Melanocyte Biology: Before, During, and After the Fitzpatrick Era

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Skin color has been a fascinating topic throughout human history, but even as late as the 17th century, explanations consisted of myths and imaginative accounts implicating divine intervention or associating dark constitutive pigmentation with intense sun and heat exposure. Well into the 20th century, little progress had been made in understanding the pigmentary process, although the finding in 1840 that the epidermis is composed of cells laid the foundation for more fruitful research. In 1946, at the First Pigment Cell Conference, data were presented that only DOPA-positive epidermal "melanoblasts" produce melanin and that the pigment is then transferred into surrounding cells (Masson, 1948). Since then, great progress has been made in our understanding of cutaneous pigmentation, and one of the pioneers was no doubt Dr Thomas B. Fitzpatrick. He made excellent use of the research tools then available—conventional biochemistry and the recently introduced electron microscope—and laid the foundation for subsequent advances largely employing molecular biology approaches. The following sections review Fitzpatrick's major contributions to the field—the central roles of tyrosinase and α-melanocyte stimulating hormone (α-MSH) in human pigmentation, identification of the melanosome as the pigmentary organelle, and the concept of the epidermal melanin unit—and briefly summarize more recent work in these key areas.

Melanogenic Enzymes

The pathway for melanin biosynthesis in invertebrates was well established by 1917, but vertebrate melanogenesis was riddled with controversies until mid-century. At that time, Fitzpatrick together with Aaron Lerner, in a series of seminal publications, identified tyrosine as the precursor molecule for melanin biosynthesis in mammals and showed that the enzyme tyrosinase is present in human skin and catalyzes the first two steps in melanin biosynthesis (Lerner *et al*, 1949, 1951; Fitzpatrick *et al*, 1950; Lerner, 1999).

Abbreviations: DAG, diacylglycerol; ET, endothelins; bFGF/FGF2, fibroblast growth factor; LAMP-1, lysosome associated membrane protein-1; MC1-R, melanocortin-1 receptor; MSH, melanocyte stimulating hormone; MITF, microphthalmia-associated transcription factor; NGF, nerve growth factor; SNARE, N-ethylmaleimide-sensitive factor attachment protein receptors; POMC, proopiomelanocrtin; PAR-2, protease activated receptor 2; PKC protein kinase C; TRP, tyrosinase related protein; UV, ultraviolet.

It was later found that the gene encoding tyrosinase belongs to a family of at least three genes thought to be duplications of the original tyrosinase gene (reviewed in Nordlund *et al*, 1998). Their products are enzymes that participate in the later steps of eumelanin biosynthesis: tyrosinase related protein (TRP)-1 (*b*-locus protein/gp75) and TRP-2 (DCT/*slaty* locus protein). TRP-1 and TRP-2 act as DHICAoxidase and DOPAchrome tautomerase, respectively, and *in vivo* appear to be complexed together with tyrosinase. TRP-1 and TRP-2 share 40% amino acid homology with tyrosinase and the divergence of *TRP-1* and *TRP-2* genes from *tyrosinase* gene is thought to have occurred prior to mammalian evolution, as these enzymes are present in all mammals studied to date. TRP-1 and TRP-2 appear to stabilize tyrosinase, optimizing its enzymatic activity and influencing melanogenic protein trafficking (Nordlund *et al*, 1998). In the years since Fitzpatrick demonstrated a central role for tyrosinase in human pigmentation, the cloning of tyrosinase has permitted identification of multiple specific mutations leading to the disease albinism (Nordlund *et al*, 1998; Oetting, 2000), and geneticists have identified more than 100 genes whose protein products modulate the melanogenic process in ways other than directly catalyzing melanin synthesis (Nordlund *et al*, 1998; Halaban *et al*, 2003).

Melanosomes

In a series of elegant experiments with Makoto Seiji, Fitzpatrick showed that tyrosinase is incorporated into special pigment granules that gradually melanize, and he coined the term "melanosomes" for them (Seiji *et al*, 1961). Subsequent studies have further characterized these lysosome-related organelles. At the earliest stage of melanosome development, tyrosinase is incorporated. Melanocytes also synthesize chaperone proteins, including calnexin and calreticulin, that specifically bind to glycoproteins such as tyrosinase and assure their appropriate folding and assembly (reviewed in Halaban *et al*, 2003). Tyrosinase, TRP-1 and TRP-2, as well as other melanosome-specific proteins like Pmel 17 (sliver protein/gp100) and the lysosome associated membrane protein-1 (LAMP-1) are then transported to the melanosomes, packaged in transport vesicles. A short cytoplasmic tail on these proteins containing a di-leucine motif protrudes from the vesicle surface and is recognized by a cargo protein complex (AP-3) that directs them into the melanosomes (Halaban *et al*, 2003).

Regulation of Tyrosinase Transcription and Activation

Following Fitzpatrick's identification of the importance of tyrosinase, others elucidated the control of tyrosinase expression and activation. The tyrosinase promoter was cloned and it was found that a short sequence of 115 bp contained all three elements necessary to positively regulate the transcription of the gene: M-box, Sp-1 site and E-box (Nordlund *et al*, 1998). The M-box is an evolutionarily conserved 11 bp sequence that is bound by the microphthalmia-associated transcription factor (MITF), a factor belonging to the family of basic helix-loop-helix leucine-zipper transcription factors (Yasumoto *et al*, 1994). Interestingly, the promoters of *TRP-1* and *TRP-2* also contain an M-box and an E-box. cAMP through the cAMP dependent-protein kinase A pathway up-regulates *tyrosinase, TRP-1* and *TRP-2* transcription through the M-box and the E-box that are present in their promoters. These effects are mediated, at least in part, through MITF as cAMP induces MITF transcription and enhances MITF binding to its consensus sequence in the promoter (Bertolotto *et al*, 1998). The ubiquitous Sp-1 transcription factor is thought to control the constitutive level of tyrosinase expression. Recognition that tyrosinase transcription is also up-regulated by the tumor suppressor protein p53 (Nylander *et al*, 2000; Khlgatian *et al*, 2002) further validated the clinically appreciated role of melanin pigmentation in preventing UV-induced epidermal injury and specifically photocarcinogenesis. Indeed, recent work identifies enhanced melanogenesis as an integral part of the cellular response to DNA damage (Gilchrest and Eller, 2001).

It gradually became apparent that tyrosinase mRNA and protein levels did not alone dictate tyrosinase activity, which in turn determines the rate of melanogenesis. Work has identified tyrosinase as a phosphoprotein requiring activation by the protein kinase C (PKC) pathway (Park *et al*, 1993). Delayed tanning, a photoprotective cutaneous response to UV irradiation, appears within 3–4 days after a single UV exposure and clinically parallels increased tyrosinase activity in melanocytes. UV-irradiation disrupts cellular membranes, cleaving the lipid bilayer to release the molecule diacylglycerol (DAG), which is the secondary messenger similarly released by growth factor or cytokine binding to cell surface receptors. DAG then activates the enzyme protein kinase C (PKC) that phosphorylates proteins on serine/threonine residues. It was found that activation of the β isoform of PKC specifically phosphorylates two serine residues in the cytoplasmic domain of tyrosinase (Park *et al*, 1999), a transmembrane protein with a "tail" that projects outside the melanosome, and this phosphorylation event is required for melanogenesis (reviewed in Nordlund *et al*, 1998).

Melanocortins and their Receptors

As early as 1912 it was known that factors present in bovine pituitary extract can darken frog skin (reviewed in Nordlund *et al*, 1998). However, in 1954 Drs Lerner and Fitzpatrick were the first to name the activity "melanocyte stimulating hormone" (MSH) and to show that when injected into humans, MSH produces skin darkening (Lerner *et al*, 1954). Since then, the melanocortins, a group of peptides derived from a precursor protein proopiomelanocortin (POMC), have been thoroughly characterized. Differential processing of POMC, a molecule now known to be produced not only in the pituitary but also in other tissues including the skin, gives rise to several peptides including γ-MSH, α-MSH, β-MSH, ACTH, γ-lipotropin and β-endorphin. Melanocortins bind their specific receptors, a family of G-protein-coupled seven-membrane spanning molecules (Nordlund *et al*, 1998). α-MSH, by binding its cognate melanocortin-1 receptor (MC1-R), activates adenylate cyclase, elevating intracellular cAMP levels and leading to *tyrosinase, TRP-1* and *TRP-2* transcription. Interestingly, it was found that loss-of-function-mutations in *MC1-R* characterize individuals with red and blond hair, implying that the synthesis of eumelanin *versus* phaeomelanin is regulated through MC1-R (reviewed in Rees, 2000). At least in mice and likely also in humans, the action of α-MSH on MC1-R can be competitively inhibited by the agouti protein that occupies but does not activate the receptor (Willard *et al*, 1995).

The Epidermal Melanin Unit

Fitzpatrick and Breathnach (1963) first coined the term "epidermal melanin unit" to describe a single basilar melanocyte surrounded by the epidermal cells, specifically keratinocytes (36 in their estimate), to which it supplies melanin. Fitzpatrick and Lerner also suggested the use of the term "melanocyte" to describe pigment producing cells of higher vertebrates, compared to the term "melanophore" for analogous cells in lower vertebrates, and eliminating the previously used term "melanoblast" that implied an undifferentiated state (Fitzpatrick *et al*, 1966; Lerner, 2004). It has since become evident that there is extensive cross-talk between keratinocytes and melanocytes and that keratinocytes affect melanocyte proliferation, melanogenesis and dendricity via paracrine stimulation. Not surprisingly, the majority of these keratinocyte-derived signals are induced by UV irradiation (reviewed in Nordlund *et al*, 1998; Halaban *et al*, 2003). These include basic fibroblast growth factor (bFGF/FGF2) (Halaban *et al*, 1988), endothelin (ET-1) (Imokawa *et al*, 1992), α-MSH (Bhardwaj and Luger, 1994), and nerve growth factor (NGF) (Yaar *et al*, 1991). Acting through their cognate receptors on the melanocyte surface, these and other keratinocyte-derived factors variously modulate cAMP levels; increase tyrosinase gene transcription; induce melanocyte proliferation, migration and dendricity; and enhance melanocyte survival after UV irradiation.

Movement of melanosomes along melanocyte dendrites and their eventual transfer to surrounding keratinocytes is necessary if melanin is to achieve its function of protecting cells in the skin from UV (and perhaps other) injury. Two cytoskeletal macromolecule-polymers, actin filaments and microtubules, direct intracellular transport of cytoplasmic organelles like melanosomes (reviewed in Marks and Seabra, 2001; Halaban *et al*, 2003). Melanosomes are transported along these pathways by small motor proteins: kinesin (Hara

et al, 2000) and dynein/dynactin (Byers *et al*, 2000), acting as short cross-bridge structures connecting the melanosome to the microtubule; and myosin Va, a member of the myosin V family, that is the actin filament-based motor protein (Wu *et al*, 1998). In addition, linker proteins have been identified that link the melanosome to myosin Va. These include the protein Rab27a that encodes a small GTPase protein and melanophilin, a member of the Rab family of proteins. Recent data establish that melanosome movement in dendrites is bi-directional, although net movement is usually outward. Kinesin promotes anterograde transport of melanosomes toward the dendrite tip, while dynein/dynactin promotes their retrograde movement toward the nucleus.

Once the melanosomes reach the dendrite tips they are transferred to keratinocytes where they preferentially localize above the nucleus forming a "protective cap", a process that relies on dynein (Byers *et al*, 2003). Three distinct modes of melanosome transfer are currently thought to occur (Marks and Seabra, 2001; Halaban *et al*, 2003). The first involves phagocytosis of dendrite tips by surrounding keratinocytes, a process mediated in part through the protease activated receptor 2 (PAR-2) that is present on keratinocytes (Seiberg *et al*, 2000); the second is through fusion of melanocyte and keratinocyte membranes, a process mediated in part by N-ethylmaleimide-sensitive factor attachment protein receptors (SNARE) that are present on the membranes of the participating cells (Scott *et al*, 2002), creating a conduit for melanosomal transfer; and the third involves exocytosis of melanosomes into the intercellular space where they are phagocytosed by keratinocytes (Marks and Seabra, 2001).

Perspectives

Over the past 50 years, our understanding of melanocyte biology has expanded remarkably. We now appreciate many of the events within the epidermal melanin unit that maintain or increase melanocyte number in response to epidermal injury, affect melanin synthesis, and enhance melanosome transfer to keratinocytes. This detailed knowledge is based largely on the seminal work of Dr Fitzpatrick and colleagues, who paved the way for new generations of investigators to explore the fascinating topic of melanocyte biology.

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