Molecular Cloning and Domain Structure of Human Myosin-VIIa, the Gene Product Defective in Usher Syndrome 1B

ZHENG-YI CHEN,* TAMÁ HASSON,† PHILIP M. KELLEY,‡ BRIAN J. SCHWENDER,*§ MARC F. SCHWARTZ,† MEENA RAMAKRISHNAN,† WILLIAM J. KIMBERLING,‡ MARK S. MOOSEKER,† AND DAVID P. COREY*§,1

*Departments of Neurobiology and Neurology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114; †Departments of Biology, Cell Biology, and Pathology, Yale University, New Haven, Connecticut 06520; ‡Center for Hereditary Communication Disorders, Boys Town National Research Hospital, Omaha, Nebraska 68131; and §Howard Hughes Medical Institute

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Myosin-VIIa is an unconventional myosin with relatively restricted expression. Cloned first from an intestinal epithelium cell line, it occurs most notably in the testis, in the receptor cells of the inner ear, and in the pigment epithelium of the retina. Defects in myosin-VIIa cause the shaker-1 phenotype in mice and Usher syndrome 1B in human, which are characterized by deafness, lack of vestibular function, and (in human) progressive retinal degeneration. Because the described cDNAs encode less than half of the protein predicted from immunoblots, we have cloned cDNAs encoding the rest of human myosin-VIIa. Two transcripts were found, one encoding the predicted 250-kDa protein and another encoding a shorter form. Both transcripts were found in highest abundance in testis, although the shorter transcript was much less abundant. Both could be detected in lymphocytes by RT-PCR. The myosin tail encoded by the long transcript includes a long repeat of ~460 amino acids. Each repeat contains a novel “MyTH4” domain similar to domains in three other myosins, and a domain similar to the membrane-associated portion of talin and other members of the band-4.1 family. © 1996 Academic Press, Inc.

INTRODUCTION

Myosin-VII is a member of the myosin superfamily, which now includes 11 or more different branches (Cheney and Mooseker, 1992; Hasson and Mooseker, 1995). It is considered an “unconventional” myosin, distinct from the conventional myosin-II family originally characterized in muscle. Two forms of this myosin, VIIa and VIIb, were first identified in a human intestinal epithelium cell line, on the basis of partial amino acid sequence of the conserved head domain (Bement et al., 1994). A portion of the myosin-VIIa head domain was also cloned from the sensory epithelium of the bullfrog inner ear (Solc et al., 1994). Larger portions of the mouse and human myosin-VIIa sequences were described when it was found that defects in myosin-VIIa cause the mouse shaker-1 phenotype and the human Usher Syndrome 1B, which are characterized by deafness, lack of vestibular function, and (in human) progressive retinal degeneration (Gibson et al., 1995; Weil et al., 1995). A still larger portion of the human sequence was described by Hasson et al. (1995), who also used a specific antibody to the tail domain to characterize the protein. Immunoblots indicated that myosin-VIIa is a protein of molecular weight 230–250 kDa which is expressed most highly in testis but also in the retina and the cochlea. Immunocytochemical labeling showed that myosin-VIIa is expressed in the receptor cells of the inner ear and the pigment epithelium cells of the retina, consistent with the sensory deficits of Usher syndrome 1B (Hasson et al., 1995).

The published sequence still accounts for less than half of the predicted coding sequence of myosin-VIIa. Whereas the “motor” head domain is like other members of the superfamily, containing the conserved actin and ATP-binding regions, the uncharacterized portion includes most of the unique tail domain that gives the molecule its specificity. To understand the interaction of myosin-VIIa with other proteins, specific probes must be generated from unique segments of the tail. In addition, gene defects in the head domain have been found in fewer than half of the families with Usher 1B. Additional defects in the tail may be expected in the
remaining families and must be identified for accurate diagnosis.

We have used probes based on the myosin-VIIa head domain (Hasson et al., 1995) and on the genomic structure of the Usher 1B locus (Kelley et al., in preparation) to clone the rest of the cDNA encoding myo-VIIa from a human testis cDNA library. Myosin-VIIa is expressed in at least two alternatively spliced transcripts, with the longer form predicting a protein of 250 kDa. The tail domain includes a repeated element of ~460 amino acids. Each element contains two domains: one (~100 aa) that is similar to domains occurring in the tails of three other myosin families and a second (~300 aa) similar to the membrane-associated portion of talin and other members of the band-4.1 family.

METHODS

Library screening. Two different probes were used to screen a random- and oligo(dT)-primed human testis cDNA library, constructed in the Lambda ZAP Express vector (Stratagene, La Jolla, CA) and plated at 2 × 10^6 plaques. Hybond-N+ (Amersham) was used for the phage lifts. Hybridization and isolation of phagemid clones were essentially as described (Chen et al., 1992a,b).

The first probe came from an 85-kb P1 clone (P-8079) obtained by screening the Dupont P1 library (Genome Systems, St. Louis MO) with a probe from a cosm id subcloned from the YAC 965F10, which mapped to this region (Kelley et al., in preparation). A 10-kb EcoRI fragment of the P1 clone that mapped just past the 3' end of U34227 was then identified and isolated by gel purification. The EcoRI fragment was preassociated with human Cot-1 DNA (GIBCO-BRL) before hybridization to eliminate background from repetitive DNA, and two overlapping clones (Nos. 4.4 and 1.13) were derived from the EcoRI fragment screen. These encompass nt 3985–7368 of myosin-VIIa transcript 1 (GenBank Accession No. U55208). RT-PCR was used to obtain an additional clone that linked clone 4.4 and U34227 (see below).

The second probe was a PCR product of 382 bp synthesized from bases 3010–3391 of our previous myosin-VIIa sequence (Accession No. U34227; Hasson et al., 1995). Two overlapping clones were derived from this screen, encompassing nt 488–4060 of myosin-VIIa transcript 2 (GenBank Accession No. U55209). The database of expressed sequence tags was screened with the probe derived from a cosmid subcloned from the YAC 965F10, my7c3.1t71, followed by a second round of PCR using primers HM7A3431F/my7c5600R and two overlapping clones (Nos. 4.4 and 1.13) were derived from these clones, using the BLAST network service at the NCBI, and 11 positives were identified. One of these (Accession No. R27660; clone 134502) was then identified and isolated by gel purification. The clone was essentially as described (Chen et al., 1992a,b).

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RESULTS

Identification of Two Transcripts

The apparent molecular weight of human myosin-VIIa is ~240 kDa, based on immunoblots, but we had previously cloned only the N-terminal half (Hasson et al., 1995). To recover cDNAs representing the rest of the myosin-VIIa message, we used a cosm id subcloned from a YAC containing the myosin-VIIa gene to isolate a P1 genomic clone and identified a 10-kb fragment that mapped just past the 3' end of the existing cDNA. This was used to screen a human testis cDNA library, and two overlapping clones (Nos. 4.4 and 1.13 of 1.7 and 2.3 kb, respectively) were recovered. Neither clone overlapped with U34227, however, so an RT-PCR was performed with human testis cDNA and a pair of primers derived from the 3' end of U34227 and the 5' end of the new clone 4.4. A product of 593 bp was amplified and subsequently subcloned. These four overlapping clones (Fig. 1A) represent 7368 bp of the transcript (transcript 1), extending to the poly(A) tail. It includes a potential polyadenylation site (CATAAA) located 20 bp upstream of the poly(A) tail. CATAAA is an uncommon form of polyadenylation signal that has been observed in less than 1% of all polyadenylation signals observed (Wickens and Stephenson, 1984).

In parallel experiments, the testis cDNA library was
also screened with a PCR product of 382 bp (3010–3391) constructed from the 3’ end of U34227. Two positive clones had 5’ ends that matched the existing myosin-VIIa sequence at different points, and both matched each other at their 3’ ends, which included a poly(A) tail and two potential polyadenylation signals (AATAAA) located 14 and 26 bases upstream of the poly(A) tail. However, these clones did not match transcript 1 at their 3’ ends; instead, they represented a second transcript that was much shorter, extending 4060 bp from the start of U34277 (Fig. 1B; transcript 2). Exhaustive screening of this library with a probe derived from U34227 never produced a clone corresponding to the longer transcript 1, perhaps due to insufficient internal priming in library construction.

Transcript 1 has an open reading frame of 6525 bp (bases 268–6792, counting from the 5’ end of U34227) which encodes a protein of 2175 amino acids with a predicted molecular weight of 250 kDa. The predicted amino acid sequence is shown in Fig. 2A and is most likely the protein studied in Hasson et al. (1995). Transcript 2 is smaller; it has an open reading frame of 3609 bp (bases 268–3876) encoding a protein of 138 kDa. The predicted amino acid sequence of transcript 2 is identical to that of transcript 1 over most of its length. At amino acid 1171, however, transcript 2 diverges entirely (indicated by an asterisk in Fig. 2) and ends 32 aa later in a stop (Fig. 2B). One clone representing transcript 2 also had a 9-bp insert, encoding 3 additional amino acids at position 1095, so that this clone read GEGEVLQAQL, where transcript 1 had GEGEVLQAOQL (insert underlined). Other clones from this library representing transcript 2 do not have the VLQ insert. Thus, there appear to be at least 3 alternative splice forms of myosin-VIIa.

**FIG. 1.** Sequence assembly and splice boundaries of two transcripts. (A) cDNA clones. For transcript 1, the positions of two cDNA clones (4.4 and 1.13) and a subcloned PCR product (C-3) are shown. (B) For transcript 2, the positions of two positive cDNA clones (clones 3.1 and 5.2) are shown relative to our previous sequence (Accession No. U34227). (C) Genomic organization in the splice region. Splicing for transcript 2 (top) includes upstream exons encoding the head domain, and exons a, b, and cd. Transcript 1 (bottom) includes exons a, b, the “c” part of exon cd, exon e, and others downstream. (D) The division between the two parts of exon cd is marked by a vertebrate splice site consensus. GenBank Accession Nos. are U55208 (transcript 1) and U55209 (transcript 2).
FIG. 2. (A) Amino acid sequence predicted from transcript 1. The head domain (aa 1–742) includes the ATP- and actin-binding sites (underlined), followed by five light-chain-binding IQ motifs (IQ 1–5), and a predicted coiled-coil region (aa 859–940). Two MyTH4 domains (aa 1016–1054//1145–1217 and 1710–1821) are indicated by dashes, and two talin-like domains (aa 1267–1568 and 1872–2167) are indicated by dots. The point of divergence of transcript 2 at aa 1171 is indicated by an asterisk. (B) The C-terminal 243 amino acids of transcript 2. The divergent portion is indicated in boldface; the alternatively spliced amino acids (VLQ) are underlined.
Expression of Myosin-VIIa

RT-PCR was also used to study the expression of the myosin-VIIa in testis and lymphocytes. With a forward primer derived from U34227 and nested reverse primers unique to transcript 1 or transcript 2, PCR products of the correct size (~600 and ~170 bp) were amplified in both lymphocytes and testis (Figs. 3A and 3B). With a different forward primer from U34227 and a single reverse primer from transcript 2 (my7ct34/my7c31t71), a product of the correct size (~440 bp) was amplified from human testis cDNA and the control transcript 2 cDNAs, confirming that this transcript is expressed in testis (data not shown).

Domain Structure of the 250-kDa Form

Figure 2 shows the predicted amino acid sequence of the larger and most abundant form of myosin-VIIa, and Fig. 4 illustrates the notable domains of both transcripts schematically. The predicted molecular weight of the larger form is 250 kDa and the pl is 8.5. The myosin head domain, IQ motifs, and coiled-coil domain have been reported previously (Gibson et al., 1995; Weil et al., 1995; Hasson et al., 1995)

An internal repeat. Myosin-VIIa has a long repeated element of about 460 amino acids (aa 1016–1568 and 1708–2167). The first element is interrupted by an insertion of about 90 amino acids (aa 1055–1145), but the elements are otherwise 28% identical at the amino acid level. Each element has two domains that are similar to domains in other proteins: the MyTH4 domain and the talin homology domain.

The MyTH4 domain. The first ~110 amino acids of each repeat element constitute a highly conserved domain found in four different branches of the myosin superfamily (Fig. 5). We have named this motif the MYTH4 domain, for myosin tail homology, and grouped it to distinguish it from homologies TH1-3 found in the tails of myosins-I (Hammer, 1994). An Acanthamoeba myosin heavy chain that has been assigned to class IV contains one domain toward the C-terminal of the tail (Horowitz and Hammer, 1990). Myosin-VIIa has two copies of the domain, as does a novel Caenorhabditis elegans myosin, encoded by a gene on cosmid F46C3 and likely to be named myosin-XII (Accession No. Z66563). Within the domain, each myosin is about 35% identical to a consensus sequence.

The talin homology domain. The last ~300 amino acids of each myosin-VIIa element are similar to a conserved domain in the band-4.1 superfamily of proteins (Algrain et al., 1993b). These regions (amino acids 1267–1568 and 1872–2167) are most similar to talin and filopodin, being about 20% identical at the amino acid level (Fig. 6). They are slightly less similar (18%) to a similar domain in the ERM branch of the band-4.1 family, which includes ezrin, radixin, moesin, and merlin, and they have limited similarity to band-4.1 itself (11%). Although the identity is not strong, the similarity is significant and extends over a long distance. These two domains in myosin-VIIa appear to be equally distant from the talins and from the ERMs: the amino acids conserved between myosin-VIIa and the talins are not consistently conserved between myosin-VIIa and the ERMs, but other amino acids are conserved between myosin-VIIa and the ERMs. Identities between each group are illustrated in Fig. 6.

**DISCUSSION**

Expression of Two Myosin-VIIa Transcripts

Analysis of cDNA sequences for myosin-VIIa has identified two different transcripts, encoding different tails associated with the same motor domain. The major cDNA for myosin-VIIa encodes a 250-kDa poly-

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**FIG. 3.** RT-PCR of myosin-VIIa transcripts 1 and 2. (A) Transcript 1. PCR primers unique to transcript 1 (HM7A3431F/my7c5600R + 4.4jR3R) were used to amplify a fragment of ~600 bp from testis cDNA and two samples of lymphocyte cDNA. (B) Transcript 2. With primers unique to transcript 2 (HM7A3431F/my7c31t71 + my7c31t73), a fragment of ~170 bp was amplified from two testis samples and two lymphocyte samples. Arrows in Fig. 1 indicate the position of PCR primers used for RT-PCR. M indicates 1-kb ladder.
 FIG. 4. Domain structure of the predicted amino acid sequences for both transcripts. The antigen used for the myosin-VIIa-tail-specific antibody (Hasson et al., 1995) is common to both transcripts, so the antibody should also recognize a protein of the transcript 2 molecular weight in tissues where that transcript is expressed.

peptide, in keeping with the identification of a 240-kDa protein by immunoblot using antibodies directed against the proximal portion of the myosin-VIIa tail (Hasson et al., 1995). Our RT-PCR data indicate that transcript 2, encoding the shorter 138-kDa form of myosin-VIIa, is expressed in testis. Using a probe specific to the unique 3' end of transcript 2, however, we were unable to detect expression of this transcript in Northern blots of testis, suggesting a low level of expression. In frogs and rodents, we have not detected the expression of the 138-kDa form in either retina or cochlea as judged by immunoblot (T. Hasson, unpublished data) although the 250-kDa form can be clearly detected. Production of 138-kDa-form-

 FIG. 5. (A) Positions of MyTH4 domains (checkered) in myosins VIIa, IV, X, and XII, and a kinesin-like protein in plants. "Motor domain" indicates myosin heads or a kinesin motor domain; the ovals indicate calmodulin or other light-chain-binding domains. (B) Sequence comparisons. Amino acids identical to the consensus are shaded (human myosin-VIIa, Accession No. U55208; Acanthamoeba myosin-IV, Accession No. M60954; C. elegans myosin-XII, Accession No. Z66563; Arabidopsis thaliana kinesin-like protein, Accession No. L40358).
FIG. 6. (A) Location of talin-homology domains in myosin VIIa, talin, filopodin, merlin, and ezrin. (B) Sequence comparisons for selected examples (mouse talin, Accession No. P26039; human merlin, Accession No. P35240). Amino acids that are identical to talin are boxed; those identical to merlin are shaded. Note that few amino acids are shared by all four sequences. The tyrosine phosphorylated in ERM family members is indicated by an asterisk. Neither myosin-VIIa nor talin has a tyrosine at this position.

specific antibodies is in progress to address the function of this novel isoform.

RT-PCR experiments indicate that myosin-VIIa is expressed at least at low levels in lymphoblasts. This is in agreement with the work of Bement et al. (1994), who identified myosin-VIIa expression by RT-PCR from peripheral blood lymphocytes. Although the protein could not be detected by immunoblot, the message could be amplified. This should simplify mutation screening in those at risk for Usher 1B since the large myosin-VIIa gene would not need to be screened exon by exon, although genomic amplification will still be needed in cases where mutations involve splice sites.

Similarity to Other Myosins

As previously reported (Gibson et al., 1995; Weil et al., 1995; Hasson et al., 1995), the head domain of myosin-VIIa is similar to that of other members of the myosin superfamily and includes the ATP-binding site and the actin-binding site (indicated on Fig. 2). This is followed by 5 IQ motifs, which are probable binding sites for regulatory light chains such as calmodulin (Cheney and Mooseker, 1992; Hasson and Mooseker, 1995). The IQ domains are followed immediately by a predicted coiled-coil domain of 80 amino acids. Although this is short in comparison to some myosins, it is probably sufficient to mediate dimerization, so that the native protein (with light chains) would occur as a complex of up to 670 kDa.

Except for coiled-coil domains, the tails of different myosin classes are thought to be quite different (Mooseker and Cheney, 1995). Thus it is especially interesting to find a large domain in the myosin-VIIa tail (MyTH4) that is shared by other myosin classes. The only other motif shared by multiple myosin classes is the SH3
domain found in myosins I and IV (Mooseker and Cheney, 1995). In contrast, the other conserved domain in the tail (talin homology) has been found primarily in the band-4.1 superfamily.

Phylogenetic analysis of myosin motor domains has shown that myosin-VIIa is most similar to the myosin-X class (D. P. Corey and R. E. Cheney, unpublished results; Mooseker and Cheney, 1995). Myosin-X has also been cloned in part from bullfrog inner ear (Solc et al., 1994) and in full from bovine aorta (D. P. Corey and R. E. Cheney, unpublished results). Bovine myosin-X has a single MyTH4 domain, followed by a talin homology domain at its C-terminal. These similarities suggest a conservation of tail function between these two classes, in addition to the motor function common to all myosin heads. Recently, a protein from Arabidopsis with a kinesin motor domain has been identified (Reddy et al., 1996). It has also a MyTH4 domain followed by a talin homology domain (Fig. 5), suggesting that the pairing of these domains in association with a motor is functionally significant.

The MyTH4 Domain

The best characterized of the four myosins carrying a MyTH4 domain is Acanthamoeba myosin-IV. Preliminary work has found that myosin-IV is a monomer with a globular myosin motor head and a 50-nm tail (Repezza et al., 1994). This suggests that the MyTH4 domain does not facilitate dimerization. Although myosin-VIIa and myosin-X both harbor the talin homology, myosin-IV and myosin-XII do not; suggesting that the MyTH4 and talin domains in the repeat element can also serve distinct functions.

The Talin Homology Domain

The two 300-amino-acid domains found in myosin-VIIa are homologous to a portion of talin that has been well characterized biochemically. Talin was first identified as a component of adhesion plaques (Burridge and Connell, 1983) and was found to be capable of interacting with membranes, actin, and the adhesion-plaque component, vinculin (Nuckolls et al., 1990; Niggli et al., 1994). The membrane binding portion of talin has been mapped to the N-terminal 47 kDa (434 aa), of which aa 94–406 constitute the talin homology region in myosin-VIIa. The actin and vinculin binding sites have been mapped to the C-terminal 220-kDa fragment and are distinct from the N-terminal domain (Nuckolls et al., 1990; Niggli et al., 1994). The membrane-binding characteristics of the 47-kDa N-terminal talin peptide have been studied extensively in vitro and have been found by a number of methods to bind acidic phospholipids specifically (for review see Nuckolls et al., 1990).

The N-terminus of talin is also homologous to the N-termini of band-4.1 and members of the ERM family of actin-binding proteins (ezrin, radixin, moesin, and merlin). Band-4.1 has also been found to bind preferentially to acidic phospholipids via its N-terminal domain (Cohen et al., 1988). Biochemical analysis of the N-terminal domain of ERM family members has not been performed, but all ERM proteins are associated with plasma membrane compartments (Algrain et al., 1993a; Sato et al., 1992; Berryman et al., 1993). These shared functional roles suggest that the two talin homology domains found in the tail of myosin-VIIa target this molecular motor to plasma membrane compartments and, in particular, to acidic phospholipid bilayers.

In addition to membrane binding activities, the talin homology domains may also serve a role as protein-binding sites. The N-terminus of band-4.1, for example, has been shown also to serve as the binding site for both glycophorin C and p55 (Marfatia et al., 1994) as well as calmodulin (Kelly et al., 1991). The N-terminus of ezrin serves a self-association role, as it can bind to the C-terminus of another ezrin molecule (Gary and Bretscher, 1995; Bretscher et al., 1995). Therefore, we cannot rule out that the talin homology domains in the myosin-VIIa tail serve a role in protein–protein interaction or dimerization.

The N-terminal domain of ERM family members has also been shown to serve a regulatory function. Upon treatment with growth factors, human epidermoid carcinoma A431 cells are stimulated to form microvilli and exhibit membrane ruffling. This activity is correlated with the phosphorylation of ezrin by a tyrosine kinase (Brescher, 1989). One of the sites, Tyr145, lies in the N-terminal domain homologous to the talin homology of myosin-VIIa (Krieg and Hunter, 1992). This amino acid is conserved in other family members, including radixin and merlin (Fig. 6), but is not conserved in talin, band-4.1, or myosin-VIIa. Therefore, it is likely that myosin-VIIa is not regulated at the level of tyrosine phosphorylation of the talin homology domains.

Preliminary biochemical analysis of the 250-kDa myosin-VIIa polypeptide shows it to be tightly associated with membrane in both testis and kidney (T. Hasson and M. Mooseker, unpublished observations). In both tissues, it is also found in close association with polarized, bundled actin filaments. This type of location is also seen in retina and cochlea where myosin-VIIa is associated with the apical actin-rich villi of the retinal pigmented epithelium and the stereocilia of the cochlear hair cells (Hasson et al., 1995). Given the domain structure of myosin-VIIa, it is possible that the tail of myosin-VIIa serves to target the motor to membranes or membrane proteins while the motor domain targets the protein to polarized actin filaments. Myosin-VIIa may therefore serve a structural role tethering the bundled, polarized actin filaments of both villi and stereocilia to the plasma membrane.

Since this paper was submitted, Weil et al. (1996) have also described the cloning of the full-length myosin-VIIa from retinal pigment epithelial cells. In addition to two small inserts, which may represent splice differences between retina and testis, their sequence
has 10 single-base differences resulting in 7 amino acid differences, which might be polymorphisms.

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