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Identification of a Novel Unconventional Myosin from Scallop Mantle Tissue

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We isolated a cDNA encoding a novel unconventional myosin from scallop mantle tissue (scallop unconventional myosin: ScunM) and determined the nucleotide sequence. It comprises 2,739 bp with 5′ and 3′-noncoding sequences and has an open reading frame of 2,334 bp that encodes 778 amino acids. While ScunM has a motor domain and a short tail domain without having light chain-binding IQ motifs like myosin XIV, the deduced amino acid sequence exhibits low homology, 30–36%, to known myosins. Phylogenetic analysis of the motor domain suggested that ScunM belongs to a novel unconventional myosin class. ScunM has an insertion of 67 amino acids in the putative actin-binding site (loop2 site). Western blot analysis with an antibody produced against the N-terminal region revealed that ScunM was strongly expressed in the mantle and mantle pallial cell layer of scallop.

Key words: cloning, mantle, scallop, unconventional myosin.

Myosins are mechanoenzymes that utilize the energy of ATP hydrolysis either to translocate along or to move actin filaments. The heavy chains of all known myosins contain ATP-binding and actin-binding sites within the head domain, followed by the neck domain, which contains multiple light chain–binding IQ motifs, and a multifunctional C-terminal domain. It has become clear that the myosins constitute a superfamily. Phylogenetic analysis of the head domains of myosins has revealed the existence of at least of 15 classes, termed either conventional and unconventional myosins (*1–3*). The myosins function in a multitude of cellu-

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Abbreviations: PAGE, polyacrylamide gel electrophoresis; SDS-PAGE, PAGE in the presence of SDS; ScunM, scallop unconventional myosin.

(A)

(1)	GESGAGKT	EASKIIMRYIAAVTNLGGQKEVERVKDVLITSNVIL	EAFGNAKT	class I
(2)	GESGAGKT	TKKVIMYLAKVACATKKKTEEGGTDKKEGSLEDQIIQANPVL	EAFGNAKT	class II
(3)	GESGAGKT	TKKVIQYLAHVAASNRPSGNRSSVSNLHIQGSNVFTQGELENQLLQANPIL	EAFGNAKT	class II
(4)	GESGAGKT	ESTKLILQFLAAVSGQESWIEQQILEAVPIN	EAFGNAKT	class VII
(5)	GESGAGKT	ESTKYMVKHLVSLCPKETGDLHERIVKINPLL	EAFGNAKT	unknown

(B)

GAGAGCACTAAATACATGGTCAAACACCTTGTGTCCTTGTGTCCGAAAGAGACTGGTGACCTCCACGAACGTATTGTCAAGATCAACCCACTTCTG

GESGAGKT E S T K Y M V K H L V S L C P K E T G D L H E R I V K I N P L L EAFGNAKT

Fig. 1. **Identification of scallop mantle myosin cDNAs.** Scallop mantle myosins were amplified by RT-PCR using degenerate primers. Sequence analysis of cloned PCR products revealed the presence of 5 different types of myosin-like fragments (A). On comparison of the deduced amino acid sequences with proteins in the GenBank database, four fragments (clones 1–4) were identified as myosin I, II, II, and VII, respectively. Although each of the four fragments exhibited 78–87% sequence identity with members of only one myosin class, one fragment (clone 5) showed low sequence identity with known myosins. The amino acid sequences corresponding to a set of degenerate primers are boxed. (B) Nucleotide and deduced amino acid sequences of clone 5. The sense and antisense primers employed for screening are underlined (see "MATERIALS AND METHODS").

lar processes such as motility, cytokinesis, phagocytosis, endocytosis, secretion, and organelle movement (*1*).

We were interested in identifying novel myosins expressed in the mantle tissue of scallop. The mantle tissue of bivalve molluscan shells consists of a muscle portion and nonmuscle portions such as mucous cells and epithelial cells. The epithelial cells are known to play roles in such as secretion of shell organic matrix proteins (*4, 5*), and migration and proliferation during the wound healing process (*6*). We postulated that the nonmuscle cells contain multiple unconventional myosin species. Using the PCR technique, we previously identified nonmuscle myosin II, which was specifically expressed in the pallial cell layer (*7*). In this paper, we report the identification of a novel unconventional myosin.

MATERIALS AND METHODS

Materials—Adductors (striated and catch muscles), mantle, gonad, and gland were prepared from scallop, *Patinopecten yessoensis*. The mantle pallial cell layer was separated from muscle by scraping with a knife.

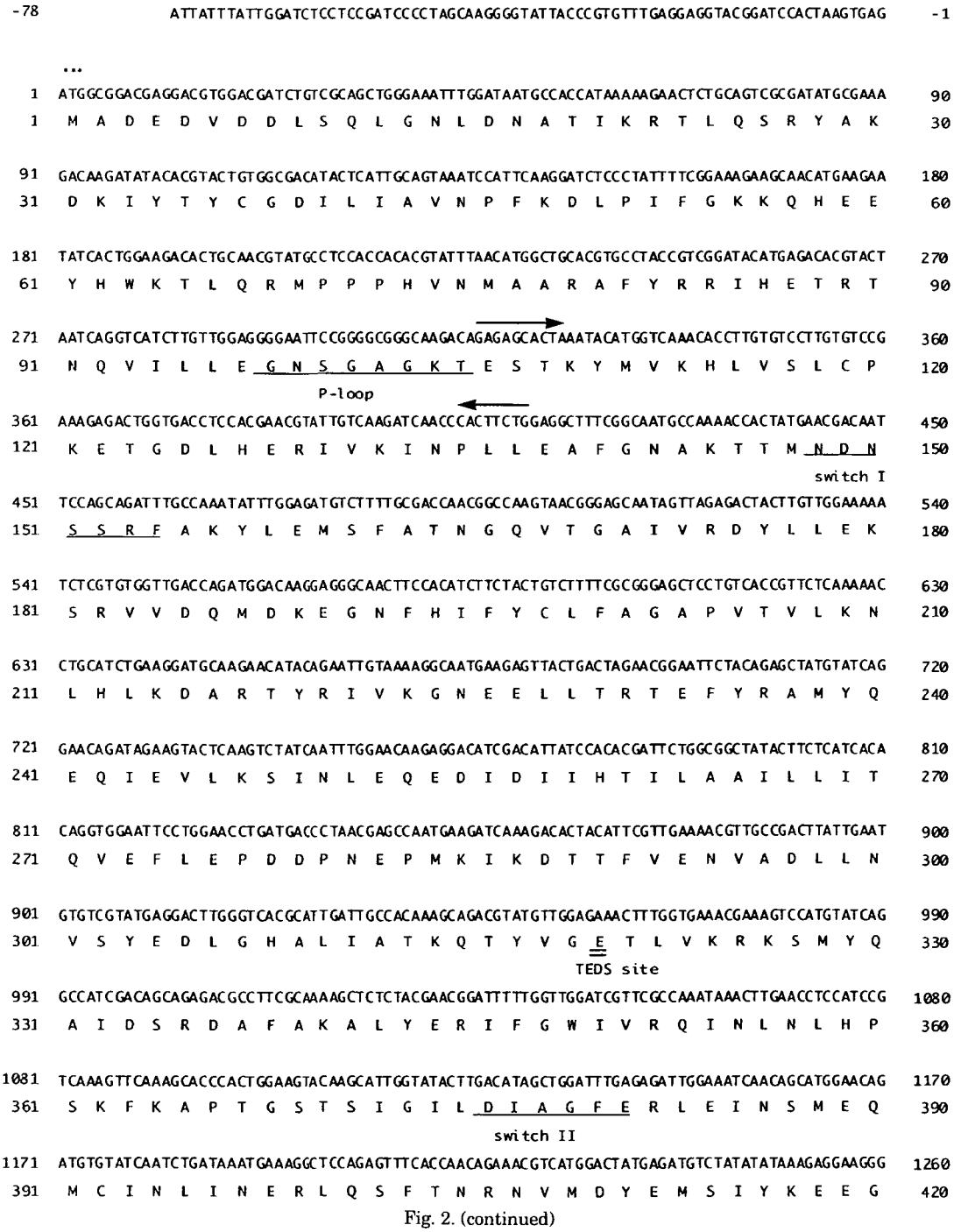

Fig. 2. (continued)

RT-PCR—Total RNA was extracted from the mantle tissue including the pallial cell layer with guanidinium isothiocyanate and purified. Aliquots (1 μg) of total RNA were subjected to RT-PCR as described previously (7). RT-PCR was carried out using degenerate myosin primers with the following sequences described by Bement *et al.* (8): 5′-GGIGA(A/G)(A/T)(C/G)IGGIGCIGGIAA(A/G)AC-3′ and 5′-GT-(C/T)TTIGC(A/G)T TICC(A/G)AAIGC(C/T)TC-3′, which correspond to the highly conserved amino acid sequences GESGAGKT and EAFGNAKT within the myosin motor domain. The amplified fragments were cloned into TA-cloning vector (INVITROGEN) as described previously (7), and the DNA sequences of each clone were determined using a Dye-Deoxy terminator cycle sequencing kit (Amersham Pharmacia) with a DNA sequencer model 310 (Perkin Elmer).

Construction of a cDNA Library—cDNA was synthesized from the total RNA using a SMART cDNA Library Construction kit (CLONTECH) according to the instruction manual, and then the cDNA library, which was inserted

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1261  ATCCACGTGACCGGTATCAAGTTTAAGAACAATGATGCGCTGTTGGACTTGTTCATGAAGAAAACATTTGGCCTGCTGCCACTCCTCGAC  1350
 421   I  H  V  T  G  I  K  F  K  N  N  D  A  L  L  D  L  F  M  K  K  T  F  G  L  L  P  L  L  D   450

1351  GAGGAGTCGAAGCTTGGACAAGGTTCCAATGAAAGATTTGTAAAAAAACTCAATGACAAGTACGATACGCACCCATGTTTCACAGAATCT  1440
 451   E  E  S  K  L  G  Q  G  S  N  E  R  F  V  K  K  L  N  D  K  Y  D  T  H  P  C  F  T  E  S   480

1441  CCACATGGTCGTGTGGAATTCGGTGTCAGACACTTCGCCGCCCAGGTCTGGTATGACGGGTCATTGTTTATTGAGAAGAACCGAGATATG  1530
 481   P  H  G  R  V  E  F  G  V  R  H  F  A  A  Q  V  W  Y  D  G  S  L  F  I  E  K  N  R  D  M   510

1531  CTGAGCCAAGATGTTACCTCCTGTATGAGAGAGAGTGACAATCCATTTGTTTCCGACCTTTTCACTGTTAAAAAGGGGCCAACAGGGACT  1620
 511   L  A  Q  D  V  T  S  C  M  R  E  S  D  N  P  F  V  S  D  L  F  T  V  K  K  G  P  T  G  T   540

1621  ATTTCAGCGACAATGCAGAACATCAGGAGGTCCAGGAAAGCAGAAGGTAGAGGTCCGAGGAAACCTATTACGGCCAGAGGACAACTTCTT  1710
 541   I  S  A  T  M  Q  N  I  R  R  S  R  K  A  E  G  R  G  P  R  K  P  I  T  A  R  G  Q  L  L   570

1711  ATGGCCGATTTAGGAAGGTCTCTAAAAGAAAGGTACGGTGAATCTGTCCAGAGCACTAATCAGGTGTACAACCCTAAAGATCACAAACA  1800
 571   M  A  D  L  G  R  S  L  K  E  R  Y  G  E  S  V  Q  S  T  N  Q  V  Y  N  P  K  D  H  K  T   600

1801  GTCATCTCATACTTCCAGAGCTCTATGAATGAACTGCTACAGAAATTGCAACGGGCAGACCCATATTATGTACGCTGTATCAAACCCAAC  1890
 601   V  I  S  Y  F  Q  S  S  M  N  E  L  L  Q  K  L  Q  R  A  D  P  Y  Y  V  R  C  I  K  P  N   630
                                                                          actin-binding

1891  ATGTTCCTAAAACCAGACAACTTCGATGACGAAAAGGTGTTGGAACAGATGCTTTATAATGGGATATCGGAAGTGGCAAAGATTAGAAAA  1980
 631   M  F  L  K  P  D  N  F  D  D  E  K  V  L  E  Q  M  L  Y  N  G  I  S  E  V  A  K  I  R  K   660
                                                    conserved glycine

1981  CTTGGTCTTCCTATTCGGAAACGGTATGACGACTTCACAAAGAGATACAGACCACTGTTTCTGGATTGTCGGAAGGCGCGATCGGACAGA  2070
 661   L  G  L  P  I  R  K  R  Y  D  D  F  T  K  R  Y  R  P  L  F  L  D  C  R  K  A  R  S  D  R   690

2071  GCTGGGGCAGAGCTGCTTCTAAAAAAAACTTTGCCAGATAAAATGATGTCAGGGATACAATTTGGCAAAACAAGGGTTTTCATGCAGGAA  2160
 691   A  G  A  E  L  L  L  K  K  T  L  P  D  K  M  M  S  G  I  Q  F  G  K  T  R  V  F  M  Q  E   720

2161  GATGTCAGTATCTGGCTAGAGAAATGTCGTGGTTTCAGGGAGCGGGCTGCCGTTGACACCATTGCTAAAAGGTGGCAGCAATATAAGATT  2250
 721   D  V  S  I  W  L  E  K  C  R  G  F  R  E  R  A  A  V  D  I  I  A  K  R  W  Q  Q  Y  K  I   750
                                                                                    ***

2251  GAAAACAAACGAAAAGAAGAATTGTCGGAAGGCGCGATCGGACAGAGCAGGGGCAGAGCTGCTTCTGAAAAAAACTTTGCCAGATAAAAT  2340
 751   E  N  K  R  K  E  E  L  S  E  G  A  I  G  Q  S  R  G  R  A  A  S  E  K  N  F  A  R          778

2341  GATGTCAGGGATACAATTTGGCAAAACAAGGGTTTTCATGCAGGAAGATGTCAGTATCTGGCTAGAGGGGAACCAAACAGCTATAGAGGT  2430
2431  AGAAACCAGGACTGTATGCTGATGTGGGCCAGGAAGGACTTCCGCTGGGACGACGACGAGTGCCACCTTCAAAGGTCGTTCATCTGTGAA  2520
2521  AGATTCGTGTTTTCAGAAAAGGTGTGCAGATTGTCGGGTGTAGGAGGGTTGACACGCCTCACAGTATATATCAAGTACCGTGTATAGGCA  2610
2611  GAAGTCACGGGATTGTAACAAGTAGAACAATAAAGAAGTTCTGAAGACACG                                          2661
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Fig. 2. **Nucleotide and deduced amino acid sequences of ScunM.** The start and stop codons are indicated by astersks. The nucleotide sequence of the original RT-PCR fragment (Fig. 1B) was identical to the corresponding sequence between the arrows. Solid lines below the amino acid sequence represent the sequences of ATP-binding (P-loop, switch I, and switch II) and actin binding sites. The glutamic acid (at position 320) that corresponds to the TEDS site and the conserved glycine (at position 651) that was proposed to act as a pivot point of the lever arm are also underlined with double lines. The insertion of loop 2 is boxed. The short tail of ScunM, rich in basic residues, is underlined with a dashed line. The di-basic and tri-basic sequences within the tail domain are indicated by double lines. The nucleotide sequence has been deposited in the DDBJ database under accession No. AB057425.

into the λTriplEX2™ phage vector, was packaged with the GigapackII Gold extract (STRATAGENE).

cDNA Cloning of Scallop Unconventional Myosin—To select positive phage clones of scallop unconventional myosin, PCR screening was carried out with the following primer pairs: 5′-TTGTGTCCTTGTGTCCGAAA-3′ as a sense primer and 5′-TGACAATACGTTCGTGGAGG-3′ as an antisense primer (Fig. 1). These primers correspond to parts of the nucleotide sequences of a fragment that was amplified using a set of degenerate primers described above. Approximately 3×10^5 phage clones of the cDNA library were screened as described previously (*7*). The pTriplEX2™ plasmid with an insert from an isolated positive clone was recovered using the *in vivo* excision feature of the λTripleEX2™, and the nucleotide sequences of both strands of an insert were determined. The determined sequence revealed that the isolated cDNA clone did not contain a 3′-noncoding region. To extend the sequence in the 3′ direction, the 3′-RACE method was applied, and an amplified fragment was subjected to DNA sequencing.

Production of Polyclonal Antibodies—The peptide covering the N-terminal region of scallop unconventional myosin (ADEDVDDLSC) was synthesized and coupled to BSA by means of maleimidobenzoyl-*N*-hydroxysuccinimide ester. A

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ScunM       ------------------------------------------------------------
MyoK        ------------------------------------------------------------
adrenal     ------------------------------------------------------------
chicken     --------ASPDAEMAAFGEAAPYLRKSEKERIEAQNKPFDAKSSVFVVHPKESFVKGTI    52
TgM-A       MASKTTSEELKTATALKKRSSDVHAVDHSGNVYKGFQIWTDLAPSFTVKEEPDLMFAKCI    60

ScunM       ------------------------------MADEDVDDLSQLGNLD--------NATIK    21
MyoK        ----------------------------MFRLFSSGVDDLVLVSNPS--------NGEVT    24
adrenal     ------------------------MESALTARDRVGVQDFVLLENFTS--------EAAFI    29
chicken     QSKEGGKVTVK-TEGGETLTVKEDQVFSMNPPKYDKIEDMAMMTHLH--------EPAVL   104
TgM-A       VQAGTDKGNLTCVQIDPPGFDEPFEVPQANAWNVNSLIDPMTYGDIFTGMLPHTNIPCVL   120

ScunM       RTLQSRYAKDKIYTYCG--DILIAVNPFKDLPIFGKKQHEEYHW---KTLQRMPPPHV-N    75
MyoK        SQIGARFDRELIYTNIGEVLIFTAVNPYKALPITGPEFIKLYQN---ASGSDASP-HIYA    80
adrenal     ENLRRRFRENLIYTYIFTGPVLVSVNPYRDLQIYSRQHMERYRG---VSFYEVPP-HLFA    85
chicken     YNLKERYAAWMIYTYSG--LFCVTVNPYKWLPVYNPEVVLAYRG---KKRQEAPP-HIFS   159
TgM-A       DFLKVRFMKNQIYTTAD--PLVVAINPFRDLGNTTLDWIVRYRDTFDLSFTKLAP-HVFY   177

ScunM       MAARAYRRIHETRTNQVILLE--GNSGAGKTESTKYMVKHLVSLCP--------------   120
MyoK        LAERAYRRMVDENESQCVIISGESGAFTGKTVSAKLILQYVTSVSPNNSSGGGIGGSGGG   140
adrenal     VADTVYRALRTERGDQAVMISFTGESGAGKTEATKRLLQFYAETCP--------------   131
chicken     ISDNAYQFMLTDRENQSILIT--GESGAGKTVNTKRVIQYFATIAA--------------   203
TgM-A       TARRALDNLHAVNKSQTIIVS--GESGAGKTEATKQIMRYFAAAKT--------------   221
                                                          loop1
ScunM       ----------------------------------------------KETGDLHERIVKINP-   135
MyoK        NGGIPQYDGGSDDRPSPPMGRGMGMPGFTMVGRGGLPTRGGGPPSRGGGPPPTRGRGGPP   200
adrenal     ---------------------------------------APERGGAVRDRLLQSNP-   148
chicken     ---------------------------------SGEKKKEEQSGKMQGTLEDQIISANP-   229
TgM-A       ---------------------------------------GSMDLRIFTQNAIMAANP-   239

ScunM       ------------------------------------------------------------
MyoK        PPIPQNRGAPPPVSNGGAPPPVARGPVAFTPPPTRGAPPTRGGGPANRGGRGGGPPPVST   260
adrenal     ------------------------------------------------------------
chicken     ------------------------------------------------------------
TgM-A       ------------------------------------------------------------

ScunM       ------------------------------LLEAFGNAKTTMNDNSSRFA--KYLEMSFA   163
MyoK        SRGGGGYGGSSKTVDVEHIKKVILDSNPLFTMEAIGNAKTVRNDNSSRFG--KYLEIQFD   318
adrenal     ------------------------------VLEAFGNAKTLRNDNSSRFFTGKYMDVQFD   178
chicken     ------------------------------LLEAFGNAKTVRNDNSSRFG--KFIRIHFG   257
TgM-A       ------------------------------VLEAFGNAKTIRNNNSSRFG--RFMQLDVG   267

ScunM       TNGQVTGAIVRDYLLEKSRVVDQM--DKEGNFHIFYCLFAGAPVTVLKNLHLKDAR----   217
MyoK        DNNAPVGGLISTFLLEKTRVTFQQ--KNERNFHIFTFYQMLGGLDQTTKS-EWGLTQATD   375
adrenal     FKGAPVGGHILSYLLEKSRVVHQN--HGERNFHIFYQLLEGGEEETLRRLGLFTERNPQS   236
chicken     ATGKLASADIETYLLEKSRVTFQL--PAERSYHIFYQIMSNKKPELIDMLLITTNP---Y   302
TgM-A       REGGIKFGSVVAFLLEKSRVLTFTQDEQERSYHIFYQMCKGADAAMKERFHILPLS----   323

ScunM       -TYRIVKGNEELLTRTEFYRAMYQEQIEVLKSINLEQEDIDIIHTILAAILLITQVEFLE   276
MyoK        FYYLAQSKCTTVEDVDDGKDFHEVKAAMETVGISRDFTEQTEIFRILAAILHVGNIRFQG   435
adrenal     YLYLVKGQCAKVSSINDKSDWKVVRKALTVIDFTEDEVED--LLSIVASVLHLGNFTTHF   294
chicken     DYHYVSQGEITVPSIDDQEELMATDSAIDILGFSAD--EKTAIYKLTGAVMHYGNLKFK-   359
TgM-A       EYKYINPLCLDAPGIDDVAEFHEVCESFTFRSMNLTEDEVASVWSIVSGVLLLGNVEVTA   383
                                                    TEDS site
ScunM       PDDPNEPMKIKDTTFVENVADLLNVSYEDLGHALIATKQTYVG-----EITLVKRKSMYQA   331
MyoK        EAP----ASVIDETPLQWAASLLGCDPTFLCQSLNHRQIQSFTGSARHTQYQVPQNPDQS   495
adrenal     AADEESNAQVTTENQLKYLTRLLGVEGSTLREALTHRKIIAKG-----EELLSPLNLEQA   349
chicken     QKQREEQAEPDGTEVADKAAYLMGLNSAELLKALCYPRVKVGN-----EFVTKGQTVSQV   414
TgM-A       TKDGGIDDAAAIEGKNLEVFKKACGLLFFTLDAERIREELTVKVSYAQNQEIRGRWKQED   443

ScunM       ID--SRDAFAKALYERIFGWIVRQINLMLHPSKFKAPTG--STSIGILDIAGFERLEIN-   386
MyoK        AG--LRDALAKTLYERIFDFIVARVNKAMSFSGN-------CKVIGVLDIYFTGFEVFER   546
adrenal     AFTYARDALAKAVYSRTFTWLVAKINRSLASKDAESPSWRSTTVLGLLGIYGFEVFQHNS   409
chicken     HN--SVGALAKAVYEKMFLWMVIRINQQLDTKQPR------QYFIGVLDIAGFEIFDFN-   465
TgM-A       GD-MLKSSLAKAMYDKLFMWIIAVLNRSIKFTPPGG----FKIFMGMLDIFGFEVFKNN-   499
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Fig. 3. (continued)

rabbit was first immunized with 500 μg of peptide with Freund's complete adjuvant. Three successive injections of 500 μg of peptide in incomplete adjuvant were performed at intervals of 2 weeks. Serum was collected regularly after the final immunization and affinity-purified over a synthetic peptide.

Electrophoresis and Immunoblotting—Each scallop tissue was homogenized in a solution containing 2% SDS, 20 mM Tris, 10% glycerol, and 0.1% 2-mercaptoethanol. After measuring protein concentrations by the Bicinchoninic acid (BCA) assay, equal amounts of tissue extracts were separated by SDS-PAGE (*9*). After electrophoresis, the proteins were transferred to nitrocellulose membranes. The membranes were blocked with 5% skim milk (w/v) in a solution containing 0.5 M NaCl, 20 mM Tris-HCl (pH 7.5), and 0.05% Tween 20 before the addition of the purified antiserum, followed by incubation with secondary antibodies, alkaline phosphatase–conjugated goat anti–rabbit IgG. Color development was performed with nitroblue tetrazolium and 5-bromo-4-chloro-3-indoyl phosphate.

RESULTS AND DISCUSSION

Identification of Myosin cDNAs from Scallop Mantle Tissue—To identify unconventional myosins from scallop mantle tissue, the RT-PCR technique was carried out using degenerate primers derived from conserved sequences in the myosin head regions (GESGAGKT and EAFGNAKT) as described by Bement *et al.* (*8*). Sequence analysis of amplified fragments yielded five kinds of products (clones 1–5). Comparison of the GenBank database with the nucleotide sequences of these fragments revealed that four products (clones 1–4) exhibited substantially higher sequence identity to members of myosin classes (class I, class II, class II, and class VII) from a variety of organisms (Fig. 1A). However, the remaining product (clone 5) did not show close similarity to known myosins (Fig. 1). We previously identified clones 2 and 4 as nonmuscle myosin II and myosin VIIA-like protein, respectively (*7, 10*). To confirm a PCR product of clone 5 was myosin, we isolated cDNA clone that covered the entire coding region from the scallop mantle cDNA library as described under "MATERIALS AND METHODS," and the isolated cDNA clone was subjected to DNA sequencing.

Primary Structure of ScunM—The determined nucleotide sequence and deduced amino acid sequence are shown in Fig. 2. It comprises 2,739 bp with 5′ and 3′-noncoding sequences and has an open reading frame of 2,334 bp that encodes 778 amino acids. The deduced amino acid sequence revealed that this gene product has characteristic ATP binding (P-loop, switch I, and switch II) and actin binding (VRCIKPN) sites, and shares many conserved regions with myosins of other classes. The calculated molecular mass of the predicted ScunM protein was 89,642 Da, which is smaller than that of class XIV myosin (TgM) from *Toxoplasma gondii,* which is known as the smallest unconventional myosin among myosins (*11*). The deduced amino acid sequence of ScunM showed that a tail of 46 amino acids is attached to the motor domain without a light chain–binding domain. The overall structure of ScunM is

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ScunM       -SMEQMCINLINERLQSFTNRNVMDYEMSIYKEEGIHVTGIKFKNNDALLDLFMKKTFGL   445
MyoK        NSFEQFCINYVNERLQQIFIDLTVRGEQREYHEEGMKWKDISFFDNKIVVDLFTIDGNKP   606
adrenal     FEFTQFCINYCNEKLQQLFIELTLKSEQEEYEAEGIAWEPVQYFNNKIICDLVEEKFKGI   469
chicken     -SFEQLCINFTNEKLQQFFNHHMFVLEQEEYKKEGIEWEFIDFGMDLAACIELIEKPMGI   524
TgM-A       -SLEQFFINITNEMLQKNFVDIVFDRESKLYRDEGVSFTSKELIFTSNAEVIKILTAKNN   558

ScunM       LPLLDEESKLGQ--------GSNERFVKKLNDKYDTHPCFTES---------PHGRVEFG   488
MyoK        PGIMRVLDDVCKTVHAVDSAAADIKFMEKLIHSIQSHPHLVIS-----NTGSSADEFTFT   661
adrenal     ISIFTLDEECLR-----PGEATDLTFLEKLEDTIKQHPHFLTHKLADQRTRKSLDRGEFR   524
chicken     FSILEEECMFPK--------ATDTSFKNKLYDQHLGKSNNFQKP----KPAKGKAEAHFS   572
TgM-A       SVLAALEDQCLAP------GGSDEKFLSTCKNALKGTTKFKPAK------FTVSPNINFL   616

ScunM       VRHFAAQVWYDG--SLFIEKNRDMLSQDVTSCMRESDNPFVSDLFTVKKGPTGTISATMQ   546
MyoK        IKHYAGEVSYS--IEEFCFKNNDNLYASIVGCLQNSTYQFIVSLFP--------------   705
adrenal     LLHYAGEVTFTYNVTGFLDKNNDLLFRNLKETMCSSENPILGQCFD--------------   570
chicken     LVHYAGTVDYN--ISGWLEKNKDPLNETVIGLYQKSSVKTLALLFA--------------   616
TgM-A       ISHTVGDIQYN--AEGFLFKNKDVLRAEIMEIVQQSKNPVVAQLFA--------------   660
                loop2
ScunM       NIRRSRKAEGRGPRKPITARGQLLMADLGRSLKERYGESVQSTNQVYNPKDHKTVISYFQ   606
MyoK        --ENIQDNKQAPTTS-------------------------------------SFFTKIR   725
adrenal     --RSELSSKKRPETV-------------------------------------ATQFKMS   590
chicken     --TYGGEAEGGGGKKGGKKKGSSFQ---------------------------TVSALFR   636
TgM-A       --GIVMEKGFTKMAKGQ-----------------------------------LIGSQFL   682
                                                conserved glycine
ScunM       SSMNELLQKLQRADPYYVRCIKPNMFLKPDNFDDEKVLEQMLYNGI--SEVAKIRKLGLP   664
MyoK        QSSSYLVTRLSACTPHYIRCIKPNDKKQPMNFVSSRVEHQVKYLGI--LENIKVKRSGFT   783
adrenal     LLELFTVEILKSKEPAYVRCIKPNDSKQPGRFDEVLIRHQVKYLGL--MENLRVRRAGFA   648
chicken     ENLNKLMANLRSTHPHFVRCIIPNETKTPGAMEHELVLHQLRCNGV--LEGIRICRKGFP   694
TgM-A       SQLQSLMELINSTEPHFIRCIKPNDTKKPLDWVPSKMLIQLHALSVFTLEALQLRQLGYS   742

ScunM       IRKR--YDDFTKRYRPLFLDCRKARSDRAGAELLLKKTLPDKMMSGIQFGKTRVFMQEDV   722
MyoK        YAYRQLKDIFLNRFGKIMDVQP---RNVQEFVEYITRTHKDINADEFEEGKTKIFVKNPE   840
adrenal     YRRKYEAFTFLQRYKSLCPETWPTWTGRRQDGVTVLVRHLGYKPEEYKMGRTKIFIRFPK   708
chicken     SRVLYADFKQRYRVLNASAIPEGQFMDSKKASEKLLG-SIDVDHTQYRFGHTKVFFKAGL   753
TgM-A       YRRPFKEFLFQFKFIDLSASENPNLDPKEAALRLLKSSKLPSEEYQLFTGKTMVFLKQAK   802

ScunM       SIWLEKCRGF                                                   732
MyoK        TIFTFVMEDL                                                   850
adrenal     TLFATEDAFT                                                   808
chicken     LGLLEEMRDD                                                   763
TgM-A       ELTQIQRECL                                                   812
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Fig. 3. **Comparison of the deduced amino acid sequence of the motor domain of ScunM with the head sequences of MyoK, adrenal gland myosinIβ (adrenal) (*26*), chicken skeletal muscle myosinII (chicken) (*27*), and TgM-A.** Dashes indicate gapped amino acids. The TEDS rule site and the conserved glycine are boxed. The loop 1 and loop 2 regions are also indicated.

similar to those of TgM and MyoK (class I myosin) from *Dictyostelium discoideum* in terms of a short and basic tail and a lack of a neck (light chain binding site) (*11, 12*). However, sequence comaparison of ScunM with TgM and MyoK revealed a distinct difference in the essential amino acid residues that affect ATPase activity and regulation. Almost all known myosins contain a phosphorylatable residue (serine or threonine) or a negatively charged residue (asparatic acid or glutamic acid) at a conserved site (TEDS rule) (*13*). In lower eukaryotes, phosphorylation of this residue has been shown to be crucial for stimulation of the ATPase activity of class I myosins (*14*). MyoK and ScunM have a phosphorylatable threonine residue and a negatively charged glutamic acid residue (at position 320) at this site, respectively (Fig. 3). On the other hand, TgM has a glutamine residue, which does not follow the TEDS rule. The conserved glycine, which has been proposed to act as a pivot point of the lever arm (*15, 16*), is substituted by a serine residue in TgM but not in MyoK or ScunM (Fig. 3). Thus, the essential amino acid residues differ among ScunM, MyoK, and TgM, suggesting that these myosins may be regulated by different mechanisms.

To classify ScunM into a myosin subclass, the sequence of the head domain of ScunM was aligned with those of typical members of the 15 already established classes of the myosin family, and a phylogenetic tree was created using the ClustalW program (Fig. 4). The numerical values at the branch points are the bootstrapping values. These values indicate the number of times that given branches clustered together in out of 1,000 bootstrap trials. ScunM does not join any of the existing branches of the trees with greater than 60% confidence, suggesting that ScunM does not fall into any of the 15 classes of myosins already identified. Therefore, ScunM constitutes a new class of myosin.

A search of the database with the tail domain sequence revealed no homology to other proteins or known motifs. The tail domain is short and basic, with a pI value of 10.36. It resembles the basic tails of some other unconventional myosins that have the ability to bind acidic phospholipids and may mediate myosin–membrane interaction (*17, 18*). Analysis of the predicted secondary structure of the tail domain of ScunM revealed an α-helix structure like that of TgM (*19*). The tail domain of TgM has four di-basic motifs (Arg-Arg, Lys-Lys, Lys-Lys, and Arg-Arg), the last motif (Arg-Arg) being responsible for plasma membrane association (*19*). The di-basic sequence, Lys-Arg, or the tri-basic sequence, Lys-Arg-Lys, observed in the tail domain of ScunM may also contribute to the membrane association.

It has been proposed that two proteolytically sensitive surface loops that lie near ATP (loop 1) and actin (loop 2) binding sites could be critical for modulation of myosin kinetic activities (*20–23*). In comparison with conventional class II myosin (chicken skeletal muscle myosin II), the ScunM sequence has an 11 amino acid deletion at the posi-

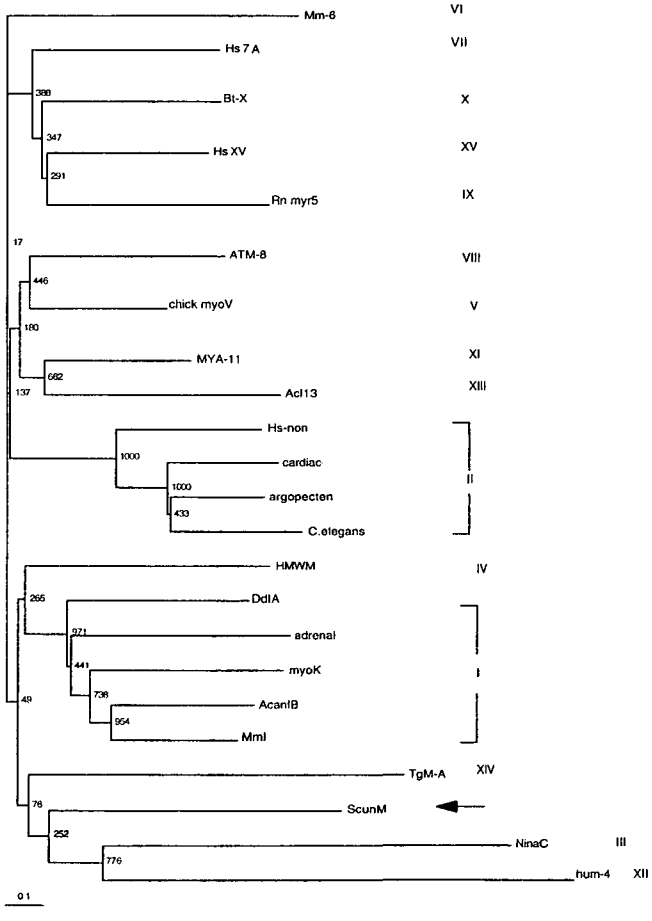

Fig. 4. **Phylogenetic tree of the myosin superfamily based on the sequences of the head domains.** The sequences from the amino terminus to the end of the head domain of typical members of each myosin class were taken. The sequences were aligned using default parameters and a bootstrap tree file was created, and then a phylogram tree was drawn with the Tree-View program. The bootstrapping values at the nodes indicate the numbers of times that given branches clustered together in out of 1,000 bootstrap trials. ScunM is indicated by an arrow. Accession numbers: Mm-6, Q64331; Hs 7A, Q13402; Bt-X, U55042; Hs XV, AF053130; Rn myr5, X77609; ATM-8, P47808; chick myoV, Q02440; MYA-11, Q39160; Acl 13, X69505; Hs-non, P35579; cardiac, P13539; argopecten, X55714; C. elegans, P12844; HMWM, P47808; DdIA, P22467; adrenal, U03420; myoK, AB017909; AcanIB, P19706; MmI, P70248; TgM-A, AF006626; NinaC, P10676; hum-4, Q20456.

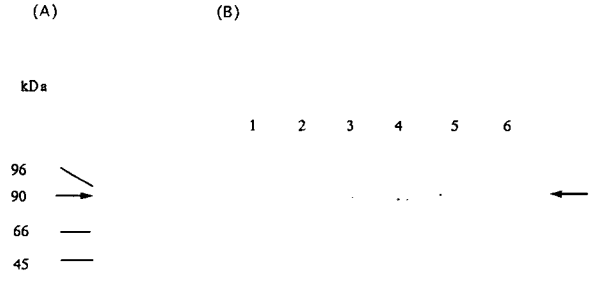

Fig. 5. **Detection of ScunM in scallop tissues on Western blotting.** Scallop tissues were extracted with 2% SDS, 20 mM Tris, 10% glycerol, and 0.1% 2-mercaptoethanol as described under "MATERIALS AND METHODS." (A) The mantle extract was immunoblotted with an affinity-purified polyclonal antibody against N-terminal domain. A ScunM band is indicated by an arrowhead. (B) Equal amounts of total extracts of different tissues were immunoblotted. Lane 1, gland; lane 2, gonad; lane 3, pallial cell layer; lane 4, mantle; lane 5, catch muscle; lane 6, striated muscle.

tion of loop1, as found in other unconventional myosins (Fig. 3). At the position of loop 2, the ScunM sequence contains a unique insertion of 67 amino acids that is highly basic. Such an insertion in the loop 2 region has also been found in myr5/myr7 (class IX myosin) (*24, 25*). The charge and length changes of the loop 2 region affect actin-activated ATPase activity and the affinity for actin. Van Dijk *et al.* (*23*) reported that the addition of four positive charges in the primary sequence of loop 2 produced a 12-fold reduction of the Kapp for actin. It is presumed that the insertion would have an important influence on the nature of the ScunM-actin interaction.

Tissue Distribution of ScunM—To investigate the tissue distribution of ScunM, polyclonal antibodies were generated against a synthetic peptide (ADEDVDDLSC) as described under "MATERIALS AND METHODS." This antibody recognized an approximately 90 kDa band of the mantle extract (Fig. 5A), which corresponds to the calculated molecular weight determined from the deduced amino acid sequence. A tissue distribution study with this antibody demonstrated that ScunM was abundant in the mantle and mantle pallial cell layer, lower levels being detected in striated muscle, catch muscle, gland, and gonad (Fig. 5B). In several tissues, the apparent molecular weight of ScunM was slightly larger, suggesting that ScunM isoforms may exist in these tissues.

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