Myosin V Colocalizes with Melanosomes and Subcortical Actin Bundles Not Associated with Stress Fibers in Human Epidermal Melanocytes

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Mutations of the gene encoding myosin V can produce a dilute or silvery hair color and various neurologic defects in mice and patients with Griscelli syndrome, leading to speculations that the myosin V motor protein plays a critical role in transporting melanosomes within melanocytes and neurosecretory vesicles within neurons. Therefore, we investigated the *in vitro* expression of myosin V in cultured normal human melanocytes, keratinocytes, and dermal fibroblasts using reverse transcriptase-polymerase chain reaction and northern blot analysis. Subcellular distribution of myosin V and proximity to actin bundles and melanosomes were determined by double indirect immunofluorescence labeling and immunogold electron microscopy. In all studied cells myosin V is expressed and treatment of melanocytes with the cyclic AMP-inducer 3-isobutyl-1-methylxanthine causes an induction of the myosin V message. In all cells myosin V colocalizes with actin bundles, concentrating in the subcortical cell zone. In the melanocyte it is closely associated with melanosomes. Quantitative analysis of myosin V labeling in melanocytes reveals a significantly higher ($p < 0.005$) presence of myosin V in the periphery of dendrites. These results suggest that myosin V is important in melanosome transport in human melanocytes. Possible roles in the other skin cells remain to be elucidated. ***Key words: cytoskeleton/dendritogenesis/melanosome movement. J Invest Dermatol 111:835–840, 1998***

Very little is known about the molecular mechanisms underlying dendrite extension and melanosome transport in human melanocytes. It is expected that cytoskeletal proteins such as actin and microtubule components with their respective motor proteins play a role in these processes. Indeed, Lacour *et al* (1992) reported that actin microfilaments play a role in dendrite formation and microtubules play a role in dendrite maintenance in cultured human melanocytes.

Myosin V is an actin-activated ATPase, a type of motor protein representing the fifth of 13 classes of unconventional myosins (for reviews see Mooseker, 1993; Hammer, 1994; Mooseker and Cheney, 1995; Hasson and Mooseker, 1996, 1997b; Titus, 1997a; Baker and Titus, 1998). The first group of myosin V members is encoded by the yeast *myo2/myo4* genes (Govindan *et al*, 1995), the mouse *dilute* gene (Mercer *et al*, 1991), the chicken *p190* gene (Espreafico *et al*, 1992), and the human *myoxin* gene (Engle and Kennett, 1994; Moore *et al*, 1995). Recently, *myr 6*, a second group of the myosin V family, was cloned and characterized in rat (Zhao *et al*, 1996). Structurally, myosin V is a two-headed, nonfilamentous protein consisting of an N-terminal head domain containing actin-binding and ATP-hydrolysis sites, a neck domain with six IQ motifs that bind calmodulin/light chains and a tail domain consisting of a coiled-coil stalk and a carboxy-terminal globular domain that is thought to bind myosin to organelles (Cheney *et al*, 1993; Nascimento *et al*, 1996).

Yeast *myo2* gene mutations result in large, unbudding cells that pack vesicles in their cytoplasm. The originally described mouse *dilute* locus mutations termed *dilute-viral* (*d*^{v}) cause a dilution of coat color (Silvers, 1979). Other more severe *dilute* alleles (*dilute-lethal*, *dL*) also produce neurologic defects with seizures, opisthotonus, and death at early age (Mercer *et al*, 1991). The human autosomal recessive Griscelli–Pruniéras syndrome (Griscelli *et al*, 1978) is characterized by partial albinism with silvery-blond hair discoloration, primary immunodeficiency, and severe neurologic disorders, with death occurring in the first decades of life. Recently, mutations in the myosin V gene of two Griscelli patients were reported by Pastural *et al* (1997). Initially, the pigment disorder seen in humans and mice was thought to be due to underdeveloped melanocyte dendrites, but recent findings (Provance *et al*, 1996; Wei *et al*, 1997) have shown that *dilute* melanocytes do not lack dendrites but display defective melanosome transport into these dendrites. These phenotypic findings have led to the hypothesis that class V myosins play a role in organelle transport or membrane dynamics. Govindan *et al* (1995) suggest that yeast *myo2* plays a role in transporting secretory vesicles from the mother cell along actin cables into the bud. Myosin V is abundantly present in neurons (Mercer *et al*, 1991), where it may participate in organelle transport along actin filament bundles in rat neuroneal growth cones (Evans and Bridgman, 1995; Evans *et al*, 1997; Prekeris and Terrian, 1997). Wang *et al* (1996) suggest a role for myosin V in filopodial extension in growth cones of chick dorsal root ganglion neurons, whereas Evans *et al* (1997) observed no necessary role of myosin V in regulating filopodial dynamics in nerve growth cones, because neurite outgrowth in *dilute-lethal* mice is normal. As for

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Abbreviation: IBMX, 3-isobutyl-1-methylxanthine.

pigment cells, Provance *et al* (1996) showed that myosin V can be immunoprecipitated from isolated melanosomes in mice, a finding that is highly suggestive for actin-based locomotion of melanosomes, confirmed by light immunofluorescence microscopy and immunoelectron microscopy by Wu *et al* (1997).

In this study of the role of myosin V in human melanocytes, we examine the role of myosin V in dendrite formation and melanosome transport by looking at myosin V expression and subcellular localization. Moreover, we investigate its expression in the other cutaneous cells, keratinocytes, and dermal fibroblasts. We find myosin V to be expressed in all studied skin cells and, in melanocytes, to be associated with melanosomes.

MATERIALS AND METHODS

Cell culture Epidermal melanocyte primary cultures were obtained from neonatal foreskins and established in M199 medium (Gibco BRL, Merelbeke, Belgium) supplemented with 2% fetal bovine serum, 10^{-9} M choleratoxin, 10 ng basic fibroblast growth factor per ml, 10 μg insulin per ml, 1.4 μM hydrocortisone, and 10 μg transferrin per ml as described earlier (Naeyaert *et al*, 1991). Post-primary cultures were cultured in low calcium (0.03 mM) M199 medium supplemented with the same factors and 10% fetal bovine serum. For the experiments, third passage melanocytes were cultured for at least 4 d in a medium free of the cAMP-enhancer choleratoxin as described by Donatien *et al* (1993). Briefly, this melanocyte growth medium is composed of two-thirds MCDB153 and one-third Iscove's modified Dulbecco's medium supplemented with whole bovine pituitary extract (47 μg per ml), epidermal growth factor (3.3 ng per ml), insulin (3.3 μg per ml), hydrocortisone (0.94 μM), and 3% fetal bovine serum. For the induction experiments, 100 μM 3-isobutyl-1-methylxanthine (IBMX) was added to these cultures for 48 h. All chemicals and growth factors noted here are from Sigma (Bornem, Belgium), except whole bovine pituitary extract which is available at Gibco BRL (Gent, Belgium).

Cultures of human keratinocytes were initiated from adult skin biopsies and cultured in growth factor- and serum-supplemented Dulbecco's modified Eagle's medium/Ham's F12 to near confluency as previously described (Beele *et al*, 1991). Fibroblasts were established as explants from dermal portions of skin biopsies and cultured in Optimem I supplemented with 2% Ultroser G and 1% glutamine (Gibco BRL, Merelbeke, Belgium).

RNA preparation Total cellular RNA was harvested from subconfluent cell cultures in Tri-reagent (Sigma) and isolated according to the manufacturer's instructions. Poly(A)$^+$ RNA was obtained using PolyA Tract mRNA Isolation System III (Promega, Leiden, The Netherlands). Purity of the obtained total RNA and mRNA samples was checked by measuring the absorbance ratio at 260/280 nm, which was >1.7 in all samples. Total RNA was DNAse treated (DNAse I, Promega, Leiden, The Netherlands) prior to use in reverse transcriptase-polymerase chain reaction (RT-PCR) to eliminate amplification of genomic DNA.

RT-PCR Approximately 1 μg of total RNA was reverse transcribed with random primers and amplified using the GeneAmp RNA-PCR kit (Perkin Elmer, Brussels, Belgium). Two oligonucleotides (P1, 5′-TGCACCTGCTTA-TGGAGAC-3′; P2, 5′-TTCGCTTCCTCTTCACTTAGT-3′) were chosen defining a 1006 bp cDNA in the neck region of the human myosin V cDNA (Engle and Kennett, 1994; Genbank accession numbers L19401 or Y07759) using Oligo 5.0 software. Considering the high probability of sequence overlap with other myosin sequences (Hasson *et al*, 1996), the oligonucleotides were carefully checked for false priming sites in the following published myosin coding sequences (Genbank): human myosin IB and IE (X98411 and X98507), human myosin IC gene (U14391), human myosin VI (U90236), human myosin VIIa (U39226), and human myosin IXb (U42391). False priming sites in human frequent sequences were excluded as well. PCR amplification conditions were 95°C for 1 min, 60.5°C for 1 min, and 72°C for 1.5 min during 35 cycles after an initial denaturation at 95°C for 2 min and terminating the reaction with 7 min at 72°C. cDNA bands were visualized on a 1.5% TAE-agarose ethidium bromide stained gel. The resulting 1006 bp cDNA was eluted from the gel (QiaEx gel extraction kit, Westburg, The Netherlands) and after purification used as a hybridization probe in northern blot.

Northern blot Northern blot was performed as previously described (Wintzen *et al*, 1996). Briefly, 2 μg of poly(A)$^+$ RNA was electrophoresed on a 1% agarose gel containing 2.2 M formaldehyde. RNA was transferred overnight onto a nylon membrane (Hybond-N+, Amersham, Gent, Belgium) and immobilized by UV irradiation in a UV-Stratalinker (Stratagene, La Jolla, CA). Prehybridization of the blot was in a solution containing 50% deionized formamide, 5×Denhardt's solution, 0.02% sodium dodecyl sulfate, and 0.2 mg salmon sperm DNA per ml at 45°C during 2 h. The 1006 bp myosin V cDNA probe was labeled with [^{32}P]-dCTP using Oligolabeling kit (Pharmacia, Roosendaal, The Netherlands) and hybridized to the blot overnight at 45°C in a solution containing 50% deionized formamide, 2.5 × Denhardt's solution, 0.02% sodium dodecyl sulfate, 5 × SSPE, and 0.02 mg salmon sperm DNA per ml together with a radiolabeled G3PDH control probe. Washes were performed at room temperature once for 5 min and three times for 20 min in 0.3 M NaCl, 0.03 M sodium citrate, 0.1% sodium dodecyl sulfate, and subsequently twice for 20 min at 45°C and twice at 55°C in 15 mM NaCl, 1.5 mM sodium citrate, 0.1% sodium dodecyl sulfate. Blots were exposed to a phosphor screen (Molecular Dynamics, Krefeld, Germany) during 48 h and the digitized images were evaluated densitometrically using Phosphor Imager Imagequant software.

Indirect immunofluorescence and confocal microscopy Cells were grown on coverslips and fixed for 20 min at room temperature with 3% paraformaldehyde in phosphate-buffered saline (PBS). For permeabilization, cells were treated for 5 min with 0.2% Triton X-100 in PBS. Double staining for myosin V proteins and actin was performed by incubating for 1 h at room temperature with a rabbit polyclonal anti-myosin V antibody DIL-2 (1:400) (Wu *et al*, 1997). After three washes in TBS, the slides were incubated in a biotinylated donkey-anti-rabbit antibody (Amersham) 1:50 dilution for 1 h at room temperature, again washed, and finally incubated in a mixture of fluorescein isothiocyanate-conjugated phalloidin (Sigma) and streptavidin-texas red for 30 min at room temperature. For double staining of myosin V and melanosomes, slides were first incubated for 1 h at room temperature in a 1:40 mouse monoclonal NKI-beteb (Monosan, Uden, The Netherlands) and a 1:400 DIL-2 dilution, followed by a second incubation step of 1 h at room temperature with an fluorescein isothiocyanate-linked swine-anti-rabbit antibody (1:20) and a biotinylated sheep anti-mouse antibody (1:50) (both of Prosan, Gent, Belgium) and a final third incubation step with streptavidin-texas red. Slides were coverslipped in Prosan fluorescence mounting fluid and confocal images (1 μm sections) were obtained with a Zeiss laser confocal microscope. For negative controls incubation with an irrelevant isotype-matched antibody was performed. The (polyclonal) antibody DIL-2 used in our immunocytochemical experiments was extensively tested on its specific interaction with a myosin V isoform (raised against myosin V heavy chain residues 910–1106) encoded by the *dilute* locus by its generators (Wu *et al*, 1997).

Immunoelectron microscopy Confluent cell cultures were fixed for 1 h at room temperature in 0.1% glutaraldehyde in 0.14 M cacodylate buffer, pH 7.3, and processed for immunogold labeling as described elsewhere (Mommaas *et al*, 1992). Briefly, cells were pelleted and embedded in 10% gelatin, cut into 1 mm^3 cubes, cryoprotected in 2.3 M sucrose for 25 min and snapfrozen in liquid nitrogen. Ultrathin cryosections were prepared and incubated with the polyclonal rabbit anti-myosin V antibody DIL-2 (cfr. supra) diluted 1:250, followed by 10 nm protein A-gold. For double labeling with actin bundles a mouse anti-human actin bundles monoclonal antibody (dilution 1:20) from Chemicon (Biognost, Wevelgem, Belgium) was used and visualized with 5 nm protein A-gold after an intermediate incubation step with a secondary rabbit anti-mouse immunoglobulin (Dako, The Netherlands). For negative controls the primary antibody step was omitted and incubation with an irrelevant isotype-matched antibody was performed. After immunolabeling, sections were embedded and contrasted in methylcellulose/uranyl acetate and viewed with a Philips EM 410 electron microscope.

Image analysis For quantitation of myosin V immunolabeling, immunoelectron microscopy photographs of whole melanocytes (n = 6) were made at a final magnification of 55,000×. Four different cell regions were considered (**Fig 1**): periphery of the dendrite, center of the dendrite, perinuclear area, and periphery of the perinuclear area. Periphery of the dendrite and of the perinuclear area was recognized as a more darkly stained peripheral area of cytoplasm that represents the 150 nm thick actin subcortical meshwork. The perinuclear area was defined as the vesicle-rich area surrounding the nucleus. These cell zones were scored quantitatively by automatical analysis of the number of myosin V colloidal gold particles per μm^2 using Kontron KS400 image processing software. Nuclear labeling was taken as background. Results obtained for various experimental groups were compared using the nonparametric Friedman test. p values <0.05 were considered to be statistically significant.

RESULTS

Myosin V is expressed in skin cells *in vitro* The three examined skin cells show the appropriately sized myosin V 1006 bp cDNA band after RT-PCR (**Fig 2**). Northern blot analysis (**Fig 3*a***) reveals the complex transcription pattern of myosin V in all cells with 7, 8, and 12 kb messages as described in the literature (Mercer *et al*, 1991).

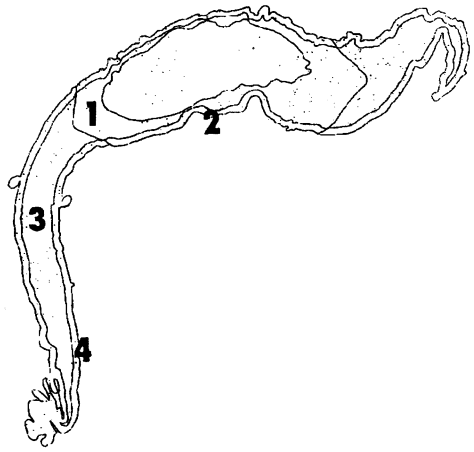

Figure 1. Schematic presentation of the subdivision of the melanocyte into four different cytoplasmic regions. 1, perinuclear area; 2, peripheral perinuclear area; 3, center of the dendrite; 4, periphery of the dendrite.

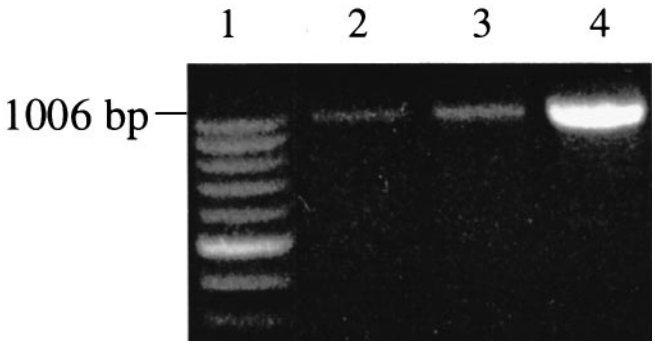

Figure 2. Myosin V is expressed in normal human skin cells. RNA was extracted from cultured human melanocytes (*lane 2*), keratinocytes (*lane 3*), and dermal fibroblasts (*lane 4*) of a normal individual. RNA was reverse transcribed and amplified by RT-PCR using myosin V-specific primers, resulting in an appropiately sized myosin V 1006 bp band. *Lane 1* represents a 100 bp DNA-ladder (Gibco BRL, Gent, Belgium) with the more intense band being 500 bp DNA.

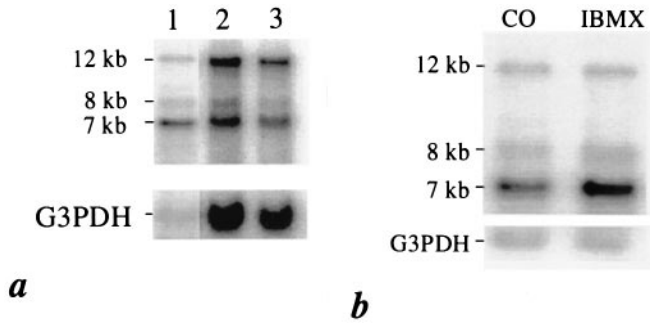

Figure 3. Northern blot analysis of different skin cell types reveals different myosin V transcripts and induction of myosin V in melanocytes under the influence of IBMX. (*a*) Melanocytes (*lane 1*), keratinocytes (*lane 2*), and dermal fibroblasts (*lane 3*) have the 7, 8, and 12 kb messages probed with a [^{32}P]-labeled fragment of human myosin V cDNA PCR product. (*b*) Two micrograms mRNA was isolated out of control melanocytes *versus* IBMX-treated melanocytes and analyzed by northern blot analysis hybridized for myosin V and G3PDH. On this representative blot, quantitation of density of the myosin V bands using a phosphorimager revealed a 5-fold induction of myosin V after correction for loading differences relative to the G3PDH mRNA intensity.

Myosin V message is induced in melanocytes after stimulation of differentiation with IBMX Melanocytes grown in melanocyte growth medium display a bipolar to tripolar slender cell body. IBMX (100 μM final concentration) changed the morphology of the bipolar to tripolar melanocytes towards cells with a higher number of dendrites (four or more) per cell body within 48 h after addition (not shown). Dendrites were also generally longer and sometimes highly branched in the treated melanocytes. Quantitative analysis of the northern blot (**Fig 3*b***) of control bipolar melanocytes *versus* highly dendritic melanocytes showed that the myosin V mRNA level increases up to 5-fold in melanocytes treated with IBMX for 48 h above control melanocytes.

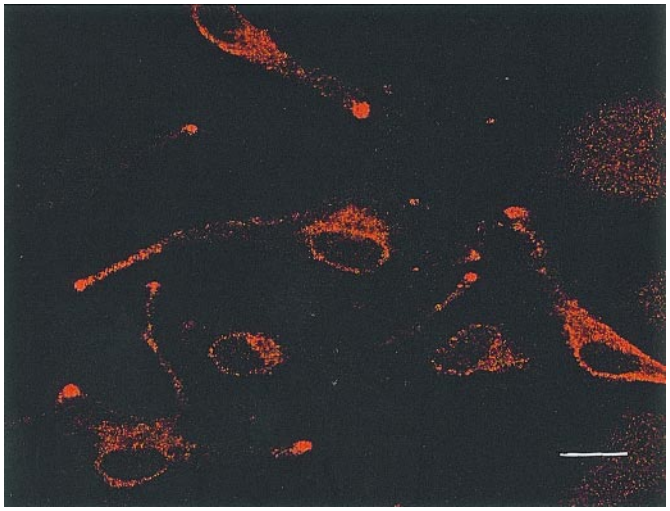

Figure 4. Indirect immunofluorescence localization of antigen recognized by the myosin V antibody in melanocytes. Human melanocytes were cultured on coverslips for 24 h, fixed, and stained with a polyclonal anti-myosin V antibody. *Scale bar*: 10 μm.

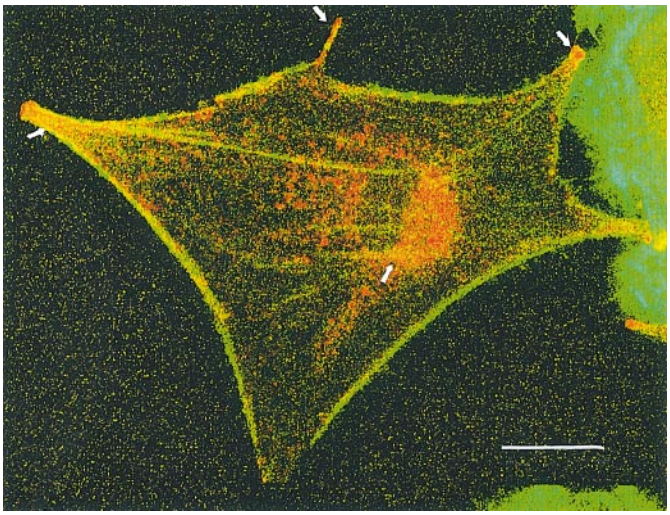

Figure 5. Double immunofluorescent staining for myosin V and actin. Human melanocytes were cultured on coverslips for 24 h, fixed, and double stained with a polyclonal anti-myosin V antibody (texas red signal) and phalloidin (fluorescein signal). In this confocal photomicrograph, yellow staining (*arrows*) indicates the presence of myosin V in the actin-rich subcortical meshwork and near actin bundles in the perinuclear area. *Scale bar*: 10 μm.

Myosin V is present throughout the cytoplasm In all cells studied, myosin V immunofluorescent images reveal a punctate-staining pattern throughout the cytoplasm with clear intensification in the perinuclear area and, in melanocytes (**Fig 4**), throughout the dendrites. Double labeling of myosin V with actin (**Fig 5**) shows that in the melanocyte myosin V colocalizes with subcortical actin. In the central cytoplasm no well-structured colocalization of myosin V with actin bundles (e.g., with the focal contacts and stress fibers) can be observed. Moreover, myosin V staining is accentuated at the tip of each melanocytic dendrite (**Figs 4**, **5**).

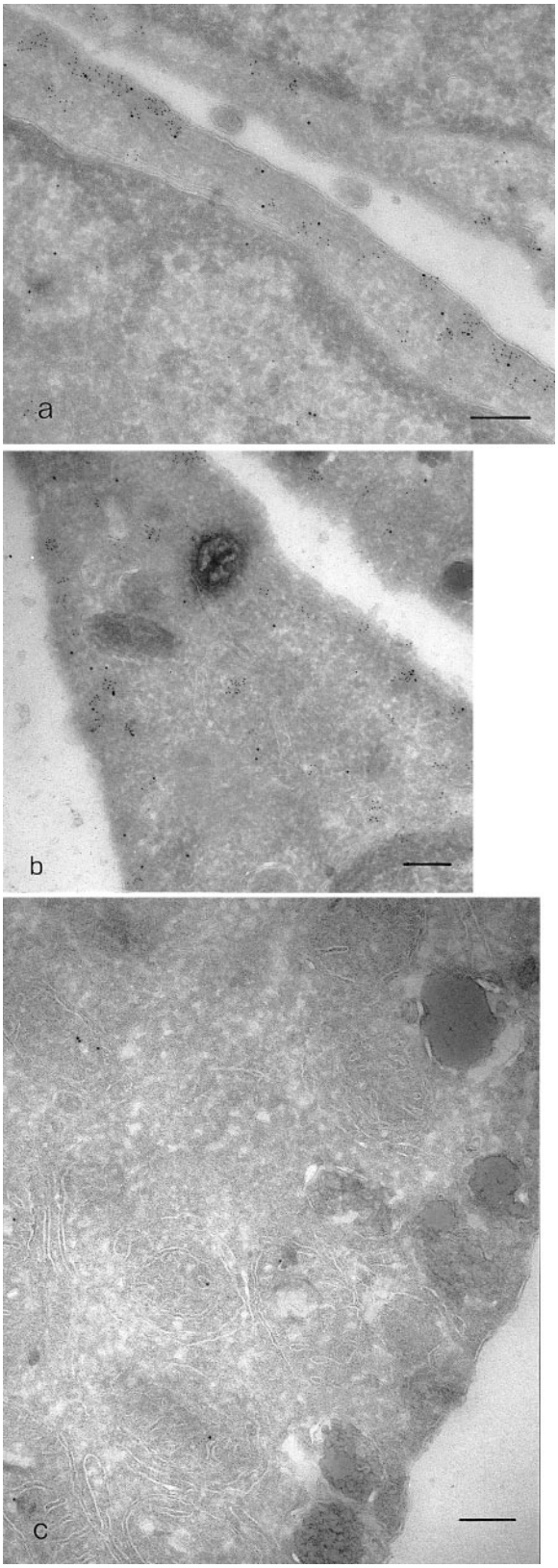

Figure 6. Melanocytes show immunocolocalization of myosin V with actin, particularly in the dense subcortical network. Glutaraldehyde-fixed ultrathin cryosections of cultured human melanocytes were subjected to an immunogold-labeling procedure with anti-myosin V (10 nm gold particles) and anti-human actin bundles (5 nm gold particles) antibodies. Colocalization is most noticeable underneath the cell membrane in the peripheral perinuclear area (*a*) and in the peripheral dendrite (*b*). Negative control results are shown in (*c*). *Scale bar*: 0.2 μm.

Table I. Myosin V is present in a significantly higher number in the subcortical region of the melanocyte

	cpg per μm^2[a]			
Melanocyte	Perinuclear area	Peripheral perinuclear area	Center of the dendrite	Periphery of the dendrite
Melanocyte 1	1.6	11.5	6.8	8.8
Melanocyte 2	4.6	12.3	1.1	12.1
Melanocyte 3	13.4	19.6	11.0	13.9
Melanocyte 4	14.3	20.7	23.3	29.0
Melanocyte 5	6.0	13.0	11.2	12.9
Melanocyte 6	7.0	11.5	6.2	9.0
Mean ± SEM[b]	7.8 ± 2.0	14.8 ± 1.7[c]	9.9 ± 3.0	14.3 ± 3.1[c]
Median	6.5	12.7	8.9	12.5

[a]The background corrected number of myosin V colloidal gold particles per μm^2 (cpg per μm^2) in four different melanocytic cytoplasmic regions.
[b]$n = 6$.
[c]$p < 0.005$.

Myosin V colocalizes with the actin cytoskeleton For better resolution the subcellular localization of myosin V was also determined with immunoelectron microscopy. In all cultured skin cells, actin immunoreactivity (5 nm gold particles) is prominent in the subcortical region where actin is also morphologically recognizable as thin filamentous bundles (**Fig 6*a***). In the melanocytic dendrite actin bundles are mainly localized in the periphery. Actin is less organized and less present in the rest of the cytoplasm, e.g., in the organelle-rich perinuclear area. In all these actin-rich areas there is a striking overlap with myosin V staining (10 nm gold particles) and in the melanocytes, colocalization of myosin V and actin is mainly seen in the periphery of the dendrites (**Fig 6*b***).

In control sections, only a few scattered gold particles were seen (**Fig 6*c***).

Quantitative analysis of myosin V labeling in the melanocyte revealed a significantly higher ($p < 0.005$; see **Table I**) presence of myosin V in the subcortical cell zone, i.e., in the periphery of the dendrite and the peripheral perinuclear zone as compared with the center of the dendrite and the perinuclear area.

In melanocytes, myosin V forms a link between melanosomes and the actin cytoskeleton In addition to the colocalization with actin, in all cells myosin V is occasionally seen in close association with cytoplasmic membranous organelles that are not mitochondria. In the melanocyte, double staining results using myosin V antibody and NKI-beteb, a (pre)melanosomal marker, indicate colocalization (yellow color) of myosin V with melanosomes in the perinuclear area and in the dendrites (**Fig 7**). At the electron microscopic level, late stage melanosomes are easily recognized as membrane-lined melanin-containing vesicles. Several melanosomes located in the dendrites of melanocytes colocalize with an actin bundle and myosin V, with myosin V seen as an intermediate molecule between the melanosome and an actin bundle (**Fig 8*a***). Melanosomes also label with myosin V without evidence for actin bundles in their neighborhood. Myosin V specific gold particles are also seen on nonmelanized organelles in the perinuclear area (**Fig 8*b***). It remains to be investigated whether these are early melanosomes (stage I melanosomes), Golgi-derived melanogenesis-related coated vesicles, or other cytoplasmic organelles, e.g., lysosomes. In the other skin cells (data not shown), myosin V can also be seen in association with membrane-lined organelles.

DISCUSSION

Myosins are expressed in many different cell types and several different myosin classes can occur in one cell type at the same time (Hasson and Mooseker, 1996). The latter fact represents a problem when performing hybridization assays for myosin V as unconventional myosins display a high degree of sequence overlap, especially at the 5′-end or head region (Mooseker, 1993). Excluding significant sequence similarity to any other known human myosin sequences in the GenBank

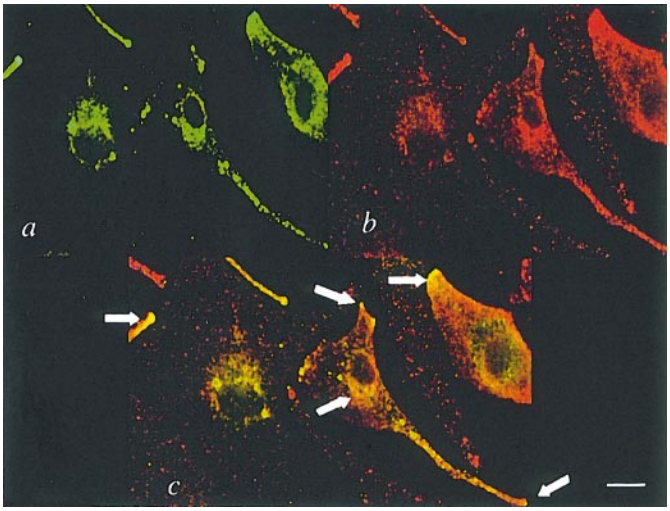

Figure 7. Melanocytes show immunocolocalization of myosin V with melanosomes. Fluorescence confocal photomicrographs represent single labeling of (*a*) DIL-2 (myosin V antibody + tertiary fluorescein isothiocyanate-conjugated), (*b*) NKI-beteb (anti-melanosome antibody + secondary Texas red-conjugated), and (*c*) double labeling with both antibodies in a melanocyte. Colocalization (yellow color; *arrows*) of melanosomes and myosin V occurs mainly in the tips of dendrites and in the perinuclear area. *Scale bar*: 10 μm.

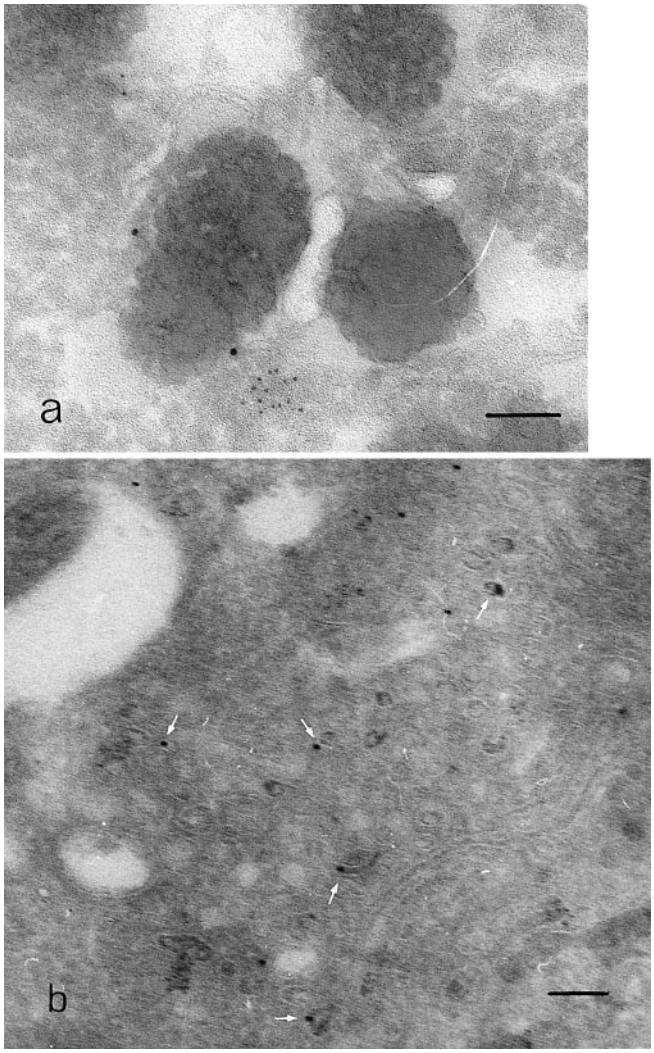

Figure 8. In melanocytes myosin V often colocalizes with actin and melanosomes, but is also seen on nonmelanized organelles. Mature melanosomes are easily recognized as the electron-dense oval structures. Myosin V (10 nm gold particles) appears in association with an actin bundle (5 nm gold particles) close to a melanosome (*a*). Myosin V is also seen on the membrane of microvesicles (*arrowheads*) in the trans-Golgi network (*b*), possibly carrying the post-Golgi melanogenic enzymes. *Scale bar*: 0.1 μm.

databases, we were able to provide the first evidence using RT-PCR and northern blot analysis that myosin V is also expressed in human epidermal melanocytes, keratinocytes, and dermal fibroblasts.

Northern blot expression and induction experiments were performed in a basal medium (melanocyte growth medium) devoid of any cAMP-inducers, as Nascimento *et al* (1997) and Wu *et al* (1997) recently reported using western blot analysis that the cAMP signaling pathway might regulate myosin V gene expression. Nascimento *et al* (1997) detected a significant 4-fold stimulation of myosin V protein expression after addition of IBMX/α-melanocyte stimulating hormone. Wu *et al* (1997) saw at least two to three times as much myosin V in B16-F10 cells after stimulation with α-melanocyte stimulating hormone. Upon stimulation of melanocytes with IBMX, an inhibitor of cAMP phosphodiesterase, we repeatedly found an up to 5-fold increase of the relative amount of myosin V message as compared with controls after 48 h; however, we saw no effect of α-melanocyte stimulating hormone on dendricity nor synergistic effect on the dendricity evoked by IBMX (result not shown), similar to the results of Hedley *et al* (1998). Our findings expand the hypothesis that myosin V expression is regulated by the cAMP signaling pathway to the transcriptional level, at least at this time point after stimulation.

Close association of myosin V with certain membranous organelles in the organelle-rich perinuclear area and with melanosomes in particular, suggests that myosin V might function more generally as an organelle transporter. Its presence on fully melanized melanosomes, early melanosomes, and lysosome-like organelles is in accordance with findings in mice (early and late melanosomes) and with the fact that lysosomes and melanosomes have a common origin (Orlow *et al*, 1993). Myosin V could interact with both organelles via the same receptor/ligand structure. Its prominent presence in the tips of the melanocytic dendrites might be an illustration of an active role in melanosome transfer to surrounding keratinocytes, or it might just be a reflection of its colocalization with actin bundles that show a highly dynamic behavior at this leading edge. Simple concentration of organelles at the narrow dendrite tips may also contribute.

Some authors (Provance *et al*, 1996; Wei *et al*, 1997) believe that the primary event in *dilute* melanocytes is not the defective extension of dendritic processes (because dendrites are present, though stubby and underdeveloped), but is mainly a problem of melanosome movement. The fact that dendrites are not fully extending could then be secondary to the lack of stimulus from arriving melanosomes. On the other hand, Wang *et al* (1996) do suggest a specific role for myosin V in filopodial extension of chicken neuroneal growth cones, indicating a possible role of myosin V in the extension of actin-rich processes, e.g., by arranging the cytoskeletal actin. The presence of myosin V in the subcortical actin meshwork in dendrites, in addition to the observation that stimulation of melanocytes with IBMX is accompanied by increased dendricity and that myosin V is at the same time upregulated, suggest that myosin V may play a role in the reorganization of actin bundles in the subcortical area. The colocalization of myosin V with actin in the subcortical area, however, may just indicate trafficking of myosin V along actin filaments, with or without an attached organelle. Also, as the resulting differentiation of the melanocyte includes increased melanogenesis (Naeyaert *et al*, 1991; Hedley *et al*, 1998), myosin V transcription and translation may be merely induced because of the greater need for melanosome transport.

Next to the actin-based organelle movement system, the melanosome translocation along microtubuli to specific destinations in the melanocyte also needs to be considered. Actin is located in the thick subcortical meshwork in the peripheral dendrite, whereas microtubuli are present in more central areas of the melanocyte like the organelle-rich

perinuclear domain and the central dendrite area (Lacour *et al*, 1992; Cole and Lippincott-Schwartz, 1995). One can indeed imagine a complementary role for the actin-based and the microtubuli-based cytoskeleton, with each system taking melanosome transport in a particular region of the melanocyte into account. We observed that melanosomes also label with myosin V in central cytoplasmic areas where actin is less abundant and less organized. It could be that myosin V, in these regions where microtubuli are described to be clearly organized (Lacour *et al*, 1992), associates passively with melanosomes that are at that point being transferred along microtubuli by their respective motors. The two best-known microtubuli-associated motor proteins kinesin and dynein have been shown to intervene in the transport of membranous organelles in neurons (Pfister *et al*, 1989; Hirokawa *et al*, 1990, 1991; Ferreira *et al*, 1992) and of pigment granules in fish melanophores (Rodionov *et al*, 1991). Evidence for an overlap in function is given by the fact that the yeast mutant *myo*2 phenotype can be suppressed by overexpression of a member of the kinesin superfamily (Lillie and Brown, 1992). Furthermore, some authors suggest that the gene product of the *dilute suppressor* gene in mice that is capable of suppressing the phenotype of the *dilute* mutation is possibly a kinesin (Moore *et al*, 1988).

In melanocytes of Griscelli patients and *dilute* mice another protein completely taking over the myosin V function in melanosome transport is obviously lacking; however, there is as yet no explanation for the fact that the albinism is only partial, with normally pigmented skin occurring next to hypopigmented patches and silvery-gray hair. These findings suggest that the presumed important function of myosin V in organelle transport might be taken over by another myosin or microtubuli-associated motor protein with a redundant function, which makes the search for the expression of other myosins and/or members of the kinesin/dynein family most interesting. On the other hand the role of myosin V might vary in importance between these different cell types with a lower treshold to produce abnormal phenotypic findings in the follicular melanocytes.

In conclusion, our findings support the hypothesis that myosin V has an important function in melanosome transport. The mechanism of myosin V activation to bind melanosomes and to direct their movement remains to be elucidated. The eventual role of myosin V in determining melanocyte dendricity and its possible role in the other skin cells needs further examination.

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