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Review

Membrane transport proteins in melanosomes: Regulation of ions for pigmentation

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ABSTRACT

Melanosomes are unique organelles in melanocytes that produce melanin, the pigment for skin, hair, and eye color. Tyrosinase is the essential and rate-limiting enzyme for melanin production, that strictly requires neutral pH for activity. pH maintenance is a result of the combinational function of multiple ion transport proteins. Thus, ion homeostasis in melanosomes is crucial for melanin synthesis. Defect of the ion transport system causes various pigmentation phenotypes, from mild effect to severe disorders such as albinism. In this review, we summarize the up-to-date knowledge of the ion transport system, such as transport function, structure, and the physiological roles and mechanisms of the ion transport proteins in melanosomes. In addition, we propose a model of melanosomal ion transport system—how the functional coupling of multiple transport proteins modulates and maintains ion homeostasis. We discuss melanin synthesis in terms of the ion transport system.

1. Introduction

Melanosome (~500 nm in diameter) is a large unique organelle found in skin and hair melanocytes, and retinal pigment epithelial cells [1]. Melanosomes are responsible for synthesis, storage, and transport of melanin. The main roles of melanin are pigmentation (skin, hair and eye color) and defense mechanism against ultraviolet radiation and mechano-stress [1–5]. Unlike retinal epithelial cells where melanosomes are matured and the pigment is settled during development, melanocytes in skin and hair continuously produce melanosomes throughout life [1]. Originating from endosomes, melanosome is a member of lysosome-related organelles (LROs) and shares some characteristics and pathways with those of lysosome; similarities include biogenesis, maturation, trafficking (exo/endocytosis), recycling and migration [6,7]. However, melanosome biogenesis exhibits unique processes and specifically takes place in melanocytes. Newly mature melanosomes migrate from melanocytes to keratinocytes, resulting in pigmentation of epithelial cells (Fig. 1) [1,6,8]. As there are multiple processes, several protein molecules are involved in melanosome biogenesis, trafficking, and migration. Alterations of each corresponding molecule undoubtedly contribute to the variation of skin and eye color as well as ocular and dermatological diseases such as retinal pigment epitheliopathy, albinism, hyperpigmentation disorders, and skin cancers [4,9,10].

Membrane transport proteins (i.e., transporters, channels and pumps) in melanosomes have shown strong correlations to melanosome biogenesis. Some of these membrane transport proteins including OCA2 (oculocutaneous II), SLC45A2 (solute carrier 45 member 2), SLC24A5 (solute carrier 24 member 5), TPC2 (two-pore channel protein 2) and ATP7A (copper-transporting ATPase 1) have been predicted to modulate melanosomal pH and contribute to the activity of tyrosinase (TYR), an essential protein for melanin production [11]. Previous studies indicated that mutations and polymorphisms of these transport proteins are strongly associated with oculocutaneous albinism (OCA) [4]. Until present, several causative mutations have been identified. However, the roles or transport functions of the transport proteins in melanogenesis are still poorly understood. In this review, we highlight the current progress of the research of membrane transport proteins in melanosomes and melanogenesis. Specifically, transport function, roles in melanogenesis, and significant causative mutation are summarized here. Furthermore, we predict 3D structures of the transport proteins and discuss the unknown functions of these membrane transport proteins in melanosomes.

Abbreviations: TYR, tyrosinase; TYRP1, tyrosinase-related protein 1; TYRP2, tyrosinase-related protein 2; OCA, oculocutaneous albinism; OCA2, oculocutaneous albinism 2; SLC45A2, solute carrier 45 member 2; SLC24A5, solute carrier 24 member 5; TPC2, two-pore channel 2; ATP7A, copper-transporting ATPase 1

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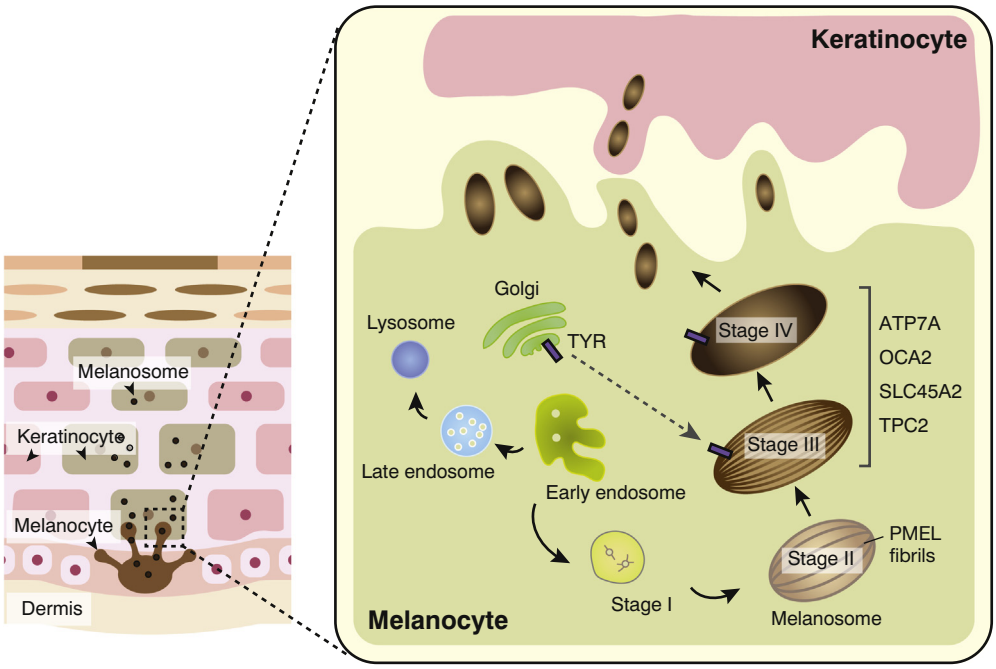

Fig. 1. Schematic of epidermis layers and melanosome biogenesis in melanocytes.
Melanocytes and keratinocytes are cells located in epidermis layers (left). Melanogenesis takes place in melanosome, a unique organelle in melanocytes. Melanosome is developed from the early endosome. Melanosome formation and maturation are called biogenesis, which is composed of four stages. Stages I–II are the nonpigmented stages of melanosomes, called premelanosomes. In premelanosomes, PMEL fibrils are formed (stage I), and completely organized as parallel sheets in ellipsoidal shape (stage II). Melanogenesis pathway begins at stage III, the stage which TYR along with TYRPs, ATP7A, OCA2, SLC45A2, and TPC2 are translocated from trans-Golgi network to melanosomes. The premelanosomes have acidic pH, but stages III–IV melanosomes have neutral pH, which may be a result of combinational function of the newly deposited ion transport proteins. Under neutral pH, TYR is functional and melanin is synthesized. Synthesized melanins fully mask on PMEL fibrils at stage IV, resulting in mature melanosome. Mature melanosomes are delivered to keratinocytes and are distributed throughout the skin.

2. Melanin and melanogenesis

Melanin is a group of pigments made up of heterogeneous polymers and is widely found in animals, plants, protozoa, eubacteria, and some fungi [5]. Melanin has a broad absorption spectrum and a high optical refractive index, enabling intriguing biological properties; such as UV light absorption and scattering, free radical scavenging, ion storage, metal chelation, and electromagnetic field sensing [2,5,12]. Animals have two major types of melanin: brown-black eumelanin and red-yellow pheomelanin. While eumelanin in the skin is the major factor contributing to skin color (dark or light skins), the ratio of eumelanin and pheomelanin in the hair determines diverse shades of hair color. Because both types of melanin have distinct chemical structures and optical properties, the response to UV radiation and reactive oxygen species (ROS) are different. Pheomelanin is less protective against UV radiation and ROS than eumelanin, leading to UV-radiation-dependent melanoma risk [2,13]. In addition, the intrinsic pheomelanin synthesis pathway produces UV-radiation-independent contributors to melanomagenesis [14]. Consequently, people who have red hair/fair skin (phenotypes of low eumelanin) are at greater risk for skin cancer.

Melanogenesis is a melanin synthesis pathway occurring in melanosomes (Fig. 2). The first step in melanogenesis is hydrolyzation of L-tyrosine to L-DOPA by tyrosinase (TYR). TYR also oxidizes L-DOPA into dopaquinone. Depending on the availability of L-cysteine and the function of downstream enzymes, different types of melanin are produced. The eumelanin synthesis pathway is quite well studied compared to the pheomelanin synthesis pathway. The production of eumelanin occurs in the absence of L-cysteine. Dopaquinone is converted into two intermediates: 5,6-dihydroxylindole-2-carboxylic acid (DHICA) and 5,6-dihydroxyl indole (DHI), then finally converted to eumelanin. These processes require enzymatic reactions of TYR and two other enzymes, dopachrome tautomerase (DCT/TYRP2/TRP2) and tyrosinase-related protein 1 (TYRP1/TRP1). In the presence of L-cysteine, dopaquinone is converted into cysteinylDOPA instead. The cysteinylDOPA passes several enzymatic reactions involving oxidation and polymerization yielding pheomelanin (Fig. 2) [4,15,16].

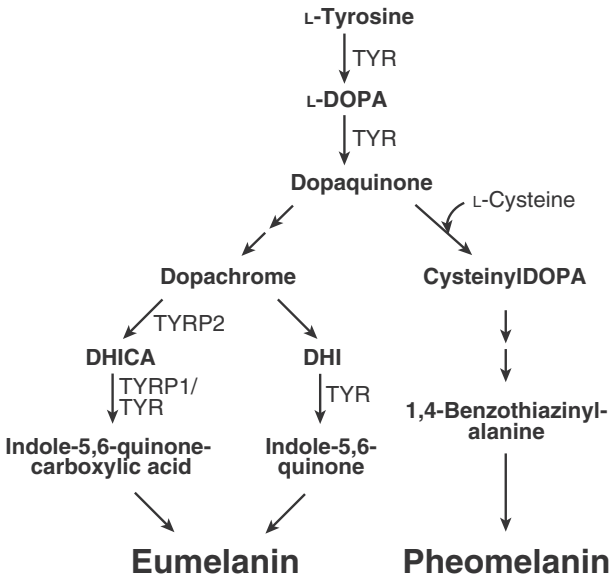

Fig. 2. Pathway of melanogenesis.
In melanin production, tyrosinase (TYR) is a rate-limiting enzyme that converts L-tyrosine to L-DOPA, and subsequently, to dopaquinone. In the absence of L-cysteine, dopaquinone is finally converted to eumelanin. TYRP1 and TYRP2 (DCT) are tyrosinase-related proteins that contribute to the processes of eumelanin synthesis. Pheomelanin is synthesized under the presence of L-cysteine via several precursors.

TYR and TYRPs belong to tyrosinase family [16]. Production of melanin (eumelanin) is dependent on the activities of TYR and TYRPs in which TYR is the rate-limiting enzyme [17]. TYR is known to be controlled by either mRNA expression or protein function. TYR mRNA expression is regulated via the melanocortin 1 receptor (MC1R)-dependent cyclic adenosine monophosphate (cAMP) signaling pathway. MC1R is a G-protein coupled receptor (GPCR) on melanocytes that

senses melanocyte stimulating hormone (MSH) and mediates cAMP signaling to regulate the microphthalmia-associated transcription factor (MITF). MITF thereby regulates the TYR mRNA expression as well as other aspects of melanosomes/melanocytes [3,18]. Functional control of TYR protein is accomplished by modulation of melanosomal pH because TYR activity is solely dependent on pH. Neutral pH preserves TYR and TYRPs functions, while acidic pH suppresses TYR function without altering its expression (discussed in later section). Zhou et al. reported that the soluble adenylyl cyclase (sAC)-dependent pathway negatively modulates melanin production by decreasing the pH of melanosomes [19].

3. Melanosome biogenesis

Both melanosomes and lysosomes originate from multivesicular endosomes. However, melanosome formation occurs through distinct pathways in melanocytes in which several unique proteins are involved [7,11,20]. Melanosome biogenesis (referred to eumelanosome biogenesis), the process of melanosome development and maturation, is composed of four different stages (Fig. 1). Melanosomes at stages I–II, also called premelanosomes, lack melanin production but contain the formation of amyloid fibrils composed of the melanocyte protein PMEL (also known as PMEL17 and gp100). The characteristic of melanosomes at stage I is similar to the early endosome, which contains intraluminal vesicles (ILVs). In this stage, PMEL is cleaved into Mα and Mβ fragments. The Mα fragments dissociate from the luminal domain and start to nucleate at the ILVs to form tiny non-rearranged fibrils. During stage II, the formation of the fibrils, called PMEL fibrils, is completed. PMEL fibrils are organized and elongated as arrays of parallel sheets in an ellipsoidal shape of melanosome, serving as a matrix for melanin deposition which is initiated at stage III and completed at stage IV (Fig. 1) [7,21]. At stage III, several enzymes (TYR, TYRP1 and TYRP2) and ion transport proteins (ATP7A, OCA2, SLC45A2 and TPC2) for melanogenesis are sorted from trans-Golgi network to melanosome, and then, melanogenesis begins [11,20,22,23]. Synthesized melanin moves on and covers the PMEL fibrils completely at stage III and thickens and darkens as shown at stage IV. In skin, the mature melanosomes enter the process of migration from melanocytes to keratinocytes resulting in the spread of skin colors [1,5,6,8]. In hair follicles, stages of melanosome biogenesis, melanogenesis and melanosome transfer are similar to those of melanocytes in epidermis. Melanosome biogenesis occurs in follicular melanocytes located in hair bulbs during the anagen stage of hair cycle. Due to the distinct anatomic compartments, follicular melanocytes have more dendritic, extensive Golgi apparatus and rough endoplasmic reticulum, and produce larger mature melanosomes than epidermal melanocytes [24].

4. Ions and ion transport proteins

Ion transport proteins, including ion channels, pumps and transporters, play significant roles in multiple cellular activities at plasma membranes and intracellular organelles. The function of a transport protein relies on the electrochemical gradients. Channels selectively facilitate passive diffusion. Active transport systems move the substances against their electrochemical gradients. Primary active transport proteins hydrolyze ATP to mediate active transport. Secondary active transport proteins utilize energy from the electrochemical gradients, such as Na^+, to drive the active transport. Tertiary active transport proteins mediate exchange or cotransport by using the energy derived from the chemical gradients generated by the secondary active transport proteins [25]. The maintenance of ion homeostasis requires the combinational function of ion transport proteins. In melanosomes, ions are indispensable factors that define the nature of the organelle for melanogenesis as well as its biogenesis. The ions and their transport proteins contribute to enzymatic reactions, redox reaction, electrical signaling, metal and ionic homeostasis, and pH maintenance. In the following section, the roles of both ions and ion transport proteins for melanosomes are discussed.

5. Ions and ion transport in melanogenesis

Ions are important for melanogenesis and melanosomal maintenance in either direct or indirect aspects. pH and Cu^{2+} are obligatory factors for TYR function. Ca^{2+} is suggested to be essential for Ca^{2+}-mediated signaling pathways and homeostasis between melanosomes and melanocytes. Here, we describe the roles of pH, Cu^{2+} and Ca^{2+} in melanosomes. The importance of other ions, Na^+, K^+, and Cl^- in melanogenesis, has not been directly revealed. However, two aspects are proposed below. The first aspect is based on the physiological function of Na^+ and K^+ for maintenance of membrane potential. The K^+ concentration gradient determines membrane potential and Na^+ is required during polarization/depolarization. Therefore, Na^+ and K^+ may contribute to the maintenance of membrane potential in melanosomes. Cl^- and Ca^{2+} may also be involved in maintaining the membrane potential. The second aspect is that Na^+, Ca^{2+}, and Cl^- are coupled substrates of ion transport proteins which are important for modulation of melanosomal pH during stages III–IV. The pH maintenance requires the functional combination of multiple ion transport proteins such as OCA2, SLC45A2, and TPC2. OCA2 mediates Cl^- conductance and TPC2 shows the properties of Na^+ and/or Ca^{2+} efflux (see in later section). Thus, it is highly possible that Na^+, Ca^{2+} and Cl^- participate in the pH maintenance of melanosomes.

5.1. pH and H^+

pH plays a critical role in melanogenesis. Melanosomes in different stages have different internal pH. Premelanosomes exhibit acidic pH while stages III–IV melanosomes show neutral pH. PMEL fibrils are rapidly formed under strict acidic condition of premelanosomes [26,27]. However, TYR and TYRPs, which are expressed in stages III–IV melanosomes, are active at neutral pH and have diminished activity in acidic conditions [28–31]. Neutral pH promotes eumelanin synthesis by maintaining the full activity of TYR as observed in melanosomes of the melanocytes from Black skin. In contrast, melanosomes of melanocytes from Caucasians are more acidic, and their TYR is largely inactive. Rescue experiments using NH_4Cl to increase melanosomal pH confirmed the importance of pH in TYR activity. Caucasian melanocytes treated with NH_4Cl show an increase of TYR activity whereas Black melanocytes do not change the TYR activity with NH_4Cl treatment [30]. Acidification also suppresses the eumelanin synthesis at the later steps after dopachrome (Fig. 2) [31]. Moreover, weak acidic pH promotes the pheomelanin production pathway [32]. These results demonstrate that during melanosome maturation at stages II–III the melanosomal pH changes from acidic to neutral thereby terminating PMEL polymerization steps and, instead, initiating TYR function.

Vacuolar H^+-ATPase (V-ATPase) is a H^+ pump expressed in all stages of melanosomes. V-ATPase mediates H^+ influx resulting in acidic pH in premelanosomes [33]. In stages III–IV melanosomes, there must be some types of machinery to modulate acidic pH into neutral pH—either suppressing V-ATPase activity or clearing out the H^+. Failure to neutralize the pH results in acidic melanosomes and lowered melanin production. The role of V-ATPase as a negative pH regulator in melanin production is confirmed by Caucasian melanocytes and animal cell models. Inhibition of V-ATPase in acidic melanosomes by bafilomycin or concanamycin rescues the failure of the melanin production but the treatment has no effect in melanosomes which have neutral pH [28–30,34,35].

In physiological condition, stages III–IV melanosomes would express a transport protein(s) that fluxes H^+ out. SLC45A2 (MATP/AIM1) is the most promising molecule. SLC45A2 belongs to the H^+/sugar co-transporter family [36]. Knockdown of SLC45A2 results in melanosomal acidification, which interrupts TYR activity in the cell models

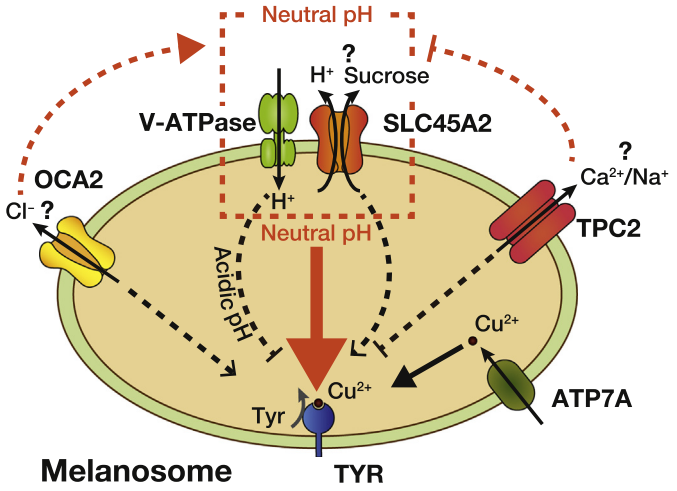

Fig. 3. Ion transport proteins in melanosomes.
V-ATPase, a H^+ pump, is expressed since stage I premelanosomes, generating acidic pH in premelanosomes. TYR along with other ion transport proteins are expressed in stages III–IV melanosomes. ATP7A is a Cu^{2+} pump, supplying Cu^{2+} as a cofactor for TYR. OCA2 and SLC45A2 are positive regulators for TYR function (black dash arrows) while TPC2 is negative regulator (black dash line with blunt end).
Transport properties and substrates of OCA2, SLC45A2 and TPC2 are proposed. Combinational function of these proteins is suggested to switches acidic pH into neutral pH (orange dash lines), which corresponds to the termination of stage II and initiation of stage III melanosomes. pH neutralization serves as the optimal environmental platform for TYR function, leading to melanin production during stages III–IV melanosomes (orange arrow).

[37]. In albino zebrafish with a *SLC45A2* mutation, the TYR activity is disrupted, but rescued by reinjection of SLC45A2 mRNA or bafilomycin treatment [35]. Although SLC45A2 transport activity in melanosomes is unclear, it is proposed that SLC45A2 effluxes H^+ coupled with substrates and neutralizes the pH of stages III–IV melanosomes (Fig. 3). Additionally, voltage-gated Cl^-/H^+ exchangers (ClCs) and Na^+/H^+ exchangers (NHEs) may be candidates participating in H^+ export. ClC-7 mediates H^+ efflux in lysosome to control lysosomal pH. Mutation in *ClC-7* gene causes hyperacidification and results in albinism [38]. Thus, it is possible that ClC-7 is also expressed in melanosomes. NHE3 and NHE7 are colocalized with TYRP1 in melanocytes, although the function of NHEs in melanosomes has not yet been demonstrated [39].

To date, the mechanism of pH regulation in melanosomes has not been revealed. However, several studies clearly indicate that multiple ion transport proteins participate in the regulation of melanosomal pH (Fig. 3). OCA type 2 (OCA2/P) and SLC45A2 (MATP/AIM1) proteins are reported to be positive regulators for pH neutralization [35,40]. Two-pore channel 2 (TPC2) was found to be a negative regulator for pH neutralization [41]. OCA2 and TPC2 are ion transport proteins but may not directly transport H^+. The proposed roles and functions of these proteins are discussed in later section.

5.2. Cu^{2+} and Zn^{2+}

Cu^{2+} and Zn^{2+} ions are indispensable in the enzymatic activities of the tyrosinase family. TYR, TYRP1, and TYRP2 share a signaling peptide-like domain, a cysteine-rich region, a single transmembrane domain at the C-termini, and residues for coordinating the binding of the two metal ions. TYR contains two Cu^{2+} binding sites, and TYRPs hold two Zn^{2+} binding sites [15,16]. The requirement of Cu^{2+} ions for TYR has been clearly demonstrated. Binding of two Cu^{2+} ions and an oxygen molecule in TYR enables the redox properties on oxidation states of monooxygenase and oxidase activities [15]. While TYR requires Cu^{2+}, TYRP2 tautomerase activity requires Zn^{2+} [42]. The binding of metal ions to TYRP1 is still controversial. TYRP1 contains Zn^{2+} binding sites but biochemical experiments have failed to prove the direct binding of Zn^{2+} [43]. Moreover, mouse and human TYRP1 seem to exhibit different enzymatic properties [16]. Nevertheless, both Cu^{2+} and Zn^{2+} are bona fide cofactors of enzymatic reactions in melanosomal tyrosinase family.

The Cu^{2+} ion in melanosomes is supplied by copper-transporting ATPase 1 (ATP7A), which is translocated from the trans-Golgi network to melanosomal membranes (Fig. 3) [44]. Deficiency of Cu^{2+} by ATP7A malfunction causes Menkes disease, an X-linked multisystemic lethal disorder of Cu^{2+} metabolism [45–47].

Transport of Zn^{2+} in melanosomes is still unclear. Members of the SLC30 family are known as organellar zinc transporters and presumably trafficked from the trans-Golgi network [48]. ZnT2 (SLC30A2) is found in lysosome and critical for lysosomal acidification [49]. It is possible that ZnT2 expresses and supplies Zn^{2+} in melanosomes, but this hypothesis needs to be verified.

5.3. Ca^{2+}

In premelanosomes, Ca^{2+} is necessary for the activity of furin-like protease, an enzyme that cleaves PMEL in the premelanosomes during fibril formation [50]. In stages III–IV melanosomes, Ca^{2+} might be one of the coupling ions for pH modulation. The role of Ca^{2+} is not limited to melanogenesis in melanosomes but is also involved in the signaling responses between melanosomes and melanocytes. Ca^{2+} is known to be a secondary messenger that contributes to signaling pathways and membrane voltage. In non-melanocyte cells, intracellular Ca^{2+} is stored in organelles such as endoplasmic reticulum, mitochondria and lysosome [51]. Melanosome is found to be another organellar Ca^{2+} store in melanocytes due to the high accumulation of Ca^{2+} in melanosomes and properties of Ca^{2+} binding to melanin [52–54]. The enriched Ca^{2+} in melanosomes is released by stimulants such as caffeine and IP_3 [53]. The α-MSH pathway, for instance, is a Ca^{2+} response mechanism for TYR expression [55]. These results support the significance of melanosomal Ca^{2+} in Ca^{2+}-mediated signaling pathways and Ca^{2+} homeostasis between melanosomes and melanocytes, which may be involved in melanogenesis.

Still, the mechanism of Ca^{2+} storage and transport are poorly understood in melanosomes. Ca^{2+} pumps such as SERCA-type calcium pumps (sarco/endoplasmic reticulum calcium ATPase) is a canonical pathway for intracellular Ca^{2+} storage [56]. Ca^{2+} accumulation in melanosomes may be the result of a calcium pump because high Ca^{2+} uptake is observed in the purified melanosomes [53].

As for the Ca^{2+} efflux mechanism, TPC2 is the main contributor (Table 1). TPC2 is identified as the nicotinic acid adenine dinucleotide phosphate (NAADP)-sensitive Ca^{2+} release channel in lysosomes [57–59]. In melanosomes, TPC2 regulates the pH balance by participating in Ca^{2+} efflux [41,60].

SLC24A5 is thought to be a K^+-dependent Na^+/Ca^{2+} exchanger based on the conserved sequences of the ion binding residues to other SLC24 members. SLC24A5 undoubtedly plays a role in melanin production, but its subcellular localization is uncertain. SLC24A5 would likely mediate Ca^{2+} transport if expressed in melanosomes [9]. Zhang et al., who found SLC24A5 in mitochondria, predicted the model of Ca^{2+} supplement from mitochondria to melanosome via SLC24A5 function in mitochondria-melanosome contact region [61].

6. Ion transport proteins in melanogenesis

Due to the significance of ions and ion transport proteins in melanogenesis, there is no doubt that impairment of the ion transport system would result in pigmentation disorders such as albinism. Albinism is a congenital disorder defined by a lack of pigmentation (both complete and partial). Two groups of albinism, oculocutaneous albinism (OCA) and ocular albinism (OA), are classified based on the diagnostic phenotypes. OCA, the most common group, describes the abnormal

Table 1
Ion transport proteins in human pigmentation.

Proteins	Substrates	Localization	Genetic disorder	A) Pathogenic mutations[a] B) Polymorphisms[b]	Phenotypes[c]
OCA2/P	Cl^-?	Melanosome stages III–IV	Type II OCA	A) V443I, A481T, P743L B) R305W, R419Q	A) Skin and hair hypopigmentation, reduced iris and retinal pigments, and ocular defect B) Blue/green/hazel eye colors
SLC45A2/MATP/ AIM1	H^+/sugar co-transport?	Melanosome stages III–IV	Type IV OCA	A) D93N, D157N, G188V, T437A, T440A, A477T B) E272K/K272E, F374L/L374F	A) Light/yellow/white/blue hairs, blue/red/brown/ gray eye colors, and some nystagmus. B) Blue/green/hazel/brown eye colors, olive/ brown/ black skin, black/brown/blond/red hair
SLC24A5/NCKX5	Na^+/Ca^{2+} and K^+ antiport?	TGN Mitochondria (Melanosome?)	Type VI OCA	A) A115E, R174K, S182R, W197X B) T111A/A111T	A) White skin, brown hair, brown iris, and underdeveloped macula B) Light skin pigmentation
TPC2/TPCN2	Ca^{2+} or Na^+?	Melanosome stages III–IV *Multiple organelles	–	B) G734E, M484L	B) Blond hair
ATP7A/MNK	Cu^{2+}	TGN Melanosome stages III–IV	Menkes disease	A) E628V, S653Y, C1002F	A) Broad phenotypes. Severe defect leads to death. Classical phenotypes: growth failure, Kinky hair, nervous system deterioration
V-ATPase	H^+	Melanosome (all stages) *Multiple organelles	–	–	–

OCA, oculocutaneous albinism; TGN, trans-Golgi network.
[a] Examples of pathogenic mutations.
[b] Examples of non-pathogenic polymorphisms.
[c] Examples of phenotypes which are corresponded to either pathogenic mutations (A) or non-pathogenic polymorphism (B).

formation of melanin in eyes, skin, and hair, while OA is the abnormality limited to eyes. OCA is an autosomal recessive disorder caused by the impairment of genes involved in melanogenesis. OCA is subdivided into seven types (type I–VII OCA) [4]. Type I and III OCA are caused by mutations of *TYR* and *TYRP1*, respectively. Type II, IV and VI OCA are caused by mutations of ion transport proteins *OCA2*, *SLC45A2*, and *SLC24A5*, respectively (Table 1). The defect of ATP7A does not cause albinism but causes Menkes disease. Symptoms of the Menkes patients vary but include abnormalities in hair and skin [62]. TPC2 defect leads to abnormalities of melanin production and pigmentation [63]; however, their corresponding disease was not reported (Table 1). Here, we summarize the current knowledge of the ion transport proteins—their physical properties, biochemical functions, physiological roles and pathological effects in melanosomes.

6.1. OCA2/P

6.1.1. Functional aspect

OCA2 (also known as *pink-eyed dilution/P*; human gene: NM_000275) gene was first identified in mouse and human as the *P* locus responsible for pink-eyed dilution [64,65]. At the same time, Ramsay et al. identified the loci at the close region of *P* locus as the associated loci for type II OCA [66]. Later, the two loci were verified to be the same gene named *OCA2/P*, which is also typically related to Prader-Willi and Angelman syndrome, a disorder resulting from partial deletion of chromosome 15q11-q13 [67]. OCA2 (838 amino acid residues) is predicted to contain 12 transmembrane domains [40,67]. OCA2 mRNA is expressed in the eyes, skin, and brain (The Human Protein Atlas project: http://www.proteinatlas.org, last update March 2020). At subcellular localization, OCA2 protein is specifically expressed in mature (stages III–IV) melanosomes [68]. Like TYR and TYRPs, OCA2 contains an acidic dileucine motif and colocalizes with BLOC-1 (a cargo-specific sorting protein for TYRP1) for sorting from Golgi apparatus to early endosomes and targets to melanosomes [23].

By utilizing immortal OCA2 mutant and knockout cell models, several studies showed the role of OCA2 as a positive regulator of pH neutralization and melanogenesis promotion (Fig. 3) [28,29,34,40,69,70]. The pH regulation by OCA2 targets V-ATPase as observed in the rescue of TYR function after V-ATPase inhibition in OCA2-knockout melanocytes [28,29,34,69,70]. Besides pH regulation, OCA2 likely facilitates TYR trafficking from ER/Golgi network to melanosomes. The OCA2-knockout melanocytes show some TYR retaining on ER/Golgi network, and the melanosomal re-localization of TYR could be rescued by pH modulation [69–72]. All data point to the *dual roles* of OCA2-dependent pH regulation—for both TYR trafficking and TYR activities.

OCA2 is predicted to be a member of the Mg^{2+}/citrate or Na^+/citrate transporter family (SLC13 family) based on amino acid sequence similarities [73]. However, OCA2 has not been classified into any existing SLC families [74]. Previous studies indicated that OCA2 is not a tyrosine transporter [75]. Bellono et al. demonstrated that OCA2 mediates Cl^--selective anion conductance by using the patch clamp method with OCA2-knockdown melanosomes dissected from *Oa1*$^{-/-}$ melanocyte (melanocytes with enlarged melanosomes). OCA2 induces large outwardly rectifying current with a negative charge of Cl^-. This Cl^- conductance is essential for maintaining neutral pH for melanosomes. In the addition, electrophysiological property has been characterized in the endolysosome of the recombinant OCA2-expressing AD-293 cells suggesting that OCA2 is an electrodiffusion anion channel [40].

6.1.2. Mutations and polymorphisms

Despite the limited knowledge of OCA2 function, mutations of *OCA2* in humans and animals have been intensively identified. *OCA2* is a causative gene for type II OCA (MIM#203200), which is the most common type of OCA worldwide. Hundreds of mutations are identified in humans (Albinism Database: http://www.ifpcs.org, last update September 2009; The Human Gene Mutation Database: http://www.hgmd.cf.ac.uk, last update April 2019). The pathogenic phenotypes include hypopigmentation of skin and hair, reduced iris and retinal pigments, and ocular defect (Lewis RA. Gene Reviews, last update August 2012: http://www.ncbi.nlm.nih.gov/books/NBK1343/). At the cellular level, the patient has small melanocytes, immature melanosomes, and no or little melanin production. Examples of pathogenic mutations are V443I, P743L, and A481T. Patients with V443I and P743L double mutations develop severe phenotypes of type II OCA and Prader-Willi syndrome [76]. A481T, a mutation associated with mild-symptom phenotypes, is commonly found in Caucasians as well as in East Asians [76–78]. Polymorphisms of *OCA2* give rise to diverse variations of eyes, hair, and skin colors such as blue eyes (Table 1) [79,80].

6.2. SLC45A2/MATP/AIM1

6.2.1. Functional aspect

Before discovering *SLC45A2* as a causative gene for albinism, *SLC45A2* (*MATP/AIM1*; human gene NM_016180) was identified as a gene named "*Absent In Melanoma*" associated to tumor suppression in melanoma model [81,82]. In 2001, Newton et al. discovered *SLC45A2* as a responsible gene for human type IV OCA (OCA4) and mouse "*underwhite*" [83]. At the same time, Fukamachi et al. found that *SLC45A2* is a gene for allele *b*, a well-known hypopigmentation allele in medaka [84]. SLC45A2 (530 amino acid residues), predicted to contain 12-transmembrane domains, is a member of H^+/sugar cotransporter family (SLC45 family). SLC45 mRNA expresses in eyes, skin, placenta, liver, and kidney (The Human Protein Atlas project: http://www.proteinatlas.org, last update March 2020) [82]. In melanocytes, SLC45A2 is expressed in stages III–IV melanosomes [37].

The function of SLC45A2 in melanosomes is still largely unclear. Recombinantly expressed mouse SLC45A2 mediates sugar transport at the plasma membrane of yeast cells, with the optimal function at a wide pH range. The sucrose transport is H^+ dependent, supporting the property of SLC45 as a H^+-coupled sugar transporter [36]. A rice-homolog protein also transports sugar in the plasma membrane of oocytes [85]. Although SLC45A2 and the ortholog are proposed to be sugar transporters, the activities were observed at the plasma membrane. Thus, physiological transport function and kinetic property of SLC45A2, expressed in melanosomes, has not been demonstrated.

The role of SLC45A2 in melanosomes is well defined. Costin et al. showed that SLC45A2 promotes TYR trafficking from the trans-Golgi network to melanosomes. The defect of SLC45A2 (*underwhite*) disrupts the TYR secretion process [86]. Dooley et al. describes that SLC45A2 regulates pH and melanogenesis. Albino zebrafish has a SLC45A2 mutation and a defect of TYR function. The phenotypes can be rescued by inhibition of V-ATPase or SLC45A2 RNA reinjection [35]. Bin et al. supports the role of SLC45A2 in pH regulation. The knockdown of SLC45A2 does not affect melanosomal morphology but induces melanosomal acidification that interrupts TYR activity [37].

The role of SLC45A2 in melanogenesis is similar to that of OCA2—to promote TYR trafficking and positively regulate pH neutralization for TYR activity. Although both aspects could be independent, the role of SLC45A2 in the pH regulation is certain. Based on the current information, SLC45A2 is predicted to efflux H^+ from melanosomes (Fig. 3) [36,37,85]. If so, the H^+ efflux by SLC45A2 reduces melanosomal H^+ concentration driven by V-ATPase, thereby neutralizing the melanosomal pH. Because SLC45A2 likely transports sugar, a significant agent for osmotic concentration, SLC45A2 may control both pH and osmolarity [87].

SLC45A2 does not only play a role in melanogenesis, but also acts as a suppressor of cancer invasion. Because *SLC45A2* was discovered to be a deleted gene in melanoma, SLC45A2 has been suggested to play a role in cancer prevention or suppression. Haffner et al. showed that SLC45A2 interacts with actin cytoskeleton to prevent actin remodeling, and then suppresses the pro-invasion and migration of the benign tumor [88]. The role of SLC45A2 in cancer could be related to its role in melanosomes.

6.2.2. Mutations and polymorphisms

Type IV OCA (MIM#606574) is an autosomal recessive hypopigmentary disorder that disrupts pigmentation in the skin, hair and eyes. Type IV OCA by *SLC45A2* mutation has been reported to be another common form, especially in Japanese patients [89]. The most frequent mutations are D93N, D157N, and G188V, which result in light/yellow/white/blue hair, blue/red/brown/gray eye color, and some nystagmus. A477V causes leucism in lions and tigers, and the mutation (A477T) has been reported once in humans [90,91]. We constructed a 3D structure model of the SLC45A2 by using lactose permease (LacY; PDB No. 5GXB) as a template (Fig. 4) [92]. The model

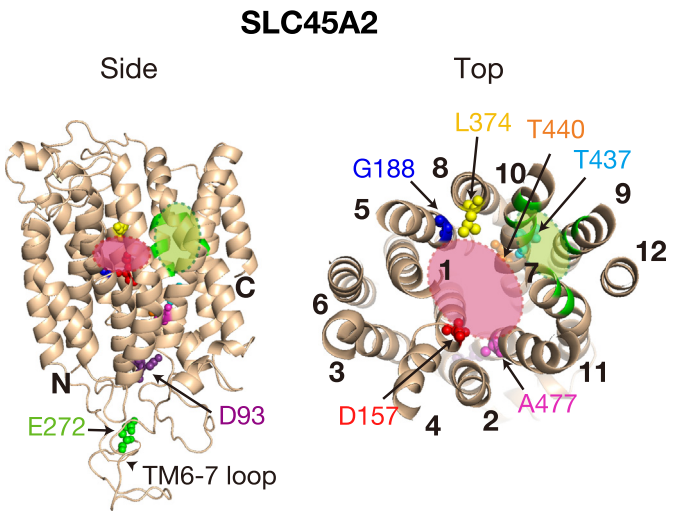

Fig. 4. 3D structural model of human SLC45A2.
Structural model of human SLC45A2 is built by SWISS-MODEL using Lac Y as template: left, side view; right, top view (neglect bottom parts of the structure). SLC45A2 contains 12 transmembrane domains (TM numbers are indicated in the top view picture). The model conserves the structural fold of the major facilitator superfamily in which TM1–6 and TM7–12 are arranged in two-fold pseudosymmetry. Some residues at TM6–7 loop are omitted in the modeling for the best fit. The substrate binding sites are predicted to be at the core center of TM1, 4, 5 and TM7, 10, 11 (magenta area), whereas the H^+ recognition sites are at TM7, 9, 10 (green area). Residues corresponding to type IV OCA mutations and polymorphisms are shown in ball-and-stick. Residues D157 and G188 are located at the area of substrate binding sites.

contains 12 transmembrane domains (TM) with the conserved structural fold of the major facilitator superfamily (MFS). Two bundles, (TM 1, 4, 5) and (TM 7, 10, 11), are arranged in pseudosymmetry and form a presumable substrate binding site, while residues on TM 7, 9 and 10 may be involved in proton coupling and transfer. In the model, residues D157 and G188 are located in the middle of TM 4 and 5, respectively, suggesting that they are key residues for substrate binding (Fig. 4).

Polymorphisms of *SLC45A2* generate different degrees of melanosomal size, shape, melanin content and melanosomal maturity, resulting in hair and skin color variety. E272K/K272E and L374F/F374L are associated with population differences in human skin colors, which may be a result of natural selection or adaptation to ultraviolet radiation [93]. Both E272 and L374 are located outside of the proposed H^+ and substrate binding sites, which may be a reason for the mild symptoms. Currently, more than 50 mutations have been reported in humans (Albinism Database: http://www.ifpcs.org, last update September 2009; The Human Gene Mutation Database: http://www.hgmd.cf.ac.uk, last update April 2019). The structural model may be beneficial in predicting the severity and pathology of type IV OCA.

6.3. SLC24A5/NCKX5

6.3.1. Functional aspect

SLC24A5 (*NCKX5*, human gene NM_205850) was first identified as a gene susceptible for *golden* (hypopigmentation) phenotype in zebrafish [94]. By next-generation sequencing, *SLC24A5* is found to be a causative gene for type VI OCA (OCA6) [95]. SLC24A5 mRNA is specifically detected in pigmentation-related tissues (The Human Protein Atlas project: http://www.proteinatlas.org, last update March 2020). Malfunction of SLC24A5 alters the melanin production and results in albino phenotypes (small sized melanosomes, less melanin content, and low pigment granules) in humans as well as animal models [95,96]. SLC24A5 is detected in the stages III–IV melanosome-enriched fraction by sucrose gradient centrifugation experiments [9]. Subsequently, imaging experiments revealed that SLC24A5 is presented in the trans-

Golgi network [97,98]. Recently, Zhang et al. demonstrated localization of SLC24A5 both in mitochondria and trans-Golgi network but not in melanosomes [61].

SLC24A5 is the most recently identified member of SLC24 family. Although little is known about SLC24A5, the functional properties of other members are well defined, especially for SLC24A2 (NCKX2) which is important for neuronal function [99]. Ca^{2+}-cation antiporter superfamily is composed of two families, Na^+/Ca^{2+} exchanger (NCX, SLC8) and K^+-dependent Na^+/Ca^{2+} exchanger (NCKX, SLC24) families. NCKXs transport 1 Ca^{2+} and 1 K^+ in exchange for 4 Na^+. NCKXs play physiological roles in Ca^{2+} homeostasis and membrane potential maintenance [99,100].

SLC24A5 is predicted to transport K^+ and Ca^{2+} in exchange with Na^+ like other NCKX members. The transport function of SLC24A5 activates both melanogenesis and melanosome biogenesis implying ion regulation and Ca^{2+} supplementation by SLC24A5 in melanosomes [61,97]. There are three proposed mechanisms for how SLC24A5 supplies the ions to melanosomes based on its subcellular localization. The first possibility is that SLC24A5 in trans-Golgi network plays a role in pH regulation by functional coupling with NHE7 and V-ATPase. This functional coupling may ensure the TYR maturation and proper trafficking of TYR from trans-Golgi network to melanosomes [97]. Another possibility is based on SLC24A5 localization in melanosomes as mentioned in the previous section; SLC24A5 supplies Ca^{2+} to promote PMEL polymerization and maintain Ca^{2+} homeostasis as well as membrane potential in melanosomes [94]. The third possibility is derived from the localization of SLC24A5 in mitochondria. SLC24A5 may transfer Ca^{2+} from mitochondria to melanosome via "mitochondrion-melanosome contact" [61,101].

6.3.2. Mutations and polymorphisms

The defect of SLC24A5 causes type VI OCA (MIM#113750), an autosomal recessive albinism. Type VI OCA patients have mild symptoms compared to other OCA. The patients have hypopigmentation, reduced visual acuity, nystagmus, hair color ranging from platinum blond to light brown, and green or blue eye color. Pathogenic mutations are, for examples, A115E, R174K, S182R, and W197X. However, these patients have non-syndromic OCA [95,102]. Polymorphism of *SLC24A5* is related to regions. A111 is found to be associated with light skin pigmentation among European and East Asians. The variation is approximately 20–40% of European-African difference in skin melanin index. T111 is commonly found in Europeans while A111 is common in Africans and East Asians [94].

Presumably, the 3D structures of NCKXs are conserved with that of NCXs [103]. The crystal structure of the archaebacterial ortholog (NCX_Mj) represents a structure composed of 10 transmembrane domains in 5–5 inverted repeats and Na^+/Ca^{2+} binding sites located in the middle of TM2–3 and TM7–8 (see also Fig. 5) [104]. Models of eukaryotic NCXs and NCKXs show a set of 10 TMs similar to NCX_Mj, and two additional regions—(1) N-terminal region, which contains a single transmembrane domain (called TM0) and a signal peptide sequence (SPase cleavage site) between TM0 and TM1, may be cleft out; and (2) large cytosolic regulatory f-loop containing two Ca^{2+} binding domains (~500 amino acids) between TM5 and TM6 which functions as a Ca^{2+}-sensing domain [100,103]. We, therefore, constructed a 3D structural model of SLC24A5 using the structure of NCX_Mj (PDB No. 3V5U) as the template. We aligned amino acid sequences of SLC24A5 to NCX_Mj, and other human NCKXs. The feasible N-terminal region and cytosolic f-loop are omitted from the model. The model shows 10 TM domains which are well aligned to NCX_Mj structure (Fig. 5).

We mapped the mutation residues (A111, A115, R174, S182, and W197) to the SLC24A5 model (Fig. 5). Notably, none of them correspond to substrate binding sites. A111, A115, R174, and S182 are located on TM4 and TM6 which are at the outer array of the core substrate binding TMs (TM2–3 and TM7–8). W197 is located at the top of TM7 but far away from the center. We suggest that these residues are not involved with the substrate recognition; therefore, the mutation may not severely cause the loss of transport activity. The position of mutation residues likely explains why the patients with A115E, R174K, S182R, and W197X exhibit mild symptoms and A111T only causes variety of hair and skin colors.

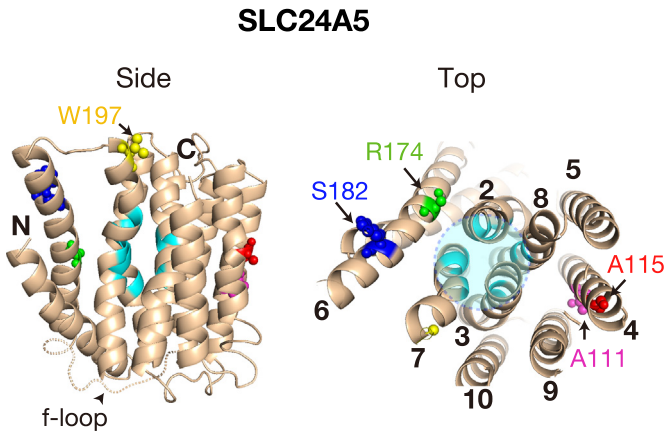

Fig. 5. 3D structural model of human SLC24A5.
Structural model of human SLC24A5 is predicted based on the structure of archaebacterial ortholog NCX_Mj; left, side view; right, top view. Seventy amino acid residues at N-terminus and residues 212–314 located between TM 5–6 covering the signaling peptides and f-loop, respectively, are omitted from the modeling for the best fit. The model contains 10 transmembrane domains in which TM1–5 and TM6–10 align in two-fold inverted repeats. Apparent f-loop (dash line) is manually drawn to connect TM5 and TM6. Amino acid sequence alignment of SLC24A1–SLC24A5 and NCX_Mj demonstrated that all ion binding sites in NCX_Mj are conserved among all SLC24 members. The predicted ion binding sites of SLC24A5 are A46, S49, S50 and E53 in TM2; S76, and, N80 in TM3; A204, T207, S208, and D211 in TM7; and S234, and D238 in TM8 (cyan area). Residues corresponding to type VI OCA mutations (A115, R174, and S182) and polymorphisms (A111) are shown in ball-and-stick. All mutation residues locate far away from the putative ion binding site and indeed the phenotypes of type VI OCA is considered as non-severe syndrome.

6.4. TPC2/TPCN2

6.4.1. Functional aspect

TPC2 (*TPCN2*, human gene NM_139075) gene was cloned based on the sequence similarity to *TPC1*, another member of two-pore channels (TPC) [59]. TPC2 (752 amino acids) is an organellar transporter localized in endosome, endolysosome, lysosome and melanosome and ubiquitously expressed in most tissues [41,58,59]. The 3D cryo-EM structure of TPC2 reveals the dimerization of TPC2 forming a *tetramer-like* voltage-sensing channel. Each subunit comprises two sets of homologous 6-TM domains. The voltage sensing domain is from TM1–4 helices. TM5 and TM6 from each set of protomers assemble to form a central ion conduction pore [105]. Unlike TPC1 and TPC3, TPC2 functions as a voltage-independent cation channel, meaning that the channel is opened in response only to the binding of its ligands, independently from membrane potential [105–107]. The opened channel allows the cation (Na^+ or Ca^{2+}) to flow across from lumen to cytosol (cation conductance).

The TPC2-regulating pathway in non-pigmented cells has been studied quite intensively, although the recognized ligands and ionic substrates of TPC2 are still controversial. TPC2 is found to be a receptor for either nicotinic acid adenine dinucleotide phosphate (NAADP) or PI(3,5)P_2 or both [108,109]. Both NAADP and PI(3,5)P_2 are intracellular second messengers in response to various stimuli. NAADP-sensing TPC2 mediates Ca^{2+} release in lysosome and endolysosome [58,59,110–114]. PI(3,5)P_2-dependent TPC activation induces Na^+ release [105,115–118]. Despite the difference of stimuli and responsive ions, most studies demonstrate the common role of TPC2 in lysosomal

stability and pH maintenance, implying the significance of TPC2 on lysosomal protein functions. Malfunction of TPC2 results in impairment of lysosomal acidification. Upstream molecules that regulate TPC2 are, for example, VEGF, mTOR, p38, and MAPK [113,119,120]. TPC2 has a wide effect on cellular activities such as cell invasion, autophagy, muscle contraction, and angiogenesis [112,118,119,121]. Notably, TPC2 is a target for Ebola virus and MERS-CoV (Middle East respiratory syndrome coronavirus) infectivity and treatment [122–124].

Both *TPC1* and *TPC2* were initially discovered as associated genes for genetic pigmentation in Europeans, but only TPC2 has been confirmed to exist in melanosomes [41,125,126]. Studies in melanosomes bring a similar debate to those in non-melanocyte's lysosomes about preferable stimulants and ions of TPC2. By using oocyte and melanocyte models, Lin et al. and Ambrosio et al. showed that TPC2 mediates Ca^{2+} release in response to NAADP [41,60]. Meanwhile, Bellono et al. demonstrated the $PI(3,5)P_2$ activates TPC2-mediated Na^+-selective conductance [126]. The response to $PI(3,5)P_2$ is further observed in a TPC2-expressing endolysosome models [63]. Nevertheless, loss of TPC2 clearly affects melanosomal pH and size, and melanin production. Likewise, TPC2 plays a role in pH maintenance by acting as a negative regulator for melanin production and pH neutralization (TPC2 promotes acidic pH) [41,126]. It is postulated that TPC2 generates membrane voltage (membrane potential) by the positive conductance, thus, controlling the function of V-ATPase for H^+ influx. The combinational function of the negative regulator TPC2 and the positive regulator OCA2 is also proposed to control melanosomal pH [126]. The anion conductance by OCA2 could be counterbalanced with the cation conductance by TPC2 in order to modulate V-ATPase function thereby maintaining ion and pH homeostasis inside the melanosomes (Fig. 3).

6.4.2. Mutations and polymorphism

TPC2 has not been reported to be associated with albinism, however, has been associated in the genetic colors and some pigmentation defect in the European nations. Genome-wide association studies (GWAS) found that TPC2 mutations in Icelandic and Dutch individuals are linked to hair color, freckles, and degrees of skin sensitivity to UV radiation. Residues G734E and M484L are significantly associated with blond hair over brown hair (Table 1) [125].

6.5. ATP7A/MNK

6.5.1. Functional aspect

ATP7A (*MNK*, human gene NM_000052), located on the X-chromosome, encodes 1500 amino acids for a P-type ATPase. ATP7A is a primary active transport protein that utilizes ATP hydrolysis to mediate Cu^{2+} transport out of the cytosol. ATP7A is ubiquitously expressed in most tissues and plays a central role in controlling Cu^{2+} balance in the cells. Cu^{2+} is an essential cofactor for enzymatic functions, however, excessive amounts of Cu^{2+} lead to tissue toxicity such as liver diseases and neurological defects [62,127,128]. Accordingly, cells need to restrict cellular Cu^{2+} homeostasis, and thus, ATP7A is critical. ATP7A has dual functions; in non-pigmented cells, ATP7A is trafficked between plasma membrane and trans-Golgi network via exocytosis and endocytosis [129]. Under normal conditions, ATP7A resides in the trans-Golgi network and supplies Cu^{2+} to the Cu^{2+}-dependent proteins (so called cuproproteins) such as cytochrome C oxidase and superoxide dismutase [130,131]. In the presence of an exceeding amount of Cu^{2+}, ATP7A is translocated to the plasma membrane and transports Cu^{2+} out of the cell. When Cu^{2+} returns to physiological level, ATP7A is relocated back to trans-Golgi network [128,132–134]. At the blood-brain barrier, ATP7A is trafficked to the basolateral membrane to promote Cu^{2+} delivery to the brain. ATP7A plays important roles in neuron activation, angiotensin-II-associated hypertension, cisplatin resistance and hepatic Cu^{2+} mobilization [62].

The 3D structure of ATP7A by homology modeling revealed the conserved core structure of P-type ATPases which is composed of transmembrane (M) domain assembled from MA, MB and TM1–6 helices, actuator (A) domain at TM2–3 loop, and phosphorylation (P) and nucleotide (N) domains located at TM4–5 loop [135]. The Cu^{2+} binding sites are located inside the M domain. All members of copper-ATPases contain N-terminal 6 heavy metal binding domains, which may be involved in autoinhibition and trafficking rather than Cu^{2+} transport [135]. Importantly, ATP7A contains a lumenal loop located between MA and MB called HM loop (His- and Met-rich loop) which is absent in ATP7B, another copper-transporting ATPase [135,136]. HM loop likely mimics the function of metallochaperones by serving itself as a platform for Cu^{2+} transferring from ATP7A to cuproproteins directly without specific chaperones. His and Met in HM loop are potential copper ligands, and they undergo His-to-Met ligand switching via His protonation in different pH. This pH-sensitive HM loop thereby accommodates conformational flexibility allowing Cu^{2+} transfer to different cuproproteins that exhibit optimal functions at different pH, e.g. metalation to TYR in neutral pH and PHM (peptidylglycine alpha-hydroxylating monooxygenase) in acidic pH [136].

In melanocyte, ATP7A is the transporter that supplies Cu^{2+} for TYR function. ATP7A is translocated from trans-Golgi network to melanosomes via BLOC-1 containing cargo which also sorts TYRP1 and OCA2 [22]. As described, Cu^{2+} is an indispensable cofactor for TYR function. Previous studies demonstrated that TYR exhibits as Cu^{2+}-bound form since it is located in trans-Golgi network (prior to translocation to the melanosome), and Cu^{2+} is continuously supplied to TYR to ensure TYR function in stages III–IV melanosomes [137]. Setty et al. confirmed that melanosomal ATP7A transports Cu^{2+} from cytosol to the lumen, thereby supplying Cu^{2+} to TYR during melanosome maturation (Fig. 3) [22]. One question remains to be addressed: how does TYR get Cu^{2+} loading during its localization in trans-Golgi network? ATP7A may be involved in this action as well because ATP7A and TYR coexist in the trans-Golgi network. Additionally, ATP7A is active in broad-range pH due to its H^+-controlling switch in HM loop [136]. Taken together, ATP7A may be functional to continuously supply Cu^{2+} to TYR at both trans-Golgi networks and melanosomes.

6.5.2. Mutations and polymorphism

Menkes disease (MIM#309400) and Occipital Horn syndrome (OHS) (MIM#304150) are the X-linked disorders of copper deficiency, which is caused by *ATP7A* [45–47]. Because *ATP7A* is located on the X-chromosome, the vast majority of Menkes disease patients are male. Since ATP7A is ubiquitously expressed and Cu^{2+} has wide functionality, the patients have various degrees of symptoms ranging from mild to death clinical syndromes [62,138,139]. Complete disrupting the protein function (immature protein or deletion) may lead to childhood fatality [140]. The *classical form* phenotype is characterized by progressive neurological degeneration, osteoporosis, connective tissue defect, spare hypopigmented hair, hypopigmented skin, and retinal degradation. The *milder form* is termed OHS, which has connective tissue abnormalities [62]. At present, more than 100 mutations have been reported.

7. Conclusion and future direction

This review summarizes the current knowledge of the ions and ion transport proteins in melanogenesis. We describe both the functional properties of ion transport proteins and the roles of the ion substrates. All ion transport proteins unite their unique functions to grant the central goal—to promote TYR function. TYR is the key enzyme for melanogenesis. TYR function is solely dependent on neutral pH and the existence of Cu^{2+} as a cofactor. All ion transport proteins play significant roles in this respect. ATP7A simply transports Cu^{2+} to supply Cu^{2+} to TYR. OCA2, SLC45A2 and TPC2 (perhaps also SLC24A5) are important for pH maintenance. Although the mechanism of pH regulation remains unclear, the goal of the transport proteins is simple—to change the acidic pH in premelanosomes to neutral pH in stages III–IV

melanosomes. To modulate pH, OCA2, SLC45A2, and TPC2 likely need to function together in a so called "*combinational function of ion transport proteins*". Based on the present information, we propose the model of combinational function of ion transport proteins for the pH maintenance in stages III–IV melanosomes (Fig. 3).

Existing research provides the key information: (1) Premelanosomes (stages I–II) has acidic pH from the function of V-ATPase, to authorize PMEL function; (2) OCA2, SLC45A2, and TPC2 along with TYR and TYRP1 are trafficked from trans-Golgi to melanosomes at stages II–III; (3) melanosomes at stages III–IV have neutral pH; and (4) TYR functions to produce melanin at neutral pH in melanosomes at stages III–IV. Together, these results suggest that the combination of OCA2, SLC45A2 and TPC2 functions serves as a "*switch*" for changing melanosomes from stage II to stage III. pH modulation leads to the termination of PMEL fibril formation indicating the end of stage II premelanosomes, and subsequently provides a neutral pH environment for initiation of TYR function indicating the beginning of stage III melanosomes. Additionally, pH appears to be critical for the trafficking of TYR and TYRPs.

The significance of the ion transport proteins in pigmentation is clear, yet the knowledge of their biological function and physiological roles is insufficient. To elucidate the combinational function of multiple ion transport proteins, understanding of the functional properties of each transport protein is crucial. Therefore, further characterization of the transport proteins is a must. Comprehensive studies of protein-protein interactions by mass spectrometry, so-called interactomics, will explain the combinational function of the ion transport proteins. Future genomic and transcriptomic studies may identify more gene candidates and fill the remaining pieces of the Jigsaw puzzle in melanogenesis. The latest advance in structure biology will give us structural information of the ion transport proteins in detail, which may suggest the transport mechanism of proteins. Most importantly, although it is not easy to dissect transport functions of organellar transport proteins, the transport function of the ion transporters can be revealed by using a classical biochemical method, the cell-free transport assay with proteoliposomes, purified protein-reconstituted liposomes as shown by Moriyama or others [141,142]. Recently, *MFSD12* (major facilitator superfamily domain containing protein 12) was identified as an associated gene in pigmentation in African population by GWAS [143,144]. In addition to the ion transport proteins we described above, MFSD12 requires characterization of its function and role in melanocytes. The melanosomal transport proteins provide a large number of open questions to us.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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