Medical Sciences. In the article "A synthetic retinoid antagonist inhibits the human immunodeficiency virus type 1 promoter" by Mi-Ock Lee, Peter D. Hobbs, Xiao-kun Zhang, Marcia I. Dawson, and Magnus Pfahl, which appeared in number 12, June 7, 1994, of *Proc. Natl. Acad. Sci. USA* (**91,** 5632–5636), the preparation of the retinoid 4-[1-(1-methoxy-2,2,2-trifluoroethyl)-5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-3-anthracenyl]benzoic acid (SR11335) given on p. 5632 should be corrected to include the step describing the reduction of the 1-trifluoroacetyl group on the tetrahydroanthracenyl ring to a 1-(1-hydroxy-2,2,2-trifluoroethyl) group, and the description of the ^{1}H NMR spectrum of SR11335 should be revised to indicate that the signal at 5.34 ppm is for a single proton. The correct synthetic procedure is as follows: 4-[1-(1-Methoxy-2,2,2-trifluoroethyl)-5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-3-anthracenyl]benzoic acid (SR11335) was prepared by acylation of ethyl 4-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-anthracenyl)benzoate (20) with CF_3COCl in $(CH_2Cl)_2$ using $AlCl_3$ as the catalyst and then reduction with $NaBH_4$ in ethanol to give ethyl 4-[1-(1-hydroxy-2,2,2-trifluoroethyl)-5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-3-anthracenyl]benzoate, followed by methylation of the trifluoromethylcarbinol group with MeI and K_2CO_3 at reflux to give the methyl ether and base hydrolysis of the ethyl ester using aqueous KOH in ethanol at 80°C for 1 h. Acidification produced SR11335 in 12% overall yield as white crystals: mp 286–288°C; IR (KBr) 3500–2350, 1688, 1608, 1426, 1296, 1170, 1140, 909, 851, 773, 702, 668 cm^{-1}; 300 MHz ^{1}H NMR (C^2HCl_3 δ 1.43 (m, 12, 5,5,8,8-CH_3), 1.81 (s, 4, 6,7-CH_2), 3.51 (s, 3, OCH_3), 5.34 (q, J = 6.6 Hz, 1, $HCCF_3$), 7.84 (d, J = 8.5 Hz, 2, ArH meta to CO_2H), 7.90 (m, 1, ArH), 7.92 (s, 1, ArH), 8.10 (m, 2, ArH), 8.23 (d, J = 8.5 Hz, 2, ArH ortho to CO_2H).

Agricultural Sciences. In the article "Milk composition and lactation of β-casein-deficient mice," by Satish Kumar, Alan R. Clarke, Martin L. Hooper, David S. Horne, Andrew J. R. Law, Jeffrey Leaver, Anthea Springbett, Elizabeth Stevenson, and J. Paul Simons, which appeared in number 13, June 21, 1994, of *Proc. Natl. Acad. Sci. USA* (**91,** 6138–6142), the authors request that the following correction be noted. E14 embryonic stem cell line was incorrectly defined in the journal as embryonic day-14 stem cell; derivation of E14 embryonic stem cell line is as referenced in the article.

Cell Biology. In the article "Identification and overlapping expression of multiple unconventional myosin genes in vertebrate cell types" by William M. Bement, Tama Hasson, Joel A. Wirth, Richard E. Cheney, and Mark S. Mooseker, which appeared in number 14, July 5, 1994, of *Proc. Natl. Acad. Sci. USA* (**91,** 6549–6553), the following correction should be noted. On p. 6549, right column, lines 21 and 22, the nucleotide sequence of the second PCR primer ("EAF-A") was missing six bases. The correct sequence of this primer is GT(C/T)TTIGC(G/A)TTICC(G/A)AAIGC(C/T)TC.

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Cell Biology

Identification and overlapping expression of multiple unconventional myosin genes in vertebrate cell types

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ABSTRACT **Myosin diversity in the human epithelial cell line Caco-2_{BBe}, the porcine epithelial cell line LLC-PK_1 (CL-4), human peripheral blood leukocytes, and human liver was analyzed. PCR amplification yielded 8–11 putative myosins (depending on the cDNA source) representing six distinct myosin classes. Analysis of clones obtained by hybridization screening demonstrated that the original PCR products correspond to bona fide myosins, based on the presence of sequences highly conserved in other myosins. RNase protection analysis confirmed mRNA expression of 11 myosins in Caco-2_{BBe} cells. Immunoblot analysis showed that at least 6 myosin immunogens are expressed in Caco-2_{BBe} cells. The results reveal the existence of at least 11 unconventional human myosin genes, most of which are expressed in an overlapping fashion in different cell types. The abundance of myosins suggests that the myosin I vs. myosin II paradigm is inadequate to explain actin-based cellular motility.**

Eukaryotes display a broad array of motility phenomena, ranging from muscle-driven movement to translocation of intracellular organelles. The mechanisms underlying these phenomena are not entirely clear, but it is generally thought that "molecular motors," such as kinesins, dyneins, and myosins, act at cytoskeletal–cytoskeletal and cytoskeletal–membrane interfaces to power organism and cellular movements (1–4). These motors are represented by multiple members in eukaryotes (4, 5), each of which is presumed to play one or more roles during the life of the organism. For example, the fruit fly is known to have at least 6 (6) and possibly as many as 30 (7) kinesins. A unicellular organism, *Dictyostelium discoideum*, has a minimum of five myosins (8, 9) and probably more than nine (10, 47).

Thus, within an organism, multiple diverse molecular motors are the rule. For myosins, phylogenetic analyses have revealed the existence of eight classes (11–13), termed either conventional (filament-forming myosins II) or unconventional (apparently nonfilamentous myosins I and III–VIII) (14). Although evidence indicates that many of the unconventional myosin classes will be represented in a given organism (5, 15), to our knowledge, in no case has the number of myosins expressed in a metazoan cell type been determined. Consequently, studies concerning actin-based motility in metazoan cells are complicated by limited information on cellular myosin diversity. We therefore analyzed myosin diversity in two epithelial cell lines, Caco-2_{BBe} (16) and LLC-PK_1 [CL-4] (17), and in human leukocytes and liver.¶ Surprisingly, these cell types express six myosin classes, most of which have at least two representatives. The results challenge the standard "myosin I vs. myosin II" paradigm for actin-based movement in the typical eukaryotic cell.

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MATERIALS AND METHODS

PCR Amplification of Myosins, Sequencing, and Library Screening. Caco-2_{BBe} cells were cultured as described (16). LLC-PK_1 (CL-4) cells were obtained from C. Slayman (Yale University), cultured in minimum essential medium, α modification (GIBCO/BRL)/10% (vol/vol) fetal calf serum/2% (wt/vol) glutamine (Sigma) and penicillin 100 (units/ml)/streptomycin (100 μg/ml)/fungizone (0.25 μg/ml) (JRH Biosciences, Lenexa, KS), and used at 4 days after confluence. Peripheral blood leukocytes were purified from whole blood of human volunteers via density gradient cell separation (18). RNA was isolated by guanidinium lysis followed by a CsCl gradient (19). cDNAs were generated using reverse transcriptase (New England Biolabs). For PCR of liver cDNAs, a λZAP cDNA library (Stratagene) prepared from normal human liver (a gift from James Mustafa Anderson, Yale University) was employed. Myosins were amplified from cDNAs using standard PCR techniques (20). Two degenerate myosin primers, "ATP-3" (upstream) [GGIGA(G/A)(A/T)(G/C)IGGIGCIGGX-AA(G/A)AC] and "EAF-A" (downstream) [GT(C/T)TT-IGC(G/A)AAIGC(C/T)TC], were designed based on the myosin consensus sequence (2). ATP-3 corresponds to the amino acid sequence GESGAGKT, a sequence found in all myosins (e.g., aa 163–170 in avian myosin V; ref. 21), and EAF-A corresponds to EAFGNAKT, a sequence found in most myosins. After amplification, products were separated on NuSieve (FMC) agarose gels, excised from the gel, and reamplified by PCR. After the second amplification, products were separated on agarose gels and purified on DEAE-cellulose paper. Purified products were ligated into T-modified Bluescript vector (22) and transformed into competent bacteria (XL1-Blue, Stratagene). Plasmid minipreps (23) were performed and insert presence was confirmed by restriction analysis. Inserts were sequenced with a kit (United States Biochemical). Larger clones were obtained by hybridization screening of the human liver library with a subset of four of the subcloned PCR products. Plasmid inserts were labeled with ^{32}P by using random priming (Boehringer Mannheim). Positive plaques were isolated and converted to phagemid by *in vivo* excision.

Expression Analysis. RNase protection probes were generated using subcloned PCR products. Antisense [^{32}P]UTP and rCTP-labeled RNA were transcribed from plasmids using T3 or T7 RNA polymerase. Probes were hybridized with 20 μg of total Caco-2_{BBe} RNA, digested with RNases A and T1 (19), and separated on 8% polyacrylamide gels, using end-labeled λ DNA digested with *Hin*fI as size markers. Because

Abbreviations: HuncM, human unconventional myosin; HConM, human conventional myosin; PuncM, porcine unconventional myosin; PConM, porcine conventional myosin.

¶The sequences reported in this paper have been deposited in the GenBank data base (accession nos. L29137–L29149 for human unconventional myosins and L29128–L29136 for porcine unconventional myosins).

probes were derived from PCR products containing inosine residues at their 5′ and 3′ ends and because primer matching need not be exact for amplification, two complications must be considered. (*i*) The probes were heterogenous at their ends, thus, protected products often resolved as several bands on autoradiographs. (*ii*) It was not possible to predict the exact size of protected products; rather, size predictions were limited to the range, with the region between the primers defining the lower limit (90–120 bp) and this region plus the primers defining the upper limit (136–166 bp). Nevertheless, RNase protection using degenerate PCR products provides a reproducible and sensitive means to assess myosin RNA expression (24).

Flasks containing Caco-2_{BBe} cells were rinsed with TBS (150 mM NaCl/50 mM Tris·HCl, pH 7.4), scraped into a polypropylene tube, pelleted, and homogenized in SDS sample buffer. Samples were separated on 5–15% polyacrylamide gels, electroblotted to nitrocellulose (16), and probed with anti-human platelet myosin II antibodies (Biomedical Technologies, Stoughton, MA), affinity-purified anti-chicken brush-border myosin I antibodies (25), affinity-purified anti-chicken myosin V antibodies (21), or affinity-purified anti-porcine myosin VI antibody (26).

RESULTS

PCR Analysis of Myosin Diversity in Human and Porcine Cells. To assess myosin diversity, PCR amplification of myosin-like sequences was performed using the human epithelial cell line Caco-2_{BBe} (16, 27). This clonally derived line was selected based on its resemblance to normal human intestinal epithelia (16, 24, 28). PCR amplification yielded 10 products that bore various degrees of homology to known human and mammalian myosins (Fig. 1*A*). Products were grouped into five previously identified classes (myosins I, II, V, VI, and VII) and a sixth group (myosin IX) that bore no similarity to any known myosin class. Most classes had at least two representatives, and, based on the nucleotide sequences that differed along the length of the products, all of the sequences, except HConM-IIA', appeared to be prod-

A. Human Myosin-like PCR Products

```
Myosins-I
   Bov BBMI (109-142)      EASKLVMSYVAAVCGKGEQVNSVKEQLIQSNPVL
   HuncM-IA                EASKLVMSYVAAVCGKGEQVNSVKEQLLQSNPVL C2
   MMIα (116-149)          EASKLVMSYVAAVCGKGAEVNQVKEQLLQSNPVL
   HuncM-IB                EASKLVMSYVAAVCGKGAEVNQVKEQLLQSNPVL LV
   DDMIB (110-143)         EAAKLIMGYVSAISGSTEKVEYVKHVILESNPLL
   HuncM-IC                VAAKYIMSYISRVSGGGTKVQHVKDIILQSNPLL C2, LK
Myosins-II
   Hum MIIA (185-218)      ENTKKVIQYLAYVASSHKSKKDQ.....GELERQLLQANPIL
   HConM-IIA               ENTKKVIQYLAYVASSHKSKKDQ.....GELERQLLQANPIL C2, LV
   HConM-IIA'              ENTKKVIQYLAYVASSPKGRKEPGVPASVSTVSYGELERQLLQANPIL C2
Myosins-V
   Dilute (171-202)        VSAKYAMRYFATVSGSASEANVEEKVLASNPIM
   HuncM-VA                VSAKYAMRYFATVGGSASETNIEEKVLASSPIM C2, LK, LV
   HuncM-VB                VSARYAMRYFATVSKSGSNAHVEDKVLASNPIT C2, LK
Myosins-VI
   PuncM-VI (159-189)      ENTKFVLRYLTESYGTGQDIDDRIVEANPLL
   HuncM-VI                ENTKFVLRYLTESYGTGQDIDDRIVEANPLL C2, LK, LV
Myosins-VII
   HuncM-VIIA              ESTKLIPQFLAAISGQHSWIEQQVLEATPIL C2, LK
   HuncM-VIIB              ETTKLILQFLATISGQHSWIEQQVLEANPIL C2, LK
Myosins-IX
   HuncM-IXA               QSTNFLIHHLTALSQKGFASGVEQIILGAGPVL C2
   HuncM-IXB               QSTNFLIHCLTALSQKGYASGVERTILGACPVL LK
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B. Porcine Myosin-like PCR Products

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Myosins-I
   DroM-IA (108-143)       EASKIIMKYIAAVTNAQGQNEIERVKNVLIQSNAIL
   PuncM-I                 EASKYIMQYIAAITNPSQRAEIERVKNMLLKSNCVL
Myosins-II
   Hum MIIA (185-218)      ENTKKVIQYLAYVASSHKSKKDQGELERQLLQANPIL
   PConM-IIA               ENTKKVIQYLAHVASSHKSKKDQGELERQLLQANPIL
   Hum MIIB (185-221)      ENTKKVIQYLAYVASSHKGRKDHNIPGELERQLLQANPIL
   PConM-IIB               ENTKKVIQYLAHVASSHKGRKDHNIPGELERQLLQANPIL
Myosins-V
   Dilute (171-202)        VSAKYAMRYFATVSGSASEANVEEKVLASNPIM
   PuncM-V                 VSARYAMRYFATVSKSSSNAHVEDKVLASNPIT
Myosins-VI
   DrM95F (159-189)        ESTKYLLKYLCYSHDSAGPIETKILDANPVL
   PuncM-VI                ENTKFVLRYLTESYGTGQDIDDRIVEANPLL
Myosins-VII
   PuncM-VIIA              ESTKLILQFLAAISGQ.....HSWIEQQVLEATPIL
   PuncM-VIIB              ESTKLILKFLSAISQQSLDLSLKEKTPSVERAILESSPIM
Myosins-IX
   PuncM-IXA               QSTNFLIHHLTALSQKGFASGVEQIILGAGPVL
   PuncM-IXB               QSTNFLIHCLTALSQKGYASGVERTILGAGPVL
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FIG. 1. (*A*) Alignment of putative myosin PCR products from human sources. Subscripts denote sources from which PCR products were obtained: C2, Caco-2_{BBe} cells; LV, human liver library; LK, human leukocytes. Products are grouped with each other by homology and, where possible, with homologues from the database (underlined). Numbers after database homologues indicate the residues spanned. Identical residues within a grouping are shown in boldface type. (*B*) Alignment of putative myosin PCR products from LLC-PK_1 cells. Bov BBMI, bovine brush border myosin I (29); MMIα, murine myosin Iα (30); DDMI, *Dictyostelium discoideum* myosin I (31); human MIIA and B, human nonmuscle myosins A and B (32); Dilute, murine myosin V (33); DroM-IA, *Drosophila* myosin IA (34); DrM95F, *Drosophila* 95F MHC (35). Unconventional and conventional human myosins are referred to as HuncMs or HConMs, respectively, and their porcine counterparts are referred to as PuncMs or PConMs. Numbers are used to indicate the apparent class to which they belong, with letters to distinguish between different class members. For example, the human homologue of brush border myosin I is HuncM-IA.

ucts of different genes. For example, the two most closely related human myosins (87% amino acid identity) HuncM-VIIA and -VIIB are 87% identical at the nucleotide level. HConM-IIA' may represent a splice form of human nonmuscle myosin IIA, since its nucleotide sequence is identical to that myosin (32) except for an insert in the middle of the PCR product, an area known to be differentially spliced in other myosins II (e.g., ref. 36).

To determine whether the myosins were cell-type-specific, PCR analysis of myosin diversity was also conducted using human peripheral blood leukocyte cDNAs or a human liver cDNA library as starting material. Most of the myosin PCR products identified in Caco-2_{BBe} cells were also found in leukocytes and liver (Fig. 1*A*). Because the leukocyte and liver cDNAs represent several cell types, myosin diversity in the LLC-PK_1 cell line was determined. As in the human cells, six groups of PCR products were identified in LLC-PK_1, including myosins IX (Fig. 1*B*). Porcine myosin IX products bore no homology to known myosins but were nearly identical to human myosin IXs obtained by PCR.

Confirmation of PCR Products as Myosins. The foregoing results suggested that vertebrate cell types express multiple myosins, most of which are not cell-type specific. It was essential, however, to demonstrate that the PCR products correspond to larger myosin sequences. Specifically, the presence of the canonical myosin sequences, GESGAGKT and EAFGNAKT, upon which the PCR primers were based, had to be independently confirmed, since any PCR-amplified products will necessarily contain these sequences. This was accomplished by screening a human liver cDNA library with four of the PCR products, including the least "myosin-like" (myosins IX). Six myosin-like sequences were thereby obtained, five (HuncM-IB, HuncM-IC, HuncM-VI, HuncM-IXA, and HuncM-IXB) that correspond to myosins identified by PCR and one (HuncM-ID) that was not found by PCR (Fig. 2). HuncM-ID was 96% identical to HuncM-IC (amino acids), but scattered nucleotide differences (85% identity) along the length of the two clones indicate that they are distinct gene products. All of the larger clones were similar to known myosins (Fig. 2), with the exception of the myosins IX. For example, HuncM-VI had 100% amino acid identity with PuncM-VI (95% nucleotide identity; ref. 26) and HuncM-IB was 100% identical to a mouse myosin I, MMIα (30), and 98% identical to a rat myosin I, myrIc (37). Further, all of the clones, including the myosins IX, possessed perfect or nearly perfect GESGAGKT and EAFGNAKT sequences (Fig. 2). Thus, based on sequence homology, all of the products obtained by PCR, including the myosins IX, represent bona fide myosin sequences.

Confirmation of Multiple Myosin mRNA and Protein Expression. The abundance of myosins revealed by PCR amplification suggested that vertebrate cells express many actin-based motors. To confirm this, total RNA from confluent Caco-2_{BBe} monolayers was analyzed by RNase protection using the subcloned PCR products. Nine of the 10 original PCR products were found to be expressed by Caco-2_{BBe} cells, as demonstrated by protection of RNA within the predicted size range (Fig. 3). (One of the products, HConM-IIA', gave a strong signal at a size smaller than predicted, possibly due to a *Taq* polymerase error in the original amplification.) HuncM-IB, which was not detected by PCR in Caco-2_{BBe} cells, was nevertheless expressed in Caco-2_{BBe} cells as assessed by RNase protection (Fig. 3) and HuncM-IXB, which was not detected by PCR or RNase protection, was identified by Northern blot analysis of total Caco-2_{BBe} RNA (Table 1). Likewise, all myosin PCR products identified in LLC-PK_1 cells were also detected by RNase protection (data not shown). To confirm that the multiple bands in the RNase protection assays reflected the presence of "ragged ends" arising from inosines in the PCR products, a portion of a myosin VI cDNA obtained by library screening was used for RNase protection. This yielded a single band of exactly the predicted size (data not shown), confirming the validity of the use of degenerate PCR products for RNase protection.

To further assess myosin diversity in Caco-2_{BBe} cells, monolayer extracts were examined by immunoblot analysis. As previously shown (16, 24), Caco-2_{BBe} cells express at least one myosin II and three myosin I immunogens of 140, 130, and 110 kDa (Fig. 4). In addition, immunoblot analysis with antibodies against avian myosin V (21) and porcine myosin VI showed that each of these is expressed in Caco-2_{BBe} cells. Thus, of those myosin classes for which antibody probes are available, all are represented by at least one protein product in Caco-2_{BBe} cells.

DISCUSSION

The results show that vertebrate cell types express a diverse array of myosin motors (Table 1). This conclusion hinges upon two assumptions: (*i*) the PCR screen yields bona fide myosins and (*ii*) the cells used are an accurate approximation of normal vertebrate cell types. The first assumption is amply supported by the results. Of the conventional myosins, one (HuncM-IIA) is identical to human nonmuscle myosin IIA (32). Of the unconventional myosins, most of those identified here are identical or highly similar to unconventional myosins from other sources. Of those PCR products that bear little homology to known myosins, sequence analysis of larger clones confirmed the presence of two regions of sequence diagnostic of myosins—the GESGAGKT sequence and the EAFGNAKT sequence. Moreover, analysis of cDNAs identified in this report as HuncM-IC and HuncM-IXB has shown that each encodes a complete myosin "head" domain (W.M.B. and J.A.W., unpublished results). Thus the sequences identified by PCR represent true myosins.

The second assumption crucial to this study is more difficult to address. Caco-2_{BBe} cells are derived from a human colonic adenocarcinoma (27); consequently, it could be argued that multiple myosins simply reflect abnormal deregulated myosin expression. Based on the following observa-

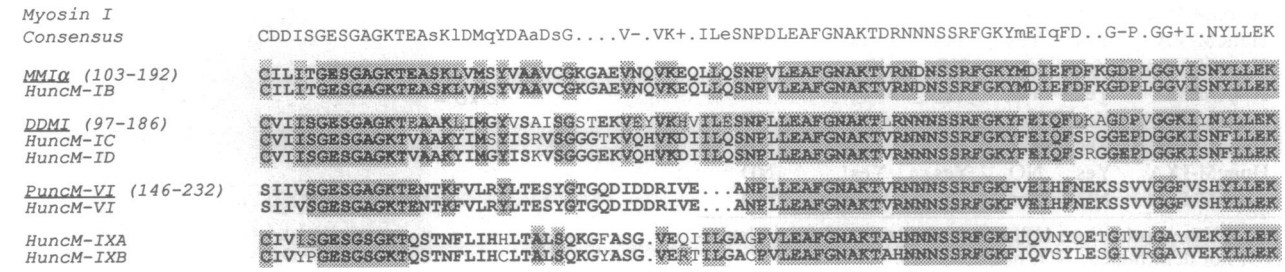

FIG. 2. Alignment of human unconventional myosins with a myosin I consensus sequence. Consensus sequence is from ref. 2. Human unconventional myosins are grouped with each other or with other myosins. Matches with the consensus are in stippled boxes; identical residues within a grouping are in boldface type.

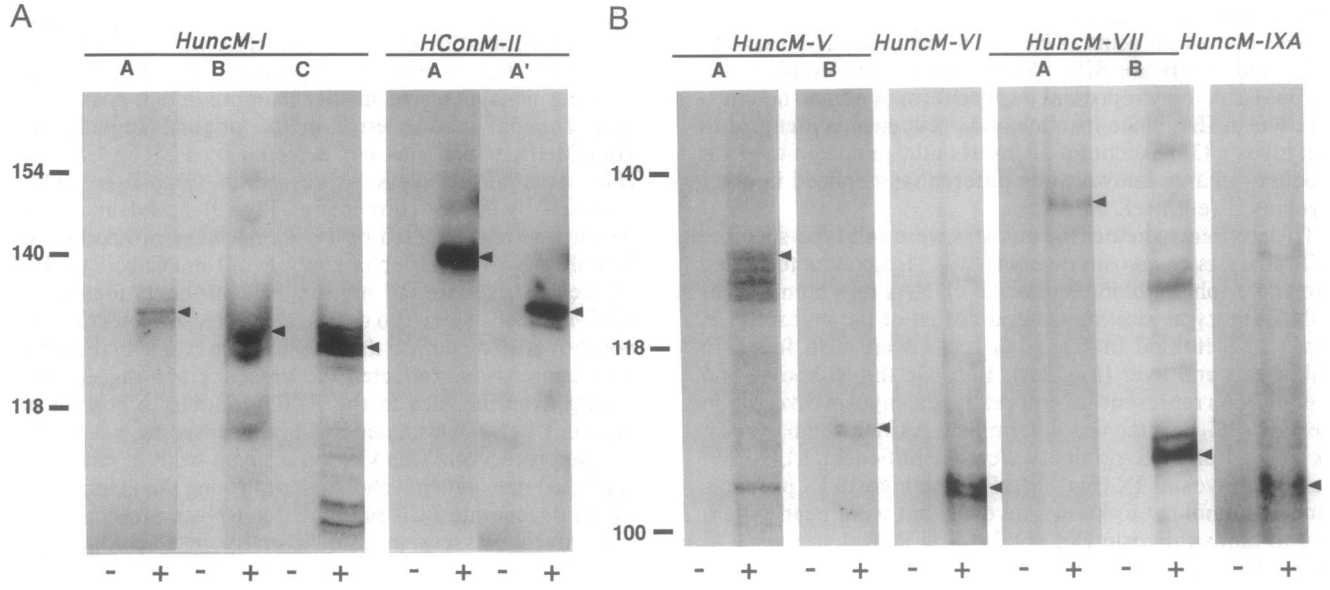

FIG. 3. RNase protection analysis of myosin expression in Caco-2_{BBe} cells. (*A*) Protection of pieces by PCR-generated probes for myosins I and II. RNase digestion of probe RNA was performed in the absence (−) or presence (+) of Caco-2_{BBe} RNA. Arrowheads indicate major protection products; minor bands result from PCR/PCR primer-mediated differences in probes. Predicted sizes: HuncM-IA, -IB, and -IC, 102–148 bp; HuncM-IIA, 108–154 bp; HConM-IID, 141–187 bp. Numbers at left indicate size standards in base pairs. (*B*) Protection of pieces by PCR-generated probes to myosins V, VI, VII, and IX. Predicted sizes: HuncM-VA and -VB, 99–145 bp; HuncM-VI, -VIIA, and -VIIB, 93–139 bp; HuncM-IXA, 85–131 bp.

tions, however, this possibility is unlikely. (*i*) No muscle myosins were detected, even though all muscle myosins possess the canonical myosin sequences upon which the PCR primers were based. (*ii*) Caco-2_{BBe} cells regulate expression of at least one myosin, human brush-border myosin I (HuncM-IA), in a fashion that closely parallels normal human gut (24). (*iii*) LLC-PK_1 cells, which are not of tumor origin (17), also express multiple myosins. (*iv*) Multiple myosins were found in normal human leukocytes and in a human liver cDNA library. (*v*) Recent studies on chicken enterocytes reveal expression and differential localization of at least four classes of myosins (38). These observations collectively suggest that expression of multiple myosins by metazoan cell types is normal. While the presence of myosin mRNAs does not guarantee expression of the corresponding proteins, the immunoblot results presented here and elsewhere (16, 24) suggest that most if not all of the myosins expressed at the RNA level are also expressed as proteins.

How many actin-based motors does a metazoan cell type have? The answer is at least 11. The first implication of these findings is that it is inappropriate to consider actin-based motility simply in terms of myosin I vs. myosin II. Most of the previously identified myosin classes (myosins I–VIII; refs. 11–13) are represented in all of the cell types analyzed. The second implication is related to the first but is more specific. The various cell types analyzed express multiple members of several myosin classes. In Caco-2_{BBe} cells, there are three myosins I, two myosins II, two myosins V, one myosin VI, two myosins VII, and two myosins IX (Table 1).

Table 1. Summary of myosins identified from human sources

Class	Myosin name	$C2_{BBe}$ PCR	HPL PCR	Liver library*	$C2_{BBe}$ RNAP	$C2_{BBe}$ immunoblot bands, kDa
I	HuncM-IA	Yes	NO	NO	Yes	110, 130, 140
	HuncM-IB	NO	NO	Yes	Yes	
	HuncM-IC	Yes	Yes	Yes	Yes	
	HuncM-IA′	NO	NO	Yes	ND	
II	HConM-IIA	Yes	ND†	Yes	Yes	220
	HConM-IID	Yes	ND	NO	Yes	
V	HuncM-VA	Yes	Yes	Yes	Yes	200
	HuncM-VB	Yes	Yes	NO	Yes	
VI	HuncM-VI	Yes	Yes	Yes	Yes	145
VII	HuncM-VIIA	Yes	Yes	Yes	Yes	ND
	HuncM-VIIB	Yes	Yes	Yes	Yes	ND
IX	HuncM-IXA	Yes	NO	Yes	Yes	ND
	HuncM-IXB	NO	Yes	Yes	ND‡	ND

RNAP, RNase protection; HPL, human peripheral blood leukocyte; ND, not determined; NO, not observed.
*Combined results of PCR and screening.
†Myosins II were not analyzed in human peripheral blood leukocytes (see ref. 32).
‡Detected by Northern blot analysis.

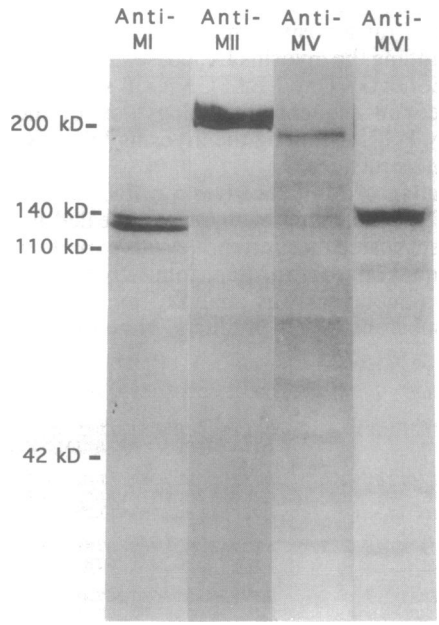

FIG. 4. Immunoblot of extracts from mature Caco-2_{BBe} cell monolayers. Lanes were probed with anti-myosin (M) antibodies as indicated.

These findings explain apparent discrepancies found in recent studies on myosin I distribution in vertebrate cells. Nusrat *et al.* (39) and Conrad *et al.* (40) found myosin I localized in punctate structures in motile epithelial cells and fibroblasts, respectively, with no leading edge staining. In contrast, Wagner *et al.* (41) found localization of myosin I at the leading edge of vertebrate cell types, including fibroblasts, similar to results from amoeboid organisms (e.g., refs. 42 and 43). It is likely that the cell types used in the above studies express several myosins I, and the various antibodies employed recognize different subsets of these.

The extensive repertoire of unconventional myosins found within different vertebrate cell types must reflect an overlying diversity of myosin genes, similar to what has been found for human conventional myosin genes, of which there are at least nine (e.g., refs. 32 and 44). The results presented here demonstrate the existence of at least 11 unconventional human myosin genes (Table 1), effectively doubling the number of known human myosin genes. This is probably an underestimate, since several mammalian myosins I have been described that were not detected in the present study (e.g., myosins Iβ and γ; ref. 30). Thus, human myosins are encoded by a superfamily of genes with more than 20 members.

Three hypotheses could explain the striking number and diversity of unconventional myosins found in vertebrate cells. (*i*) Each myosin may mediate a specific motile function. If so, there may be several undiscovered actin-based motilities within vertebrate cells. (*ii*) Myosins within a given class may overlap in function, as indicated by gene disruption experiments performed in *Dictyostelium* (e.g., ref. 45). (*iii*) In contrast to what is generally believed, some of the myosins may play roles more subtly related to their mechanochemical potential, such as regulation of membrane channels and enzymes. While the last hypothesis remains speculative, a myosin I immunogen has been localized to the tip of stereocilia where it is thought to tether calcium channels to underlying actin filaments, thereby regulating channel position and activity (46). Since all unconventional myosins studied in detail have membrane binding domains and display intracellular distributions characteristic of membrane binding proteins (2, 5, 14), it may be that some unconventional myosins serve as regulatable crosslinkers between membrane proteins and the cytoskeleton. In any case, the remarkable number and diversity of myosins expressed in metazoan cells suggest that conventional notions about myosin function must be reevaluated.

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1. Warrick, H. M. & Spudich, J. A. (1987) *Annu. Rev. Cell Biol.* **3**, 379–421.
2. Pollard, T. D., Doberstein, S. K. & Zot, H. G. (1991) *Annu. Rev. Cell Physiol.* **53**, 653–681.
3. Endow, S. A. & Titus, M. A. (1992) *Annu. Rev. Cell Biol.* **8**, 29–66.
4. Skoufias, D. A. & Scholey, J. M. (1993) *Curr. Opin. Cell Biol.* **5**, 95–104.
5. Titus, M. A. (1993) *Curr. Opin. Cell Biol.* **5**, 77–81.
6. Stewart, R. J., Pesavento, P. A., Woerpel, D. N. & Goldstein, L. S. B. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 8470–8474.
7. Endow, S. A. & Hatsumi, M. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 4424–4427.
8. Titus, M. A., Warrick, H. M. & Spudich, J. A. (1989) *Cell Regul.* **1**, 55–63.
9. Jung, G. & Hammer, J. A., III (1990) *J. Cell Biol.* **110**, 1955–1964.
10. Jung, G., Fukui, Y., Martin, B. & Hammer, J. A., III (1993) *J. Biol. Chem.* **268**, 14981–14990.
11. Cheney, R. E., Riley, M. A. & Mooseker, M. S. (1993) *Cell Motil. Cytoskel.* **24**, 215–223.
12. Goodson, H. V. & Spudich, J. A. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 659–663.
13. Knight, A. E. & Kendrick-Jones, J. (1993) *J. Mol. Biol.* **231**, 148–154.
14. Cheney, R. E. & Mooseker, M. S. (1992) *Curr. Opin. Cell Biol.* **4**, 27–35.
15. Mooseker, M. (1993) *Curr. Biol.* **3**, 245–248.
16. Peterson, M. D. & Mooseker, M. S. (1992) *J. Cell Sci.* **102**, 581–600.
17. Hull, R. N., Cherry, W. R. & Weaver, G. W. (1976) *In Vitro* **12**, 670–677.
18. English, D. & Anderson, B. R. (1974) *J. Immunol. Methods* **5**, 249–252.
19. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G. & Struhl, K. (1989) *Current Protocols in Molecular Biology* (Wiley, New York).
20. Innis, M. A., Gelfand, D. H., Sninisky, J. J. & White, T. J. (1990) *PCR Protocols: A Guide to Methods and Applications* (Academic, New York).
21. Espreafico, E. M., Cheney, R. E., Matteoli, M., Nascimento, A. A. C., De Camilli, P. V., Larson, R. E. & Mooseker, M. S. (1992) *J. Cell Biol.* **119**, 1541–1557.
22. Marchuk, D., Drumm, M., Saulino, A. & Collins, F. S. (1991) *Nucleic Acids Res.* **19**, 1154.
23. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.
24. Peterson, M. D., Bement, W. M. & Mooseker, M. S. (1993) *J. Cell Sci.* **105**, 461–472.
25. Carboni, J. M., Conzelman, K. A., Adams, R. A., Kaiser, D. A., Pollard, T. D. & Mooseker, M. S. (1988) *J. Cell Biol.* **107**, 1749–1757.
26. Hasson, T., Thompson, L. & Mooseker, M. S. (1993) *Mol. Biol. Cell* **4**, 39 (abstr.).
27. Pinto, M., Robine-Leon, S., Appay, M.-D., Kedinger, M., Triadou, N., Dussaulx, E., Lacroix, B., Simon-Assmann, P., Haffen, K., Fogh, J. & Zeibaum, A. (1983) *Biol. Cell.* **47**, 323–330.
28. Peterson, M. D. & Mooseker, M. S. (1993) *J. Cell Sci.* **105**, 445–460.
29. Hoshimaru, M. & Nakanishi, S. (1987) *J. Biol. Chem.* **262**, 14625–14632.
30. Sherr, E. H., Joyce, M. P. & Greene, L. A. (1993) *J. Cell Biol.* **120**, 1405–1416.
31. Jung, G., Saxe, C. L., III, Kimmel, A. R. & Hammer, J. A., III (1989) *Proc. Natl. Acad. Sci. USA* **86**, 6186–6190.
32. Simons, M., Wang, M., McBride, O. W., Kawamoto, S., Yamakawa, K., Adelstein, R. S. & Weir, L. (1991) *Circ. Res.* **69**, 530–539.
33. Mercer, J. A., Seperack, P. K., Strobel, M. C., Copeland, N. G. & Jenkins, N. A. (1991) *Nature (London)* **349**, 709–713.
34. Strom Morgan, N., Skovronsky, D., Artavanis-Tskonas, S. & Mooseker, M. S. (1994) *J. Mol. Biol.*, in press.
35. Kellerman, K. A. & Miller, K. G. (1992) *J. Cell Biol.* **119**, 823–834.
36. Takahashi, M., Kawamoto, S. & Adelstein, R. S. (1992) *J. Biol. Chem.* **267**, 17864–17871.
37. Ruppert, C., Kroschewski, R. & Bahler, M. (1993) *J. Cell Biol.* **120**, 1393–1403.
38. Heintzelman, M. B., Hasson, T. & Mooseker, M. S. (1993) *Mol. Biol. Cell* **4**, 42 (abstr.).
39. Nusrat, A., Delp, C. & Madara, J. L. (1992) *J. Clin. Invest.* **89**, 1501–1511.
40. Conrad, P. A., Giuliano, K. A., Fisher, G., Collins, K., Matsudaira, P. T. & Taylor, D. L. (1993) *J. Cell Biol.* **120**, 1381–1391.
41. Wagner, M. C., Barylko, B. & Albanesi, J. P. (1992) *J. Cell Biol.* **119**, 163–170.
42. Fukui, Y., Lynch, T. J., Brzeska, H. & Korn, E. D. (1989) *Nature (London)* **341**, 328–331.
43. Baines, I. C., Brzeska, H. & Korn, E. D. (1992) *J. Cell Biol.* **119**, 1193–1203.
44. Yoon, S.-J., Seiler, S. H., Kucherlapati, R. & Leinwand, L. A. (1993) *Proc. Natl. Acad. Sci. USA* **89**, 12078–12082.
45. Titus, M. A., Wessels, D., Spudich, J. A. & Soll, D. (1993) *Mol. Biol. Cell* **4**, 233–246.
46. Gillespie, P. G., Wagner, M. C. & Hudspeth, A. J. (1993) *Neuron* **11**, 581–594.
47. Titus, M., Kuspa, A. & Loomis, W. F. (1994) *Proc. Natl. Acad. Sci. USA* **91**, in press.