GENOMICS 36, 431–439 (1996)
ARTICLE NO. 0488

Mapping of Unconventional Myosins in Mouse and Human

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Received March 22, 1996; accepted June 28, 1996

Myosins are molecular motors that move along filamentous actin. Seven classes of myosin are expressed in vertebrates: conventional myosin, or myosin-II, as well as the 6 unconventional myosin classes -I, -V, -VI, -VII, -IX, and -X. We have mapped in mouse 22 probes encompassing all known unconventional myosins and, as a result, have identified 16 potential unconventional myosin genes. These genes include 7 myosins-I, 2 myosins-V, 1 myosin-VI, 3 myosins-VII, 2 myosins-IX, and 1 myosin-X. The map location of 5 of these genes was identified in human chromosomes by fluorescence *in situ* hybridization.

INTRODUCTION

Myosins are molecular motors that, upon interaction with actin filaments, utilize energy from ATP hydrolysis to generate mechanical force. Phylogenetic analysis of the myosin motor domains has identified 11 distinct classes, 7 of which are expressed in vertebrates (for review see Mooseker and Cheney, 1995). These 7 vertebrate myosin classes include conventional myosin, or myosin-II, and 6 less-well-characterized unconventional myosin classes, myosins-I, -V, -VI, -VII, -IX, and -X. Each myosin has a conserved N-terminal motor domain, being 25–40% identical at the amino acid level, that contains both ATP-binding and actin-binding sequences. Following the motor domain is a light-chain-binding "neck" region containing 1–6 copies of a repeat element (the IQ motif; Cheney and Mooseker, 1992) that serves as a binding site for calmodulin or other members of the EF-hand superfamily of calcium-binding proteins (Kretsinger, 1980). At the C-terminus, each class has a distinct tail domain that serves in dimerization, membrane binding, protein binding, and/or enzymatic activities and targets each myosin to its particular subcellular location (for review see Mooseker and Cheney, 1995).

In recent years, analysis of the myosin superfamily has centered on the cloning of cDNAs for the unconventional myosins from a variety of species (for review see Mooseker and Cheney, 1995). To date upward of 39 different full-length or partial cDNAs, representing at least 14 distinct unconventional myosins, have been identified from vertebrates. Each myosin characterized thus far exhibits a distinct tissue expression profile and subcellular location, suggesting different functions for each molecular motor. Of the 14 proposed unconventional myosin genes, only 3 have been mapped: myosin-V (*Myo5a*) and myosin-VIIa (*Myo7a*) (in humans and mice) and myosin-VI (*Myo6*) (in mice) and all have been associated with known mouse mutations.

Myosin-V is encoded by the mouse dilute (*d*) locus (Mercer *et al.*, 1991). The melanocytes of homozygous *d* mice appear adendritic. As a result, there is an uneven deposition of pigment granules within the developing hair shaft and the mice exhibit a lighter coat color (summarized in Silvers, 1979). Other alleles of *d*, termed dilute-lethal, produce a postnatal neurological disorder characterized by opisthotonos and convulsions in addition to a lighter coat color (Silvers, 1979). These and other results suggest that myosin-V may be required for the proper maintenance of melanocyte and neuronal cell functions. Myosin-V was recently mapped in humans to a syntenic region on chromosome 15 (Engle and Kennett, 1994; Moore *et al.*, 1995), but a human disease has not been mapped to this position.

Myosin-VIIa is encoded by the mouse shaker-1 (*sh1*) locus (Gibson *et al.*, 1995). Homozygous *sh1* mice exhibit the head-tossing, circling, and hyperactivity phe-

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0888-7543/96 $18.00

notypes characteristic of vestibular dysfunction in addition to a complete degeneration of the cochlear sensory epithelium (Schnerson *et al.*, 1983). Myosin-VIIa defects have also been identified in human Usher syndrome type 1B (USH1B) patients (Weil *et al.*, 1995). USH1B is characterized by profound neurosensory deafness, vestibular dysfunction, and blindness due to retinitis pigmentosa. Myosin-VIIa is expressed in the hair cells of the cochlea as well as the pigmented epithelial cells of the retina, the two primary cell types affected in USH1B patients (Hasson *et al.*, 1995). The function of myosin-VIIa in these cell types is, however, unknown.

Myosin-VI is encoded by the mouse Snell's waltzer (*sv*) locus (Avraham *et al.*, 1995). Homozygous *sv* mice display an inner ear phenotype that is very similar to that observed in *sh*1 mice, and myosin-VI, like myosin VIIa, is expressed in the cochlea exclusively in hair cells (Avraham *et al.*, 1995). These and other results suggest that regulated expression of myosin VI and myosin VIIa is critical for hair cell survival.

The analysis of these 3 unconventional myosin genes emphasizes the importance of actin-based motilities in specialized cell types such as melanocytes, neurons, and cochlear hair cells. To begin to elucidate the biologic functions of the rest of the unconventional myosin superfamily, we have mapped 22 unconventional myosin probes in mouse, identifying 16 genes. We have also mapped the location of 5 of these unconventional myosin genes in human.

MATERIALS AND METHODS

Interspecific Mouse Backcross Mapping

Interspecific backcross (IB) progeny were generated by mating (C57BL/6J × *Mus spretus*) F1 females and C57BL/6J males as described (Copeland and Jenkins, 1991). A total of 205 N2 mice were used to map the myosin loci (see below for details). DNA isolation, restriction enzyme digestion, agarose gel electrophoresis, Southern blot transfer, and hybridization were performed essentially as described (Jenkins *et al.*, 1982). All blots were prepared with Hybond-N+ nylon membrane (Amersham). The probes and restriction fragment length polymorphisms (RFLPs) used in the mapping studies are listed in Tables 1 and 2 and they were labeled with [α-32P]dCTP using a nick-translation labeling kit (Boehringer Mannheim) or a random-primed labeling kit (Amersham); washing was performed to a final stringency of 0.1–1.0× SSCP, 0.1% SDS, at 55 or 65°C. In all cases, the presence or absence of the *M. spretus*-specific fragments was followed in backcross mice.

A description of the probes and RFLPs for nearly all the loci linked to the *Myo* genes has been reported. These include *Il1r1, Gls,* and *Ctla4* on Chromosome 1 (Pathak *et al.*, 1996b); *Tyr, Omp,* and *Pth* on Chromosome 7 (Pathak *et al.*, 1996a; Wang *et al.*, 1996); *Jundl* and *Mlr* on Chromosome 8 (Terajima *et al.*, 1994); *Csk, Rora, Myo5* (dilute), *Gsta,* and *Myo6* (Snell's waltzer) on Chromosome 9 (Kingsley *et al.*, 1989; Avraham *et al.*, 1995; Giguère *et al.*, 1995, Pecker *et al.*, 1996); *Ifg, Gli,* and *Erbb3* on Chromosome 10 (Justice *et al.*, 1990; Copeland *et al.*, 1995); *Myhs, Zfp3, Nf1,* and *Scya1* on Chromosome 11 (Ashworth *et al.*, 1989; Buchberg *et al.*, 1989; Youn *et al.*, 1995); *Ghr* and *Hspg1* on Chromosome 15 (Spring *et al.*, 1994); *Pim1* on Chromosome 17 (Siracusa *et al.*, 1991); *Ttr, Apc, Fgf1, Dcc,* and *Mbp* on Chromosome 18 (Justice *et al.*, 1992); and *Mxi1* and *Aop1* on Chromosome 19 (Steingrímsson *et al.*, 1995; Tsuji *et al.*, 1995).

Three loci, neuropeptide Y receptor Y1 (*Npy1r*), 5-hydroxytryptamine (serotonin) receptor (*Htr1b*), and phosphoglycerate kinase 2 (*Pgk2*), have not been reported previously for the Frederick IB. The *Npy1r* probe was a 595-bp *Eco*RI/*Pst*I rat cDNA that was kindly provided by Herbert Herzog. The probe detected major *Taq*I fragments of 2.8 (C57BL/6J) and 3.6 kb (*M. spretus*). The *Htr1b* probe was a 2.24-kb *Sac*I/*Bgl*II mouse genomic clone that was kindly provided by René Hen. The probe detected *Sph*I fragments of 4.6 (C57BL/6J) and 9.0 (*M. spretus*) kb. The *Pgk2* probe was a 1.85-kb fragment of mouse cDNA that was kindly provided by Michael McBurney. The probe detected major *Bgl*II fragments of 6.6 (C57BL/6J) and 5.3 (*M. spretus*) kb. Recombination distances were calculated as described (Green, 1981) using the computer program SPRETUS MADNESS. Gene order was determined by minimizing the number of recombination events required to explain the allele distribution patterns.

Fluorescence in Situ Hybridization

MYO10. A 5.4-kb cDNA encoding the tail of bovine myosin-X (Tables 1 and 2) was biotinylated using the BioNick labeling kit (BRL), and fluorescence *in situ* hybridization (FISH) was performed according to Heng *et al.* (1992) and Heng and Tsui (1993). Slides were washed, detected, and amplified as described in Heng and Tsui (1994) and Heng *et al.* (1994). The FISH signal and DAPI banding pattern were recorded separately, and assignment of the FISH mapping location was achieved by superimposition of FISH signal with DAPI-banded chromosomes (Heng and Tsui, 1993).

MYO1A, MYO1E, MYO1F, and MYO7A. The human myosin cDNA fragments for myosin-IA, myosin-IC, myosin-ID, and myosin-VIIa (Tables 1 and 2) were biotinylated and hybridized simultaneously with a digoxigenin-labeled probe specific for the *Alu* family of repeats (Matera and Ward, 1992). Labeled chromosomes were counterstained with DAPI and visualized as described in Reid *et al.* (1992).

RESULTS

The mouse chromosomal location of 22 clones, representative of all 6 classes of mammalian unconventional myosins, was determined using an interspecific backcross mapping panel derived from crosses of [(C57BL/6J × *M. spretus*)F1 × C57BL/6J] mice. This mapping panel has been typed for over 2100 loci that are well distributed among all 19 mouse autosomes and the X chromosome (Copeland and Jenkins, 1991; N.G.C. and N.A.J., unpublished results). C57BL/6J and *M. spretus* DNAs were digested with several different restriction enzymes and analyzed by Southern blot hybridization for informative RFLPs using the clones described in Table 1. The strain distribution pattern (SDP) of each RFLP was then determined for the backcross mice. All backcrosses were to C57BL/6J and, as expected, backcross progeny were either homozygous for the C57BL/6J allele or heterozygous for the C57BL/6J and *M. spretus* alleles. The presence or absence of RFLPs specific for *M. spretus* was followed in backcross mice. The chromosomal location of each locus was then determined by comparing its SDP with the SDPs for all other loci already mapped in the backcross. The mapping results are summarized in Fig. 1; the probes and RFLPs used for mapping are shown in Table 2.

The 22 probes identified 16 loci that are well distributed in the mouse genome (Fig. 1). In accordance with mouse nomenclature, the loci have been designated

TABLE 1

Summary of Unconventional Myosins Mapped in Mouse and Human Chromosomes

Mouse locus[a]	Clones mapped	Reference for clone	Mouse map location[b]	Predicted human homology region[b]	Human map location[c]
Myo1a	Human Myosin-IA Bovine Myosin-Ihc	Skowron *et al.* (1994) Hoshimaru and Nakanishi (1987)	10	12q13	12q13–q15
Myo1b	Mouse Myosin-Iα Human Myosin-IB	Sherr *et al.* (1993) Bement *et al.* (1994a)	1	2q12–q34	
Myo1c	Rat Myosin-I Beta	Sherr *et al.* (1993)	11	17pter–17q11	
Myo1d	Rat Myosin-Iγ Porcine Myosin-I	Sherr *et al.* (1993) Bement *et al.* (1994a)	11	17q11–q12	
Myo1e	Human Myosin-IC	Bement *et al.* (1994b)	9	15q21–q22	15q21–q22
Myo1f	Human Myosin-ID	Bement *et al.* (1994a) This manuscript[d]	17	6p12 or 19p13.3	19p13.3–p13.2
Myo1f-rs1	Human Myosin-ID	Bement *et al.* (1994a)	19	10q24–q26	
Myo5a	Mouse dilute Human Myosin-VA Human Myosin-VB Porcine Myosin-VA	Mercer *et al.* (1991) Bement *et al.* (1994a) Bement *et al.* (1994a) Bement *et al.* (1994a)	9	NA	15q21
Myo5b	Human Myosin-VA	Bement *et al.* (1994a)	18	18q21.1–q23	
Myo6	Mouse Myosin-VI Porcine Myosin-VI	Avraham *et al.* (1995) Hasson *et al.* (1995)	9	6p12–q16.3	
Myo7a	Human Myosin-VIIA Porcine Myosin-VIIA	Hasson *et al.* (1995) Bement *et al.* (1994a)	7	NA	11q13.5
Myo7b	Human Myosin-VIIB	Bement *et al.* (1994a)	18	18q12.1 or 5q21–q22	
Myo7b-rs1	Human Myosin-VIIB	Bement *et al.* (1994a)	11	17pter–p12	
Myo9a	Human Myosin-IXA	Bement *et al.* (1994a)	9	15q21–q25	
Myo9b	Human Myosin-IXB	Wirth *et al.* (1996)	8	4q31–q32 or 19p13.1	
Myo10	Mouse Myosin-X Porcine Myosin-VIIB	This manuscript[e] Bement *et al.* (1994a)	15	5p14–p12 or 8q22–q23	5p15.1–p14.3

[a] Loci were defined by the data shown in Table 2 and Fig. 1.
[b] Data were taken from Fig. 1. NA; not applicable.
[c] The human chromosomal location of two unconventional myosins has been reported [*MYO5*, (*MYH12*) Engle and Kennet (1994); Moore *et al.* (1995); *MYO7A*, (*MYU7A; USH1B*) Weil *et al.*, 1995].
[d] The Genbank Accession No. for human Myosin-ID is U57053.
[e] The Genbank Accession No. for mouse Myosin-X is U55210.

Myo, followed by the number of the class represented. When more than one locus per class was identified, a letter designation was added. The 9 class I clones mapped to 6 mouse autosomes (Fig. 1, Table 1). As expected, clones thought to be orthologs based on sequence analysis did indeed define the same locus. For example, probes for the orthologs mouse myosin-Iα and human myosin-IB detected very similar or identical restriction fragments, mapped to the same position on mouse Chromosome 1 and defined the *Myo1b* locus (Fig. 1; data not shown). One class I myosin probe, human myosin-ID, detected 2 loci, one in the proximal region of Chromosome 17 and one in the distal region of Chromosome 19 (Fig. 1; Table 2). Based on the human mapping data (see below), we have designated the Chromosome 17 locus *Myo1f* and the Chromosome 19 locus as *Myolf-rs1*. Little is known about human myosin-ID but amino acid comparisons have suggested it is the ortholog of chicken brush border myosin-IB (Knight and Kendrick-Jones, 1993; Mooseker and Cheney, 1995).

The structural gene for *Myo5* maps to the central region of mouse Chromosome 9 (Mercer *et al.*, 1991). However, one of the four Myosin-V probes used in this study, human myosin-VA, detected a second locus in the distal region of mouse Chromosome 18 (Fig. 1, Table 2). This locus has been designated *Myo5-rs1*. Human myosin-VA is thought to be the ortholog of a newly identified class-V member, rat myr 6 (Provance *et al.*, 1994), and therefore, *Myo5-rs1* may well indicate a second class-V gene, which we would call *Myo5b*.

As reported previously, the structural gene for *Myo6*

TABLE 2

Loci Mapped in Interspecific Backcross Mice

Locus	Probe[a]	Enzyme	Fragment sizes (kb)[b] C57BL/6J	Fragment sizes (kb)[b] *M. spretus*
Myo1a	~2.2-kb *Eco*RI fragment of human cDNA	*Sac*I	15.5, 3.9	9.0, 3.9
Myo1b	~750-bp *Xho*I/*Eco*RI fragment of mouse cDNA	*Taq*I	2.4, 1.2	1.4, 0.7, 0.5
Myo1c	~1.0-kb *Eco*RI/*Xho*I fragment of rat cDNA	*Sac*I	8.4, 4.1	9.6
Myo1d	~1.2-kb *Nsi*I/*Bam*HI fragment of rat cDNA	*Sca*I	5.8	4.7
Myo1e	~3.3-kb *Eco*RI fragment of human cDNA	*Hin*dIII	19.0, 10.5, 9.4, 7.6, 6.3, 4.3, 3.8, 2.6, 2.3, 0.8	~23.0, 10.5, 9.6, 7.6, 5.6, 4.4, 2.6, 0.7
Myo1f	~3.2-kb *Eco*RI fragment of human cDNA	*Bgl*II	7.8, 3.3	4.0[c], 3.0[c]
Myo5	~130-bp *Eco*RI/*Hin*dIII fragment of human cDNA	*Hinc*II	8.4, 3.0	8.8[d], 1.1[d]
Myo6	~159-bp fragment of mouse cDNA	*Pst*I	5.3	7.3
Myo7a	~3.5-kb human cDNA	*Bam*HI	21.0, 9.2, 7.4, 4.6	15.5, 9.2, 4.6
Myo7b	~130-bp *Eco*RI/*Hin*dIII fragment of human cDNA	*Hin*dIII	6.4, (5.6), 1.7	(11.5)[e], 6.6,[e] 2.5[e]
Myo9a	1.9-kb *Eco*RI fragment of human cDNA	*Bgl*II	5.8	6.8
Myo9b	3.0-kb *Eco*RI fragment of human cDNA	*Kpn*I	16.0, 6.8	>23.0, 7.6
Myo10	~400-bp *Eco*RI/*Bam*HI fragment of mouse cDNA	*Bgl*I	~23.0, 1.5	15.0, 1.5

[a] In some cases, more than one probe was mapped. The probe shown here was used to calculate the data shown in Fig. 1. Additional probes that were mapped are listed in Table 1; all probes for each locus cosegregated unless otherwise noted.

[b] With the exception of the human Myosin-VIIB probe, only the major restriction fragments detected with each probe are listed. The fragments that were followed in the backcross analysis are underlined. When more than one fragment was followed, the fragments cosegregated unless otherwise noted.

[c] The probe detected two independently segregating loci: the 4.0-kb *Bgl*II fragment mapped to mouse Chromosome 17 and defined *Myo1f*, and the 3.0-kb *Bgl*II fragment mapped to mouse Chromosome 19 and defined *Myo1f-rs1*.

[d] The probe detected two independently segregating loci: the 8.8-kb *Hinc*II fragment mapped to mouse Chromosome 9 and did not recombine with other probes listed in Table 1 for *Myo5a*. The 1.1-kb *Hinc*II fragment mapped to mouse Chromosome 18 and defined *Myo5b*.

[e] Using low-stringency hybridization, we detected major *M. spretus*-specific *Hin*dIII fragments of 6.6 and 2.5 kb. The 2.5-kb fragment mapped to Chromosome 18 and defined *Myo7b*. A minor *M. spretus*-specific *Hin*dIII fragment, indicated in parentheses, of ~11.5 kb mapped to mouse Chromosome 11 and defined *Myo7b-rs1*.

maps to the central region of Chromosome 9 (Avraham *et al.*, 1995; Fig. 1) and the structural gene for *Myo7a* maps to the central region of mouse Chromosome 7 (Brown *et al.*, 1992; Gibson *et al.*, 1995; Fig. 1). There are at least two other mouse myosin-VII genes. The human Myosin-VIIB probe detected a locus in the proximal region of mouse Chromosome 18 (*Myo7b*) (Fig. 1, Table 2). In addition, an 11.5-kb *Hin*dIII *M. spretus*-specific fragment (Table 2), which could only be scored in approximately 40 animals, appears to map in the vicinity of *Zfp3* on Chromosome 11 (Fig. 1). Finally, there are two identified class IX genes, *Myo9a* and *Myo9b*, and a single class X gene (*Myo10*) (Fig. 1).

Human Mapping Studies

cDNA fragments encoding human myosin-IA, myosin-IC, myosin-ID, and myosin-VIIA (Table 1) were

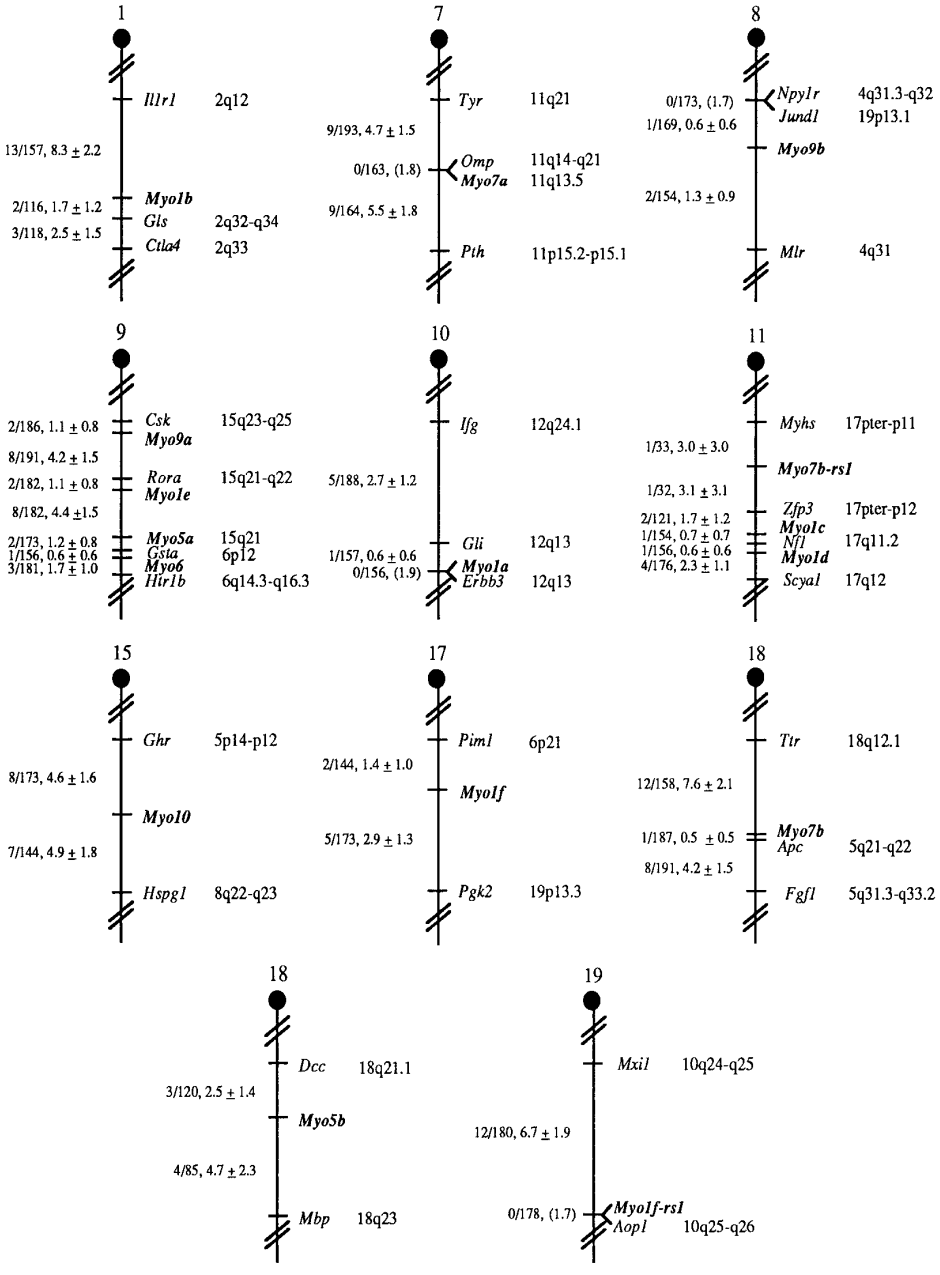

FIG. 1. Partial chromosome linkage maps showing the locations of the unconventional myosin loci in mouse. The genes were mapped by interspecific backcross analysis. Partial chromosome linkage maps showing the locations of the Myo genes in relation to linked loci are shown. The number of recombinant N2 animals over the total number of N2 animals typed plus the recombination frequencies, expressed as genetic distance in centimorgans (± one standard error), is shown for each pair of loci on the left of the chromosome maps. Where no recombinants were found between loci, the upper 95% confidence limit of the recombination distance is given in parentheses. The positions of loci in human chromosomes, where known, are shown to the right of the maps. References for the map positions of human loci described in this study can be obtained from GDB (Genome Data Base), a computerized database of human linkage information maintained by The William H. Welch Medical Library of The Johns Hopkins University (Baltimore, MD).

used as probes to locate the corresponding genes on human metaphase chromosomes using fluorescence *in situ* hybridization. Once identified, these genes were given the prefix MYO, in accordance with the Human Nomenclature Committee, and given a corresponding number (for the class) and letter (for the locus) according to the mouse locus name. For example, human myosin-IC cDNA, which identified the mouse *Myo1e* locus, defines the human *MYO1E* locus. In addition to the human cDNA probes, a cDNA clone encoding the tail portion of bovine myosin-X cDNA (D. P. Corey and R. Cheney, manuscript in preparation) was used as a probe to identify the human *MYO10* gene. Biotinylated human probes were hybridized simultaneously with a digoxigenin-labeled probe specific for the *Alu* family of repeats (Matera and Ward, 1992), while the biotinylated bovine probe was assigned chromosomal location by comparison of FISH signal with DAPI-banded chro-

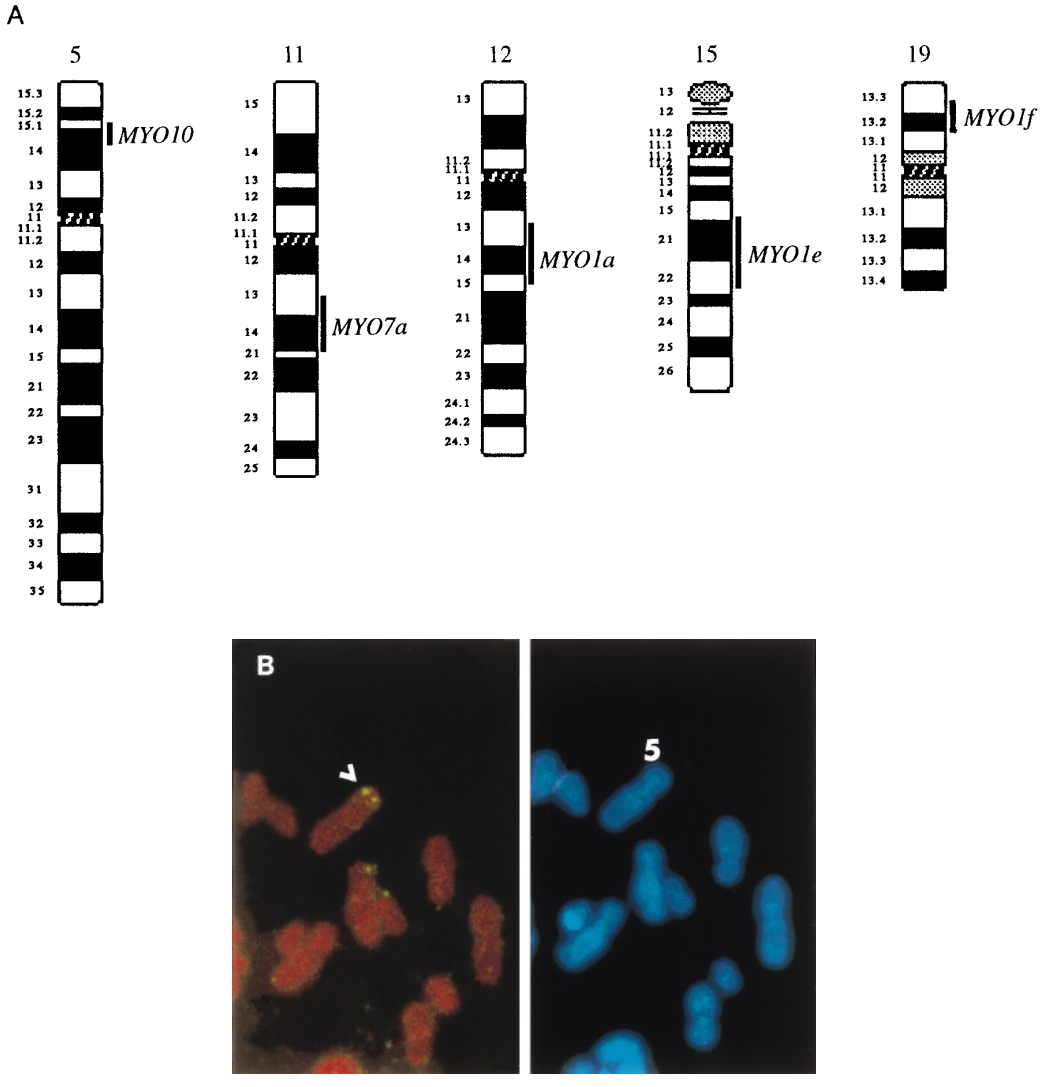

FIG. 2. Mapping of human unconventional myosin genes. (A) Idiograms of human Chromosomes 5, 11, 12, 15, and 19. The cytological banding pattern of the human chromosomes is marked on the left and the sites of myosin genes mapped are marked on the right. The Genome Data Base designation for the unconventional myosin genes is *MYO*. (B) Metaphase spread showing chromosomal localization of the *MYO10* gene by fluorescence *in situ* hybridization. The biotinylated bovine myosin-X probe is detected with FITC-avidin. The hybridization signals are indicated by an arrowhead in the left panel. Chromosomes are counterstained with DAPI in the right panel.

mosomes (Heng and Tsui, 1994). By these methods, the five probes identified five different loci (Fig. 2A, Table 1), each corresponding to the syntenic region suggested by mouse mapping. An example of a metaphase spread for myosin-X is shown in Fig. 2B. An arrowhead points out fluorescent signal placing *MYO10* on chromosome 5 region p14.3–p15.1.

DISCUSSION

We have mapped 22 unconventional myosin clones to 16 loci in mouse chromosomes. These include 7 loci for class I myosins, 2 for class V, 1 for class VI, 3 for class VII, 2 for class IX, and 1 for class X. Six of these loci have also been mapped in humans by fluorescence *in situ* hybridization. This mapping study, based on Table 1 and our unpublished data, locates the genes for all members of all vertebrate unconventional myosin classes identified to date; however, it remains possible that additional myosin genes will be identified by other techniques.

To determine if there are any mouse mutations that map in the vicinity (within ~10 cM) of these myosin loci that might have a phenotype expected for a mutation in one of these genes, we compared our interspecific backcross maps with composite mouse maps that report the approximate map location of many uncloned mouse mutations (provided from Mouse Genome Database, a computerized database maintained at The Jackson Laboratory, Bar Harbor, ME). Coat color mutations were found near three loci; *Myo1e* (ashen; *ash*), *Myo10* (underwhite, *uw*), and *Myolf-rs1* (ruby, *ru*). Neurological mutations were found near four loci: *Myolb* (tumbler, *tb*), *Myo1c* (tipsy, *ti*), *Myo7b* (ataxia, *ax*), and

Myo7b-rs1 which mapped near the shaker-2 (*sh2*) deafness mutation. This mutation is very similar in pathology and behavior to *sh1*, except that the symptoms have an earlier onset in *sh2* mice (summarized in Green, 1989). Given that *Myo7a* encodes *sh1*, this *Myo7b*-related locus is a very attractive candidate for *sh2*. *Myo7b* is also located near a mutation with an inner ear phenotype (twirler; *Tw*). *Tw* mice show the typical headshaking and circling behavior of inner ear mutants but are not deaf. This phenotype has been attributed to morphological abnormalities of the inner ear including irregularities in the semicircular canals and a reduction or absence of otoliths (summarized in Green, 1989). Additional studies will be required to determine whether any of these mutations correspond to alterations in an unconventional myosin locus.

Two unconventional myosins had been previously mapped in humans, *MYO5* (*MYH12*, Moore *et al.*, 1995) and *MYO7A* (*MYU7A, USH1B;* Weil *et al.*, 1995). We have confirmed the *MYO7A* location and mapped four new unconventional myosin loci in humans, *MYO1A, MYO1E, MYO1F,* and *MYO10*. An analysis of human diseases mapping to these four myosin loci did not identify any obvious human diseases that might be due to defects in these unconventional myosins.

Because of the coat color defects and seizures observed in dilute mice, *MYO5* has been considered a potential candidate for Griscelli syndrome, a disease characterized by partial albinism with immunodeficiency (Griscelli *et al.*, 1978; Klein *et al.*, 1994) or neurological disorders (Hurvitz *et al.*, 1993). In addition, *MYO5* has been considered a candidate for neuroectodermal melanolysosomal disease, a disease characterized by partial albinism and profound central nervous system dysfunction (Elejalde *et al.*, 1979). However, neither of these diseases has been mapped so it remains to be seen whether myosin-V defects are associated with either syndrome.

The remaining nine unconventional myosins have not been mapped in humans. However, based on the syntenic relationships between mouse and human chromosomes, we can predict where the loci will map in humans. A summary of these predictions is listed in Table 1. For example, it is likely that *MYO1B* will map to human chromosome 2q12–q34 with the most likely position at 2q32–q34 (Table 1, Fig. 1). This map location makes the human *MYO1B* gene a good candidate for juvenile amyotrophic lateral sclerosis (ALS2) type 3. Juvenile ALS2 type 3 maps to 2q33–q35 (Hentati *et al.*, 1994) and is characterized by progressive spasticity of limbs and facial and pharyngeal muscles due to a defect in motor neurons with onset between the ages of 3 and 23 years (Ben Hamida *et al.*, 1990). Myosin-IB protein and mRNA have been shown in rats and mice to be highly expressed in brain both during development and in the adult and to be specifically expressed by neurons (Ruppert *et al.*, 1993; Sherr *et al.*, 1993). Analysis of the myosin-IB gene in juvenile ALS2 patients is in progress to assess whether this unconventional myosin does indeed underlie this disease. The remaining myosin genes do not map in the vicinity of previously identified human disease loci.

The human gene for myosin-VI, *MYO6*, will likely map to chromosome 6p12–q16. As mice with mutations at the *Myo6* locus exhibit inner ear defects, *MYO6* is a potential candidate for human recessive nonsyndromic deafness although no deafness genes to date have been mapped to chromosome 6. Epidemiological studies have suggested that there are 36–200 genes for recessive deafness in human (Chung and Brown, 1970; Morton, 1991). DFNB2, a nonsyndromic recessive deafness, has been mapped to 11q13 at the same position as *MYO7A* and *USH1B* and may reflect a different class of myosin-VIIa mutations. Of the remaining predicted MYO loci, three, *MYO7B-rs1, MYOID,* and *MYOIC,* map in the vicinity of DFNB3, a recessive nonsyndromic deafness mapped to chromosome 17 (Friedman *et al.*, 1995). The protein encoded by *MYOIC*, myosin-Iβ, has been studied in a number of species (for review see Mooseker and Cheney, 1995), but studies in frog have shown that the protein is located at the tips of sensory hair cell stereocilia (Gillespie *et al.*, 1993). Therefore, myosin-Iβ is a candidate for the tip link motor required for hair cell mechanotransduction (Gillespie *et al.*, 1993) and, as such, is a good candidate for a human recessive deafness gene. The other two myosins, *MYO7B-rs1* and *MYO1D*, have not been characterized as to their expression in the inner ear.

In addition to the 16 unconventional myosin genes discussed here, there are also 10 previously identified myosin-II loci (*MYH1, 2, 3, 4, 6, 7, 8, 9, 10, 11*). Mutations in the β chain of cardiac myosin-II (*MYH7*) cause familial hypertrophic cardiomyopathy, a dominant cardiac disease characterized by left ventricular hypertrophy and, in many cases, sudden death (Fananapazir and Epstein, 1994; reviewed in Vikstrom and Leinwand, 1996). Diseases have not been associated with other myosin-II heavy chains, perhaps due to their essential roles in cell division, cell morphogenesis, and development (reviewed in Warrick and Spudich, 1987).

In summary, we have identified 16 genes encoding unconventional myosins using 22 probes directed against class-I, class-V, class-VI, class-VII, class-IX, and class-X myosins. Including the previously identified class-II myosins, this brings the total number of myosin genes in mammals to 26. These new loci should provide a basis for identification of potential human diseases that result from myosin mutations.

ACKNOWLEDGMENTS

We thank Kari Jensen for providing myosin-IXB cDNA, Bruce Derfler for sequencing bovine myosin-X, and Nurit Mokady, Deborah B. Householder, Mary Barnstead, and Brian Cho for excellent technical assistance. This research was supported by National Institute of Health Grant DK25387, Program Project Grant DK38979 (J. Morrow, P.I.), and a Basic Research Grant from the Muscular Dystrophy Association for T.H., J.F.S., W.M.B., and M.S.M., by National Institute of Health Grant NS33689 to L.A.G., by a grant from the Deaf-

ness Research Foundation to Z.Y.C., and by the National Cancer Institute, DHHS, under contract with A.B.L. D.P.C. is an associate investigator of the Howard Hughes Medical Institute.

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