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Ion transport in pigmentation

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Abstract

Skin melanocytes and ocular pigment cells contain specialized organelles called melanosomes, which are responsible for the synthesis of melanin, the major pigment in mammals. Defects in the complex mechanisms involved in melanin synthesis and regulation result in vision and pigmentation deficits, impaired development of the visual system, and increased susceptibility to skin and eye cancers. Ion transport across cellular membranes is critical for many biological processes, including pigmentation, but the molecular mechanisms by which it regulates melanin synthesis, storage, and transfer are not understood. In this review we first discuss ion channels and transporters that function at the plasma membrane of melanocytes; in the second part we consider ion transport across the membrane of intracellular organelles, with emphasis on melanosomes. We discuss recently characterized lysosomal and endosomal ion channels and transporters associated with pigmentation phenotypes. We then review the evidence for melanosomal channels and transporters critical for pigmentation, discussing potential molecular mechanisms mediating their function. The studies investigating ion transport in pigmentation physiology open new avenues for future research and could reveal novel molecular mechanisms underlying melanogenesis.

Introduction

Melanin is the primary pigment in mammals that colors eyes, skin, and hair. Melanin is critical for human health, protecting the eyes and skin against harmful solar ultraviolet radiation (UVR). Melanin is synthesized, stored, and transported in unique lysosome-related organelles called melanosomes. Melanosomes are present in mammalian skin melanocytes, uveal melanocytes, retinal pigment epithelial (RPE) cells, and in melanophores, a class of pigment-containing cells in non-mammalian vertebrates. Defects in the complex mechanisms involved in melanin synthesis and regulation result in impaired visual system development, vision defects, and pigmentation deficits that increase the susceptibility of the skin and eye to cancer (1, 2). Mouse coat-color, and zebrafish pigmentation mutants, together with population genetics have proven useful in identifying genes that regulate melanogenesis, including genes that encode putative ion transport proteins (3, 4). These

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genes often regulate basal pigmentation; mutations that disrupt their function result in oculocutaneous albinism or other pigmentation disorders (3, 5). Although many genes encoding ion transport proteins have been identified as key regulators of melanin synthesis, ion transport physiology remains a largely understudied component of pigment cell biology.

Ion transport across cellular membranes is involved in nearly every aspect of biology, including neuronal communication, immune function, development, and many others. Conventionally, the proteins able to transport ions across membranes have been divided into two groups: channels, which allow ions to diffuse down their electrochemical gradient at more than a million ions per second, and transporters, including pumps and carriers, which undergo conformational changes to transport ions against electrochemical gradients, hence producing and maintaining differences in ion concentrations between cellular compartments. Although increasing evidence of functional overlap made this classification of ion channels and transporters more ambiguous in recent years (6), both forms of ion transport remain critical for biological function.

The molecular mechanisms by which ion transport regulates melanogenesis are poorly understood. It is well established, however, that transport of ions across cellular membranes is capable of regulating important cellular functions by modulating enzymatic activity, gene transcription, and other cellular processes. Recent studies began to elucidate how ion transport contributes to melanocyte physiology and melanogenesis. Such studies have changed and will continue to transform our understanding of the molecular mechanisms underlying pigmentation.

In this review we discuss ion transport across the plasma membrane of melanocytes, between the extracellular environment and cytosol, the channels and transporters that mediate it and the ions they carry. In the second part we consider ion transport across the membrane of organelles, with emphasis on the pigment cell-specific melanosomes, where melanin is synthesized and stored. We then discuss how membrane voltage, which is regulated by ion transport, and calcium (Ca^{2+}) ions could modulate melanogenesis in pigment cells.

Ion transport across the plasma membrane of melanocytes

Several ion channels in melanocytes have been shown to function at the plasma membrane and modulate basal or facultative pigmentation. Three of these channels belong to the Transient Receptor Potential (TRP) family: TRPM1, TRPM7 and TRPA1, and the fourth is the Ca^{2+} release activated Ca^{2+} (CRAC) channel, which allows Ca^{2+} to enter the cell in response to receptor-mediated Ca^{2+} release from endoplasmic reticulum (ER). TRP channels form a large family of ion channels with diverse functions (7). Many TRP channels, including TRPM1, TRPM7, and TRPA1, allow positively charged ions to enter the cell in a nonselective manner. One of the cations passing through the pore of TRP channels is Ca^{2+} . Ca^{2+} is kept at very low concentrations in the cytosol by complex homeostasis mechanisms and often serves as a signaling ion responsible for mediating important downstream effects. Thus, TRP channels are referred to as nonselective and Ca^{2+} -permeant.

TRPM1, the founding member of the melastatin (M) family of TRP channels, was identified as a gene downregulated in aggressive melanoma (8). TRPM1 ion channels function in ON bipolar cells, which are neurons in the retina that depolarize in response to light signals transmitted from photoreceptors, thus mediating transmission of visual stimuli to the brain. TRPM1 channels are negatively regulated by light in ON bipolar cells via a mechanism not well understood (reviewed in (9) (10)). TRPM1 has also been implicated in melanocyte pigmentation, a fact first revealed by the Appaloosa Horse phenotype: light coat color with spotting patterns associated with congenital stationary night blindness (11). Patch-clamp recordings of ON bipolar cells from TRPM1 deficient mice (12-14) and of human melanocytes with reduced TRPM1 levels (using short interference RNA (siRNA)) (15) suggest that TRPM1 mediates a nonselective, Ca^{2+} -permeable current.

In melanocytes TRPM1 expression is regulated by microphthalmia-associated transcription factor (MITF) (16), which is essential for melanocyte differentiation. Reducing TRPM1 expression in human epidermal melanocytes results in decreased cellular melanin content (15), suggesting that TRPM1 levels correlate with basal pigmentation. TRPM1 has also been suggested to play a role in facultative pigmentation in response to UVR. Exposure to UVB leads to the activation of p53, MITF-mediated upregulation of TRPM1 expression, increased Ca^{2+} uptake, and increased pigmentation (17), (Fig. 1). These findings are challenged by the fact that humans with TRPM1 mutations have congenital stationary night blindness, but no pigmentation defects (18-20). Until the molecular mechanisms that connect TRPM1 function and pigmentation are elucidated in more detail, the contribution of TRPM1 to melanin content and regulation *in vivo* remains hypothetical.

TRPM7 is a ubiquitously expressed member of the melastatin TRP subfamily that forms a nonselective channel fused to a C-terminal alpha kinase of unknown function (21). TRPM7 channels are nonselective cation channels that mediate a current that is inhibited by Mg^{2+} (22) (Fig. 1). TRPM7 mutations in zebrafish lead to death of melanophores (23) and targeted disruption of TRPM7 in neural crest during mouse embryo development results in loss of pigment cells (24), suggesting that TRPM7 is important for the development of pigment cells, but its function in pigmentation remains unknown.

TRPA1 channels form the lone member of the mammalian ankyrin (A) TRP subfamily, named for the presence of multiple ankyrin repeats in the N-terminus of the channels. TRPA1 is expressed and functions in human epidermal melanocytes (25-27). TRPA1 mediates a non-selective, Ca^{2+} -permeant current activated by chemical irritants and reactive oxygen species and modulated by Ca^{2+} and other cellular messengers (28). In human epidermal melanocytes, physiological UVA doses activate TRPA1 channels downstream of a G protein-coupled signaling cascade. Interestingly, this signaling cascade requires retinal, a vitamin A derivative present in the serum, which is a critical component of light-sensitive receptors named opsins (26, 29). TRPA1 activation causes Ca^{2+} influx and an increase in the membrane potential of human melanocytes, both of which contribute to the rapid increase in cellular melanin content in response to UVA (26, 29, 30) (Fig. 1). Higher UVA doses also activate TRPA1, albeit through a different mechanism involving reactive oxygen species (31), which has not been shown to regulate cellular melanin content.

STIM/ORAI, the molecular determinants of the Ca^{2+} release activated Ca^{2+} (CRAC) channels, are expressed in many tissues, including human epidermal melanocytes, where they appear to play a role in endothelin-induced melanogenesis (32). Endothelin-1 is released by epidermal keratinocytes in response to UVR exposure and activates endothelin receptors on neighboring melanocytes (32-35). Activation of endothelin receptors leads to Ca^{2+} release from internal stores, which triggers Ca^{2+} influx via the ORAI1/STIM pathway, causing a prolonged Ca^{2+} response that increases the activity of the melanogenic enzyme tyrosinase and melanin content (32) (Fig. 1). SiRNA-mediated downregulation of ORAI1 in melanocytes inhibits endothelin-1-induced increases in cellular melanin content (32), suggesting that ORAI1 expression contributes to UVR-induced facultative pigmentation.

Ion transport across membranes of intracellular organelles

Intracellular organelles like endosomes and lysosomes (collectively called endolysosomes) have complex functions and regulatory mechanisms that often involve ion transport across their membranes. Until recently, most of what we knew about the function of endolysosomal ion channels and transporters was based on phenotypes resulting from mutations in these molecules. Recent modifications of the classical patch-clamp technique have enabled direct recordings of ion transport in endolysosomes (48, 49), allowing for electrophysiological characterization of a number of organellar ion channels and elucidation of their signaling mechanisms. Melanosomes are lysosomal-related organelles derived from early-endosomes (50, 51). Melanosomes have a number of proteins in common with lysosomes and endosomes, but also express many specific channels and transporters that have been identified as critical regulators of melanin production and storage. Below we first discuss endolysosomal ion channels/transporters that affect pigmentation and then consider melanosome-specific ion transport mechanisms.

Endolysosomal ion transport relevant to pigmentation

TRPML channels belong to the mucolipin (ML) subfamily of TRP channels and have recently been found to regulate endosomal and lysosomal functions, as mutations in these channels result in lysosomal storage disorders (52). Combinations of the three TRPML isoforms can form heteromeric channels and have different localization patterns: TRPML1 and TRPML2 localize primarily to intracellular compartments, including late endosomes and lysosomes, while TRPML3 is found both in endosomal compartments and at the plasma membrane (52). TRPMLs are nonselective channels that allow Na^+ , Ca^{2+} , Fe^{2+} and potentially other cations, to pass from the lumen of the organelles into the cytosol (Fig. 2). Functionally, TRPMLs have been implicated in iron homeostasis (49), membrane and organelle trafficking (53), and exocytosis (54). A gain-of-function mutation in TRPML3, which is highly expressed in melanocytes, is responsible for the varitint-waddler phenotype in mice, characterized by hearing and pigmentation defects (55). Melanocytes expressing mutated TRPML3 exhibit cytosolic Ca^{2+} overload that results in loss of adhesion, membrane integrity, and ultimately in cell death. The contribution of other TRPML isoforms to melanocyte physiology has not been investigated, but their localization and roles in iron homeostasis and vesicular fusion make them likely candidates to regulate melanosomal function.

TPC (two-pore channels) are intracellular channels expressed in acidic organelles (56). The TPC1 and TPC2 isoforms are expressed ubiquitously in human and rodent cells, where they localize to late endosomes and lysosomes and were initially characterized as intracellular NAADP-regulated Ca^{2+} release channels (56, 57). However, direct patch-clamp recordings of TPC1 and TPC2 activity in endolysosomes showed that they function as Na^{+} -selective channels modulated by the vesicular phosphatidylinositol bisphosphate PI(3,5)P₂ (58) (Fig. 2). TPC channels are activated by decreases in intracellular ATP concentration triggered by depletion of extracellular nutrients via a mechanistic target of rapamycin (mTOR)-dependent pathway (59). Interestingly, inhibition of mTOR in human primary melanocytes leads to elevated transcription of melanogenic enzymes and increased formation of mature melanosomes (60). Taken together, these findings suggest that mTOR modulation of TPC activity could regulate melanosome maturation.

TPC1 controls the excitability of endolysosomal membranes in a cytosolic pH dependent manner (61), while TPC2 has been implicated in organelle fusion and pH regulation (62). Recent evidence suggests that the discrepancies between TPCs ion permeability and modulation could be due to their regulation by Mg^{2+} and protein kinases, which can unmask the direct regulation by NAADP (63). The localization and function of TPC channels in pigment cells has not yet been investigated, but single nucleotide polymorphisms (SNPs) in TPC2 correlate with human hair color (64). TPC subcellular localization together with the TPC2 effect on pigmentation suggests that these channels could be regulators of melanosomal physiology and pigmentation.

CIC-7 (chloride (Cl^{-}) channel, isoform 7) is a member of the CIC family that mediates $\text{Cl}^{-}/\text{H}^{+}$ exchange in lysosomes, conducting Cl^{-} into the cytosol and transporting H^{+} inside the lumen (65) (Fig. 2). CIC-7 requires the membrane protein Ostm1 as a subunit for exchanger activity and proper function in lysosomes (66, 67). In melanocytes, CIC-7 expression is regulated by MITF (66, 68) and mutant mice lacking CIC-7 or Ostm1 have reduced coat pigmentation in addition to bone defects (*grey-lethal* mouse) (66, 68, 69), suggesting that CIC-7 is important for melanocyte survival or/and function. The $\text{Cl}^{-}/\text{H}^{+}$ exchanger function of CIC-7 suggests that it primarily contributes to the acidification of lysosomes. However, replacing wild-type CIC-7 with a single amino acid mutant unable to transport H^{+} , rendering CIC-7 solely as a Cl^{-} conductor, rescues the grey-lethal pigmentation phenotype in CIC-7^{-/-} mice (70). The role of CIC-7 mediated Cl^{-} transport in pigmentation is challenged by a recent report in which a mutant CIC-7 unable to transport neither measurable H^{+} nor Cl^{-} exhibits no pigmentation phenotype (71). This result implies that the CIC-7 expression, independent of ion transport activity, is sufficient to rescue the pigmentation defects in *grey-lethal* mice. These findings suggest that while H^{+} exchange is important for pH regulation, mere CIC-7 expression in lysosomes and potentially melanosomes is critical for organelle function, suggesting protein interactions may be responsible for the function of CIC-7 in pigmentation (72).

Luminal Cl^{-} and pH are critical regulators of organellar biogenesis. Luminal Cl^{-} concentrations vary dramatically in the endosomal pathway and inversely correlate with luminal pH (73), therefore it is possible that Cl^{-} transporters or channels modulate pH gradients to influence endosomal function and maturation. In early endosomes luminal Cl^{-}

concentration is predicted to be ~20 mM and pH 6 (72), while in lysosomes Cl^- concentration is estimated at >80 mM and pH at < 5 (72). In addition to direct modulation of pH, luminal Cl^- may modulate cationic channels (48), which, in turn, can affect luminal pH (74). Melanosomes are lysosome-related organelles hypothesized to have acidic pH at early stages, important for melanosome formation, and alkalinize as they mature and become melanized (76). Considering the possible regulatory effect of luminal Cl^- concentration on pH in endosomal-related organelles, melanosomes may use melanosome-specific Cl^- channels or transporters to modulate luminal Cl^- concentration and pH, which might affect melanosomal differentiation within the endosomal pathway.

Melanosomal ion transport proteins

Among the naturally occurring pigmentation associated mutations in mice and zebrafish, at least three genes code for putative melanosomal transporters: OCA2 (p-protein), SLC45A2 (MATP), and SLC24A5 (NCKX5) (77, 78). Mutations in all three genes affect melanin synthesis and result in oculocutaneous albinism in humans, characterized by severe skin and eye pigmentation defects and increased melanoma risk (79-81). In addition, non-pathological polymorphisms in these genes have been associated with pigmentation variation in human populations (81). Despite the immense importance of OCA2, SLC45A2, and SLC24A5 to pigmentation, their function remains largely unknown.

OCA2 (p-protein) is mutated in the *pink-eye dilution* mouse, characterized by hypopigmentation of the fur and eyes, and oculocutaneous albinism type 2 (OCA2) in humans (79, 82-84). OCA2 encodes a melanosomal protein with a predicted structure consisting of 12 transmembrane domains, shares homology with bacterial anion transporters (79, 84-86) (Fig. 2), and has been implicated in the regulation of melanosomal pH and trafficking of tyrosinase to melanosomes (85, 87, 88). OCA2-deficient melanocytes lack melanin, perhaps due to poor tyrosinase targeting and low enzymatic activity of the tyrosinase found in melanosomes, both of which could be regulated by OCA2-mediated changes in melanosomal pH.

SLC45A2 (membrane-associated transporter protein (MATP), also known as AIM-1) is the gene mutated in the *underwhite* mouse, characterized by severely hypopigmented fur and eyes (89), and causes oculocutaneous albinism type 4 (OCA4) in humans (80, 90, 91). It encodes a putative melanosomal transporter, the localization and function of which remains unclear. The predicted SLC45A2 protein structure consists of 12 transmembrane domains that share homology with sucrose/proton symporters, including a conserved sugar recognition sequence in a cytosolic loop (90, 92) (Fig. 2). SLC45A2 is also involved in the regulation of melanosomal pH (93), tyrosinase trafficking, and the regulation of melanosomal structure (94).

SLC24A5 (NCKX5) is a member of the K^+ -dependent $\text{Na}^+/\text{Ca}^{2+}$ exchanger family (NCKX) (95) (Fig. 2). Mutations in SLC24A5 underlie the zebrafish *golden* phenotype, characterized by hypopigmentation (78), and cause oculocutaneous albinism type 6 (OCA6) in humans (5). A SNP in SLC24A5 resulting in a point mutation correlates with natural human pigmentation variation (78). SLC24A5 may localize to the melanosome or trans-Golgi

membranes and appears to regulate melanosome maturation and melanogenesis (96, 97). Because increases in intracellular Ca^{2+} elicit changes in cellular melanin content (26, 32, 98), the $\text{Na}^+/\text{Ca}^{2+}$ exchanger activity of SLC24A5 (96) may provide a link between cytosolic and melanosomal Ca^{2+} signaling by regulating Ca^{2+} transport from cytosol to melanosome lumen.

ATPases: **ATP7A** is a copper transporting ATPase that localizes to melanosomes and supplies copper (Cu^{2+}) to the Cu^{2+} -dependent enzyme tyrosinase, thus sustaining melanin synthesis (99) (Fig. 2). Vacuolar ATPases (**V-ATPase**) are H^+ pumps present in the melanosomal membrane that are critical for regulating melanosomal pH (Fig. 2), an important modulator of melanin synthesis due to the pH-dependence of tyrosinase, which has the highest enzymatic activity at near neutral pH (75, 100). Interestingly, pharmacological inhibition of the V-ATPase partially rescues the phenotype of OCA2 and SLC45A2 mutants in zebrafish and cultured melanocytes (93, 101-103). These results suggest that pH dysregulation is a common mechanism for OCA2- and SLC45A2-related pigmentation defects.

OA1 (ocular albinism 1) is named after the disease caused by mutations in the OA1 gene, which result in lack of pigmentation in the eyes. OA1 is a unique organellar G protein-coupled receptor (GPCR) critical for melanosome biogenesis (104-107) (Fig. 2). L-DOPA, a melanin precursor, has been hypothesized to activate melanosomal OA1, but its signaling pathway remains elusive (108). GPCRs are important regulators of ion channels at the plasma membrane; their presence in subcellular organelles has been demonstrated, but their intracellular signaling mechanism remains poorly understood. It is conceivable that OA1, similar to many plasma membrane GPCRs, might couple to a melanosomal ion channel and regulate ion transport in melanosomes (Fig. 2) to mediate organelle transport, fusion, or fission processes that are likely to be dysfunctional in the OA1 knockout phenotype (106).

Organelle ion channels modulate ionic gradients and membrane potential likely important in fusion and fission events to regulate endocytosis, exocytosis, protein trafficking, or organelle biogenesis (53, 72, 109). Interestingly, melanosomes were recently shown to form mitofusin 2-dependent contacts with mitochondria, involved in melanosome maturation and melanogenesis (110). Melanosomal ion channels and transporters might be important for triggering melanosome-mitochondria interactions, as well as the signals generated by organelle interactions. Further investigation into pigment cell intracellular ion channels will help discern inter-organelle or plasma membrane-to-organelle signaling pathways important for melanosomal function.

Ca^{2+} as a melanogenic signal

The plasma membrane ion channels mentioned above for their contribution to pigmentation share a common trait: they all increase intracellular Ca^{2+} when activated. Ion channel-mediated Ca^{2+} responses appear to contribute to increases in cellular melanin, but the mechanism connecting the two remains poorly understood. Interestingly, the prolonged Ca^{2+} response elicited upon activation of either TRPA1 or STIM/ORAI in melanocytes is required for the increase in melanin (30), (32). Because Ca^{2+} is a pleiotropic messenger

controlling a diverse array of intracellular events, it could regulate melanogenesis using different signaling pathways.

One possibility is that opening of ion channels provides the Ca^{2+} necessary for activation of protein kinase C β (PKC β), which is expressed in melanocytes and can be activated in response to UVR (36), (37) (Fig. 1). PKC β is a Ca^{2+} and diacylglycerol dependent kinase that has been shown to modulate pigment levels in melanocytes by phosphorylating the cytoplasmic domain of tyrosinase, the key melanosomal enzyme responsible for melanin synthesis, and thus increasing its enzymatic activity (37-41). Phosphorylation also induces formation of a protein complex between tyrosinase and tyrosinase-related protein, which further enhances melanin synthesis (42).

Ca^{2+} could also act in melanosomes, modulating the synthesis and storage of pigment. Melanosomes are enriched in Ca^{2+} (111-114), possibly due to the ability of melanin to bind and chelate Ca^{2+} (43, 44), thus melanosomes could serve as Ca^{2+} stores. In such a scenario, Ca^{2+} influx through the plasma membrane could be imported into melanosomes via Ca^{2+} pumps (Fig. 1). Ca^{2+} transport across the melanosomal membrane would likely regulate membrane voltage, luminal pH, and could modulate the enzymatic activity of melanosomal enzymes.

Because skin pigmentation requires the transfer of melanin from epidermal melanocytes to neighboring keratinocytes, Ca^{2+} could contribute to pigment transfer between the two cell types, the mechanisms of which remain poorly understood (45). Ca^{2+} responses were evoked in keratinocytes by interaction with melanocytes (46) and local Ca^{2+} signals might occur in melanocytes at sites of melanin release. Melanin is transferred in melanosomes and one potential mechanism is melanosome exocytosis from melanocytes and endocytosis by keratinocytes (47) (Fig. 1). In many biological systems, including neurons and endocrine cells, vesicles are exocytosed in a Ca^{2+} dependent-manner: Ca^{2+} ions rush into the cell through Ca^{2+} -permeable channels and create high concentration Ca^{2+} microdomains, which trigger the binding and fusion of vesicles to the plasma membrane. Similarly, Ca^{2+} -permeable melanocyte ion channels might localize at the contact sites with keratinocytes and orchestrate the release of melanosomes by modulating the local Ca^{2+} concentration.

Membrane voltage as a melanogenic signal

Ion transport across membranes not only provides ionic messengers that could modulate pigmentation, but also initiates a powerful cellular signal, changes in membrane voltage. Dramatic and rapid changes in membrane voltage are a characteristic of excitable cells where they mediate action potentials that allow rapid communication between neurons, muscle cells, and endocrine cells. More subtle membrane potential dynamics are also critical for cellular signaling in nonexcitable cells, such as pigment cells.

As discussed above, changes in intracellular Ca^{2+} regulate melanin content in pigment cells. The amplitude and duration of Ca^{2+} responses in epidermal melanocytes is shaped by membrane depolarization. UVA-induced melanin synthesis requires a prolonged Ca^{2+} response, which is mediated by TRPA1-dependent membrane depolarization (26, 30) (Fig. 1). While TRPA1 is necessary for the depolarization to occur, other ion channels might

contribute. This depolarization, in turn, delays TRPA1 inactivation to keep channels open longer (30, 115), generating the sustained Ca^{2+} response necessary for melanogenesis.

Activation of K^+ channels often leads to changes in membrane potential. Although K^+ channels have not been implicated in mammalian pigment cell physiology, a unique type of potassium channel is functionally important in zebrafish melanophores. Inwardly rectifying K^+ channels (Kir), which mediate a K^+ flux into the cytosol, underlie pigment pattern formation in zebrafish (116). Kir channels are responsible for contact-dependent-depolarization of melanophores by interaction with another pigment cell, xanthophores (117). Activation of Kir channels leads to depolarization of the plasma membrane and pigment aggregation through an unknown mechanism.

Protein targeting to melanosomes and proper segregation from lysosomes is imperative for melanosome biogenesis and subsequent melanin synthesis (118). Membrane voltage could provide a signal for intracellular organelle homotypic or heterotypic fusion processes. The recently reported changes in lysosomal membrane potential via TPC1 channels (61), as well as the role of membrane potential in mitochondrial fusion (119), suggest that ion transport in melanosomes, similar to other organelles, may alter the melanosomal membrane voltage to facilitate fusion between melanosomes and other organelles, the plasma membrane, or protein transport vesicles (Fig. 1). A similar mechanism could also initiate melanosome transfer in skin, which is dependent on melanocyte-keratinocyte membrane interaction.

Future directions

This review focuses on recent advances in identifying and elucidating the function of ion transport proteins and ionic signaling in pigmentation. As demonstrated by the limited number of studies on ion channel and transporter physiology in pigment cells, there is a large gap in our knowledge of how ionic signaling regulates melanogenesis. Recent advances in organelle ion channel research will likely provide new insights into signaling between melanosomes, other organelles, and the plasma membrane, providing a physiological basis for the previously identified ion transport genes implicated in pigmentation. Furthermore, as melanosomal ion transport protein function becomes better understood, melanocytes may provide a useful model for studying inter-organelle signaling due to their diversity of endosome-related organelles and unique protein trafficking systems.

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Highlights

- Ion transport is critical for the development and function of pigment cells in the skin and eyes.
- Plasma membrane ion channels modulate pigmentation via Ca^{2+} influx.
- Mutations in ion channels and exchangers in the membrane of endolysosomes affect pigmentation.
- Putative ion transporters in the melanosomal membrane are required for pigmentation.
- Ion transport leads to changes in membrane potential that could regulate pigmentation.

Figure 1. Melanocyte plasma membrane ion channels with potential roles in pigmentation

Schematic representation of ionic currents through TRP channels (TRPM1, TRPM7 and TRPA1) and mediated by the interaction of the ER protein complex STIM with the plasma membrane ORAI1, that regulate pigmentation. Pigmentation could be modulated by changes in membrane voltage ($+V_m$), shown to occur in response to TRPA1 activation. Alternatively, increased cytosolic Ca^{2+} mediated by influx through these channels, could lead to Ca^{2+} transport into melanosomes, modulate pigmentation by activating PKC β to increase tyrosinase (TYR) activity, regulate organelle interaction events, or trigger melanin transfer.

Figure 2. Putative melanosomal ion transport proteins

(Left) Schematic representation of endolysosomal ion channels with potential role in pigmentation. TRPML and TPC mediate cation efflux from melanosomes and mutations or SNPs in these channels suggest that they might also function in melanosomes. The CIC-7 exchanger regulates the luminal pH of endolysosomes, mediates anion transport, and its expression regulates pigmentation. V-ATPases contribute to proton influx into melanothomes and pH regulation. **(Right)** Schematic representation of melanosomal ion channels with role in pigmentation. Putative melanosomal transporters OCA2, SLC45A2 and SLC24A5 are mutated in types of human oculocutaneous albinism (OCA), but little is known about how they function. Here we show predicted functions based on homology to other transporters with characterized functions. The putative melanosomal G protein coupled receptor OA1 could activate an ion channel, thereby regulating ion transport activity. V-ATPase and ATP7A contribute to acidification of the lumen and copper (Cu^{2+}) transport, respectively. These ion channels and transporters could modulate pigmentation by directly or indirectly regulating the enzymatic activity of tyrosinase (TYR), which is critical for melanin synthesis. Bidirectional arrows represent anti- or co-transporters and unidirectional arrows represent uniporters or pumps.