Kinesin Participates in Melanosomal Movement along Melanocyte Dendrites

Masahiro Hara,[1] Mina Yaar, H. Randolph Byers, David Goukassian, Richard E. Fine,* Jessica Gonsalves, and Barbara A. Gilchrest

Departments of Dermatology and *Biochemistry, Boston University School of Medicine, Boston, Massachusetts, U.S.A.; Bedford VA Hospital, Bedford, Massachusetts, U.S.A.

Movement of melanosomes along melanocyte dendrites is necessary for the transfer of melanin pigment from melanocytes to basal and suprabasal keratinocytes, an event critical to epidermal photoprotection and maintenance of normal skin color. Recent murine data suggest that in melanocyte dendrites the microtubule-associated melanosome movement is bidirectional and that actin-associated myosin V secures the peripheral melanosomes, preparing them to be transferred to surrounding keratinocytes. We now report that human melanocytes express high levels of kinesin, a molecule that participates in microtubule-associated transport of organelles in other cell types, and that ultrastructurally kinesin molecules are closely associated with melanosomes. To determine whether kinesin participates in melanosomal transport, cultured melanocytes were treated with sense or antisense oligonucleotides complementary to kinesin heavy chain sequences. Antisense oligonucleotides decreased kinesin protein levels and inhibited the bidirectional movement of the melanosomes, promoting their backward movement. Furthermore, guinea pigs were exposed to ultraviolet B irradiation, known to enhance transport of melanosomes from melanocytes to epidermal keratinocytes, and then were treated with kinesin sense or antisense oligonucleotides. The areas that were treated with kinesin antisense oligonucleotides showed significantly less pigmentation clinically and histologically than control (sense) oligonucleotide-treated areas. As observed ultrastructurally, in antisense-treated areas melanosomes remained in melanocyte dendrites but over several days were not transferred to the surrounding keratinocytes. Our study supports a major role for kinesin in microtubule-associated anterograde melanosomal transport in *human* melanocyte dendrites. ***Key words: motor proteins/organelle transport/time-lapse microscopy. J Invest Dermatol 114:438–443, 2000***

Melanocytes, neural crest derived cells, comprise 1%-2% of the epidermal cell population (Jimbow *et al*, 1999) and are solely responsible for the production and transfer of melanin, the major determinant of skin color and protection against ultraviolet (UV) irradiation (Gilchrest *et al*, 1998; Jimbow *et al*, 1999). Melanin is synthesized and packaged in cytoplasmic organelles called melanosomes, which are transferred from their site of origin in the perikaryon to the tips of melanocyte dendrites and eventually into surrounding keratinocytes (Jimbow *et al*, 1999).

Extensive evidence suggests that cellular organelle transport is controlled by two classes of microtubule-associated motor proteins: kinesins and cytoplasmic dyneins. Both motor proteins act as short cross-bridge structures connecting the organelle to the microtubules (reviewed in Hirokawa, 1998). Both are composed of two heavy chains and several light chains. Each heavy chain has a globular adenosine-5′-triphosphate binding domain that also binds the microtubules, and rod-like domains that bind cellular organelles (Vale *et al*, 1985a, b; Bloom *et al*, 1988; Vallee *et al*, 1988; Rodionov *et al*, 1990; Yang *et al*, 1990; Skoufias and Scholey, 1993; Bloom and Endow, 1995; Kull *et al*, 1996). Studies of nerve axons have determined that centrifugal, anterograde organelle movement is mediated primarily by kinesin, whereas their centripetal movement is controlled by cytoplasmic dynein (Hirokawa *et al*, 1991; Bloom, 1992; Endow and Titus, 1992; Gelfand and Scholey, 1992; Coy and Howard, 1994; Okada *et al*, 1995).

Studies examining melanosomal transport in mice suggest that their microtubule-dependent movement is bidirectional (Wu *et al*, 1998), consistent with a cooperative forward and backward pull of kinesin and cytoplasmic dynein, respectively. For melanosomes with net centrifugal movement, the bidirectional movement appears to terminate with myosin-Va-dependent melanosomal capture in the actin-rich periphery of the dendrites (Wu *et al*, 1998). Consistent with this background, we now report that human melanocytes express kinesin protein, that kinesin colocalizes with melanosomes, and that addition of kinesin antisense oligonucleotides to cultured melanocytes promotes their centripetal movement within dendrites. Furthermore, application of kinesin antisense oligonucleotides to UV-irradiated guinea pig skin

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Reprint requests to: Dr. Barbara A. Gilchrest, Boston University School of Medicine, Department of Dermatology, 609 Albany Street, J-Bldg, Boston, MA 02118-2394.

Abbreviation: TPBS, 0.05% Tween-20 in phosphate-buffered saline.

[1]Current address: Tohoku University School of Medicine, Department of Dermatology, Seiryo-machi 1–1, Aobaku, Sendai, 980, Japan.

decreases epidermal tanning clinically and decreases the number of melanosomes present in keratinocytes, a result consistent with decreased transfer of existing melanosomes from melanocyte dendrites to basal keratinocytes as assessed histologically. Our study demonstrates a major role for kinesin in melanosomal transport and in cutaneous tanning following UV irradiation.

MATERIALS AND METHODS

Melanocyte culture and melanin Neonatal foreskins obtained within 24 h of elective circumcision were used to culture human melanocytes as previously described (Gilchrest *et al*, 1984; Park *et al*, 1993). In brief, the epidermis was separated from the dermis after overnight incubation in 0.25% trypsin at 4°C. Primary cultures were then established in Medium 199 (Gibco BRL, Grand Island, NY) supplemented with 10 ng epidermal growth factor per ml (Bethesda Research Laboratories, Gaithersburg, MD), 10^{-9} M triiodothyronine (Sigma, St Louis, MO), 10 μg transferrin per ml (Sigma), 10 μg insulin per ml (Sigma), 1.4×10^{-6} M hydrocortisone (Calbiochem Bering, La Jolla, CA), 10^{-9} M cholera toxin (List Biological, Campbell, CA), 10 ng basic fibroblast growth factor per ml (Amgen, Thousand Oaks, CA), and 5%–10% fetal bovine serum. All post-primary cultures were maintained in a low calcium (0.03 mM) version of this defined melanocyte growth medium known to selectively support melanocyte growth (Naeyaert *et al*, 1991). For all experiments, subconfluent melanocytes at passage 3–6 were used. In experiments investigating the effect of sense/antisense oligonucleotides on melanosomal transport, cells were stimulated with 12-tetra-phorbol-decanoate 13-acetate (50 ng per ml) for 24 h, a treatment that induces melanocyte dendricity but has no effect on melanocyte growth rate (Gilchrest and Friedmann, 1987). Then, oligonucleotides (40 μM) were added 24 h prior to time-lapse microscopy (Ferreira *et al*, 1992).

Time-lapse microscopy Computer-assisted time-lapse recording was performed as previously described (Byers *et al*, 1991) with minor modifications. Briefly, melanosomal transport was observed with the 10× or 40× objective under phase or bright field illumination, respectively, with a Nikon Diaphot inverted microscope. The microscope stage was enclosed in a hermetically sealed Plexiglas housing and the stage temperature was maintained constant at 37°C with a thermocouple attached to a Nikon Np-2 incubator. A gas flow mixer (Shel-lab Cornelius, Oregon) that maintained a 5% CO_2/95% air atmosphere within the housing was used to maintain the medium at a constant pH. The side port of the microscope was attached to a Cohu high performance CCD camera (San Diego, CA) connected to a Power Macintosh 7100/66 with an LG-3 scientific frame grabber card (Scion, Frederick, MD). Image analysis and time lapse were performed with the IPLab Spectrum software version 3.0 (Scanalytics, Fairfax, VA). Melanosomal movement within dendrites was observed during a 1–5 min period. Mean speeds of melanosomal centrifugal and centripetal movements were calculated using digital image morphometric analysis following calibration of pixels using an Olympus micrometer slide etched at 10 μm intervals.

Western blot analysis Total cellular proteins were harvested as described by Coy and Howard (1994), and 60 μg per lane were separated on 10% sodium dodecyl sulfate/polyacrylamide gel electrophoresis and transferred to nitrocellulose membrane at 100 V for 1 h. The membrane was blocked overnight with 5% milk/1% bovine serum albumin (BSA) in 0.05% Tween-20 in phosphate-buffered saline (TPBS). Incubations with primary mouse anti-kinesin IgG (SUK4) (1:1000 dilution) were performed in 1% milk/0.2% BSA in TPBS for 2 h. Blots were then washed once for 15 min and three times for 5 min in TPBS followed by a 1 h incubation with horseradish-peroxidase-conjugated rabbit antimouse IgG (Amersham, Arlington Heights, IL) at 1:1000 dilution in 1% milk/0.2% BSA in TPBS. After extensive washing (once for 15 min and four times for 5 min in TPBS), bands were detected on Kodak XAR-5 film using the ECL western blotting detection kit (Amersham). After transfer the gels were stained with 0.1% Coomassie Blue R-250 in 40% ethanol/10% acetic acid to verify equal loading of the lanes.

Histologic analysis Four mm punch biopsies taken from guinea pigs were fixed in 10% neutral-buffered formalin for 24 h, dehydrated, and embedded according to standard histologic protocols. Vertical cross-sections (3–5 μm) were stained with Fontana-Masson silver nitrate to detect melanin. The amount of interfollicular epidermis occupied by melanin was quantitated by computer image analysis as previously described (Bhawan *et al*, 1991). The entire length of each section, excluding hair follicle ostia, was analyzed to determine mean percentage epidermal area per section occupied by melanin. Three to four interfollicular areas were analyzed in each section. Ten to 15 sections were analyzed per biopsy.

Immunocytochemistry for electron microscopy Cells were plated and fixed as above. The fixed cells were then briefly washed with Tris(hydroxymethyl)-aminomethane-buffered saline (TBS), pH 7.4, treated with TBS/0.2% Triton X-100 for 30 min, a treatment sufficient to permeabilize both plasma and melanosomal membranes, and incubated with 2% normal goat serum for 30 min to block nonspecific binding. Then specimens were incubated with SUK4 antibody (1:200 dilution) in TBS/1% BSA for 3 h. The cells were washed twice for 10 min in TBS, pH 7.2, and then three times for 10 min in TBS, pH 8.2. The secondary antibody (goat antimouse IgG) coupled to 5 nm gold particles (Sigma) was diluted 1:10 in TBS/1% BSA and the reaction was continued for a total of 12 h at room temperature and/or at 4°C. After washing five times for 10 min in TBS, pH 8.2, the cells were fixed in 1% glutaraldehyde/0.2% tannic acid in 0.1 M phosphate-buffered saline, pH 7.2, for 30 min, washed in the same buffer, fixed in 0.5% osmium tetroxide at 4°C for 10 min, and washed extensively with distilled water. In control experiments the step using the specific antikinesin antibody was skipped and cells were incubated only with secondary antibody.

Samples were stained with 1% uranyl acetate for 30 min, dehydrated, and embedded in Epon 812. Epon-embedded sections 0.1 μm thick were analyzed by transmission electron microscopy (300, Phillips, Holland) (Simmons *et al*, 1990).

Preparation of oligonucleotides Oligonucleotides were synthesized as described previously (Yamazaki *et al*, 1995) and stabilized by sulfur-modified phosphate linkages. The antisense oligonucleotide, 5′-GCCGGTCCGCCATCTTTCTGGCAG-3′, is the inverse complement of nucleotides −11 to +14 in the rat kinesin heavy chain sequence (Yamazaki *et al*, 1995). The sense oligonucleotide is the exact inverse complement of the antisense oligonucleotide.

Application of oligonucleotides to guinea pig skin Three American short-hair pigmented guinea pigs (Allan *et al*, 1995) were obtained from Kuiper Rabbit Ranch, Chicago. The animals were prepared for irradiation (see below) by shaving and the remaining stubble was removed with a depilatory (Nair). At the end of the irradiation period, when visual inspection revealed profound even darkening of the irradiated areas, 200 μM of sense and antisense kinesin oligonucleotide preparations were applied once daily for 14 d to the center of the irradiated areas. Vehicle alone was applied to a third test site. All preparations were coded and applied without knowledge of the identity of the preparation. Photographs and biopsies were taken 2 d after the final application.

UV irradiation The irradiation source consisted of two Sylvania FS40 UVB bulbs. Irradiance was adjusted to 2.6×10^{-4} W per cm^2 and metered at 285 ± 5 nm with a radiometer (model IL1700A, International Light, Newburyport, MA) fitted with a UVB probe (detector SSE 240, diffuser W). Guinea pigs were irradiated once a day Monday through Friday for 2 wk with 70 mJ per cm^2, equivalent to 60% of an average minimal erythema dose in these animals, a protocol known to produce a distinct tanning response (Allan *et al*, 1995).

Quantitation of melanosomal transfer to keratinocytes Prints were made of electron microscopy sections. Ten to 20 basal and suprabasal representative keratinocytes were selected in each section, and melanosome number in each keratinocyte was determined.

RESULTS

Expression and localization of kinesin in cultured melanocytes To examine kinesin expression in melanocytes, total cellular proteins were processed for western blot analysis using an antibody directed against the kinesin heavy chain. The ≈120 kDa kinesin heavy chain band was strongly expressed in melanocytes (**Fig 1*A***).

To determine the ultrastructural localization of kinesin, immunoelectron microscopic studies were performed. A large majority of the gold particles that bound the kinesin antibody colocalized with melanosomes (**Fig 1*B***). Measurements of the distance between gold particles and the nearest melanosome for more than 500 particles revealed that 67% were separated by less than 50 nm, the reported length of the kinesin rod domain

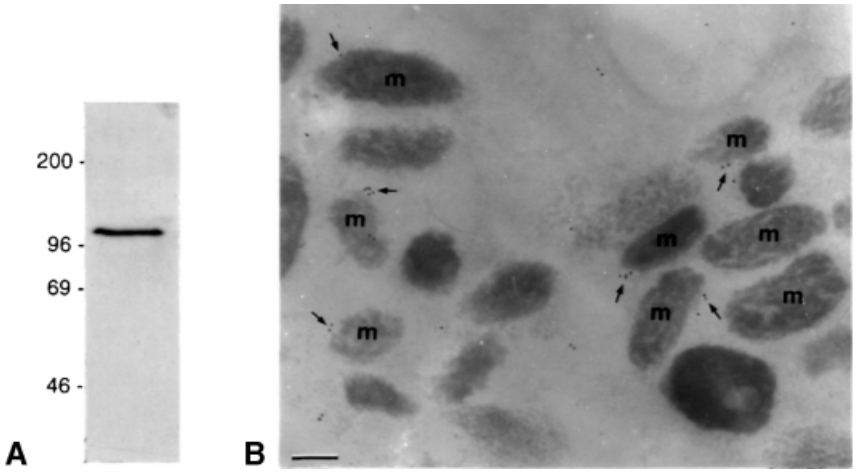

Figure 1. Kinesin is expressed in human melanocytes. (*A*) Total cellular proteins of cultured melanocytes were processed for western blotting using an antikinesin heavy chain antibody. A single band of approximately 120 kDa, the reported size of the kinesin heavy chain (Amaratunga *et al*, 1993), was detected. (*B*) Cultured melanocytes were processed for immunoelectron microscopic studies using antikinesin heavy chain antibody followed by gold-tagged secondary antibody. The majority of the gold particles (*arrows*) were localized within 50 nm of the melanosomes (m). *Scale bar:* 0.35 μm.

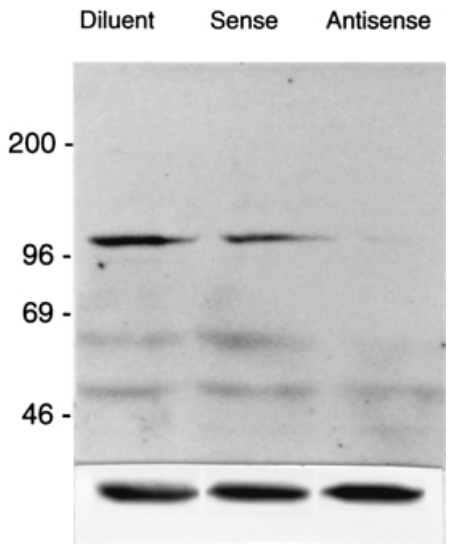

Figure 2. Kinesin heavy chain suppression by antisense oligonucleotides. Human melanocytes were maintained in serum-free melanocyte medium containing sense or antisense kinesin heavy chain oligonucleotides or diluent alone. Western blot analysis of total melanocyte proteins shows that kinesin heavy chain is hardly detectable in cultures treated with antisense oligonucleotides, compared with cultures treated with diluent alone or with sense oligonucleotides. The lower panel displays Coomassie Blue staining of the residual high molecular weight proteins on the gel, confirming equal loading of the lanes.

separating the conserved microtubule-associated end of the molecule that binds the antibody from its variable end that binds the transported protein (Andrews *et al*, 1993). In contrast, the average intermelanosomal distance was far greater. In control preparations in which the kinesin antibody was omitted, only very sparse gold particles (bound to the secondary antibody) were observed and their distribution pattern did not correlate with the melanosomes (data not shown). These data are consistent with a functional linkage and implicate kinesin as a motor protein for melanosome transport.

Kinesin plays a role in melanosomal transport To further determine if kinesin plays a role in melanosomal transport, melanocytes were first maintained in the presence of sense or antisense oligonucleotides for the kinesin heavy chain to assess the impact of these treatments on kinesin protein expression. A western blot of total proteins in antisense treated cultures, reacted with antikinesin heavy chain antibody and analyzed by densitometry, confirmed that kinesin levels were reduced by more than 80% compared with diluent-treated or sense-treated cells (**Fig 2**).

Next, melanocytes were treated with kinesin sense or antisense oligonucleotides and melanosomal movement was determined by time-lapse microscopy. In sense-treated cultures 88% of the melanosomes displayed bidirectional movement along the dendrites and none consistently moved in the centripetal direction (backward). In contrast, only 22% of melanosomes in antisense-treated cultures had bidirectional movement and 57% consistently moved backward. Interestingly, in sense-treated cultures, over the entire recorded time period, the melanosomes displayed a net forward movement of 0.72 ± 0.66 μm per min. In contrast, in antisense-treated cultures the melanosomes displayed a net backward movement of 0.21 ± 0.42 μm per min. Representative time-lapse microscopic images of movement in sense- and antisense-treated cultures are shown in **Fig 3**.

To determine the effect of kinesin downregulation on UV-induced tanning *in vivo*, guinea pigs were UV-irradiated as described in the *Materials and Methods* to produce a prominent distinct tanning response that was clearly observable at the end of the irradiation period, day 12 after the first exposure (Allan *et al*, 1995). Beginning on day 13, sense or antisense kinesin heavy chain oligonucleotides were applied topically to the center of each tanned area daily for 14 d, a period during which the tan and associated rapid melanosome transfer to keratinocytes is expected to continue (Allan *et al*, 1995). Two days after the final application, the animals were photographed and biopsies were taken from the center of each treated area. UV-irradiated areas that were treated with antisense oligonucleotides had hypopigmented centers, whereas areas treated with sense oligonucleotides or areas treated with vehicle alone had the same degree of increased pigmentation throughout the entire UV-irradiated area (**Fig 4*A***). Fontana-Masson stained biopsies showed that control areas treated with sense oligonucleotides contained many pigmented cells in the basal layer of the epidermis, consistent with staining of both melanocytes and neighboring melanin-containing keratinocytes (**Fig 4*B***). Melanin granules were also observed in suprabasal keratinocytes, and the melanin was predominantly arranged as nuclear caps, a pattern characteristic of tanned skin. In contrast, in areas treated with antisense oligonucleotides only occasional pigmented cells, consistent with staining of melanocytes alone, were observed in the basal layer of the epidermis and hardly any melanin was seen in basal or suprabasal keratinocytes (**Fig 4*C***). The amount of interfollicular epidermis occupied by melanin was quantified with an image analyzer and was found to be significantly higher in sense-treated areas compared with antisense-treated areas ($p < 0.013$, paired *t* test, $n = 3$ different animals).

Duplicate biopsies were processed for transmission electron microscopy. In the epidermal basal layer of sense-treated control

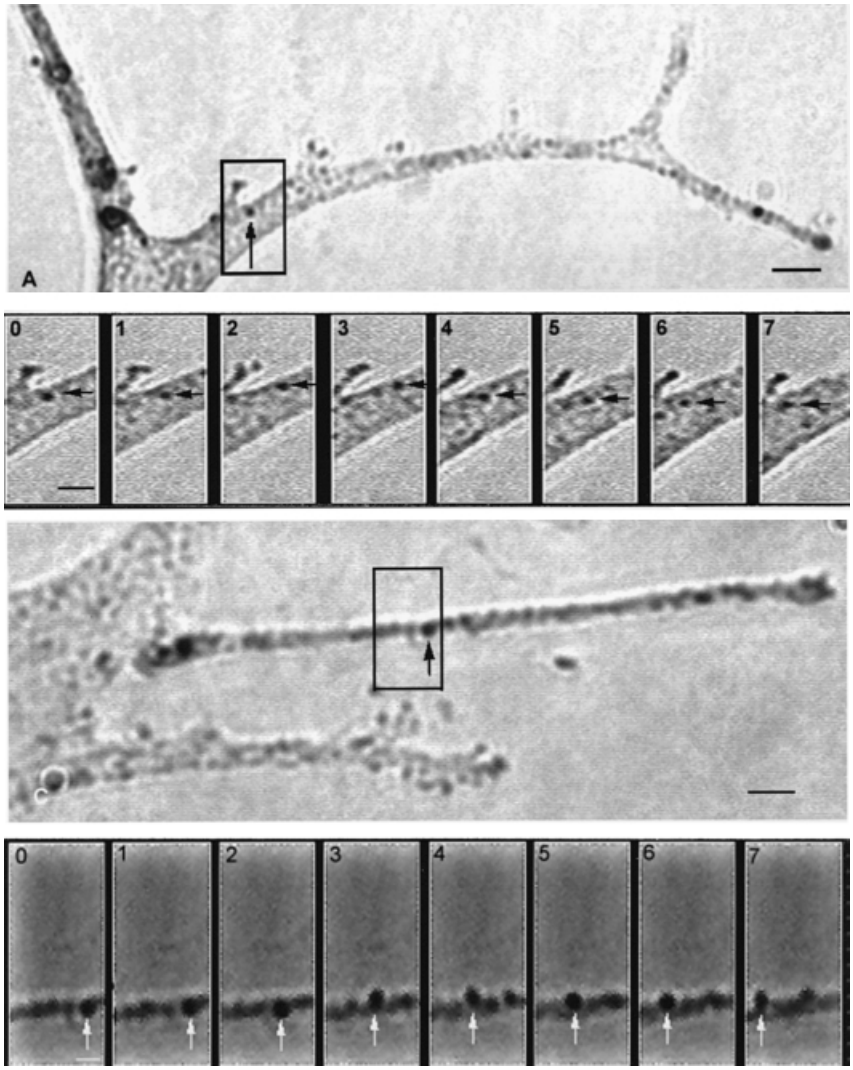

Figure 3. Kinesin antisense promotes backward movement of melanosomes in melanocyte dendrites. (*A*, *C*) Typical dendrites in melanocyte culture. The boxes indicate the analyzed area and the arrow shows the examined melanosome. (*B*, *D*) Successive time-lapse microscopic images. (*B*) Representative bidirectional melanosomal movement in sense-treated melanocyte cultures. The forward distance covered by the melanosome is 4.8 μm (*frames 0–3*). The backward distance is 6.4 μm (*frames 4–7*). (*D*) Representative centripetal movement in antisense-treated cultures. The backward distance covered by the melanosome is 14 μm. *Scale bar:* (*A*) 9 μm; (*B*) 5.6 μm; (*C*) 13 μm; (*D*) 5.4 μm.

areas, basal keratinocytes contained many melanosomes, and few melanosomes were observed in melanocyte dendrites (**Fig 4*D***), consistent with rapid melanin transfer. In contrast, in antisense-treated areas, fewer melanosomes were observed in basal keratinocytes and melanocyte dendrites were filled with melanosomes (**Fig 4*E***), suggesting decreased melanosomal transfer in these sites. Quantitation revealed that basal keratinocytes in sense-treated areas had 25 ± 9 melanosomes per keratinocyte whereas in antisense-treated areas basal keratinocytes had 14 ± 4 melanosomes per keratinocyte.

DISCUSSION

Kinesin is a member of a superfamily of rod-shaped motor molecules with ATPase activity that utilize the adenosine-5′-triphosphate energy to generate movement of intracellular organelles. Kinesins are expressed in many cell types and in most tissues and are consistently found in close association with the α- and β-tubulin subunits of cellular microtubules (Hollenbeck, 1989; Navone *et al*, 1992; Bloom and Endow, 1995; Nilsson *et al*, 1996; Tucker and Goldstein, 1997). All members of the kinesin superfamily are homologous in their microtubule binding domain and are variable in the domains that convey specific attachment to different cytoplasmic organelles (Bloom and Endow, 1995; Case *et al*, 1997). Each kinesin is thought to transport a specific cellular organelle, although there is some redundancy (Bloom and Endow, 1995; Hirokawa, 1996; Nakagawa *et al*, 1997).

We now report that in melanocytes approximately two-thirds of the kinesin molecules are localized within the immediate vicinity (50 nm) of melanosomes. Indeed, the antibodies employed in our experiments bind to the distal portion of the approximately 40–52 nm long rod-shaped kinesin protein at its microtubule attachment site (Andrews *et al*, 1993). Thus, the observed proximity of kinesin to melanosomes strongly supports the hypothesized role of this motor protein in melanosomal transport. The data further indicate that the kinesin molecule and not a kinesin-like protein participates in melanosomal transport, in that the oligonucleotides used in the antisense experiments differ significantly in sequence from the known kinesin-like proteins (Goodson *et al*, 1994; Moore and Endow, 1996; Periera *et al*, 1997) and thus would not be expected to interfere with their synthesis.

The oligonucleotides that we used are complementary to rat heavy chain kinesin sequences (Ferreira *et al*, 1992). They differ in two nucleotides from the published human kinesin heavy chain sequence (Navone *et al*, 1992): C instead of G in position 308 and G instead of T in position 324. The guinea pig sequence is unknown. Antisense oligonucleotide, however, when added to human melanocytes *in vitro*, inhibited the synthesis of kinesin heavy chain, whereas the sense oligonucleotide had no effect; and the same sequences, when previously used in other *in vivo* experiments on rabbits, efficiently blocked kinesin synthesis (Amaratunga *et al*, 1993). These facts, in combination with the lack of effect of control sense oligonucleotides, make it highly likely that the observed effect was indeed due to significant interference with translation of kinesin protein in our *in vivo* experiments.

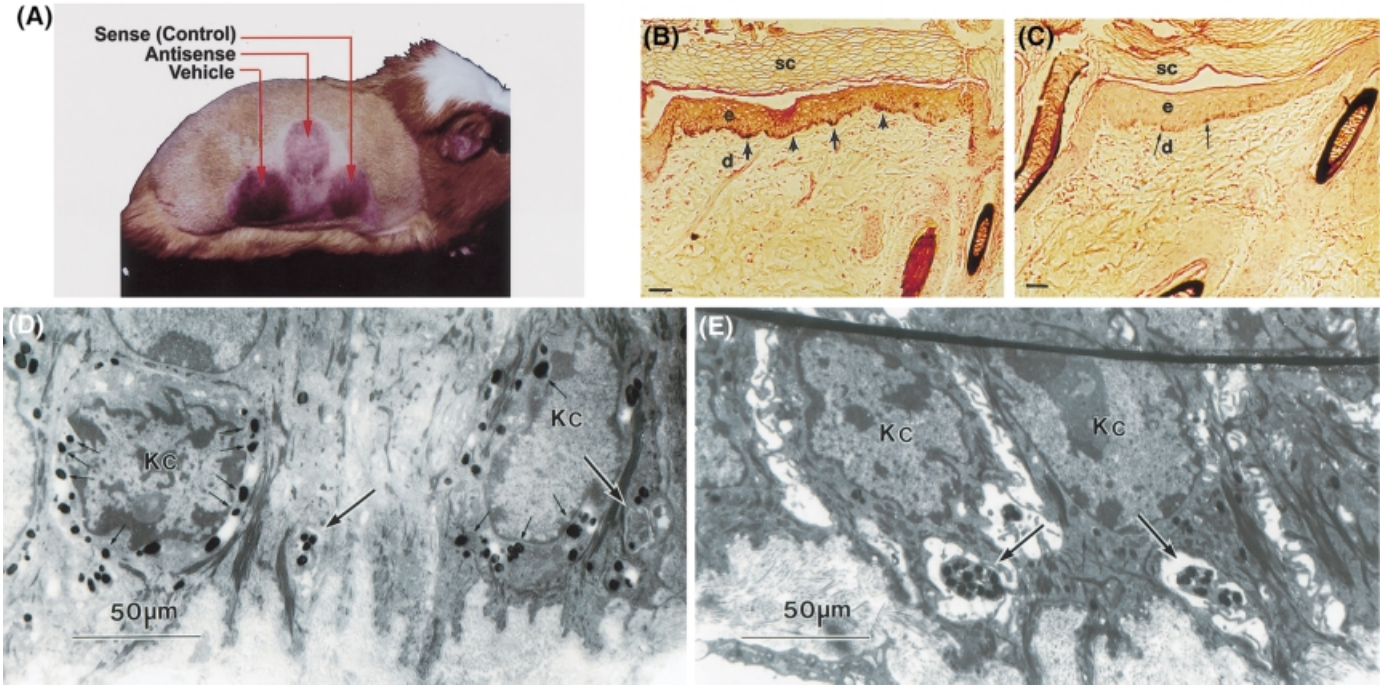

Figure 4. Effect of kinesin suppression on pigmentation and melanosome distribution *in vivo*. (*A*) Guinea pigs were irradiated daily 5 d per wk for 2 wk in three defined areas. At the end of the irradiation period, 200 μM of sense or antisense kinesin heavy chain oligonucleotides or vehicle alone were applied daily for 14 d to the center of each irradiated area. A representative animal is shown 2 d after the last oligonucleotide application. The center test site (antisense oligonucleotides) is less pigmented in the center, whereas the other test sites (sense oligonucleotides or vehicle alone) are evenly hyperpigmented. (*B*) Light microscopic appearance of a cross-section of guinea pig skin treated with sense kinesin heavy chain oligonucleotides. There is abundant melanin in the basal layer of the epidermis (e), as shown by the arrows. Melanin is also clearly present throughout upper epidermal layers. *Scale bar*: 0.1 mm. sc, stratum corneum; d, dermis. (*C*) A control cross-section obtained from skin treated for 14 d with antisense oligonucleotides. Melanin is hardly visible in either basal or suprabasal keratinocytes. Occasional darker cells are presumptive melanocytes (*arrows*). *Scale bar*: 0.1 mm. (*D*) By electron microscopy, in areas treated with sense oligonucleotides, the majority of the melanosomes are found in basal keratinocytes (*small arrows*). Melanosomes are also present in melanocyte dendrites (*large arrows*). (*E*) In contrast, in antisense-treated areas, very few melanosomes are present in the keratinocytes and melanosomal dendrites appear to be packed with melanosomes (*arrows*).

In this study we have shown that kinesin participates in melanosomal transport in human cutaneous melanocytes. Treatment with antisense kinesin oligonucleotides resulted in the majority of melanosomes displaying consistently backward movement, in contrast to sense-treated cultures in which the majority of melanosomes displayed bidirectional movement. Furthermore, as expected, the net melanosomal movement in sense-treated cultures was forward whereas the net melanosomal movement in antisense-treated cultures was backward. The large standard deviations most probably reflect the bidirectionality of the movement. Our findings are consistent with reported data that centrifugal organelle movement, away from the cell body, is driven mainly by kinesin, whereas centripetal or backwards movement is driven by cytoplasmic dynein (Hirokawa *et al*, 1991; Bloom, 1992; Endow and Titus, 1992; Gelfand and Scholey, 1992; Coy and Howard, 1994; Okada *et al*, 1995). Our data also support the findings of Wu *et al* (1998) in murine melanocytes, in which the microtubule-dependent melanosomal movement is bidirectional. Our data support and expand previous reports showing that kinesin contributes to anterograde movement along microtubules in normal human melanocytes.

Our studies are also in agreement with recent reports demonstrating that both kinesin and dynein are associated with melanosomal membranes in cod melanophores (Nilsson *et al*, 1996), but differ from the findings of Rogers *et al* (1997), who reported in Xenopus melanophores that anterograde melanosome movement is mediated by kinesin II. Also, recent work from our laboratory clearly demonstrates that cytoplasmic dynein participates in retrograde melanosomal movement (Byers *et al*, in press) indicating that in normal human melanocytes the bidirectional microtubule-associated melanosomal movement is controlled by cytoplasmic dynein and kinesin.

In vivo, topical treatment with kinesin antisense oligonucleotides decreased the UV-induced tan of guinea pig skin. *In vivo* administration of kinesin antisense oligonucleotides could certainly affect other aspects of the tanning response in addition to melanosomal movement. For example, kinesin facilitates early organelle transport between the Golgi complex and the endoplasmic reticulum (Thyberg and Moskalewski, 1999) and is required for maintaining the integrity of the Golgi complex (Burkhardt, 1998). Thus, kinesin may participate in early as well as later stages of melanosome formation and also in transport of the melanogenic enzymes to the premelanosomes. Hence, decreased tanning *in vivo* could reflect in part decreased melanosome formation. Still, the electron microscopic data show that fully formed pigmented melanosomes are trapped within melanocyte dendrites, suggesting that their movement along the melanocyte dendrite and their ultimate transfer to keratinocytes have been affected.

In conclusion, our results strongly support a major role for kinesin in melanosomal movement within the melanocyte dendrite and in the tanning response in skin.

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