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Review

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).[1] 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 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.

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[1] *Abbreviations used:* UVR, ultraviolet radiation; RPE, retinal pigment epithelial; TRP, transient receptor potential; ER, endoplasmic reticulum; MITF, microphthalmia-associated transcription factor; SNPs, single nucleotide polymorphisms; MATP, membrane-associated transporter protein; GPCR, G protein-coupled receptor.

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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 formed by a channel fused to a C-terminal alpha kinase of unknown function [21]. TRPM7 channels are nonselective cation channels that mediate a current 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 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