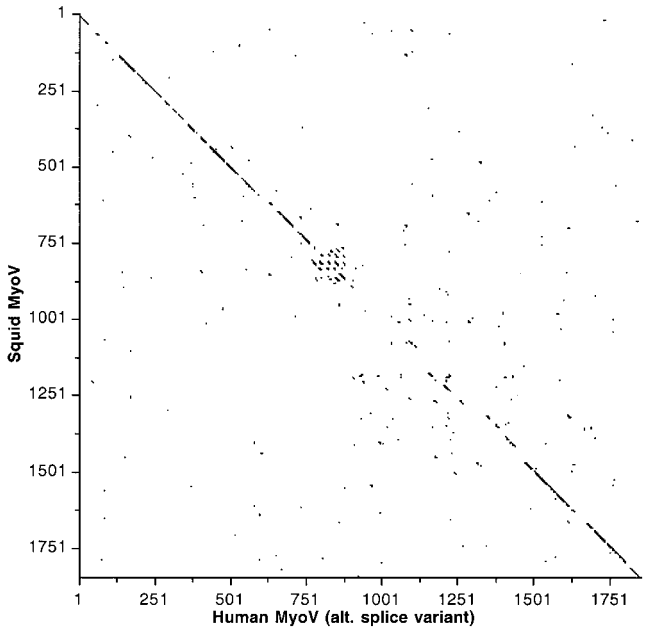


Fig. 5. Dot matrix plot of human MyoV and squid MyoV was generated by Gene Inspector [TextCo, 1999] using a Blossum62 amino acid table. Parameters were set to a conservative value, which allows the differences between the two proteins to be seen clearly. A diagonal line indicates regions of strongest conservation between the two proteins. The alignment is made using a 10-residue window, therefore repeat-containing regions will appear adjacent to the diagonal line (the IQ domains appear clearly). Similarities and identities between human and squid MyoV proteins are as follows: head (aa1–775) 50% identity, 65% similarity, proximal tail (aa906–1450) 21% identity, 39% similarity, globular tail (aa1451–1849) 49% identity, 71% similarity. Note that the highest divergence between squid and human MyoV sequence occurs in the first coil region (aa906–1088) immediately after the IQ domains (aa765–884).

differences in amino acid selection within the conserved consensus motifs.

The divergence suggests that the amino acid usage in squid is different, but that secondary structure is conserved. This unusual amino acid selection in the longfin squid is clearly seen in a comparison of the motor protein kinesin (squid kinesin heavy chain accession number: J05258). In general, the kinesin heavy chain is highly conserved from species to species; however squid kinesin shows an unusual pattern of residue substitutions, some of which are conserved and others that are not. We postulate that this pattern of amino acid substitutions seen in squid is the main reason that there is any appreciable difference in the primary amino acid sequence between squid and the mammalian myosin V proteins. Because both squid and vertebrate MyoV proteins show strong sequence similarities and structural motifs in the highly conserved functional domains, we believe that MyoV structure and function is highly conserved across species, although the pattern of amino acid usage may be different.

The globular tail contains a motif found in gene products of human AF6 and *Drosophila* canoe and is referred to as the AF6/cno or DIL motif. The subregion within this sequence that binds kinesin [Huang et al., 1999] is conserved, although surrounding sequences in the globular tail are relatively divergent.

In a previous study, an antibody (QLLQ) was generated to a synthetic peptide in the AF6/cno region of squid myosin V (black arrow indicates QLLQ site in Fig. 2). It reacted strongly to purified squid brain myosin V and the equivalent band in axoplasm [Tabb et al., 1998] but not to squid brain myosin II, thus showing high titer and specificity for squid myosin V. The QLLQ antibody inhibited actin-based vesicle movement when added to axoplasm of the squid giant axon, but had no significant affect on microtubule-based motility. An antibody raised against a recombinant bacterially expressed head domain of chicken myosin V [Espreafico et al., 1992] was also tested for its ability to inhibit movement. This antibody was previously shown to block MyoV motor activity in a sliding filament assay [Wolenski et al., 1995]. In squid axoplasm, the chicken MyoV head antibody also inhibited actin based motility by 98% [Tabb et al., 1998].

These results demonstrated that squid myosin V is the motor responsible for the movement of vesicles on actin filaments in squid axoplasm. This is the most conclusive evidence to date that class V myosins are directly involved in vesicle transport in neurons. It has previously been shown that one of the populations of vesicles observed to move on actin is the ER [Tabb et al., 1998]. In future studies, we plan to identify other populations of vesicles that are transported by myosin V as well as examine the method of docking the myosin to the membrane and the intrinsic regulation of the motor activity.

The cold marine environment of the longfin squid is likely to be a contributing factor that has influenced the pattern of amino acid usage in the *L. pealii* MyoV protein. Too few genes from squid have been sequenced to allow informed conclusions about squid genomics; however, the squid MyoV protein has properties that make it an excellent candidate for modeling neuronal vesicle transport in vertebrates. Not only does the squid MyoV protein show sequence homology to mammalian MyoV proteins, it maintains activity in motility assays when isolated on ER vesicles. The in vitro motility assay, in conjunction with further genetic and biochemical studies will greatly facilitate our understanding of the mechanism and regulation of neuronal vesicle transport.

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