Integrative and Comparative Biology

Integrative and Comparative Biology, volume 61, number 4, pp. 1546–1555
doi:10.1093/icb/icab094

Society for Integrative and Comparative Biology

SYMPOSIUM

Melanin Transfer and Fate within Keratinocytes in Human Skin Pigmentation

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From the symposium "SICB-ide Symposium: The integrative biology of pigment organelles" presented at the virtual annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2021.

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Synopsis Human skin and hair pigmentation play important roles in social behavior but also in photoprotection from the harmful effects of ultraviolet light. The main pigments in mammalian skin, the melanins, are synthesized within specialized organelles called melanosomes in melanocytes, which sit at the basal layer of the epidermis and the hair bulb. The melanins are then transferred from melanocytes to keratinocytes, where they accumulate perinuclearly in membrane-bound organelles as a "cap" above the nucleus. The mechanism of transfer, the nature of the pigmented organelles within keratinocytes, and the mechanism governing their intracellular positioning are all debated and poorly understood, but likely play an important role in the photoprotective properties of melanin in the skin. Here, we detail our current understanding of these processes and present a guideline for future experimentation in this area.

Introduction

Skin and hair pigmentation in humans is an important contributing factor to social interactions but also plays an important role in photoprotection from the harmful effects of solar ultraviolet (UV) radiation. As in other mammals, the main pigments in the skin and hair of humans are the melanins—black and brown eumelanins, and red and yellow pheomelanins (D'Alba and Shawkey 2019). The processes of melanin synthesis and distribution in the skin and hair are divided among two cell types: melanocytes synthesize melanins, and then transfer them to keratinocytes in which pigments are retained to provide the skin and hair with color (Lambert et al. 2019).

Melanocytes in the skin primarily sit at the basal layer of the epidermis where they extend dendrites into the epidermis and contact up to 30–40 keratinocytes, forming what is referred to as the epidermal melanin unit (Hadley and Quevedo 1966). Similarly, in the hair bulb, melanocytes sit at the base of the growing hair shaft and extend dendrites into proliferating keratinocytes as they enter the shaft, forming the follicular melanin unit (Tobin 2008). Processes within melanocytes that contribute to the synthesis of melanins within organelles called melanosomes

Advance Access publication May 21, 2021

are described in the companion paper by Le et al. (2021). This review will cover the ensuing processes from melanin transfer to keratinocytes to the formation and positioning of the melanin-positive organelle within keratinocytes for optimal photoprotection. To date, these processes are still debated and not well understood. We aim here to detail our current understanding and will raise some fundamental, but still unsolved, questions that underlie the cell biology of the melanin pigment in keratinocytes.

Melanin transfer

Four distinct concepts for one melanin transfer

For skin and hair to appear colored, the fully pigmented melanosomes that are synthesized in melanocytes must be transferred to neighboring keratinocytes. Although this process is not well understood, it is essential for homogenous skin coloration and efficient photoprotection. The mechanism of pigment transfer from melanocytes to keratinocytes has long been debated and several hypotheses have been proposed based on observations in different cellular models (Wu and Hammer 2014; Tadokoro and Takahashi 2017; Lambert et al. 2019; Benito-Martinez et al. 2020). Four main hypotheses for the mechanistic basis of melanin transfer are briefly outlined here (Fig. 1).

The exocytosis—endocytosis model postulates that fusion of mature melanosomes with the melanocyte plasma membrane results in exocytosis of their intraluminal contents—that is, the pigment cores devoid of a limiting membrane (a.k.a. melanocores, or MCs)—into the extracellular space (Fig. 1C). The secreted MCs are then internalized by keratinocytes, resulting in a novel membrane-bound compartment that is referred to here as a MC-positive (MC+) organelle. Key support for the exocytosis–endocytosis model comes from *in situ* ultrastructural analyses by electron microscopy of two-dimensional (2D) sections of human skin (Tarafder et al. 2014; Hurbain et al. 2018). In particular, extracellular MCs were closely apposed to both the melanocyte and keratinocyte plasma membranes, while some others appeared to be being engulfed by the keratinocyte. Additional MCs were detected in single-membrane bound compartments in keratinocyte cytoplasm (Tarafder et al. 2014; Hurbain et al. 2018; Fig. 1). Accordingly, the limiting membrane of the newly formed MC+ compartments was devoid of melanosomal proteins such as TYRP1 (Tarafder et al. 2014). These *in situ* data were supported by cell culture studies using a mouse keratinocyte cell line documenting preferential physiological uptake of naked MCs relative to membrane-bound melanosomes (Tarafder et al. 2014; Correia et al. 2018; Moreiras et al. 2020). In this model, internalized MCs would initially behave like any exogenous large particulate material captured by a cell. However, unlike most internalized particles which are subject to phagolysosomal degradation, internalized MCs are long-lived and at least in part protected from degradation within keratinocytes (see below and Tarafder et al. 2014; Hurbain et al. 2018). Hence, these data together suggest that the MC+ compartments represent a novel pigmented "hybrid" organelle in which the luminal content originates from the melanocyte and the limiting membrane originates from the keratinocyte.

The cytophagocytosis model proposes that the keratinocyte engulfs a protruding melanocyte dendrite or filopodium containing multiple pigmented melanosomes (Fig. 1B). Support for this model comes from observations of cultured keratinocytes and melanocytes of newborn guinea pig skin (Wikswo and Szabo 1972; Okazaki et al. 1976; Lambert et al. 2019) or human skin (Singh et al. 2010). This model suggests that the internalized pigments within keratinocytes first reside in a compartment limited by three membranes of different origins; the innermost membrane corresponds to the limiting membrane of the melanosome of the melanocyte, and the outer membranes correspond, respectively, to the two plasma membranes of the melanocyte and the keratinocyte (Fig. 1B). This model was supported by occasional observations of pigmented compartments with double membranes in ultrastructural analyses of skin sections (Wolff 1973). However, it remains unclear if these data reflected cytophagocytosis at the cell periphery or a 2D cross-section of a melanocyte dendrite in close apposition to the keratinocyte. Indeed, double membrane-containing melanin compartments, which would potentially be melanosomes from melanocytes enclosed in a keratinocyte-derived membrane, are not robustly observed *in situ* in more quantitative ultrastructural analyses of keratinocytes within normal or synthetic human skin epidermis (Tarafder et al. 2014; Hurbain et al. 2018; Domingues et al. 2020).

The tunneling nanotube hypothesis proposes that the plasma membranes of melanocytes and keratinocytes fuse and create a thin conduit through which intact pigmented melanosomes pass through to the keratinocyte (Fig. 1D). This model was based on observations in human epidermal keratinocyte (HEK)/human epidermal melanocyte (HEM) co-culture systems in which fusion of melanocyte-

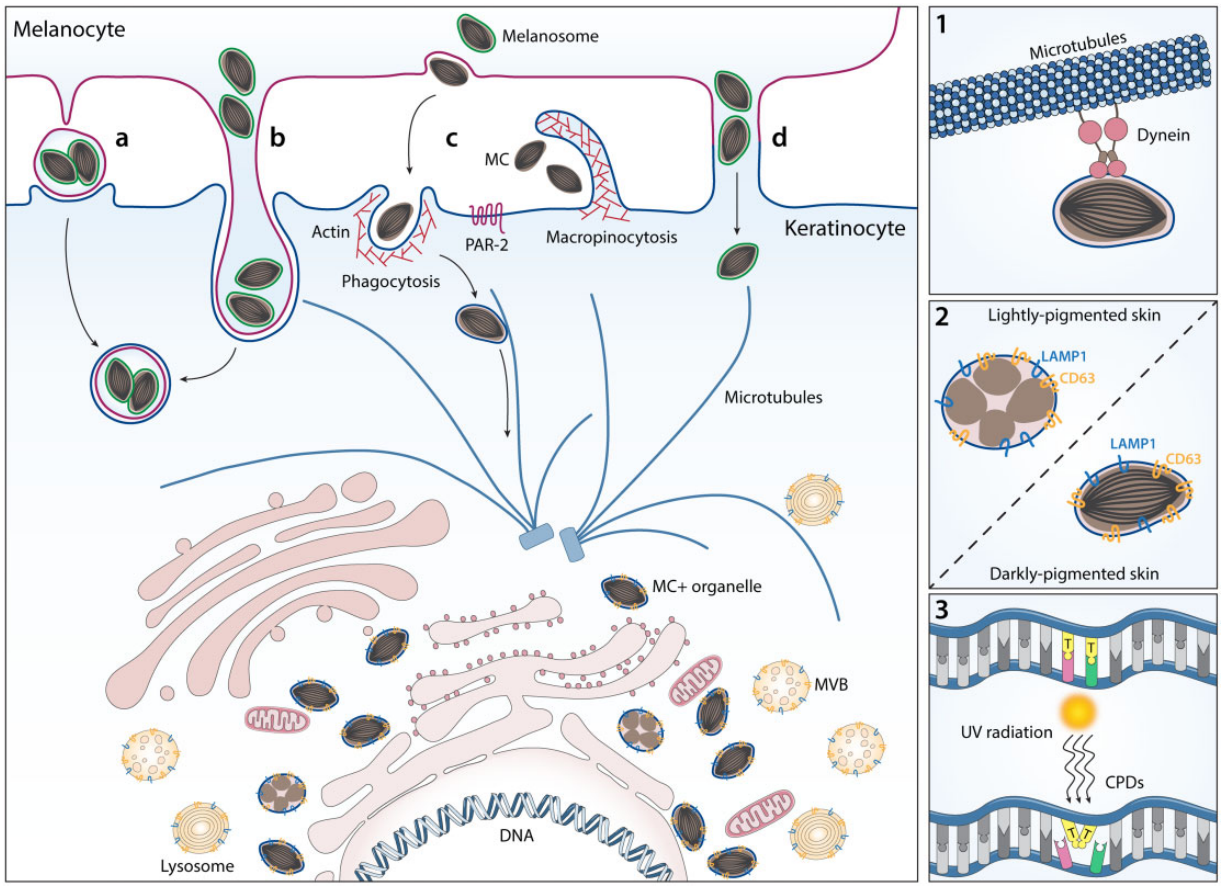

Fig. 1. Model of melanin transfer and the MC+ organelle in human skin keratinocytes. Four different models are proposed to explain the transfer of melanin from melanocytes to keratinocytes: (**a**) the melanocyte sheds a large vesicle that contains melanosomes and is internalized by the keratinocyte; (**b**) the keratinocyte employs cytophagocytosis to internalize a melanocyte dendrite or filopodium that contains multiple melanosomes; (**c**) the melanocyte emits by exocytosis the intraluminal content of melanosome, called the MC, and the MC is then internalized by the keratinocyte; and (**d**) tunneling nanotubes support the exchange of melanosomes from melanocytes to keratinocytes upon fusion of their plasma membranes (red and blue, respectively). While models **a**, **b** and **d** propose the transfer of intact melanosomes (limiting membrane in green), model **c** suggests that the pigmented transferred structure is a MC. The **a**, **b**, and **c** models suggest that the plasma membrane of the keratinocyte must be extensively remodeled by the dynamic rearrangement of actin filaments (red lines) in order to engulf melanin particles through a process of phagocytosis or macropinocytosis. These modes of internalization appear to be regulated by PAR-2 on the plasma membrane of the keratinocyte. Once MCs are endocytosed, they reside in a membrane-bound compartment (MC+ organelle; limiting membrane in blue) that is rapidly distributed to the perinuclear area where they establish close contacts with mitochondria and endoplasmic reticulum. This is likely mediated by centripetal dynein-dependent microtubule-based transport (arrows, **Box 1**). MC+ organelles bear lysosomal components (LAMP1, CD63; **Box 2**), but remain distinct from late endosomes/multivesicular bodies (MVBs) and lysosomes. MC+ organelles in darkly pigmented skin contain one MC, whereas those in lightly pigmented skin contain several MCs (**Box 2**). The main role of the MC+ organelles in keratinocytes is to support photoprotection of the genetic material that can occur through UV-dependent DNA damage, including CPDs (**Box 3**).

derived filopodia with the keratinocyte plasma membrane was reported (Scott et al. 2002). This model, like the cytophagocytosis model, predicts that keratinocytes receive intact melanosomes and not MCs. Although these models are inconsistent with the lack of melanosomal components in MC+ organelles within human keratinocytes *in situ* (Tarafder et al. 2014), it cannot be excluded that melanosome-associated components are rapidly degraded within the keratinocyte cytosol.

The shedding-vesicle model proposes that melanosomes are packaged into large vesicles or "globules" that bud from the melanocyte plasma membrane to be subsequently internalized by keratinocytes (Fig. 1A). This model is supported by imaging data in cell cultures in which pigment-filled globules were produced by HEM, possibly emerging from the tips of melanocyte dendrites (Ando et al. 2011). Moreover, globule-rich fractions isolated from the cultures and incubated with HEK were internalized (Ando et al. 2012), supporting the notion that melanosomes can be transferred, as for the cytophagocytosis model, as intact organelles within a double-membrane bound compartment (Fig. 1A). Days after

ingestion, pigment globules within the HEK tended to disappear and to be replaced by single membrane pigment organelles dispersed throughout the cytosol (Ando et al. 2012). These data suggested that the globule limiting membranes are actively digested to release their melanosome contents. Similar observations were made in mouse and chicken cultured cell models, including live cell imaging of the shedding and capture process (Wu et al. 2012; Tadokoro et al. 2016). As with the two previous models, this model does not explain the lack of melanosome-derived membrane contents within the keratinocyte pigment organelle.

Why might there be multiple transfer routes for one pigment?

Each of these models for melanin transfer is supported by data using distinct experimental systems, suggesting that the differences observed might reflect either peculiarities of the experimental system or true mechanistic differences between the systems. For instance, the exocytosis–endocytosis model (Fig. 1C) was consistently reported in ultrastructural studies of human skin or synthetic human epidermis (unpublished observations; Domingues et al. 2020), but the other proposed models could co-exist depending on the skin area, phototypes, or exposure to solar radiation (skin tanning). It is also possible that distinct mechanisms operate in skin versus hair, or in different species. A deeper understanding of the mechanism(s) of melanin transfer will likely emerge from characterizing molecular components that positively and negatively regulate the process. For example, melanin secretion by melanocytes is facilitated by two RAB GTPases (RAB11B and RAB17; Beaumont et al. 2011; Tarafder et al. 2014) and the exocyst tethering complex (Moreiras et al. 2020). Also, the melanosome positioning to the melanocyte periphery is regulated positively by the tripartite complex Myosin VA-Melanophilin-RAB27A and negatively by melanoregulin (O'Sullivan et al. 2004; Wu et al. 2012).

The intercellular melanocyte–keratinocyte dialogue involves more than just pigment exchange. For instance, keratinocytes secrete both soluble ligands for receptors on melanocytes that promote pigmentation (Yamaguchi and Hearing 2009; Nguyen and Fisher 2019) and extracellular vesicles, such as endosomal-derived exosomes (van Niel et al. 2018), that control melanocyte pigmentation by tuning expression levels of "pigmentation genes" (Lo Cicero et al. 2015). These extracellular cues modulate plasma membrane-associated structures in melanocytes called caveolae (Domingues et al. 2020), which in most types regulate lipid homeostasis, signaling, and mechanotransduction (Lamaze et al. 2017). Eliminating caveolae renders melanocytes unresponsive to keratinocyte stimulation and prevents their ability to form dendrites, establish cell contacts, and transfer pigment in 2D co-cultures and 3D synthetic human epidermis (Domingues et al. 2020). In this manner, keratinocytes can modulate the capacity of melanocytes to transfer pigment. Additional studies are needed to reveal how melanocytes integrate extracellular stimuli into intracellular events required for pigmentation.

Melanin entry into keratinocytes

Whereas the past two decades have provided major insights into melanin production by melanocytes and modest insights into melanin transfer from melanocytes, the mechanisms promoting pigment internalization by keratinocytes remain almost completely unknown. Given the large size of melanosomes (~0.3–0.5 μm in diameter), melanins are most likely internalized by phagocytosis and/or micropinocytosis (Fig. 1). Phagocytosis is receptor-mediated and leads to the internalization of large particles, while macropinocytosis can be either constitutive or induced in response to stimuli and involves the uptake of extracellular fluid that can include particles. Both mechanisms are mediated by dynamic local rearrangements of the actin cytoskeleton and on small GTPases, which together promote shaping of the phagocytic/macropinocytic cup and consequent closure (Swanson 2008). Indeed, melanin entry in guinea pig skin-derived keratinocytes co-cultured with melanocytes required an intact actin cytoskeleton network (Wikswo and Szabo 1972).

Keratinocytes can ingest beads (Wolff and Konrad 1972) or bioparticles (Sharlow et al. 2000), indicating that they can employ phagocytosis and/or macropinocytosis. Melanin might be internalized by either process, depending on the cell model and/or the conditions studied. The phagocytic activity of keratinocytes requires the protease-activated receptor-2 (PAR-2, Fig. 1) (Seiberg 2001), a G-protein-coupled receptor that is highly homologous to the thrombin receptors PAR-1, -3, and -4 (Nystedt et al. 1994; Santulli et al. 1995). Keratinocytes express both PAR-1 and PAR-2, (Marthinuss et al. 1995; Santulli et al. 1995; Seiberg et al. 2000b), both of which are activated by the serine protease-dependent cleavage of their extracellular amino terminal domain. The resulting amino termini function as tethered ligands that undergo a conformational change to bind to

and activate the receptor. Activation of PAR-2, but not of PAR-1, by trypsin or its specific peptide agonist potentiates keratinocyte pigmentation *in vitro* and *in vivo* (Seiberg et al. 2000b), at least in part by activating the Rho GTPase and adenylate cyclase (Scott et al. 2003). PAR-2 activation in macrophages and fibroblasts increases phagocytic activity (Sharlow et al. 2000); accordingly, in keratinocyte cell lines and HEK, PAR-2 activation promotes the uptake of synthetic melanin, purified melanosomes, beads, or *Escherichia coli* (Seiberg et al. 2000a, 2000b; Sharlow et al. 2000). These data suggest that PAR-2 activation might stimulate non-specific particle uptake by macropinocytosis. By contrast, in a mouse keratinocyte cell line, PAR-2 activation stimulated, while PAR-2 silencing impaired, the specific uptake of MCs, but not of membrane-bound melanosomes (Correia et al. 2018)—more suggestive of receptor-mediated phagocytosis. Much of the variability among studies might be linked to the use of different stimuli, cell models, and/or cell sources (e.g., lightly, intermediate, or darkly pigmented skin). Future studies should consider these factors to determine if a specific melanin receptor exists on keratinocytes. To date, it is still unclear whether the specificity of the pigment transfer system *in vivo* relies on the physical proximity between melanocytes and keratinocytes within the epidermis, and/or whether these cells are equipped with a molecular machinery optimized for that function.

How does PAR-2 activation result in melanin uptake? Although the physiological process leading to PAR-2 activation in the skin is not known, addition of a PAR-2 agonist to cultured cells increased secreted protease activity (Sharlow et al. 2000). This could create a positive feedback loop to stimulate phagocytosis. PAR-2 might thus function either as a receptor or as a co-receptor. Interestingly, UV-B radiation potentiates pigmentation at least in part by stimulating PAR-2; PAR-2 inhibition with serine protease inhibitors results in depigmentation *in vivo* and prevents UV-B-induced pigmentation (Seiberg et al. 2000b). This effect is mediated at least in part by upregulation of PAR-2 expression in keratinocytes (Scott et al. 2001; Enomoto et al. 2011) and/or by down-regulation of a serine protease inhibitor that prevents PAR-2 activation (Abts et al. 1999). In addition to PAR-2, keratinocyte growth factors (Cardinali et al. 2005) or toll-like receptor 3 (Koike et al. 2019) promote phagocytic activity in keratinocytes through activation of Rho family GTPases. The specific signaling events triggered by receptor activation remain elusive.

In moderately and lightly pigmented skins, MCs are predominantly found grouped in clusters enclosed by a single membrane (Fig. 1, Box 2) only within keratinocytes of the most basal epidermal layers (Szabó et al. 1969). By contrast, in highly pigmented skins, a single MC enclosed within a limiting membrane predominates within keratinocytes all over the epidermis (Szabó et al. 1969; Minwalla et al. 2001; Thong et al. 2003; Yoshida et al. 2007; Hurbain et al. 2018; Fig. 1, Box 2). Both isolated and clustered distributions co-exist in skin with intermediary phototypes, such that the ratio of MC clusters to single MC is inversely proportional to the level of skin pigmentation—that is, the more pigmented the skin, the more isolated the pigment (Hurbain et al. 2018). While this differential distribution appears to be determined by the source of the keratinocyte, it is not clear whether it represents a different mechanism of uptake (e.g., phagocytosis vs. macropinocytosis) or differential distribution following internalization. Kinetic studies of melanosome or MC uptake in keratinocytes from skins with distinct phototypes will be valuable to better understand these processes.

MC compartment trafficking and positioning within keratinocytes

Melanin pigments that have been internalized by keratinocytes are not randomly distributed, and accumulate in the perinuclear region (Fig. 1) to form a cap over the nucleus that is sometimes called a microparasol (Kobayashi et al. 1998; Gibbs et al. 2000). Microparasol formation can be stimulated *in vitro* by UV irradiation (Gibbs et al. 2000), but very little is known regarding the intracellular trafficking mechanisms and machineries that drive the pigment to its final perinuclear destination (Gibbs et al. 2000; Byers et al. 2003; Hurbain et al. 2018).

To travel long distances from the plasma membrane to the perinuclear area, the pigment is likely transported along cytoskeletal paths by motor proteins. In keratinocytes, the centripetal transport of pigment organelles was proposed to rely on dynein (Byers et al. 2003), a minus end-directed microtubule-based motor (Fig. 1, Box 1). This conclusion was based on co-distribution of the dynein intermediate chain with the melanin supranuclear cap *in situ* and with perinuclear pigment aggregates *in vitro*, and by the dispersion of melanosomes in cells depleted of dynein heavy chain (Byers et al. 2003). A following study proposed that dynactin—a protein complex that cooperates with dynein in centripetal cytoplasmic motility—is also required for the positioning of fluorescent microspheres to the perinuclear area of

keratinocytes (Byers et al. 2007), suggesting that both the natural and synthetic ingested particles by keratinocytes are fated for perinuclear positioning. Further studies using more physiological pigment sources are needed to define the directionality, speed, and timing of pigment organelle movement and the potential role of other cytoskeletal elements, such as actin, intermediate filaments, or septin filaments in defining pigment organelle positioning.

The nature and maturation of the MC organelle in keratinocytes

As described above for melanin uptake, the cellular and molecular events controlling the biogenesis and maintenance of the MC+ organelle in keratinocytes are poorly understood. Contributing to our knowledge deficit is the lack of robust and validated physiological models of keratinocyte pigmentation. For instance, the cellular behavior of ingested beads has been extensively used as a proxy to mirror melanin processing in keratinocytes, but it is not clear whether such beads are handled in the same way as melanin pigments (Correia et al. 2018). By contrast, recent studies have provided insight into the organization of physiological pigment in keratinocytes *in situ* (Hurbain et al. 2018) and *in vitro* (Correia et al. 2018).

Consistent with earlier qualitative analyses (Szabó et al. 1969), the MC+ organelles of keratinocytes in human skins of different phototypes vary in number and ultrastructure depending on the pigmentation level (Hurbain et al. 2018); in addition to their differential distribution throughout epidermal layers, they are more numerous and isolated in darker skins, and less numerous but more clustered in lighter skins (Fig. 1, Box 2). Nevertheless, all MC+ compartments are enclosed by a single limiting membrane in organelles that are often perinuclear and closely apposed to endoplasmic reticulum and/or mitochondrial membranes (Fig. 1). Despite the presence of lysosomal membrane proteins such as LAMP1 and CD63 (Fig. 1, Box 2), these organelles are neither degradative nor acidic, and lack markers of autophagosomes (Hurbain et al. 2018); similar observations were made in a cultured mouse keratinocyte cell line (Correia et al. 2018). Recent data suggest that these organelles are enriched in a specific subset of RAB GTPases (Marubashi and Fukuda 2020). These data would suggest that the MC+ organelles are, like the melanosomes in melanocytes, members of the lysosome-related organelle (LRO) family and function as protective structures dedicated to melanin integrity.

The studies cited above detail features of MC+ organelles at steady-state, but a molecular description of the formation and maturation of the organelle is still lacking. A number of key questions remain. How is the internalization of pigment and the acquisition of the lysosome-like signature of the resulting MC+ organelle spatiotemporally orchestrated? Does the initial internalized structure mature through sequential interactions with the endolysosomal system like phagosomes in macrophages (Fairn and Grinstein 2012)? The early endosomal RAB5 is required for MC entry (Correia et al. 2018), but does this reflect a requirement for fusion of the internalized MC+ structure with early endosomes or an indirect requirement for endosomes in organelle maturation? Are the MC+ structures observed at steady state unique LROs (Delevoye et al. 2019) or are they post-lysosomal structures from which the acidification machinery and proteolytic enzymes have been removed, as observed after autophagolysosome formation (Rong et al. 2012)? Members of the LRO family share features such as the presence of CD63 and RAB27, the intersection of the endosomal and/or secretory pathways, and in most cases (but not all) the stimulus-dependent exocytosis of their contents (Delevoye et al. 2019). While a clear identification of the MC+ organelle as an LRO has yet to be demonstrated, the MC+ organelle can acquire endosomal components such as RAB5, RAB7, CD63, and LAMP1 (Correia et al. 2018; Hurbain et al. 2018); whether any components derive from the secretory pathway is not yet known. Transfer of pigment from keratinocyte to keratinocyte has been observed *in vitro* (Scott et al. 2002), and we hypothesize that keratinocyte-to-keratinocyte MC transfer could occur physiologically upon some stimulus, such as sun exposure, in order to efficiently distribute the pigment to upper epidermal keratinocyte layers. Future in depth studies in a robust model system are necessary to address these knowledge gaps.

Another major question in the field is the fate of the MC+ compartments in keratinocytes from different phototypes. A common fate of the internalized content of phagosomes or macropinosomes in other cell types is degradation upon fusion with lysosomes, which is accompanied by luminal acidification and activation of hydrolases (Fairn and Grinstein 2012). Indeed, experiments using co-cultured melanocytes and keratinocytes suggest that the melanin transferred to keratinocytes can be degraded, and more so in cells from light skin than in cells from dark skin (Ebanks et al. 2011); this difference has been ascribed to higher autophagic activity in keratinocytes from light skin relative to dark skin (Murase

et al. 2013). Recent studies suggest that RAB7B (a.k.a. RAB42; Marubashi and Fukuda 2020) and cathepsin V (Homma et al. 2018), perhaps through autophagy-related processes (Kim et al. 2020), promote melanin degradation in cultured keratinocytes. However, in human skin samples, MC+ organelles are likely long-lived within non-degradative, non-acidic compartments within keratinocytes of the different human skin layers (Hurbain et al. 2018). This raises several fundamental questions. Is melanin degradable? If so, is MC+ organelle maturation stopped prior to lysosome fusion, or are the organelles that accumulate within keratinocytes derived from lysosomes that have subsequently lost their degradative capacity? If the pigment escapes degradation, does it reflect a chemical property of melanin, or are keratinocytes able to "sense" melanins and consequently protect them? Does the pigment source drive its own intracellular fate? These questions warrant rigorous experimental attention.

Melanin at the heart of photoprotection

A major biological function of melanin in the skin is photoprotection from UV exposure that can cause skin cancers. Skin cancers are common forms of cancer with increasing incidence (Tran et al. 2008) and include melanoma, basal cell carcinoma, and squamous cell carcinoma. Exposure to UV from chronic sun exposure or artificial sources such as tanning beds can result in the appearance of precancerous skin lesions such as actinic keratoses that, if not rapidly treated, may give rise to carcinoma (Ratushny et al. 2012), or to mutations in melanocytes or melanoblasts that result in melanoma (Garibyan and Fisher 2010). UV acutely causes DNA photodamage, such as cyclobutane pyrimidine dimers (CPDs; Fig. 1, Box 3) and pyrimidine (6-4) pyrimidone photoproducts (64PP; Brash 2016), that can be sustainably generated in pigmented melanocytes (Premi et al. 2015) and lead to mutagenesis and subsequent carcinogenesis (Tadokoro et al. 2003; Cadet et al. 2015). Keratinocytes are the first skin cells to be exposed to such threats, and thus have evolved UV-protective strategies that include active DNA repair mechanisms and pigmentation (Tran et al. 2008).

Epidemiological studies have demonstrated that darker skin individuals have lower incidence of skin cancer than those of lighter skin (Worswick et al. 2008). Skin pigmentation protects against UV damage through the photoprotective and absorptive properties of melanin. Melanins absorb UV photons, and thus reduce the physical exposure to sensitive biomolecules such as DNA. Moreover, eumelanins absorb reactive oxygen species produced after photo-oxidation of lipids from cellular membranes (Gilchrest et al. 1999); by contrast, pheomelanins can generate reactive oxygen species (Wenczl et al. 1998). For more detailed information about the photoprotective properties of melanins, UV irradiation, and consequences on skin pigmentation, we refer readers to several relevant reviews (Brenner and Hearing 2008; Ito et al. 2018; Nguyen and Fisher 2019). As a consequence of the photoprotective properties of melanins, dark skin has a sun protection factor (SPF) of 10–15, whereas light skin has an SPF approaching 2.5 (Kollias et al. 1991). This likely reflects not only higher total melanin content but also the eumelanin/pheomelanin ratio (Micillo et al. 2016; Swope and Abdel-Malek 2018). While excessive UV solar radiation is a harmful environmental stress, moderate levels of UV radiation can be considered as a physiological stimulus. Indeed, the relationship between pigmentation and photoprotection is much more complex, particularly given that melanogenesis is initiated by CPDs and their repair by keratinocytes upon UV irradiation (Liu and Fisher 2010). Hence, melanogenesis in melanocytes can be seen as a response to DNA damage in keratinocytes.

Conclusions and perspectives: Is the cell biology of melanin organelles important for photoprotection?

Most aspects of the cell biology of the MC+ organelles in keratinocytes remain uncertain. Therefore, whether or not the biogenesis, trafficking, and positioning of the organelles contribute to photoprotection of the keratinocyte DNA is still unknown. The positioning of the MC+ organelles to the microparasol above the nuclei is consistent with the theory that their primary function is to protect the keratinocyte DNA from damage. However, although the quantity of DNA damage photoproducts inversely correlates with skin tone and quantity of supranuclear melanin caps (Kobayashi et al. 1998; Del Bino et al. 2006), the field still lacks a formal causative link between MC+ organelle content, organelle positioning, and photoprotection and an experimental approach to test it. A better understanding of the cell biological processes underlying the entry, transport, biogenesis, positioning, and maintenance of the pigment organelles in keratinocytes is therefore crucial to address whether these processes influence skin photoprotection. For example, the observation that the MC+ organelle has properties of an LRO suggests that patients with LRO biogenesis diseases, such

as the Hermansky-Pudlak syndromes or Chediak-Higashi syndrome, might impact not only the formation of melanins in melanocytes but also the maturation of the MC+ organelle in keratinocytes. Finally, defining whether melanin itself drives some of these fundamental processes and how the genetic background of the keratinocyte alters them will help to define new experimental approaches to dissect the links between MC+ organelle structure and function. One of the first challenges faced by researchers in the field is to establish robust, flexible, and easy to use experimental cell model systems that accurately reflect keratinocyte pigmentation *in situ*. Such a gold standard will be an invaluable tool for future research, much as robust cultured melanocyte systems (Benito-Martinez et al. 2020) have allowed new advances in understanding melanosome biogenesis in pigment producing cells (Le et al. 2021).

Acknowledgments

We thank past and present members of the Raposo and Marks laboratories for many contributions and discussions, and Leslie B. King for careful editing of the manuscript.

Funding

The following funding sources contributed to this work: National Institutes of Health grants R01 EY015625 (to M.S.M. and G.R.) from the National Eye Institute, R01 AR076241 (to M.S.M., Sarah Tishkoff, and Elena Oancea) and R01 AR071382 (to M.S.M. and Elena Oancea) from the National Institute for Arthritis, Musculoskeletal and Skin Diseases; the Fondation pour la Recherche Médicale (FRM team label EQU201903007827 to G.R. and FDT202001010801 to S.B.-M.), the Fondation ARC pour la Recherche sur le Cancer (ARCPJA22020060002267 to C.D.), the Agence Nationale de la Recherche (ANR; "MYOACTIONS" ANR-17-CE11-0029-03 to C.D. and Anne Houdusse), GENESPOIR (to C.D.), LabEx Cell(n)Scale (ANR-11-LABX-0038), L'Oréal Research and Development, Institut National de la Santé et de la Recherche Médicale (INSERM), Institut Curie and the Centre National de la Recherche Scientifique (CNRS). S.B.-M. has received funding from the European Union's Horizon 2020 Research and Innovation Programme under the Marie Sklodowska-Curie grant agreement No. 666003.

Conflict of interest

The authors declare no conflict of interest.

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