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

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

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egawa@ ple light chain-binding IQ motifs, and a multifunctional Cterminal 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-

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 multi-

(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)

GAGAGCACTAAATACATGGTCAAACACC<u>TTGTGTCCTTGTGTCCGAAA</u>GAGACTGGTGA<u>CCTCCACGAACGTATTGTCA</u>AGATCAACCCACTTCTG 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.

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1	ATGGCGGACGAGGACGTGGACGATCTGTCGCAGCTGGGAAATTTGGATAATGCCACCATAAAAAGAACTCTGCAGTCGCGATATGCGAAA	90
1	M A D E D V D D L S Q L G N L D N A T I K R T L Q S R Y A K	30
91	GACAAGATATACACGTACTGTGGCGACATACTCATTGCAGTAAATCCATTCAAGGATCTCCCTATTTTCGGAAAGAAGCAACATGAAGAA	180
31	DKIYTYCGDILIAVNPFKDLPIFGKKQHEE	60
181	TATCAC TGGAAGACAC TGCAACGTATGCC TCCACCACACGTATT TAACA TGGCTGCACGTGCCTACCGTCGGATACA TGAGACACGTACT	270
61	Y H W K T L Q R M P P P H V N M A A R A F Y R R I H E T R T	90
2 71		260
91		170
51		120
361		450
121	KETGDLHERIVKINPLLEAFGNAKTTM <u>ND</u>	150
	switch I	
451	TCCAGC AGAT T TGCCAAA T AT TT GGAGA T GTCT T TT GCGACCAA CGGCCAA G TAAC GGGAGCAA TA G TT AGA GACT ACT TGT TGGAAAAA	540
151	<u>S S R F</u> A K Y L E M S F A T N G Q V T G A I V R D Y L L E K	180
541		630
101	S R V D Q M D R E G N F H I F Y L F A G A P V I V L K N	210
631	C T G C A T C T G A A G G A T G C A A G A A C A T A C A G A A T G T A A A A G G C A A T G A A G A G T T G T A C A G A G C T A T G T A T C A G A G G C T A T G T A T C A G A G C T A T G T A T C A G A G C T A T G T A T C A G A G C T A T G T A T C A G A G C T A T G T A T C A G A G C T A T G T A T C A G A G C T A T G T A T C A G A G C T A T G T A T C A G A G G C T A T G T A T C A G A G C T A T G T A T C A G A G C T A T G T A T A A G G C A A T G A A G G C A A T G A A G G C T A T G T A T C A G A G C T A T G T A T C A G A G A G A T G A T C A G A G A G A G A G A T G A A G G G C A A T G A A G G G C T A T G T A T C A G A G A G A G A G A T G A A G G G C A A T G A A G G G C A A T G A G A G G G T A T G A A G G G C A A T G A G A G G G T A T G A A G G G C A A T G A G A G A G A G A G A G A G A G	720
211	L H L K D A R T Y R I V K G N E E L L T R T E F Y R A M Y Q	240
721	GAACAGAT AGAAGTACTCAAGTCTATCAATTTGGAACAAGAGGACATCGACATTATCCACACGATTCTGGCGGCTATACTTCTCATCACA	810
241	E Q I E V L K S I N L E Q E D I D I I H T I L A A I L L I T	270
811	CAGGT GGAAT T CC TG GAAC CT GAT GACCC TAAC GAGCC AAT GAAGAT CAAAGACAC TACAT T CGTT GAAAAC GT TGCCGACT TAT TGAAT	900
271	Q VEFLEPD DPNEPMKIKDTTFVEN VADLLN	300
901		990
301	V S Y E D L G H A L I A T K O T Y V G E T L V K R K S M Y Q	330
	TEDS site	-
991	GCCAT C GACA G CA GAC G CC TT C GCAAA AGCT C TC TA C GAAC G GA TT T TT G GT TG G AT CG T TC GC C AAAT AAA CT T GA ACCT C C AT C C G	1080
331	A I D S R D A F A K A L Y E R I F G W I V R Q I N L N L H P	360
1081	T CAAA G TT CAA AG CAC CC A CT GG A AG TA C AAGC A TT GG T AT AC T TG AC A TAG CT GG A TT TG AG AG AT TG GAAAT CAACAG CA TG G AACAG	1170
361	SKFKAPTGSTSIGIL <u>DIAGFE</u> RLEINSMEQ	390
	switch II	
1171	A IG IG I AI CAA IC IGA TAAAT GAAAGGCT CCAGAGT TT CACCAACAGAAAC GTCAT GGACT AT GAGAT GTCT AT AT AAAGAG GAAGGG	1260
391	MUINLINEKLŲSFINRNVMDYEMSIYKEEG Fig. 2. (continued)	420

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'-GG-IGA(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

1261	ATC	CAC	GTG	ACC	GGT	ATC	AAG	ΠΤ	AAG	AAC	AAT	GAT	GCG	G	ат е	GAC	T TG	ттс	ATG	AAG	AAA	ACA	тπ	GGC	стG	CTG	CCA	стс	СТС	GAC	1350
421	I	н	v	т	G	I	к	F	к	N	N	D	A	L	L	D	L	F	м	к	к	т	F	G	Ł	L	Ρ	L	L	D	450
1351	GAG	GAG	TCG	AAG	стт	GGA	CAA	GGT	тсс	AAT	GAA	AGA	π	GTA		A A A	стс	AAT	GAC	AAG	тас	GAT	ACG	CAC	CCA	TGT	ттс	ACA	GAA	тст	1440
451	E	Ε	S	к	L	G	Q	G	s	N	E	R	F	v	к	к	L	N	D	к	Y	D	т	н	Ρ	c	F	т	E	s	480
1441	CCA	CAT	GGT	CGT	GTG	GAA	ттс	GGT	ൺ	AGA	CAC	ттс	GCC	GCC	CAG	бтс	тGG	τάτ	GAC	GGG	тса	T TG	тπ	ATT	GAG	AAG	AAC	CGA	GAT	ATG	1530
481	Ρ	н	G	R	v	E	F	G	۷	R	Н	F	A	A	Q	۷	W	Y	D	G	S	L	F	I	E	к	N	R	D	м	510
1531	стg	AGC		GAT	GTT	АСС	тсс	TGT	ATG	AGA	GAG	AGT	GAC	AAT	CCA	π	GΠ	тсс	GAC	стт	ттс	АСТ	GΠ		AAG	GGG	CCA	ACA	GGG	АСТ	1620
511	ι	A	Q	D	v	т	s	с	м	R	E	s	D	N	Р	F	v	s	D	L	F	т	v	к	к	G	Ρ	т	G	т	540
1621	ATT	TCA	.GC G	ACA	ATG	CAG	AAC	ATC	AGG	AGG	псс	AGG	A A A	GCA	GAA	GGT	AGA	ണ	cc G	AGG	AAA	сст	ΑΤΤ	ACG	GCC	AGA	GGA	CAA	стт	cττ	1710
541	I	S	A	т	М	Q	N	I	R	R	S	R	к	A	E	G	R	G	Ρ	R	к	Ρ	I	т	A	R	G	Q	٤	ι	570
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1711	ATG	GCC	GAT	TTA	GGA	AGG	τα	СТА	AAA	GAA	AGG	TAC	GGT	GAA	тст	GTC	C AG	AGC	ACT	AAT	CAG	GTG	TAC	AAC	сст	AAA	GAT	CAC	A A4	ACA	1800
571	M	A	D	L	G	R	Ş	L	к	E	R	Y	G	Ε	\$	۷	Q	S	т	N	Q	۷	Y	N	Ρ	К	D	н	к	т	600
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1801	GTC	ATC _		TAC	TTC	CAG	AGC	тст	ATG	AAT	GAA	CTG	СТА	CAG	AAA	TTG		C GG	GCA	GAC	CCA	TAT	TAT	GTA	CGC	TGT	атс -	AAA	-	AAC	1890
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1891 631	ATG M	ΠC F	L L	А АА К	CCA P	GAC D	A AC N	TT C F	GAT D	GAC D	GAA E	A AG K	стс v	с Г	GAA E	C AG	ATG M	cπ ι	TAT Y	AAT N	دده 	ATA I	TCG S	GAA E	GTG V	GCA A	AAG K	TTA I	AGA. R	AAA K	1980 660
1891 631	ATG M	ΠC F	L	AAA K	P	GAC D	AAC N	ττc F	D	GAC	GAA E	A AG K	GT G V	L	GAA E	C AG Q	ATG M	ст L	TAT Y	AAT N con		ATA I ved	TCG S 91	GAA E yci	GTG V ne	GCA A	AAG K	I I	AGA R	AAA K	1980 660
1891 631 1981 661	ATG M CTT	F F GGT		K K CCT	CCA P ATT	GAC D CGG	AAC N AAA	ττc F CGG	GAT D TAT	GAC D GAC	GAA E GAC	AAG K TTC	GT G V AC A	L L	GAA E AGA	Q TAC	ATG M AGA	ст L сса	TAT Y CTG	AAT N con TTT		ATA I ved GAT	тсс S I g1 тст	GAA E yci CGG	GTG V ne AAG	GCA A GCG	AAG K CGA	ATT I TCG	AGA R GAC	AAA K AGA	1980 660 2070
1891 631 1981 661	ATG M CTT L	TTC F GGT G	CTA L CTT L	ааа К ССТ Р	P ATT I	GAC D CGG R	AAC N AAA K	TTC F CGG R	GAT D TAT	GAC D GAC D	GAA E GAC D	AAG K TTC F	GT G V AC A T	L L L L AAG K	GAA E AGA R	CAG Q TAC Y	ATG M AGA R	CTT L CCA P	TAT Y CTG L	AAT N con TTT F	GGG G ISER CTG L	ATA I ved GAT D	тсс S I g1 тст С	GAA E yci CGG R	GTG V ne AAG K	GCA A GCG A	AAG K CGA R	ATT I TCG S	AGA R GAC D	AAA K AGA R	1980 660 2070 690
1891 631 1981 661 2071	ATG M CTT L	F F GGT G	CTA L CTT L	ААА К ССТ Р GAG	P ATT I	GAC D CGG R	А АС N А АА К С Т А	F F CGG R	GAT D ΤΑΤ Υ	GAC D GAC D	GAA E GAC D	AAG K TTC F	GT G V AC A T	ітс L L К К	GAA E AGA R R	CAG Q TAC Y	ATG M AGA R	CTT L CCA P	TAT Y CTG L	AAT N con TTT F		ATA I ved GAT D	TCG S 91 TGT C	GAA E yci CGG R	GTG V ne AAG K	GCA A GCG A GTT	AAG K CGA R TTC	ATT I TCG S ATG	AGA R GAC D	AAA K AGA R GAA	1980 660 2070 690 2160
1891 631 1981 661 2071 691	ATG M CTT L GCT	F F GGT G GGG G	CTA L CTT L	AAA K CCT P GAG	CCA P ATT I CTG	GAC D CGG R CTT	а ас N А аа К С ТА	TTC F CGG R AAA	:GAT D :TAT Y .AAA	GAC D GAC D ACT	GAA E GAC D TTG	AAG K TTC F CCA P	GT G V AC A T GA T	ттс L L К К	igaa E iaga R R M	CAG Q TAC Y ATG M	ATG M AGA R TCA S	CTT L CCA P GGG	TAT Y CTG L ATA T	AAT N con TTT F CAA	ССС <u>С</u> СТС СТС СТС Г Г Г	ATA I Ved GAT D GGC	тс s g т с ААА К	GAA E yci CGG R ACA	GTG V ne AAG K AGG	GCA A GCG A GTT V	AAG K CGA R TTC F	ATT I TCG S ATG M	AGA R GAC D CAG	AAA K AGA R GAA E	1980 660 2070 690 2160 720
1891 631 1981 661 2071 691	ATG M CTT L GCT A	F F GGT G GGG G	CTA L CTT L	AAA K CCT P GAG E	CCA P ATT I CTG L	GAC D CGG R CTT L	А АС N А АА К С ТА L	F F CGG R AAA K	GAT D TAT Y AAAA K	GAC D GAC D ACT T	GAA E GAC D TTG L	AAG K TTC F CCA P	GTG V ACA T GAT D	ттс L АААС К К	igaa E iaga R R M	CAG Q TAC Y ATG M	ATG M AGA R TCA S	CTT L CCA P GGG G	TAT Y CTG L ATA I	AAT N con TTT F CAA Q	GGG G Ser CTG L TTT F	ATA I Ved GAT D GGC	TCG S Ig1 TGT C AAA K	GAA E yci CGG R ACA T	GTG V ne AAG K AGG R	GCA GCG A GTT V	AAG K CGA R TTC F	ATT I TCG S ATG M	AGA R GAC D CAG Q	AAA K AGA R GAA E	1980 660 2070 690 2160 720
1891 631 1981 661 2071 691 2161	ATG M CTT L GCT A GAT	TTC F GGT G GGG GTC	CTA L CTT L GCA A	AAAA K CCT P GAG E	CCA P ATT I CTG L	GAC D CGG R CTT L CTA	AAC N AAA K CTA L GAG	TTC F CGGG R AAAA K	GAΤ D TAΤ Υ ΑΑΑΑ Κ	GAC D GAC D ACT T CGT	GAA E GAC D ⊤TG L	AAG K TTC F CCA P TTC	GTG V ACA T GAT D	БТТ (L лаа (К Хаа а К	GAA E GAGA R R M GCGG	CAG Q TAC Y ATG M	ATG M AGA R TCA S GCC	CTT L CCA P GGG GTT	TAT Y CTG L ATA I GAC	AAT N con TTT F CAA Q	GGG <u>G</u> JSER CTG L TTT F ATT	ATA I Ved GAT D GGC G	т с	GAA E yci. CGG R ACA T	GTG V ne AAG K AGG R	GCA A GCG A GTT V CAG	AAG K CGA R TTC F	ATT I TCG S ATG M TAT	AGA R GAC D CAG Q	AAA K AGA R GAA E ATT	1980 660 2070 690 2160 720 2250
1891 631 1981 661 2071 691 2161 721	ATG M CTT L GCT A GAT D	TTC F GGT G GGG GTC V	CTA L CTT L GCA A AGT S	AAA K CCT P GAG E ATC I	CCA P ATT I CTG L TGG W	GAC D CGG R CTT L CTA L	AAC N AAA K CTA L GAG E	TTC F CGG R AAAA K K	GATI D ITATI Y AAAA K .TGTI C	GAC D GAC D ACT T CGT R	GAA E GAC D ⊤TG L GGT G	AAG K TTC F CCA P TTC F	GTG V ACA T GAT D AGG	БТТ (L L L AAA (К К С ААА А К С Б GA (E	GAAA E GAGA R NATG M CGGG B	CAG Q TAC Y ATG M GCT A	ATG M AGA R TCA S GCC A	CTT L CCA P GGG G GTT <u>V</u>	TAT Y CTG L ATA I GAC	AAT N con TTT F CAA Q ACC I	GGG G Iser CTG L ITT F ATT	ATA I ved GAT D GGC G GCT	ТСG S Ig1 TGT С АААА К АААА К	GAA E yci CGG R ACA T AGG <u>B</u>	GTG V ne AAGG K TGG	GCA A GCG A GTT V CAG Q	AAG K CGA R TTC F CAA Q	ATT I TCG S ATG M TAT <u>Y</u>	AGA: R GAC: D CAG Q AAG: K	AAAA K AGGA R GAAA E ATTT J	1980 660 2070 690 2160 720 2250 750
1891 631 1981 661 2071 691 2161 721	ATG M CTT L GCT A GAT D	דד כ F GGT G G G G T C V	CTTA L CTTT L GCA A XAGT S	AAA K CCT P GAG E ATC I	CCA P ATT I CTG L TGG	GAC D CGG R CTT L CTA L	AAC N AAA K CTA L GAG E	TTC F CGGG R AAAA K K	GATI D TATI Y AAAA K TGTI C	GAC D GAC D ACT T CGT R	GAA E GAC D ⊤TG L GGT G	AAG K TTC F CCA P TTC F	GTG V ACA T GAT D AGG <u>B</u>	L L L L L L L L L L L L L L L L L L L	GGAA E GAGA R AATG M GCGG B	CAG Q TAC Y ATG M GCT A	ATG M AGA R TCA S GCC A	CTT L CCA P GGG G GTT <u>V</u>	TAT Y CTG L ATA I GAC D	AAT N con TTT F CAA Q Q ACC I	GGGG GSER CTG L TTT F ATT I	ATA I ved GAT D GGC G GCT	TCG S Ig1 TGT C AAAA K <u>K</u>	GAA E yci. CGG R ACA T AGG <u>B</u>	GTG V ne AAGG K TGG <u>W</u>	GCA A GCG A GTT V CAG Q	AAG K CGA R TTTC F CAA	ATT I TCG S ATG M TAT <u>Y</u>	AGA: R GAC: D CAG Q AAG: <u>K</u>	AAA K AGA R GAA E ATT J	1980 660 2070 690 2160 720 2250 750
1891 631 1981 661 2071 691 2161 721 2251	ATG M CTT L GCT A GAT D GAA	TTC F GGT G GGG GTC V AAAC	CTA L CTT L GCA A AGT S	AAAA K CCT P GAG E ATC I	CCA P ATT I CTG L TGG W	GAC D CGG R CTT L CTA L GAA	AAC N AAA K CTA L GAG E	TTC F CGG R AAAA K TTG	GATI D ITATI Y AAAA K TGTI C	GAC D GAC D ACT T CGT R GAA	GAA E GAC D TTG L GGT G G	AAG K TTC F CCA P TTC F	GTG V ACA T GAT D AGG R ATC	E L AAAG K K GGAG E	GAAA E GAGA R NATG M CGGG B	CAG Q TAC Y ATG M GCT A	ATG M AGA R TCA S GCC A AGG	CTT L CCA P GGG GTT ⊻ GGC	TAT Y CTG L ATA I GAC D AGA	AAT N con TTT F CAA Q ACC I GCT	GGGG GECTG L TTTT F ATTT I GCT	ATA I Ved GAT D GGC G GCT A TCT	TCG S Ig1 TGT C AAAA K <u>K</u> GAA	GAA E yci CGG R ACA T AGG <u>B</u>	GTG V ne AAGG K TGG ¥ AAC	GCA A GCG A GTT V CAG Q TTT	AAG K CGA R TTTC F CAA Q GCC	ATT I TCG S ATG M TAT <u>Y</u> AGA	AGA: R GAC: D CAG Q AAG 	AAA K AGA R GAA E ATT J AAT	1980 660 2070 690 2160 720 2250 750 2340
1891 631 1981 661 2071 691 2161 721 2251 751	ATG M CTT L GCT A GAT D GAA <u>E</u>	F F GGT G GGG G GTC V AACC	CTA L CTT L GCA A AGT S	AAAA K CCT P GAG E ATC I CGA	CCA P ATT I CTG L TGG W AAA	GAC D CGG R CTT L CTA L GAA <u>-</u> E	AAC N AAAA K CTA L GAG E GAA	TTC F CGGG R AAAA K AAAA K	GATI D ITATI Y AAAA K .TGTI C TCG	GAC D GAC D ACT T CGT R GAA	GAA E GAC D TTG L GGT G G G G G G G G G G	AAG K TTC F CCA P TTC F GCG	GTG V ACA T GAT D AGG <u>R</u> ATC	БТТ (L К К К К К С С С С С С С С С С С С С	GGAA E GAGA R AATG M GCGG B CGG B	CAG Q TAC Y ATG M GCT A GCT	ATG M AGA R TCA S GCC A GCC A GCC	стт L ССА Р GGGG GTT <u>У</u> GGCC	TAT Y CTG L ATA I GAC D AGA	AAT N con TTT F CAA Q Q ACC I GCT	GGGG Generation CTG L TTT F ATT I GGCT	ATA I Ved GAT D GGC G GCT A TCT	TCG S I g1 TGT C AAAA K GAA GAA	GAA E yci CGG R ACA T AGG <u>B</u> AAA	GTG V ne AAGG K AGG R TGG <u>W</u>	GCA A GCG A GTT V CAG Q TTT	AAG K CGA R TTTC F CAA Q GCC	ATT I TCG S ATG M TAT Y AGA	AGA. R GAC. D CAG Q Q AAG. K 	AAA K AGA R GAA E ATT J	1980 660 2070 690 2160 720 2250 750 2340 778
1891 631 1981 661 2071 691 2161 721 2251 751	ATG M CTT L GCT A GAT D GAA	F F GGT G GGG G GTC V AAC	CTA L CTT L GCA A AGT S	AAAA K CCCT P GAG E ATC I CGA	CCA P ATT I CTG L TGG W AAA	GAC D CGG R CTT L CTA L GAA	AAC N AAA K CTA L GAG E GAA	TTC F CGG R AAAA K TTG	GAT D TAT Y AAAA K TGT C TCG	GAC D GAC D ACT T CGT R GAA	GAA E GAC D TTG L GGT G GGC	AAG K TTC F CCA P TTC F GCG <u>A</u>	GTG V ACA T GAT D AGG <u>R</u> ATC <u>I</u>	ала G К К СААА К Б GGA G Е СGGA	GAAA E GAGA R AATG M GCGG B ACAG	CAG Q TAC Y ATG M GCT A GCT A GC	ATG M AGA R TCA S GCC A AGG	ст L ССА Р G G T S G C S G T S S C C S S S S S S S S S S S S S S S	TAT Y CTG L ATA I GAC <u>D</u> AGA	AAT N con TTT F CAA Q ACC I GCT	GGGG <u>G</u> Ser CTG L TTT F ATT I GCT <u>A</u>	ATA I Ved GAT D GGC G GCT A TCT	TCG S Ig1 TGT C AAAA K GAA	GAA E yci CGG R ACA T AGG <u>B</u> AAA	GTG V ne AAGG K TGG <u>W</u> AACC	GCA A GCG A GTT V CAG Q TTT E	AAG K CGAA R TTTC F CAA Q GCC <u>A</u>	ATT I TCG S ATG M TAT <u>Y</u> AGA	AGA R GAC D CAG Q AAG X TAA	AAAA K AGGA R GAAA E AATT J AAAT	1980 660 2070 690 2160 720 2250 750 2340 778
1891 631 1981 661 2071 691 2161 721 2251 751 2341	ATG M CTT L GCT A GAT D GAA GAA	TTC F GGT GGGG GGGG GTC AACC	CTA L CTT L GCA A AGT S AAA A	AAAA K CCT P GAG E ATC I CGA	CCA P ATT I CTG L TGG W AAA AAA AAA	GAC D CGG R CTT L CTA L GAA - <u>E</u> ATT	AAC N AAA K CTA L GAG E GAA TGG	TTC F CGG R AAAA K TTG <u>T</u> TG CAA	GAT D TAT Y AAAA K TGT C TCG	GAC D GAC D ACT T CGT R GAA GAAG	GAA E GAC D TTG L GGT G GGT	AAG K TTC F CCA P TTC F GCG <u>A</u> TTT	GTG V ACA T GAT D AGG <u>R</u> ATC <u>I</u> CAT	L L K AAAG K K GGAG E CGGA GCA	GGAA E GAGA R AATG M GCGG B ACAG 2 Ω GGGA	CAG Q TAC Y ATG M GCT A GCT A GCT	ATG M AGA R TCA S GCC A GCC A GCC A TGT	СТТ L ССА Р GGG G G Т САG САG	TAT Y CTG L ATA I GAC D AGA	AAT N CON TTT F CAA Q ACC I ACC I GCT	GGGG GGG CTG L TTT F ATT I GCT GCT GCT	ATA I Ved GAT D GGC G G G CT T CT S AGA	TCG S Ig1 TGT C AAAA K AAAA <u>K</u> GAA <u>F</u> GGGG	GAA E yci CGG R ACA T AGG <u>B</u> AAA <u>K</u> GAA	GTG V ne AAG K AGG R TGG <u>W</u> AAC <u>N</u> CCA	GCA A GCG A GTT V CAG Q TTT E	AAG K CGA R TTC F CAA Q GCC AGC	ATT I TCG S ATG M TAT <u>R</u> TAT	A GA: R GAC: D CAG Q XAAG. K TAA	AAAA K AGGA R GAA E AATT J GGT	1980 660 2070 690 2160 720 2250 750 2340 778 2340 778
1891 631 1981 661 2071 691 2161 721 2251 751 2341 2431	ATG M CTT L GCT A GAT GAT GAA GAT AGA		CTA L CTT L GCA A AGT S AAA A GG CAG	AAAA K CCT P GAG E ATC I CGA	CCA P ATT I CTG L TGG W AAAA <u>K</u> AAAA	GAC D CGG R CTT L CTA L GAA <u>-</u> <u>E</u> ATT ATG	AAC N AAA K CTA L GAG E GAA TGG CTG	TTC F CGG R AAAA K AAAA K TTG <u>T</u> G CAA	GATI D ITATI Y AAAA K TGT C ITCG	GAC D GAC D ACT T CGT R GAA GGAA GCC	GAA E GAC D TTG C GGT G GGT GGT GGT	AAG K TTC F CCA P TTC F GCG <u>A</u> TTT	GTG V ACA T GAT D AGG <u>B</u> ATC <u>I</u> CAT	L L L L L L L L L L L L L L L L L L L	GAAA E GAGA R AATG M CGG B CGG CGG CGG CGG	CAG Q TAC Y ATG M GCT A GCT A GCT A GCT	ATG M AGA R TCA S GCC A GCC A GCC	CTT L CCA P GGGC GTT <u>Y</u> GGCC <u>G</u> CAG	TAT Y CTG L ATA I GAC D AGA TAT GAC	AAT N CON TTT F CAA Q ACC I ACC I GAG		ATA I Ved GAT D GGC G G G C T C T C T C A G A G A G A G A G A C A C	TCG S Ig1 TGT C AAAA K AAAA <u>K</u> GAA <u>G</u> AA	GAA E yci CGG R ACA T AGG <u>B</u> AAA <u>A</u> GAA	GTG V ne AAG K AGG R TGG <u>W</u> AAC <u>N</u> CCA	GCA A GCG A GTT V CAG Q TTTT E AAC	AAG K CGA R TTCC F CAA Q GCCC AGCC	ATT I TCG S ATG M TAT <u>R</u> TAT ATC	A GA: R GAC: D CAG Q AAG K TAA: A GA	AAA K AGA R GAA E J AATT J GGT GAA	1980 660 2070 690 2160 720 2250 750 2340 778 2430 2520
1891 631 1981 661 2071 691 2161 721 2251 751 2341 2431 2521	ATG M CTT L GCT A GAT D GAA GAA AGA	TTC F GGT GGG G GTC V AAC TTC	CTA L CTTT L GCA A AGT S AAAA AGG CAG GTG	AAAA K CCT P GAG E ATC I CGA GAT GAT	CCA P I CTG L TGG W AAAA <u>K</u> TGT TCA	GAC D CGG R CTT L CTA L GAA ATT ATG GAA	AAC N AAA K CTA L GAG E GAA TGG CTG AAG	TTC F CGG R AAAA K AAAA K TTG <u>T</u> CAA ATG	GATT D TATT Y AAAA K TGT C TCG TCG TGG	GAC D GAC D ACT T CGT R GAA GAA GCC AGA	GAA E GAC D TTG L GGT GGT GGT GGT AGG TTG	AAG K TTC F CCA P TTC F GCG <u>A</u> TTT AAG	GTG V ACA T GAT D AGG B ATC <u>I</u> CAT GAC	I AAAG K K AAAA K GGAG E GGAA GCA TTC GTA	GAAA E GAGA R AATG M CGGG CGGA CGGA	CAG Q TAC Y ATG M GCT A GC <u>S</u> AGA TGG GGG	ATG M AGA R TCA S GCC A GCC A GCC TGT GAC TTG	CTT L CCA P GGG G GTT <u>Y</u> GGC <u>G</u> CAG GAC	TAT Y CTG L ATA GAC D AGA C CGC	AAT N con TTT F CAA Q ACC I ACC I GAC GAC CTC		ATA I Ved GAT D GGC. G GCT. A GCT. A GAGA CAC	TCG S Ig1 TGT C AAAA K AAAA K GAA <u>K</u> GAA GGG GGG CTT TAT	GAA E yyci. CGG R ACA. T AGG <u>B</u> AAA AGG GAA GAA	GTG V ne AAGG R TGG <u>W</u> AACC <u>N</u> CCA	GCA A GCG A GTT V CAG Q TTT <u>E</u> AAC TCG	AAG K CGA R TTCC F CAA GCCC <u>A</u> CGT	ATT I TCG S ATG M TAT Y AGA TAT ATC GTA	AGA: R GAC: D CAG Q AAG K TAA: TAA: TGT TAG	AAA K AGA R GAA E AATT J J GGT GGA GCA	1980 660 2070 690 2160 720 2250 750 2340 778 2340 778 2430 2520 2610
1891 631 1981 661 2071 691 2161 721 2251 751 2341 2431 2521 2611	ATG M CTT L GCT A GAT D GAA GAA GAA GAA	TTC F GGT GGGG GGC V AACC TTC GTC	CTA L CTT L GCA A AGT S AAAA AGG GTG ACG	AAAA K CCT P GAG E ATC I CGA GAT GGA	CCA P ATT I CTG L TGG W AAAA <u>K</u> TGT	GAC D CGG R CTT L CTA L GAA ATT ATG GAA	AAC N AAA K CTA L GAG E GAA TGG CTG AAG CAA	TTC F CGGG R AAAA K AAAA K TTG <u>T</u> CAAA ATG GTG	GATT D TATT Y AAAA K TGT C TCG TGG TGG	GAC D GAC D ACT T CGT R GAA GCC AGA	GAA E GAC D TTG L GGT G GGT GGT GGT AGG TTG	AAG K TTC F CCA P TTC F GCG <u>A</u> TTT AAG	GTG V ACA T GAT D AGG <u>I</u> CAT GAC GGT	AAAG K AAAA K GGAG E GGAA GCA C GTA	GAAA E GAGA R AATG M GCGG B CGGG GGAA	CAG Q TAC Y ATG GCT A GCT <u>S</u> AGA TGG GGG	ATG M AGA TCA S GCC A GCC A GACG TTG ACG	CTT L CCA P GGG G GTT ⊻ GGC GAC ACA	TAT Y CTG L ATA GAC D AGA <u>D</u> TAT GAC CGC	AAT N con TTT F CAA Q CAA Q ACC I GAC CTC GAG CTC		ATA I Ved GAT D GGC G G T CT GTA	TCG S 91 91 TGT C AAAA K GAAA <u>E</u> GGGG CTT TAT	GAA E yyci: CGGG R ACA T AGGG <u>B</u> AAAA <u>K</u> GAA GAA	GTG V ne AAGG K TGG TGG <u>W</u> CCA AAGG	GCA GCG A GTT V CAG Q TTT <u>E</u> AAC TCG	AAG K CGA R TTCC F CAA Q GCCC AGCC TTCC CGT	ATT I TCG S ATG M TAT Y AGA TAT AGA	A GA: R GAC: D CAG Q AAG K TAA: TGT TAG	AAA K AGA R GAA E AATT J GGT GGA GCA	1980 660 2070 690 2160 720 2250 750 2340 778 2340 778 2430 2520 2610 2661

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.

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into the λ TriplEX2TM 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 pTriplEX2TM plasmid with an insert from an isolated positive clone was recovered using the *in vivo* excision feature of the λ TripleEX2TM, 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

C		
ScunM		
MYUN		
chickon		52
TgM-A	MASKTTSEELKTATALKKRSSDVHAVDHSGNVYKGFQIWTDLAPSFTVKEEPDLMFAKCI	52 60
ScunM	NATIK	21
МуоК	NGEVT	24
adrenal	EAAFI	29
chicken	QSKEGGKVTVK-TEGGETLTVKEDQVFSMNPPKYDKIEDMAMMTHLHEPAVL	104
TgM-A	VQAGTDKGNLTCVQIDPPGFDEPFEVPQANAWNVNSLIDPMTYGDIFTGMLPHTNIPCVL	120
ScunM	RTLQSRYAKDKIYTYCGDILIAVNPFKDLPIFGKKQHEEYHWKTLQRMPPPHV-N	75
МуоК	SQIGARFDRELIYTNIGEVLIFTAVNPYKALPITGPEFIKLYQNASGSDASP-HIYA	80
adrenal	ENLRRRFRENLIYTYIFTGPVLVSVNPYRDLQIYSRQHMERYRGVSFYEVPP-HLFA	85
chicken	YNLKERYAAWMIYTYSGLFCVTVNPYKWLPVYNPEVVLAYRGKKRQEAPP-HIFS	159
TgM-A	DFLKVRFMKNQIYTTADPLVVAINPFRDLGNTTLDWIVRYRDTFDLSFTKLAP-HVFY	177
ScunM	MAARAYRRIHETRTNQVILLEGNSGAGKTESTKYMVKHLVSLCP	120
МуоК	LAERAYRRMVDENESQCVIISGESGAFTGKTVSAKLILQYVTSVSPNNSSGGGIGGSGGG	140
adrenal	VADTVYRALRTERGDQAVMISFTGESGAGKTEATKRLLQFYAETCP	131
chicken	ISDNAYQFMLTDRENQSILITGESGAGKTVNTKRVIQYFATIAA	203
Igm-A	IARRALDNLHAVNKSQIIIVSGESGAGKIEAIKQIMRYFAAAKI loop1	221
ScunM	KETGDLHERIVKINP-	135
МуоК	NGGIPQYDGGSDDRPSPPMGRGMGMPGFTMVGRGGLPTRGGGPPSRGGGPPPTRGRGGPP	200
adrenal	APERGGAVRDRLLQSNP-	148
chicken	SGEKKKEEQSGKMQGTLEDQIISANP-	229
TgM-A	GSMDLRIFTQNAIMAANP-	239
ScunM		
МуоК	PPIPQNRGAPPPVSNGGAPPPVARGPVAFTPPPTRGAPPTRGGGPANRGGRGGGPPPVST	260
adrenal		
chicken		
Igm-A		
ScunM	LLEAFGNAKTTMNDNSSRFA~~KYLEMSFA	163
МуоК	SRGGGGYGGSSKTVDVEHIKKVILDSNPLFTMEAIGNAKTVRNDNSSRFGKYLEIQFD	318
adrenal	VLEAFGNAKTLRNDNSSRFFTGKYMDVQFD	178
chicken	LLEAFGNAKTVRNDNSSRFGKFIRIHFG	257
TgM-A	VLEAFGNAKTIRNNNSSRFGRFMQLDVG	267
ScunM	TNGQVTGAIVRDYLLEKSRVVDQMDKEGNFHIFYCLFAGAPVTVLKNLHLKDAR	217
МуоК	DNNAPVGGLISTFLLEKTRVTFQQKNERNFHIFTFYQMLGGLDQTTKS-EWGLTQATD	375
adrenal	FKGAPVGGHILSYLLEKSRVVHQNHGERNFHIFYQLLEGGEEETLRRLGLFTERNPQS	236
chicken	ATGKLASADIETYLLEKSRVTFQLPAERSYHIFYQIMSNKKPELIDMLLITINPY	302
IGM-A	KEGGIKFGSVVAFLLEKSKVLIFIQDEQEKSTHIFTQMCKGADAAMKEKFHILPLS	323
ScunM	-TYRIVKGNEELLTRTEFYRAMYQEQIEVLKSINLEQEDIDIIHTILAAILLITQVEFLE	276
МуоК	FYYLAQSKCTTVEDVDDGKDFHEVKAAMETVGISRDFTEQTEIFRILAAILHVGNIRFQG	435
adrenal	YLYLVKGQCAKVSSINDKSDWKVVRKALTVIDFTEDEVEDLLSIVASVLHLGNFTTHF	294
chicken	DYHYVSQGEITVPSIDDQEELMATDSAIDILGFSADEKTAIYKLTGAVMHYGNLKFK-	359
ТдМ-А	EYKYINPLCLDAPGIDDVAEFHEVCESFTFRSMNLTEDEVASVWSIVSGVLLLGNVEVTA TEDS site	383
ScunM	PDDPNEPMKIKDTTFVENVADLLNVSYEDLGHALIATKQTYVG (E TLVKRKSMYQA	331
МуоК	EAPASVIDETPLQWAASLLGCDPTFLCQSLNHRQIQSFTGSARHTQYQVPQNPDQS	495
adrenal	AADEESNAQVTTENQLKYLTRLLGVEGSTLREALTHRKIIAKGEELLSPLNLEQA	349
chicken	QKQREEQAEPDGTEVADKAAYLMGLNSAELLKALCYPRVKVGNEFVTKGQTVSQV	414
TgM-A	TKDGGIDDAAAIEGKNLEVFKKACGLLFFTLDAERIREELTVKVSYACHQEIRGRWKQED	443
ScunM	IDSRDAFAKALYERIFGWIVRQINLMLHPSKFKAPTGSTSIGILDIAGFERLEIN-	386
МуоК	AGLRDALAKTLYERIFDFIVARVNKAMSFSGNCKVIGVLDIYFTGFEVFER	546
adrenal	AFTYARDALAKAVYSRTFTWLVAKINRSLASKDAESPSWRSTTVLGLLGIYGFEVFQHNS	409
chicken	HNSVGALAKAVYEKMFLWMVIRINQQLDTKQPRQYFIGVLDIAGFEIFDFN-	465
TgM-A	GD-MLKSSLAKAMYDKLFMWIIAVLNRSIKFTPPGGFKIFMGMLDIFGFEVFKNN-	499

Fig. 3. (continued)

rabbit was first immunized with 500 μ g of peptide with Freund's complete adjuvant. Three successive injections of 500 μ g 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 METH-ODS," 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

ScunM	-SMEQMCINLINERLQSFTNRNVMDYEMSIYKEEGIHVTGIKFKNNDALLDLFMKKTFGL	445
МуоК	NSFEOFCINYVNERLOOIFIDLTVRGEOREYHEEGMKWKDISFFDNKIVVDLFTIDGNKP	606
adrenal	FEFTOFCINYCNEKLOOLFIELTLKSEQEEYEAEGIAWEPVOYFNNKIICDLVEEKFKGI	469
chicken	-SFEQLCINFTNEKLQQFFNHHMFVLEQEEYKKEGIEWEFIDFGMDLAACIELIEKPMGI	524
TgM-A	-SLEQFFINITNEMLQKNFVDIVFDRESKLYRDEGVSFTSKELIFTSNAEVIKILTAKNN	558
ScunM	LPLLDEESKLGOGSNERFVKKLNDKYDTHPCFTESPHGRVEFG	488
МуоК	PGIMRVLDDVCKTVHAVDSAAADIKFMEKLIHSIQSHPHLVISNTGSSADEFTFT	661
adrenal	ISIFTLDEECLRPGEATDLTFLEKLEDTIKOHPHFLTHKLADORTRKSLDRGEFR	524
chicken	FSILEEECMFPKATDTSFKNKLYDOHLGKSNNFOKPKPAKGKAEAHFS	572
TgM-A	SVLAALEDQCLAPGGSDEKFLSTCKNALKGTTKFKPAKFTVSPNINFL	616
ScunM	VRHFAAOVWYDGSLFIEKNRDMLSODVTSCMRESDNPFVSDLFTVKKGPTGTISATMO	546
MyoK	IKHYAGEVSYSIEEFCFKNNDNLYASIVGCLONSTYOFIVSLFP	705
adrenal	LLHYAGEVTFTYNVTGFLDKNNDLLFRNLKETMCSSENPILGOCFD	570
chicken	LVHYAGTVDYNISGWLEKNKDPLNETVIGLYOKSSVKTLALLFA	616
TgM-A	ISHTVGDIQYNAEGFLFKNKDVLRAEIMEIVQQSKNPVVAQLFA	660
Sound		606
Scurm		775
nyun		723 E00
abielien		590
		020
Тдм-А		002
ScunM	SSMNELLOKLORADPYYVRCTKPNMELKPDNEDDEKVLEOMLYNGTSEVAKTRKLGLP	664
MyoK	OSSSYLVTRI SACTPHYTRCTKPNDKKOPMNEVSSRVEHOVKYLKTLENTKVKRSGET	783
adrenal	LI ELETVETI KSKEPAYVRCTKPNDSKOPGREDEVLTRHOVKYLKIMENI RVRRAGEA	648
chicken	ENINKI MANI RSTHPHEVRCTTPNETKTPGAMEHELVI HOLRCNOVLEGTRTCRKGEP	694
TgM-A	SQLQSLMELINSTEPHFIRCIKPNDTKKPLDWVPSKMLIQLHALQVFTLEALQLRQLGYS	742
ScunM	TRKRYNDETKRYRPI EI DCRKARSDRAGAELLI KKTI PDKMMSGTOEGKTRVEMOEDV	777
Myok		840
adrenal	YRRKYFAFTFI ORYKSI CPETWPTWTGRRODGVTVI VRHI GYKPEFYKMGRTKTETREPK	708
chicken	SRVI YADEKORYRVI NASATPEGOEMDSKKASEKI I G-STDVDHTOYREGHTKVEEKAGI	753
TgM-A	YRRPFKEFLFQFKFIDLSASENPNLDPKEAALRLLKSSKLPSEEYQLFTGKTMVFLKQAK	802
ScunM	STWI FKCRGF	737
MyoK	TIFTEVMEDI	850
adrenal	TI FATEDAFT	808
chicken	I GLI FEMRDD	763
ToM-A	FLTOTORECI	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 myosinI β (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 threeonine) 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 threeonine residue and a negatively charged glutamic acid residue (at position 320) at this site, respectively (Fig. 3). On the other hand, TgM has



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.

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. 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 "MATERI-ALS 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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