

Melanosome transfer to and translocation in the keratinocyte

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Abstract: Complexion coloration in humans is primarily regulated by the amount and type of melanin synthesized by the epidermal melanocyte. However, additional and equally contributing factors consist of (1) efficient transfer of melanin from the melanocytes to the neighboring keratinocytes and (2) distribution and degradation of the transferred melanosomes by the recipient keratinocytes. Once synthesized in the cell body of the epidermal melanocyte, pigmented melanosomes are translocated down the dendrites and captured at the dendritic tips via various cytoskeletal elements. Molecules recently identified that participate in this process consist of Rab27a, myosin-Va and melanophilin. Eventually, these peripherally localized melanosomes are transferred to keratinocytes by a presently undefined mechanism. The protease-activated receptor-2 (PAR-2) and unidentified surface lectins and glycoproteins facilitate this transfer process. Once incorporated into the keratinocytes, melanosomes are distributed individually or as clusters, aggregated towards the apical pole of the nucleus, and degraded as the keratinocytes undergo terminal differentiation and desquamation. Ultraviolet irradiation (UVR) can modulate the process of melanosome transfer from the melanocytes to the keratinocytes. UVR can upregulate expression of PAR-2 and lectin-binding receptors and increase phagocytic activity of cultured keratinocytes. Therefore, many cellular and molecular events that occur after melanogenesis contribute to skin color.

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Introduction

Pigmentation of the skin, also referred to as complexion coloration, results from a complex process of melanin synthesis within melanocytes of the interfollicular epidermis, and the subsequent transfer, translocation, and degradation of this melanin to, in, and by the recipient keratinocytes, respectively. Therefore, skin pigmentation is a combination of type and amount of melanin synthesized by the melanocyte factory and the handling of the melanin product by the keratinocyte consumer. The synthesis of constitutive pigmentation by the melanocyte is controlled primarily by the tyrosinase gene family of proteins (tyrosinase, TYRP1 and TYRP2), which regulate the type of melanin synthesized (1). Mutations affecting these melanogenic proteins result in various forms of oculocutaneous albinism; respectively OCA1, OCA3, and slaty (in the murine system) (2). Additional proteins that also regulate the synthesis of melanin in the melanosome (i.e. pmel 17, P protein, OA1 protein) have been identified. Constitutive pigmen-

tation can be modulated by various environmental factors such as ultraviolet light (3), and numerous physiological factors (4). Various autocrine and paracrine hormones/cytokines produced both in the skin and extracutaneously modulate this constitutive pigmentation, a process termed facultative pigmentation.

The type and amount of melanin produced is packaged into melanosomes that are subsequently translocated down the dendrites along cytoskeletal elements via motor proteins. Melanosomes reaching the tips of the dendrites are then transferred to the neighboring keratinocytes in both the interfollicular epidermis of the skin and the epidermis of the hair bulb. Once the keratinocytes receive these melanosomes, they incorporate the granules individually or as clusters, aggregate them over the nucleus, and degrade them as the keratinocytes undergo terminal differentiation. This keratinocyte process of incorporation, aggregation, and degradation of melanin varies per individual and correlates with complexion coloration. This review

will present the current state of knowledge pertaining to the postmelanogenic processes responsible for complexion coloration, as summarized in Fig. 1.

Melanosome transfer along dendrites

Melanosome biogenesis and the onset of melanin synthesis within the melanosome occur predominantly within the cell body of the melanocytes. Once melanin formation is underway, the pigmented melanosomes are translocated along the dendrites to the peripheral tips for transfer to neighboring keratinocytes. Cytoskeletal elements play a significant role in facilitating the movement of melanosome down the dendrites. Pioneering work in the 1970s demonstrated that in non-mammalian melanocytes, microfilaments (5) and microtubules (6) were responsible for trafficking the melanosomes both outward from the cell body to the dendritic tip (i.e. centrifugal) and from the distal ends of the dendrites back to the cell body

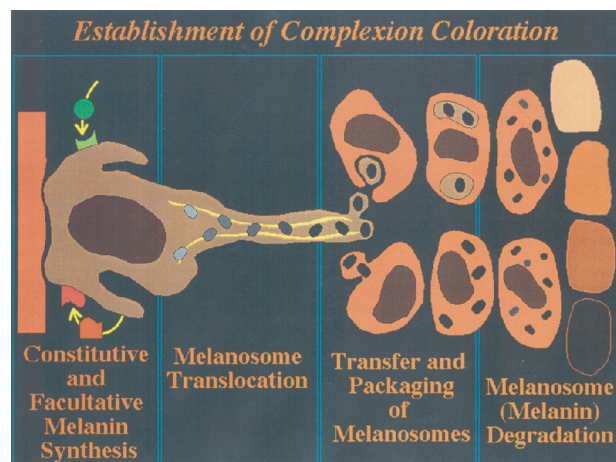


Figure 1. Schematic outline of the cellular events underlying the establishment of complexion coloration. Melanocytes (cell at the left of the illustration) of the interfollicular epidermis of human skin reside on the basement membrane (bar at left-hand edge of illustration) in the stratum basale layer, where they are responsible for transferring melanosomes to approximately 36 neighboring keratinocytes. The constitutive type and amount of melanin synthesized by the melanocyte is genetically determined. This constitutive pigment can be altered (facultative) by environmental cues and autocrine/paracrine mediators. Once melanin is synthesized within melanosomes, they are translocated down dendrites and captured at the dendritic tips. Melanosomes are then transferred to keratinocytes by endocytosis of released granules and/or cytophagocytosis of dendrites. Incorporated melanosomes are then retained as individual granules in dark skin or packaged into membrane-bound clusters in light skin by the keratinocyte. These melanosomes are then preferentially aggregated over the apical pole of the nucleus. As the keratinocyte undergoes terminal differentiation, the melanosomes are subsequently degraded.

(centripetal). Melanophores of *Xenopus* express kinesin-II, a microtubule-associated plus-end-directed motor, and dynein, a microtubule-associated minus-end-directed motor (7). In addition, the actin-based motor protein myosin V functions as a 'molecular ratchet' to favor centrifugal transport of *Xenopus* melanosomes (8). The bi-directional movement of melanosomes along dendrites in lower vertebrates is relatively rapid. In addition, melanosomes can be preferentially localized to polar areas of the melanocytes. This selective translocation of melanosomes within the cell can result in either aggregation of melanosome in the cell body or dispersion of melanosomes throughout the dendrites, which in turn correlates with light vs. dark skin coloration, primarily for environmental adaptation. Mediation of this type of melanosome movement is regulated primarily by melanocyte-stimulating hormone (MSH) (9).

In contrast, movement of melanosomes along dendrites in mammalian melanocytes does not occur by the regulated bi-directional method responsible for the rapid skin color changes that occur in lower vertebrates. Instead, melanosomes are targeted preferentially to the ends of the dendrites for transfer out to neighboring keratinocytes. However, bi-directional movement of melanosomes does occur during trafficking of the granules along the dendrites, as recently demonstrated by Hammer et al. (10). Using time-lapse photography, bi-directional movement of melanosomes from the cell center to the periphery and back again was visualized. This movement was halted when cells were treated with the microtubule depolymerizer nocodazole. Motor proteins appear to be responsible for this up-and-down movement. Kinesin propels the melanosomes towards the plus ends of the filaments (11), whereas dynein putatively moves the melanosomes in the opposite direction (12). Melanosomes that reach the microtubule ends at the peripheral tips of the dendrites must be ultimately captured and retained to prevent their centripetal re-traffic. This capturing is facilitated by myosin Va, which colocalizes with both actin and melanosomes at the distal ends of the dendrites (13,14). In addition, melanosomes within melanocytes of *dilute* mice, containing mutations within the myosin Va locus, do not accumulate at the dendritic tips (10). The capture of melanosomes in the actin-rich periphery of the dendrites relies on Rab27a, a member of the family of Rab GTPases involved in vesicular movement/fusion during intracellular membrane trafficking, and the gene product of the murine *ashen* locus (15). Rab27a appears to recruit myosin onto the melanosome surface

(16,17). Recently, the gene product of the murine *leaden* locus (*Mlph*), melanophilin (a member of the RAB effector family), has been demonstrated to regulate melanosome movement along dendrites (18). Melanophilin also appears to be necessary for the association of myosin Va with the melanosome (19, 20). It was proposed from these studies that a molecular complex of at least Rab27a, Myo-5, and *Mlph*, and possible other proteins, facilitate the transport of melanosomes along the dendrites and their ultimate capture at the distal ends. The summation of these forces results in the melanosomes reaching and being retained in the tips of the dendrites, positioned for transfer.

Several human diseases that result in disrupted translocation/capture of melanosomes in the dendritic tip have recently been identified. The form of Griscelli syndrome combining partial albinism with severe immunodeficiency results from mutations in *RAB27A* (21), while the form of Griscelli syndrome associated with neurological disease results from mutations in *MYO5A* (22,23). The corresponding murine models for these forms of Griscelli syndrome are the *ashen* mouse (*ash*) (15) and the *dilute* mouse (*d*) (24), respectively.

Transfer of melanosomes from the melanocyte to the keratinocyte

After being captured and aggregated at the tips of the dendrites, the melanosomes in the epidermal melanocytes are extruded and incorporated into neighboring keratinocytes. It has been calculated that in the interfollicular human epidermis, one melanocyte transfers melanosomes to approximately 36 keratinocytes (25) in all races and ethnic groups in what is termed the epidermal melanin unit (26). The actual processes involved in this transfer have not been delineated clearly. Various mechanisms have been proposed [reviewed by Jimbow and Sugiyama (27)], which consist of: (i) the release of the melanosomes by the melanocytes and the subsequent endocytosis of the released granules by the keratinocyte; (ii) the keratinocyte engulfing the dendritic tips of the melanocytes by active phagocytosis and incorporating portions of the melanocytes within them (i.e. cytophagocytosis) (28); (iii) the active transfer or injection of melanosomes directly into the keratinocyte by the melanocytes; and (iv) a continuous pore developing between the plasma

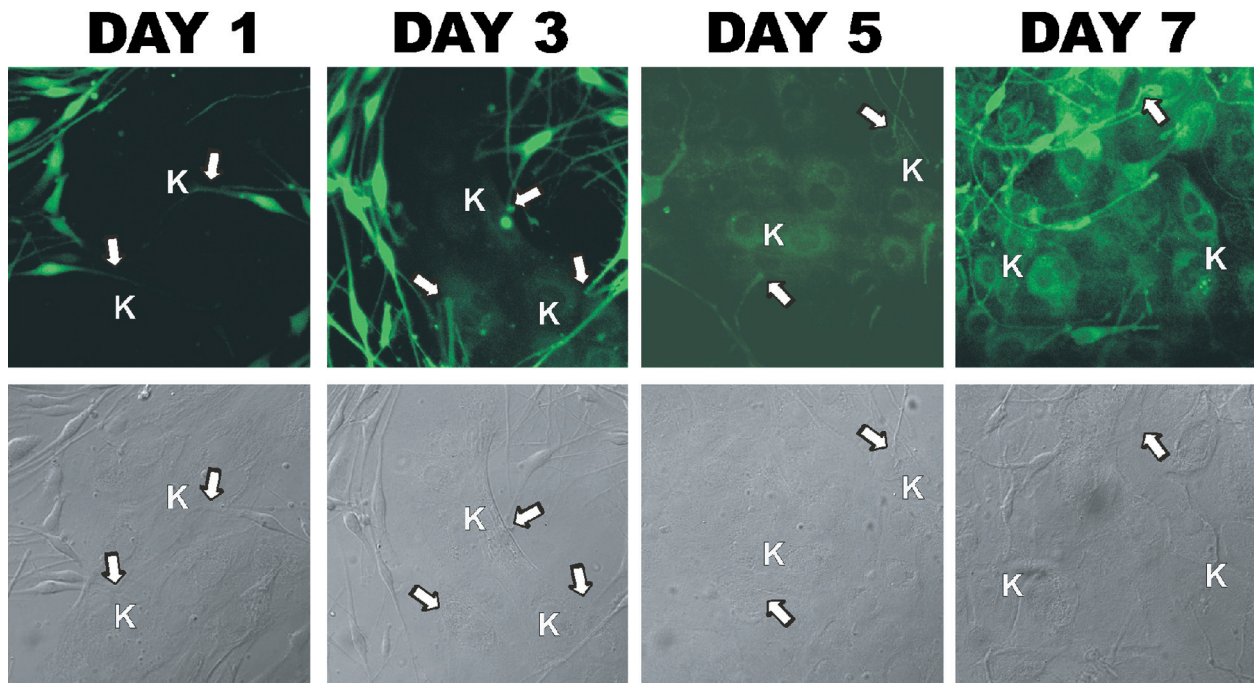


Figure 2. Transfer of material from melanocytes to keratinocytes in coculture as assessed by confocal microscopy. Melanocytes were labeled with succinimidyl ester of carboxyfluorescein diacetate (CFDA), a colorless probe that passively diffuses into cells, and which has acetate groups that are cleaved by intracellular esterases to yield a highly fluorescent, amine-reactive fluorophore. CFDA-labeled melanocytes were subsequently cocultured with keratinocytes, and observed on days 1, 3, 5 and 7 by confocal microscopy with fluorescent illumination (top row) and concurrently with differential interference contrast illumination (bottom row). Panels demonstrate that dye transfer from melanocytes to the colonies of keratinocytes (K) increased from no apparent transfer at day 1 to extensive transfer at day 7. Arrows indicate juxtaosition of melanocytes dendrites with keratinocytes. [Illustration adapted from Minwalla et al. 2001 (37).]

membrane of the melanocytes and the keratinocytes, through which the melanosomes are passed. In support of the first proposed mechanism is the fact that keratinocytes are readily phagocytic and efficiently engulf polystyrene beads (29) or isolated melanosomes (30–32) when cocultured with these particles. An extensive electron microscopic evaluation of melanosome transfer in human skin demonstrated images that were interpreted to represent all of the mechanisms proposed above, and the authors concluded that these various mechanisms coexist (33).

Recently, Hearing et al. investigated the process of melanosome transfer in an *in vitro* model of murine melanocytes and keratinocytes (34). They demonstrated that α -MSH could induce exocytosis of melanosomes and membrane ruffling in cultured melanocytes and that ultraviolet radiation (UVR) induced accumulation of melanosomes in melanocytes. Concurrently, the phagocytic activity of cultured keratinocytes was stimulated by both α -MSH and UVR. Interpretation of these data suggests that transfer of melanosomes to keratinocytes occurs predominantly via the first postulated mechanism, i.e. phagocytosis of released melanosomes by keratinocytes.

Molecular mediators of the process of melanosome transfer are being elucidated. It has been hypothesized that specific plasma membrane molecules of both melanocytes and keratinocytes must exist to facilitate the recognition, physical interaction and subsequent transfer of material between these two cell types. Plasma membrane glycoproteins and lectins are categories of putative mediators. A decade ago, Kieda et al. demonstrated that various lectins or neoglycoproteins could inhibit melanosome transfer between human melanoma cells and squamous cell carcinoma-derived keratinocytes in coculture (35,36). We have recently developed an *in vitro* model in which melanosome transfer between normal human skin-derived melanocytes and keratinocytes in coculture can be monitored and quantitated (37). In this model, melanocytes were prelabeled with a fluorescent dye (succinimidyl ester of carboxy fluorescein diacetate), and in subsequent cocultures with keratinocytes, the transfer of dye was monitored by confocal microscopy (Fig. 2) and quantified by flow cytometry. The transfer of melanosomes was subsequently confirmed by electron microscopy. With this model system, we confirmed the observations of Kieda et al. (35,36) that various lectins and neoglycoproteins can inhibit the transfer of melanosomes to keratinocytes. In addition, we have also demonstrated that niacinamide, the physiologically active

amide of niacin known as vitamin B3, which has been reported anecdotally to have skin-lightening effects, also inhibits melanosome transfer in our model system (38).

A major regulator of melanosome transfer is the protease-activated receptor-2 (PAR-2), a seven transmembrane G-protein-coupled receptor on the plasma membrane that is cleaved extracellularly by serine proteases resulting in self-activation (39). Seiberg et al. have demonstrated through a series of elegant experiments that the PAR-2 receptor is on the membrane of keratinocytes and mediates melanosome transfer (40). Activation of PAR-2 results in increased phagocytic activity of cultured keratinocytes towards isolated melanosomes (41) or microspheres (42). Increase in melanosome transfer via PAR-2 appears to be mediated by alterations in the number and length of membrane projections in keratinocytes (43). Seiberg et al. also demonstrated that upregulation of PAR-2 activity with a synthetic peptide (SLIGRL) or downregulation with trypsin inhibitors resulted in respective darkening or lightening of *in vitro* epidermal equivalents, pigmented skin of Yucatan swine, and human skin transplanted onto SCID mice (40–42).

Therefore, recent studies have indicated that membrane glycoproteins, PAR-2, and niacinamide may modulate the recognition and transfer of melanosomes between the melanocyte and the keratinocyte. However, additional molecular regulators of melanosome transfer between the melanocytes and the keratinocytes are predicted to exist.

Translocation, distribution, and degradation of melanosomes by the keratinocyte

Once melanosomes are transferred into the recipient keratinocytes they are selectively and predominantly translocated to the apical pole of the keratinocyte where they can effectively absorb incident ultraviolet light and protect the underlying nucleus from mutagenic damage (Fig. 3). Again, the cytoskeletal elements and microtubule-associated motor proteins appear to mediate this trafficking. Byers and Maheshwary (44) have reported that dynein colocalizes with phagocytosed melanosomal aggregates throughout the cytoplasm, predominantly at the microtubule-organizing center in keratinocytes.

The distribution of recipient melanosomes within the keratinocytes varies according to complexion coloration (Fig. 4), as demonstrated over a quarter of a century ago (45,46). In dark-skinned

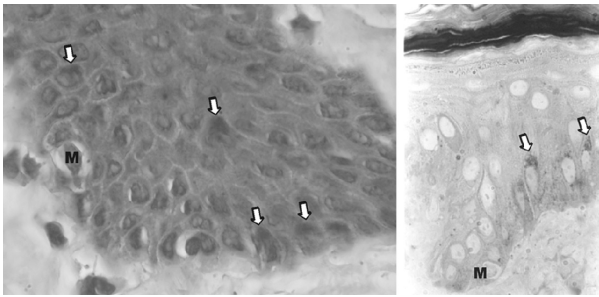


Figure 3. Melanosomes preferentially aggregate over the apical pole of the nucleus in keratinocytes. Light microscopy of human skin from dark pigmented individuals demonstrating the predominant localization of melanin over the nucleus in several keratinocytes (arrows). M: melanocytes.

individuals, melanosomes are approximately $0.8\mu\text{m}$ in diameter and are maintained as individual organelles throughout the cytosol of the keratinocyte. In contrast, in light-skinned individuals, melanosomes are significantly smaller than $0.8\mu\text{m}$, and are aggregated into membrane-bound clusters of 4–8 organelles. It is uncertain whether these distinct distribution patterns are determined by factors within the transferred melanosome or

are innate to the recipient keratinocytes. In a recent study, melanosome distribution in keratinocytes of an *in vitro* skin reconstruction model utilizing combinations of keratinocytes and melanocytes from different complexion colorations was assessed, and the investigators concluded that the distribution pattern of recipient melanosomes was dictated by the type of donor melanocyte (47). In contrast, we have recently demonstrated in melanocyte/keratinocyte cocultures that the distribution pattern of transferred melanosomes is regulated by the skin type from which the recipient keratinocyte was derived, and is unrelated to melanosome size (48). However, both of these experimental approaches utilize relatively artificial situations and therefore regulation of distribution in the skin remains unresolved.

As the keratinocyte undergoes terminal differentiation, the recipient melanosomes undergo degradation. Eventually, when the keratinocyte becomes a corneocyte, melanosome structures no longer exist except rarely in very darkly pigmented skin (49). Even in these situations, the number of surviving melanosomes apparent by electron microscopy is significantly reduced (Fig. 5). The

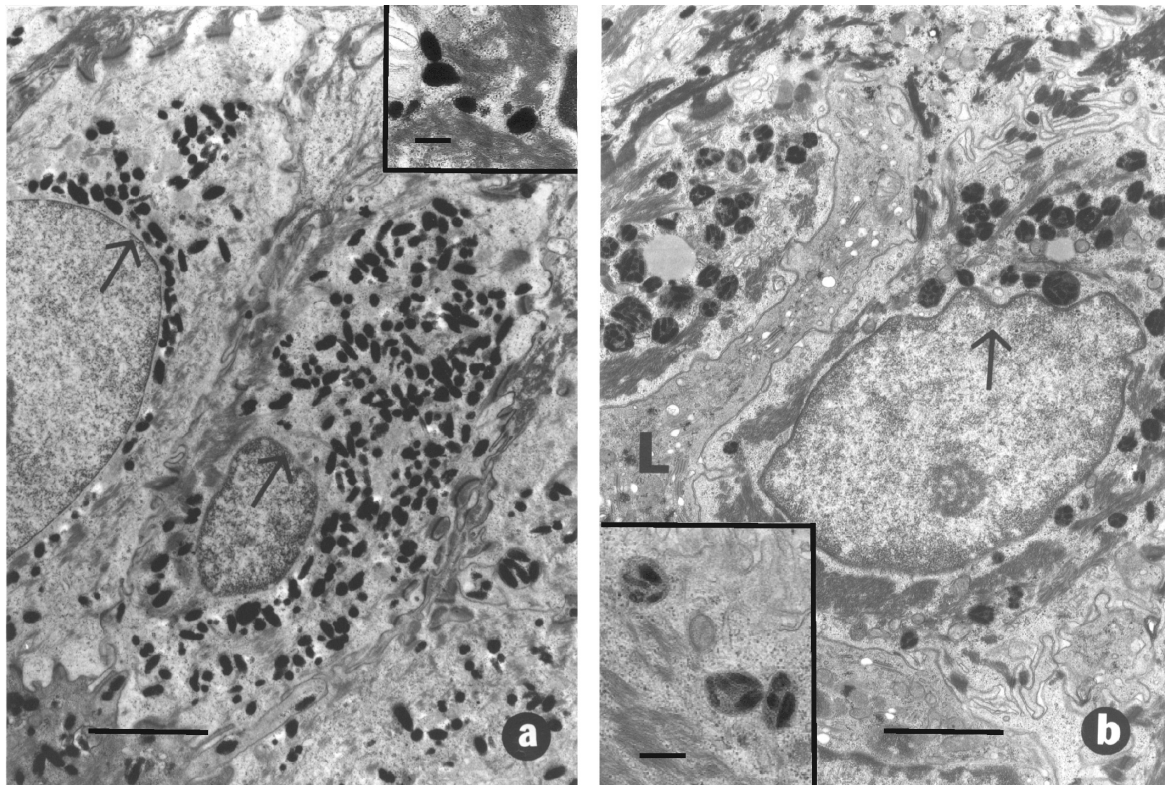


Figure 4. Differences in the distribution of melanosomes within keratinocytes of dark and light skin individuals. Melanosomes in dark skin (a) are singly distributed throughout the cytoplasm of epidermal keratinocytes. In contrast, melanosomes in light skin (b) are distributed as membrane-bound clusters. Melanosomes in both skin types are frequently concentrated over the nucleus (arrows). L: Langerhans cell. Scale bar = $5\mu\text{m}$ (inset = $1\mu\text{m}$). [Illustration adapted from Minwalla et al. (48).]

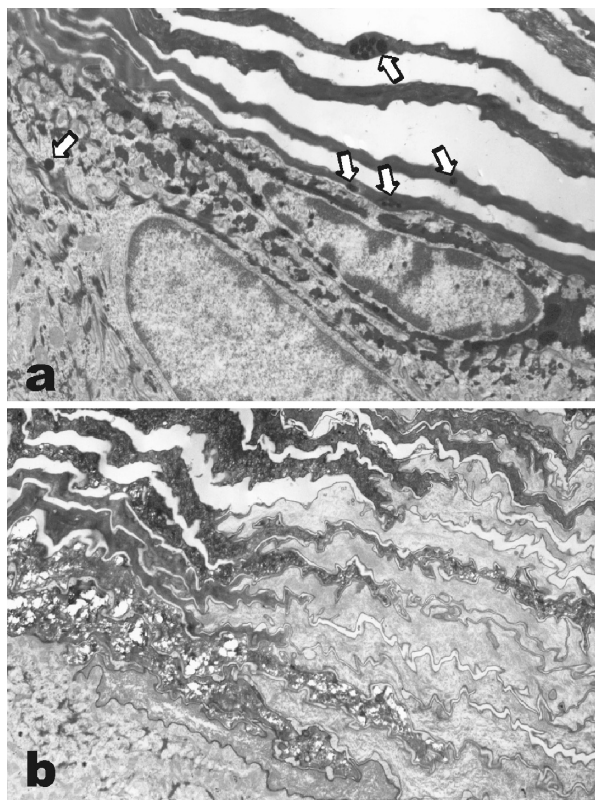


Figure 5. Melanosomes are generally degraded by the time the keratinocytes had differentiated into corneocytes. Interface between the stratum granulosum and the stratum corneum demonstrating few melanosomes (arrows) remaining in corneocytes of dark skin (a) and no apparent melanosomes remaining in corneocytes of light skin (b).

melanin polymer within the melanosome is a dense structure and by virtue of this density, purification of melanin and the molecular composition of the finalized melanin polymer has been elusive. The hydrolytic processes utilized by the keratinocytes in order to degrade the dense melanosome/melanin have yet to be identified. However, hydrolytic enzymes have been implicated in melanosome degradation by the keratinocyte (50).

Effect of ultraviolet irradiation

UVR of the skin results in increased cutaneous pigmentation by immediate pigment darkening (IPD) and delayed pigment darkening (DPD) mechanisms [for review see Gilchrist et al. (3)]. The primary consequence of UVR resulting in DPD occurs via the upregulation of melanin synthesis within the melanocytes of the irradiated skin and the subsequent increase in melanin transfer to, and content of, the epidermal keratinocytes (51). In contrast, the relatively transient IPD appears to result from rapid changes in orientation of cytoskeletal elements, translocation of the

melanosomes down the dendrites, and transport of melanosomes to, and altered distribution of melanosomes within, the keratinocytes (52). Therefore, one of the mechanisms responsible for UVR pigmentation of the skin is an increase in the immediate and/or delayed transfer of melanosomes to neighboring keratinocytes. UVR has been demonstrated to increase melanocyte dendricity in the skin (53), possibly mediated by endothelin-1 released by irradiated keratinocytes (54).

Mediators of melanosome transfer from the melanocyte to the keratinocyte are also affected by UVR. Expression of lectin-binding receptors on melanoma cells is upregulated by UVR (55). The function of PAR-2, responsible for mediating melanosome transfer by regulating keratinocyte phagocytosis, also appears to be influenced by UVR. It has previously been demonstrated that inhibition of PAR-2 prevents UVR induced pigmentation (41,43). Recently, Scott et al. demonstrated that expression of PAR-2, generally restricted to the basal epithelial layers, is dramatically upregulated throughout the epidermis upon UVR in skin types II and III and delayed in skin type I (56). In addition, cultured keratinocytes demonstrate an increase in PAR-2 cleavage activity after UVR (56). Finally, UVR increases the phagocytic activity of cultured murine keratinocytes (34).

Conclusion

Skin pigmentation/complexion coloration is regulated by the constitutive amount and type of melanin synthesized by the melanocyte, the facultative regulation of this melanin synthesis, and the transfer and maintenance of melanosomes in the keratinocytes. To date, a few regulators of this latter process have been alluded to or identified. Further experimentation is needed to define more accurately the molecular/cellular process of melanosome transfer from the epidermal keratinocyte. Changes of skin pigmentation resulting from altered melanosomal transfer may be the basis for several skin diseases. The hypomelanotic lesions of nevus depigmentosus exhibit melanosomes with irregular dendrites and keratinocytes with fewer melanosomes (57). Melanocytes with minimal short dendrites and keratinocytes with relatively few melanosomes are also characteristic of the hypopigmented lesions of patients with Griscelli-Prunieras disease (58). It has also been suggested that in tuberous sclerosis, melanosome transfer is disrupted in the associated hypopigmented sites (59). Regulation of complexion coloration is a desired consumer need in that lighter skin is

desired in various Eastern societies as representing beauty, while darker/tanned skin, representing improved solar protection, is desired in various Western societies. Regulation of complexion coloration and correction of lesion hypopigmentation by modulating melanosome transfer to, and maintenance by, the keratinocytes could conceivably be developed once the cellular/molecular mechanisms of these processes are clearly delineated.

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