Partial cDNA Sequence Analysis of Myosin Va from Rainbow Trout (*Oncorhynchus mykiss*) and Its Relationship to Myosin V Isoforms from Other Vertebrates

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Abstract: Partial cDNA sequences of myosin V from rainbow trout *Oncorhynchus mykiss* were analyzed and showed high similarity to MVa from other vertebrates. Phylogenetic analysis has shown that events resulting in the formation of paralogous copies of myosin Va, Vb, and Vc occurred before the divergence of vertebrates into different classes. Expression analysis of myosin Va, Vb, and Vc in different *O. mykiss* tissues revealed MVa exclusively expressed in hypophysis and brain whereas Vb and Vc were expressed in practically all tissues analyzed. The nucleotide sequence for myosin V was explored in a fish species for the first time and these results represent an important start in understanding the organization, evolution, and expression of myosins in early vertebrates. The data presented here represent contributions to the knowledge of rainbow trout genome. A better understanding of this economically important species could assist in development of improved strains of this fish for aquaculture.

Key Words: Nucleotide sequence, Amino acid sequence, Evolution, Tissue expression, Fish.

INTRODUCTION

Myosins make up a large superfamily of molecular motors that appeared early in eukaryotic evolution [1,2). They are mechanoenzymes that bind to actin and hydrolyze ATP to produce mechanical force; their members participate in activities as diverse as cytokinesis, muscle contraction, and organelle motility [3].

Myosins are typically composed of three functional subdomains: (1) the motor domain which interacts with actin and binds ATP, (2) the neck domain which binds light chains or calmodulin, and (3) the tail domain involved in protein-protein interactions. Although most myosins have the same general structure, the nonmotor regions vary enormously. This structural diversity reflects functional diversity [4]. Myosin heavy chains have been categorized into 18 structurally distinct protein classes, mostly based on comparisons and phylogenetic analysis of the conserved motor domain. However, current studies of the myosin gene family have revealed 37 myosin types with different protein domain combinations and scattered taxonomic distribution [5].

Class V myosin is one of the most ancient and extensively studied group of the myosin superfamily and the wide range of species in which it has been identified suggests that myosin V is a fundamental component of organelle transport in all higher eukaryotes [6]. It was initially characterized as an unusual calmodulin binding protein from the brain with a number of myosin-like biochemical properties [7,8,9]. Subsequently, myosin V heavy chain genes were cloned from mouse, yeast, and chicken, thus defining the fifth class of actin-based motors [10,11,12,13,14]. Vertebrate class V myosins have three members, Va, Vb, and Vc. Myosin Va has undergone the most investigation and is expressed at high levels essentially in the brain and melanocytes. The second member, myosin Vb has been cloned from rat [15], but has received relatively little attention. The third member, myosin Vc was discovery and cloned recently and is relatively abundant in many secretory and glandular tissues, where it is predominantly expressed in epithelial cells [16].

The presence of the three class V members of myosins among vertebrates leads to many questions on their relative roles within the cell related to expression and tissue distribution. Considering that little attention has been direct to myosin genes in ancient vertebrates, in this study we investigated myosin V-encoding cDNAs in *Oncorhynchus mykiss* rainbow trout aiming to contribute to the basic knowledge on its functions and evolutionary behavior in early vertebrates. Raibow trout originate from western North America and were widely introduced in several coutries of the world, including Brazil [17]. Although genetic linkage maps and physical chromosome maps have been constructed for rainbow trout [18], genome information of this species are still needed to assist in development of improved strains of this fish for aquaculture.

MATERIALS AND METHODOLOGY

Adult rainbow trout (*O. mykiss*) specimens were obtained from Núcleo Experimental de Salmonicultura de Campos do Jordão (São Paulo, Brazil). This trout stock was originated from imported animals from Mount Shasta, California, USA. The animals were sacrificed with an overdose of benzocaine followed by spinal transection (Protocol 01204 – Ethical Committee on Animal Experimentation – Instituto de

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Table 1. Primer Sequences and Annealing Region on Myosin V Domains

Functional Domains	Primer Sets
Head	BMV-F (5'-CTGGTGGGTGAGAATGACCT-3') BMV-R1 (5'-CTTGATGCAGCGCACATAGT-3')
Neck	MyRC1 (5'-AAGACTGTTGGCTGCCAGTT-3') BMV-R (5'-GCAGCTGCATGATCTTGTTT-3')
Tail Va, Vb, and Vc	Tail-Va-F1 (5'-TAGAAGTGGGGCAGATGGAG-3') Tail-Va-R1 (5'-ACTGGAGGTTCGCTTTCTGA-3') Tail-Vb-F1 (5'-TGAGAGTATCCAGGGGCTGT-3') Tail-Vb-R1 (5'-AGCATCCTCCTGGGTCTTCT-3') Tail-Vc-F1 (5'-CCAAGCAACTTTTGGAGAGC-3') Tail-Vc-R1 (5'-CTGCTTGCTTCTTTGGGAAC-3')

Biociências, UNESP, Botucatu, Brazil) and hypophyses, brains, aortas, hearts, gills, muscles, stomachs, livers, kidneys, intestines, and spleens were dissected and frozen in liquid nitrogen for subsequent RNA extraction.

Total RNA samples were obtained using TRIzol reagent (Invitrogen) as per manufacturer's instructions. RNA was quantified by spectrophotometry (Biophotometer Eppendorf) at 260nm and 280nm, using the RNA correction factor. First-strand cDNA synthesis reaction was performed with "SuperScriptTM First-Strand Synthesis System for RT-PCR" commercial kit (Invitrogen Life Technologies) using random hexamer primers. The cDNA amplification was performed using BM5-F and BM5-R1 primer sets for the head domain, and MYCR1 and BMV-R for the neck domain of myosin Va, and Tail-Va-F1 and Tail-Va-R1, Tail-Vb-F1 and Tail-Vb-R1 and Tail-Vc-F1 and Tail-Vc-R1 for the tail domains of myosin Va, Vb, and Vc, respectively (Table **1**). The primer sets employed were designed from published chicken myosin Va cDNA sequence (GenBank accession number NM205300), genomic nucleotidic sequence of zebrafish (GenBank accession number BX088562), and rat myosin V cDNA sequences (GenBank accession numbers AB035736, U60416, and XM236411).

RT-PCR products were visualized by 1% agarose gel electrophoresis and cloned in plasmid pCR2.1 (kit TA Cloning – Invitrogen) and pGEM-T (Promega) and used to transform *E. coli* DH5α strain competent cells (Invitrogen Life Technologies). The recombinant plasmids containing genes of interest were purified with the "Wizard Plus Minipreps DNA Purification System" kit (Promega) and submitted to nucleotide sequencing on an ABI 377 Automated DNA Sequencer (Applied Biosystems).

Table 2. Species, Myosin Isoforms and Domains Analyzed, and Accession Numbers of Amino Acid Sequences from Several Organisms Obtained from NCBI Databases

Species	Myosin Isoform and Domain	Accession Numbers
Bos taurus	Va head and neck	X_P615219
Bos taurus	Vc head and neck	XP_611694
Bos taurus	Va tail	XM_615219
Bos taurus	Vc tail	XM_611694
Canis familiaris	Va head and neck	XP_535487
Canis familiaris	Vb head and neck	XP_537345
Canis familiaris	Vc head and neck	XP_544680
Canis familiaris	Va tail	XM_535487
Canis familiaris	Vb tail	XM_537345
Canis familiaris	Vc tail	XM_544680
Danio rerio	Va head and neck	CAK04124
Danio rerio	Vb head and neck	XP_695789
Danio rerio	Vc head and neck	XP_691143
Gallus gallus	Va head and neck	CAA77782
Homo sapiens	Va head and neck	NP_000250
Homo sapiens	Vb head and neck	XP_944193
Homo sapiens	Vc head and neck	EAW77446

Table 2. contd…..

Species	Myosin Isoform and Domain	Accession Numbers
Homo sapiens	Va tail	NM_000259
Homo sapiens	Vb tail	XM_939100
Homo sapiens	Vc tail	NM_018728
Macaca mulatta	Va head and neck	XP_001084476
Macaca mulatta	Vb head and neck	XP_001090434
Macaca mulatta	Va tail	XM_00108476
Macaca mulatta	Vb tail	XM_001090668
Mus musculus	Va head and neck	NP_034994
Mus musculus	Vb head and neck	NP_963894
Mus musculus	Vc head and neck	XP_988841
Mus musculus	Va tail	NM_010864
Mus musculus	Vb tail	NM_201600
Mus musculus	Vc tail	XM_983747
Pan troglodytes	Vc head and neck	XP_510411
Pan troglodytes	Va tail	XM_001170332
Pan troglodytes	Vb tail	XR_024243
Pan troglodytes	Vc tail	XM_510411
Rattus norvegicus	Va head and neck	NP_071514
Rattus norvegicus	Vb head and neck	NP_058779
Rattus norvegicus	Vc head and neck	XP_236411
Rattus norvegicus	Va tail	NM_022178
Rattus norvegicus	Vb tail	NM_017083
Rattus norvegicus	Vc tail	XM_001057675
Sus scrofa	Va head and neck	BAE03307
Sus scrofa	Va tail	AB209957
Tetraodon nigroviridis	Va head and neck	CAG00830
Tetraodon nigroviridis	Vb head and neck	CAG01035
Xenopus laevis	Va head and neck	AAH45050
Strongylocentrotus purpuratus	V head and neck	NP_999655
Caenorhabditis elegans	V head and neck	NP_505433
Drosophila melanogaster	V head and neck	AAC99496

The sequences from myosin V head and neck were processed by CAP3 software, available at http://pbil.univ-lyon1.fr/cap3.php, to obtain a unique contiguous sequence for this region. It was not possible to connect sequences from the myosin V tail portion with the head-neck segment. The resulting cDNA and predicted aa sequences were subjected to Blast/n and Blast/x searches, respectively [19] at the NCBI website http://www.ncbi.nlm.nih.gov/blast. A further search for similarity to myosin V was performed against sequences contained in NCBI databse. Selected and representative sequences from different vertebrates were aligned using ClustalX [20] and molecular evolutionary analyses were conducted with MEGA version 3.1 [21], using the rainbow trout predicted aa sequences from the head and neck myosin domains and NCBI database myosin V aa sequences from other organisms (Table **2**).

RESULTS AND DISCUSSION

RT-PCR amplification of *O. mykiss* myosin V from total RNA extracted from brain and other tissues allowed the amplification of a 2545bp band corresponding to MVa head and neck domains, and bands of 650bp for MVa, 400bp for MVb and 500bp for MVc from the tail domains. The RT-PCR DNA fragments were cloned and sequenced. Nucleotide sequence analysis through NCBI database searches by Blast/n indicated that rainbow trout isolated cDNA nucleotide sequences were highly similar to MV sequences of other vertebrates.

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284  CTGGTGGGTGAGAATGACCTCACAGCTCTTAGCTACCTCCACGAGCCGGCCGTCCTGCACAACCTTAAAGTCCGCTTCACCGACTCCAAACTCATCTACA
      L  V  G  E  N  D  L  T  A  L  S  Y  L  H  E  P  A  V  L  H  N  L  K  V  R  F  T  D  S  K  L  I  Y
384  CATACTGTGGTATTGTGCTGGTGGCCATTAATCCCTATGAGAACCTTCCCATCTACGGTAGTGACATCATCAATGCGTACAGTGGCCAGAACATGGGAGA
      T  Y  C  G  I  V  L  V  A  I  N  P  Y  E  N  L  P  I  Y  G  S  D  I  I  N  A  Y  S  G  Q  N  M  G  D
484  CATGGACCCTCATATCTTTGCTGTGGCTGAGGAGGCTTACAAACAGATGGCCAGAGATGAGAGGAACCAGTCTATAATCGTAAGCGGTGAGTCAGGAGCA
       M  D  P  H  I  F  A  V  A  E  E  A  Y  K  Q  M  A  R  D  E  R  N  Q  S  I  I  V  S  G  E  S  G  A
584  GGGAAGACTGTCTCAGCTAAATACGCCATGCGCTACTTTGCTACGGTCAGTGGAGCAGCCACAGAGGCCAACGTAGAGGAGAAGGTCCTCGCATCCAANC
      G  K  T  V  S  A  K  Y  A  M  R  Y  F  A  T  V  S  G  A  A  T  E  A  N  V  E  E  K  V  L  A  S  X
684  CCATCATGGAGGCGATAGGAAACGCTAAGACGACTCGTAACGACAACAGCAGTCGTTTTGGGAAGTACATTGAGATTGGCTTCGACAAACGCTACCGTAT
      P  I  M  E  A  I  G  N  A  K  T  T  R  N  D  N  S  S  R  F  G  K  Y  I  E  I  G  F  D  K  R  Y  R  I
784  CATTGGAGCTAACATGAGGACATATCTACTGGAGAAGTCTAGAGTGGTGTTTCAGGCGGACGAGGAGAGGAACTATCATATATTCTACCAGCTGTGTGCG
       I  G  A  N  M  R  T  Y  L  L  E  K  S  R  V  V  F  Q  A  D  E  E  R  N  Y  H  I  F  Y  Q  L  C  A
884  TCCTCCCATCTACCAGAGTTCAAGAATCTGAAGCTAGGTAGTGCTGATGTCTTCCACTGCACCAATCAGGGCCGGAACCCGGTCATCGATGGAGTCGACG
      S  S  H  L  P  E  F  K  N  L  K  L  G  S  A  D  V  F  H  C  T  N  Q  G  R  N  P  V  I  D  G  V  D
984  ATGCTAAAGAGATGTGCACAACACAACATGCCTTCTCACTGCTAGGCATCAACGAGTTGAACCAGAAGGGGCTGTTCCAGGTCCTGGCTGCCATCTTACA
      D  A  K  E  M  C  T  T  Q  H  A  F  S  L  L  G  I  N  E  L  N  Q  K  G  L  F  Q  V  L  A  A  I  L  H
1084 TCTGGGCAACGTGGAGATCAAGGACCGAGACTCTGACAGCAGCATCATACCTCCCAATAACCGCCACCTGACGGTGTTCTGTGAGCTGATGGGCGTGACC
       L  G  N  V  E  I  K  D  R  D  S  D  S  S  I  I  P  P  N  N  R  H  L  T  V  F  C  E  L  M  G  V  T
1184 TACCAGGACATGTCTCATTGGCTGTGCCACAAGAAGCTGAAGACAGCCCAGGAGACCTACATCAAGCCCATCCCTCGGCTGCAAGCCTCCAATGCCCGCG
      Y  Q  D  M  S  H  W  L  C  H  K  K  L  K  T  A  Q  E  T  Y  I  K  P  I  P  R  L  Q  A  S  N  A  R
1284 AAGCGCTCGCCAAACACATTTACGCCAAAGTCTTCAACTGGATCGTGGACCATGTCAACAAATCTCTACGCGCCACCGTCAAACAGCACTCCTTCATAGG
      E  A  L  A  K  H  I  Y  A  K  V  F  N  W  I  V  D  H  V  N  K  S  L  R  A  T  V  K  Q  H  S  F  I  G
1384 AGTCCTGGATATCTATGGGTTTGAGACGTTTGAGATCAACAGTTTTGAACAGTTCTGTATCAACTATGCTAACGAGAAACTGCAACAGCAGTTTAACATG
       V  L  D  I  Y  G  F  E  T  F  E  I  N  S  F  E  Q  F  C  I  N  Y  A  N  E  K  L  Q  Q  Q  F  N  M
1484 CATGTGTTCAAGCTGGAGCAGGAGGAGTATTTGAAGGAGCAGATTCCCTGGACTCTGATAGACTTCTATGATAACCAGCCATGTATCAACCTCATAGAGG
      H  V  F  K  L  E  Q  E  E  Y  L  K  E  Q  I  P  W  T  L  I  D  F  Y  D  N  Q  P  C  I  N  L  I  E
1584 CCAAGATGGGGGTACTCGACCTACTGGATGAAGAGTGCAAGATGCCTNNTAAAGGTTCAGATGACTCGTGGGCCCAGAAGCTGTGTAACACCCATCTGAA
      A  K  M  G  V  L  D  L  L  D  E  E  C  K  M  P  X  K  G  S  D  D  S  W  A  Q  K  L  C  N  T  H  L  K
1684 GACCTGCTCTCTGTTTGAGAAACCTCGCATGTCCAACAAAGCCTTCATCATACAGCACTTCGCTGACAAGGTGCAGTACCAGTGTGATGGCTTTCTGGAG
       T  C  S  L  F  E  K  P  R  M  S  N  K  A  F  I  I  Q  H  F  A  D  K  V  Q  Y  Q  C  D  G  F  L  E
1784 AAGAACAAGGACACAGTGAATGAAGAGCAGATCAATGTCCTGAAGGCCAGCAAGTTGGACCTGCTAGTGGAGCTGTTTCAGGATGAGGAGAAAGTGACCA
      K  N  K  D  T  V  N  E  E  Q  I  N  V  L  K  A  S  K  L  D  L  L  V  E  L  F  Q  D  E  E  K  V  T
1884 GTCCAACAGGGACCGCTCCAGGAGGTCGAACACGACTCAGCGTCAAACCAAAAGAGGTCAGATCTGGAGCAAGCAGCAAGGAGCACAAGAAGACTGTTGG
      S  P  T  G  T  A  P  G  G  R  T  R  L  S  V  K  P  K  E  V  R  S  G  A  S  S  K  E  H  K  K  T  V  G
1984 CTTGCAGTTCCGGAACTCTCTGGCTATGTTGATGGAGACTCTGAATGCAACCACTCCTCACTATGTGCGCTGCATCAAGCCCAACGACCTCAAGTTCCCC
       L  Q  F  R  N  S  L  A  M  L  M  E  T  L  N  A  T  T  P  H  Y  V  R  C  I  K  P  N  D  L  K  F  P
2084 TTCACGTTTGACCCTAAGCGAGCGGTGCAGCAGCTCAGAGCCTGTGGTGTTCTGGAGACCATCCGCATCTCAGCCGCAGGCTTCCCATCCAGATGGACCT
      F  T  F  D  P  K  R  A  V  Q  Q  L  R  A  C  G  V  L  E  T  I  R  I  S  A  A  G  F  P  S  R  W  T
2184 ACCAGGAGTTCTTCAGTCGTTACCGGGTTCTGATGAAGCAGAAGGACGTCCTGTCGGACAGGAGGCTGACCTGTAGGAACGTCCTGGAGAAACTGGTGCA
      Y  Q  E  F  F  S  R  Y  R  V  L  M  K  Q  K  D  V  L  S  D  R  R  L  T  C  R  N  V  L  E  K  L  V  Q
2284 GGACCAGGACAAGTACCAGTTTGGTAAGACTAAGATCTTCTTCAGGGCTGGTCAGGTTGCCTACCTGGAGAAGCTGAGGGCTGATAAGCTGAGGAAGGCG
       D  Q  D  K  Y  Q  F  G  K  T  K  I  F  F  R  A  G  Q  V  A  Y  L  E  K  L  R  A  D  K  L  R  K  A
2384 TGCGTCCGTATCCAAAAGACCATCCGCTGCTGGCTGGTAAGCCAAGAAAGTACCTCCGAAAGAAGCATGCTTGCCATCACCATCCAGAGATACACCCGCG
      C  V  R  I  Q  K  T  I  R  C  W  L  V  S  Q  E  S  T  S  E  R  S  M  L  A  I  T  I  Q  R  Y  T  R
2484 GACACCAGGCCCGCTGTCTGGTTAAGTACATGCGTCAGACCCTGGCAGCCATCACTATCCAGAAGTTCCAGAGGATGTGTGTCCAGAGGAAAGTGTACCT
      G  H  Q  A  R  C  L  V  K  Y  M  R  Q  T  L  A  A  I  T  I  Q  K  F  Q  R  M  C  V  Q  R  K  V  Y  L
2584 ACAGAAGCAGGCTGCTGCCCTGGTCATGCAGACTATCCTCAGAGCGTACATGGCCCGACAGAAATACCAAGGGTTGCTGCGTAACCACAATGCTGTTTC
       Q  K  Q  A  A  A  L  V  M  Q  T  I  L  R  A  Y  M  A  R  Q  K  Y  Q  G  L  L  R  N  H  N  A  V  F
2684 ATCCAGAAACACGTGCGTGGCTGGCTGGCCAGACAGCGGTACAAGCGCTCCCTGCGAGCCATCGTCTATNTGCAGTGCTGCATCAGGAGGATGAAGGCCA
      I  Q  K  H  V  R  G  W  L  A  R  Q  R  Y  K  R  S  L  R  A  I  V  Y     Q  C  C  I  R  R  M  K  A
2784 AGAAAGAGCTGAAGAAGCTGAAAATCGAGGCCCGCTCCGTTGAAC
      K  K  E  L  K  K  L  K  I  E  A  R  S  V  E
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Fig. (1). Nucleotide and amino acid deduced sequences from trout brain myosin Va head and neck domains. The ATP-binding site is enhanced in gray and the actin-binding site in black. The underlined amino acid sequence denotes the myosin neck domain containing six IQ-motifs. The nucleotide sequence is deposited in NCBI under accession number EF592540.

Brain MVa head and neck domain nucleotide sequences were obtained for four clones, edited with CAP3 software and manually, and resulted in fragments with 2545bp (Fig. **1**). The deduced *O. mykiss* MVa head and neck aa sequence corresponds to aa residues 68 to 935 with high similarity to MVa from other vertebrates. Genetic distance analysis between the four cloned fragments of brain MVa head and neck domains revealed variant forms of MVa. The four isolated fragments were highly similar to each other (mean genetic distance of 0.0105). The variant transcripts detected could be related to polymerase errors during PCR or even sequence reading errors. On the other hand, variant transcripts related to alternative MVa splicing were detected in vertebrate tissues and were related to specific cellular process roles [22,23,24,25].

All myosins share a core of conserved residues in their motor domains, many of which are known to participate in actin binding [26]. The ATP-binding site for MVa was highly conserved and no variation in nucleotide or aa sequences was observed in several non-related vertebrates. This is probably due to the fundamental role of this region in energy conversion for the mechanical force required by the head domain for protein movement [6]. The actin-binding site was also well conserved for vertebrates with only two amino acid substitutions in residues 644 and 645 (A/H or Q and M/L). In the first substitution, a hydrophobic amino acid (alanine) was substituted by histidine or glutamine, which are both more water soluble, in the sequences of several organisms [27]. The second alteration occurs between aa with same properties, which should not cause damage in the protein structure because both are hydrophobic and important in stabilizing the protein structure by promoting hydrophobic interactions in its core [27]. Analyzed sequences showed the neck domain to be a more variable region, with several aa residue substitutions. However, most variable residues are neutral or basic. Even with such a variation, six imperfect aa repetitions can be recognized; these are called IQ-motifs [28,29], a feature of class V myosins, represented by the consensus sequence IQXXXRGXXXR which are calmodulin and other light chain binding sites [31]. Comparative analy-

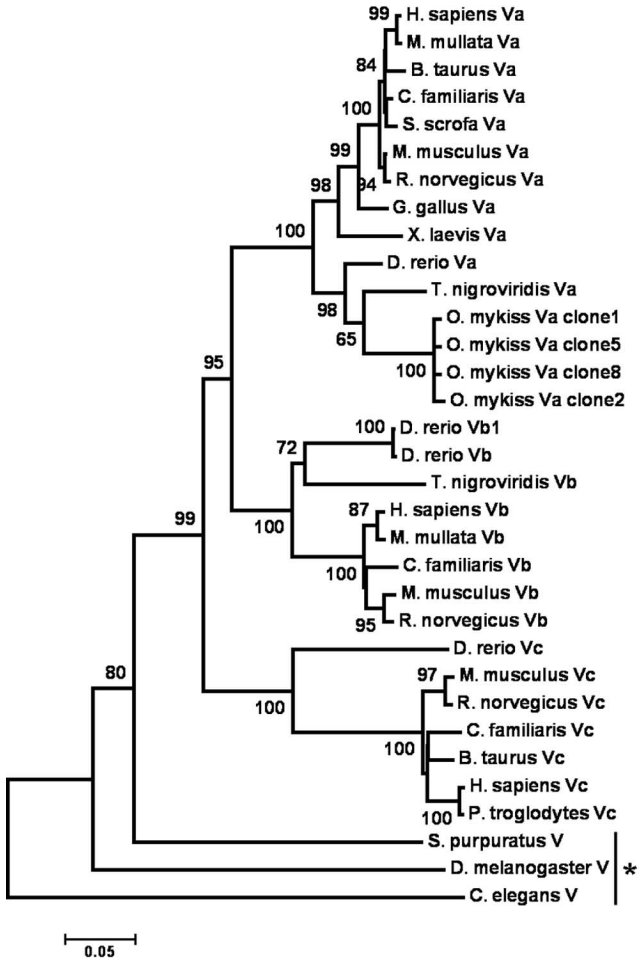

Fig. (2). Phylogenetic tree obtained from neighbor-joining analysis of amino acid sequences from rainbow trout brain and other vertebrate myosin Va head and neck domains. Sequences of nematodes, arthropods, and echinoderms (asterisk) were included as out-groups. Branch lengths are proportional to evolutionary distance (scale bar). Bootstrap resampling (2,000 trials) was used to judge the node robustness. Species NCBI accession numbers are described in Material and Methods.

sis of the myosin V head and neck domains amino acid frequency in several organisms and rainbow trout myosin Va showed a high frequency of neutral and basic amino acids such as Leucine and Lysine.

Genetic distance analyzes between myosin V from several vertebrates showed a mean genetic distance of 0.118 for MVa, 0.187 for myosin Vb, and 0.122 for myosin Vc. Comparative analysis of myosin V showed that rainbow trout MVa had a mean genetic distance of 0.118 in relation to MVa from other vertebrates, 0.278 to myosin Vb, and 0.299 to myosin Vc. Rainbow trout MVa is closely related to the MVa and quite distant from MVb and MVc of several vertebrates. Trout MVa had lower genetic distance values than other fish species compared to other vertebrates. Such data can also be seen in phylogenetic reconstruction based on aa sequences from several vertebrates including rainbow trout (Fig. **2**). Distant non-related species such as nematodes, arthropods, and echinoderms were included in the analysis as out-groups. The degree of similarity between sequences from different animal groups follows their phylogenetic distance. The event that resulted in the formation of paralogous Va, Vb, and Vc myosin copies occurred in a common ancestor before vertebrates diverged into different classes. This evolutionary event generated two copies of myosin V which diverged, one originated MVc in several vertebrates and the other generated the Va and Vb isoforms which were distributed in different vertebrate classes. It is interesting to note that, even within a specific vertebrate clade, all the myosin V members underwent evolutionary events which lead to divergences in a specific myosin class between different groups. All these evolutionary gene duplication and divergence events are supported in phylogenetic analyses by high bootstrap values (Fig. **2**).

Tail domain nucleotide sequences for MVa, MVb, and MVc were submitted to searches by similarity in GenBank databases using the Blast/N program. Results showed a high similarity mainly for MVa sequences, with lesser values for MVb and MVc (Table **3**). MVa tail was RT-PCR amplified in hypophysis and a very discreet band was also seen in brain (Fig. **3a**); this agrees with literature which indicates that isoform is mainly expressed in nervous tissue [16,32]. Immunochemistry analysis suggest that MVa is involved in cell process such neurotransmission [33,34,35], growth cones motility in growing neurons [36] and translocation of cell organelles [37,38]. Mutations in the MVa cause the *dilute* pheno-

Table 3. Partial cDNA Nucleotide Sequence Similarity Levels (%) for Myosin Va, Vb and Vc Between Rainbow Trout Tail Domain and Several Organisms Obtained from NCBI

	***O. mykiss* Va**		***O. mykiss* Vb**		***O. mykiss* Vc**
***B. taurus* Va**	80	*B. taurus* Vb	--	*B. taurus* Vc	66
***R. norvegicus* Va**	89	*R. norvegicus* Vb	59	*R. norvegicus* Vc	83
***M. musculus* Va**	86	*M. musculus* Vb	54	*M. musculus* Vc	79
***H. sapiens* Va**	81	*H. sapiens* Vb	50	*H. sapiens* Vc	68
***P. troglodytes* Va**	81	*P. troglodytes* Vb	50	*P. troglodytes* Vc	68
***M. mulatta* Va**	81	*M. mulatta* Vb	51	*M. mulatta* Vc	--
***S. scrofa* Va**	81	*S. scrofa* Vb	--	*S. scrofa* Vc	--
***C. familiaris* Va**	80	*C. familiaris* Vb	50	*C. familiaris* Vc	68

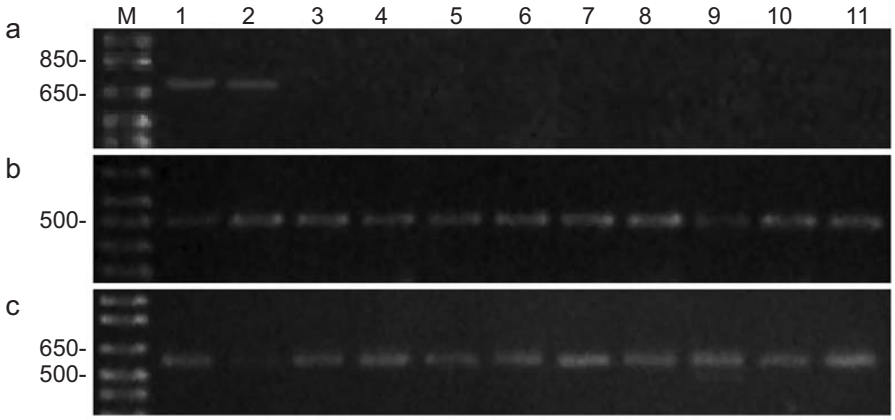

Fig. (3). 1% agarose gel demonstrating MVa (a) MVb (b), and MVc (c) expression in different rainbow trout tissues. Legend: 1, hypophysis; 2, brain; 3, aorta; 4, heart; 5, gill; 6, muscle; 7, stomach; 8, liver; 9, kidney; 10, intestine; 11, spleen; and M, molecular weight marker in bp.

type in mice, which is associated to several neurological disorders that lead to death up to three weeks after birth [10]. The *dilute* fenotype causes de Griscelli's syndrome in humans that is characterized by neurological damages [39]. MVb and MVc isoforms were found in practically all tissues analyzed (Fig. **3b** and **3c**), with Vc more widely distributed and with a higher expression level in epithelial tissues. MVc is an actin-based motor protein involved in membrane trafficking of many physiologically crucial tissues of the human body [16]. Although the three MV classes are involved with membrane trafficking, several evidences have showed that the different myosin V isoforms are associated with a specific set of membrane trafficking events [16].

CONCLUSIONS

Comparative analysis of MVa between different vertebrate species clearly demonstrated that the evolution of myosins has accompanied the divergence of the main vertebrate groups. These data support the idea that conserved MV genes phylogeny is accompanied by conserved cellular functions. This is the first study to reveal the cDNA nucleotide sequence and expression of MV isoforms in rainbow trout. The data presented here represent new contributions to the knowledge of the genome of rainbow trout. A better understanding of this economically important species could assist in development of improved strains of this fish for aquaculture.

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ABBREVIATIONS

aa	=	amino acid
ATP	=	Adenosine triphosphate
Blast/N	=	Basic local alignment search tool for nucleotide databases
Blast/X	=	Basic local alignment search tool for protein databases
bp	=	base pair
CAP3	=	Contig Assembling Program
DDBJ	=	DNA Data Bank of Japan
EMBL	=	European Molecular Biology Laboratory
GB	=	GenBank Data Base
IQ-motif	=	consensus sequence IQXXXRGXXXR that binds to calmodulin
MEGA	=	Molecular Evolutionary Genetics Analysis
MVa	=	Myosin Va
NCBI	=	National Center for Biotechnology Information
RT-PCR	=	Reverse Transcriptase – Polymerase Chain Reaction

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