Melanocytes are pigment-producing cells that originate from the dorsal portions of the closing neural tube in vertebrate embryos (Fig. 70-1). They derive from pluripotent neural crest cells that differentiate into numerous cell lineages, including neurons, glia, smooth muscle, craniofacial bone, cartilage, and melanocytes (reviewed in refs. 2 and 3). Progenitor melanoblasts migrate dorsolaterally between the mesodermal and ectodermal layers to reach their final destinations in the hair follicles and the skin as well as inner ear cochlea, choroids, ciliary body, and iris (reviewed in refs. 2 and 4). Pigment-producing cells can be found in fetal epidermis as early as the fiftieth day of gestation.

Melanoblast migration and differentiation into melanocytes is influenced by a number of signaling molecules produced by neighboring cells. These include Wnt, endothelin-3 (ET3), bone morphogenetic proteins (BMPs), steel factor (SF; stem cell factor (SCF), c-Kit ligand), and hepatocyte growth factor (HGF)/scatter factor. By interacting with their specific cell surface receptors, these molecules induce intracellular and intranuclear signaling to influence gene transcription and protein synthesis.

**Wnt**

Wnt is expressed in the dorsal neural tube during neural crest cell migration and directs the maturation of pluripotent neural crest cells into melanoblasts. The Wnt family is composed of 16 different secreted glycoproteins. They bind and activate Frizzled, a transmembrane heptahelical G protein–linked receptor, and induce the accumulation of β-catenin (Fig. 70-2A). Under baseline conditions, when Wnt does not bind Frizzled, cytosolic β-catenin is complexed with the enzyme glycogen synthase kinase 3β (GSK3β) that induces rapid ubiquitin-mediated degradation of β-catenin by cellular proteosomes. Binding of Wnt to its receptor Frizzled inhibits GSK3β activity, leading to β-catenin accumulation in the cytosol followed by its translocation to the nucleus (Fig. 70-2B). In the nucleus, β-catenin binds specific transcription factors, and together the complex induces the transcription of microphthalmia-associated transcription factor (Mitf). Mitf affects melanoblast differentiation by inducing the transcription of three enzymes that regulate melanin synthesis: tyrosinase, tyrosinase-related protein-1 (TRP-1), and 3,4 dihydroxyphe-nylanine (DOPA)chrome tautomerase (TRP-2) (reviewed in ref. 12).

**Bone Morphogenetic Proteins**

BMPs are disulfide-linked dimeric proteins produced as large precursors. They include more than 20 secreted proteins all sharing amino acid homology (reviewed in ref. 14). BMPs belong to the transforming growth factor-β family of secreted growth factors, and their signaling suppresses neural crest cell differentiation into melanoblasts and thus may be viewed as Wnt antagonists. Accordingly, there is a decrease in BMP expression in the dorsal neural tube at the time of melanoblast migration.

**Endothelins**

ETs are a family of peptides, 21 amino acids long, originally identified by their...
vasoactive properties, that include three members, ET1, ET2, and ET3 (reviewed in ref. 15). They are produced via proteolysis of larger precursor molecules. Like Wnt, ETs bind and activate heptahelical transmembrane G protein–linked receptors EdnrA and B. ET3, which is synthesized by ectodermal cells, and its EdnrB receptor are particularly important during melanoblast migration along the dorso-lateral pathway (reviewed in ref. 15), and their proper expression is required for survival, proliferation, and/or migration of melanoblasts.

Defects in either ET3 or its EdnrB receptor result in prominent melanocyte loss. It appears that the receptor plays a more critical role in melanocyte development as compared to its ligand, as ET3 null mice do not have as severe depigmented phenotype as EdnrB null mice. Because ETs and Ednrs also affect the development of neural crest cells other than melanoblasts, defects in ET3 or EdnrB lead to disease symptomatology beyond pigmentary disorders, such as that present in type IV Waardenburg syndrome and in Hirschsprung syndrome (see also Chap. 71).

**Steel Factor**

Early melanoblast development requires the presence of the cytokine SF (mast/SCF, c-Kit ligand) and its tyrosine kinase transmembrane receptor c-Kit. SF is expressed by epidermal keratinocytes, and, as soon as c-Kit is expressed on melanoblasts, they begin their migration to their final destination (reviewed in ref. 18).

Piebaldism is an autosomal dominant disorder that results from mutations of c-Kit or SF and leads to melanoblast failure to migrate to the skin and/or survive there (see Chap. 71). Affected individuals display broad depigmented patches, most prominent on the central forehead and trunk. Interestingly, the ventral aspect of the body is more frequently affected than the dorsal aspect, supposedly because it is the area farthest from the dorsally located neural crest where melanoblast migration begins. Complete absence of melanocytes as well as abnormalities of the reproductive and hematopoietic systems, whose development also depends on SF/Kit receptor, have been observed in mice with homozygous loss of SF or c-Kit.

**Hepatocyte Growth Factor**

HGF (scatter factor) is the ligand for the transmembrane tyrosine kinase receptor Met. In vitro studies performed on murine neural crest cells show that HGF induces melanoblast proliferation and allows their differentiation into mature melanocytes. In addition, HGF regulates cadherin expression in melanocytes, specifically downregulating E-cadherin, thus affecting melanoblast homing (see Cadherins). Interestingly, in mice, Met null mutations do not appear to affect melanoblast number or migration, and no decrements in Met function have been identified to date in human pigmentary disorders. Thus, the role of HGF/Met signaling during melanocyte development is still unclear.
**Cadherins**

Cadherins are a family of transmembrane glycoproteins (E-, P-, and N-) that promote calcium-dependent cell-to-cell adhesion (reviewed in ref. 25). In melanoblasts, their cytoplasmic domains bind β-catenin. Murine studies show that E-cadherin is induced in melanoblasts before their entry into the epidermis. Later, when melanoblasts migrate into the hair follicle, E-cadherin expression is muted, and melanoblasts begin to express P-cadherin. It is thought that coordinate expression of E-cadherins by epidermal keratinocytes and melanocytes plays a role in suppressing melanocyte proliferation in the epidermis. Interestingly, at times, some epidermal melanocytes switch from E-cadherin to N-cadherin, and this switch allows them to escape the keratinocyte-mediated growth suppression and proliferate/aggregate in nests to form neovascular nevi. Generally, cadherin expression by melanocytes matches cadherin expression of surrounding cells.

**SITE-SPECIFIC MELANOCYTES**

**Melanocyte Stem Cells**

Generally, stem cells are defined by their undifferentiated state and their capacity to develop into several differentiated cell types. They are quiescent, slow-cycling cells that frequently are found in niches where they are surrounded by differentiated cells that affect their behavior through the secretion of cytokines and growth factors (reviewed in refs. 29 and 30).

Melanocyte stem cells reside in the hair follicle bulge (Fig. 70-5). They express TRP-2 as well as the neural crest stem cell intermediate filament nestin in addition to other neural crest stem cell markers, including the transcription factors Sox10 and Pax5 that participate in Sox10 and Pax5 that participate in differentiation and proliferation. In addition to other neural crest stem cell markers, Sox10 and Pax5 are also present in the neural crest. In addition to other neural crest stem cell markers, Sox10 and Pax5 are also present in the neural crest.

**Cutaneous Melanocytes**

The largest number of melanocytes is present in the skin and hair follicles. In most furred mammals, melanocytes are found only in the hair follicle, but in humans melanocytes are also present in interfollicular epidermis, specifically in the basal layer (reviewed in ref. 28). There is approximately one melanocyte per five or six basal keratinocytes. Melanocytes synthesize melanin, a pigmented polymer that is stored in cytosolic organelles called melanosomes. They are transferred to keratinocytes through melanocyte dendritic processes (Fig. 70-4). As keratinocytes are continuously being desquamated, there is a constant need for synthesis and transfer of melanosomes from melanocytes to keratinocytes to maintain cutaneous pigmentation.

The term "epidermal melanin unit" describes a single epidermal melanocyte surrounded by several epidermal keratinocytes (Fig. 70-5). Interestingly, signals from keratinocytes substantially regulate epidermal melanocyte survival, dendricity, melanogenesis, and the expression of cell surface receptors (see Signaling Pathways Regulating Melanocyte Function). Most keratinocyte-derived signals are induced by ultraviolet (UV) irradiation.

Melanocyte density/mm² ranges from approximately 550 to > 1200, with the highest concentrations found in the genitalia and face. Melanocyte density is the same in individuals of different ethnic backgrounds, and thus cutaneous pigmentation does not depend on melanocyte number, but rather on melanogenic activity within the melanocyte, the proportion of mature melanosomes, and/or their transfer and distribution within the keratinocytes (reviewed in ref. 35). Indeed, in light-skinned individuals, melanosomes are smaller and are present in clusters within the keratinocytes, whereas in ethnic groups with darker complexion, melanosomes are larger.

![Figure 70-3](image1.png) **Melanocyte stem cells in the hair follicle bulge.** A stem cell melanocyte is shown in the hair follicle bulge, indicated by an arrow in the high-power insert. These cells stain positive for TRP-2 (green fluorescence), an early marker of commitment to the melanocyte lineage, but are negative for the proliferation marker Ki-67 (red fluorescence) that characterizes melanocytes migrating down the follicle to the dermal papilla during the anagen phase of the hair cycle. (From Botchkareva NV et al: SCF/c-kit signaling is required for cyclic regeneration of the hair pigmentation unit. *FASEB J* **15**:645, 2001, with permission.)

![Figure 70-4](image2.png) **Melanosomes are organized into supranuclear “caps” within keratinocytes.** Note melanized dendritic melanocytes and adjacent keratinocytes with the supranuclear “caps.” Melanin silver stained (Fontana-Masson) section of a heavily melanized human epidermis. (Bar = 50 μM.) (From Byers HR et al: Role of cytoplasmic dynein in perinuclear aggregation of phagocytosed melanosomes and supranuclear melanin cap formation in human keratinocytes. *J Invest Dermatol* **121**:813, 2003; with permission.)
darker, and are individually dispersed within the keratinocytes.\(^{36}\)

**Hair Follicle Melanocytes**

In contrast to interfollicular epidermal melanocytes, the follicular melanin unit undergoes cyclic modifications in coordination with the hair cycle (Fig. 70-6). Melanocytes are located in the proximal hair bulb during anagen, and there is a ratio of 1:5 between melanocytes and keratinocytes and 1:1 between melanocytes and basal layer keratinocytes.\(^{37}\)

Melanocytes proliferate, migrate, and undergo maturation during early to mid anagen. Melanogenesis and melanin transfer to keratinocytes occur throughout anagen. Melanocytes eventually apoptosis during late catagen. In mice, melanocyte proliferation and differentiation during anagen depends on c-Kit expression by melanocytes and SF synthesis by keratinocytes.\(^{38}\)

Similar to their role in the epidermis, in hair, melanocytes transfer melanin to differentiated keratinocytes that ultimately form the hair shaft. They thus determine hair color by the amount of melanin transferred, as well as by the ratio of eumelanin (black-brown) to pheomelanin (red-yellow) (see Melanin Biosynthesis) (reviewed in ref. 39).

In hair, melanin does not appear to have a protective effect, as UV irradiation does not reach the hair follicle. Still, in furry animals hair color plays an important role in camouflage, mimicry, and social communication.\(^{40}\) It is also speculated that melanocytes restrain keratinocyte proliferation, affect calcium homeostasis, and protect against reactive oxygen species (ROS) during the rapid proliferation and differentiation of the hair follicle.\(^{37}\)

**Ocular Melanocytes**

Ocular melanocytes are found in the uveal tract (reviewed in ref. 28). Unlike cutaneous melanocytes, ocular melanocytes are in contact only with each other, and they do not transfer their melanosomes (Fig. 70-7). It is proposed...
that ocular melanocytes are exposed to high oxygen tension and melanin serves as protection against oxidative damage. Iris melanin may be required to protect capillaries, muscles, and motor nerves that control pupil contraction.28 Melanocytes appear to be essential for the development and function of the eye and optic nerve (reviewed in ref. 41). It is thought that they may play a role in the proper routing of ipsilateral and contralateral neural fibers in the optic chiasm during early development of the optic cup,42,43 as in the absence of melanin, the path of the highly intermixed optic nerve axons is deranged, resulting in disruption of the topographic relationship of the nerve fibers.44,45 The importance of melanin for proper ocular function is demonstrated by the visual abnormalities observed in patients with albinism.44

Otic Melanocytes

Melanocytes reside in the cochlea28 and are important for hearing, as loss of otic melanocytes leads to deafness. It is thought that otic melanocytes help in the maintenance of the endolymph through the regulation of potassium transport.46 The endolymphatic fluid in the cochlea is one of the few extracellular fluids with high concentrations of positively charged potassium, and melanocytes are believed to play a critical role in its maintenance, probably by transporting potassium from areas of high concentration to areas of low concentration through plasma membrane ionic channels.47 In the absence of cochlear melanocytes, the endolymphatic potential is low, leading to deafness.48 Loss of melanocytes at any age can eventually lead to deafness, and patients with Waardenburg syndrome type 2 (see Chap. 71) are deaf because of lack of melanocytes in the inner ear.49,50 Interestingly, the preservation of hearing in albinos indicates that melanin production within otherwise viable melanocytes is not essential for hearing. However, it appears that because albinos are more susceptible than the normal population to hearing loss from noise and/or exposure to toxic agents,51,52 melanin must provide some protective effect.

Cephalic Melanocytes

Melanocytes are dispersed throughout the meninges and are particularly dense...
in the leptomeninges above the pons and the medulla oblongata.53 They are thought to function as scavengers for toxic cations and ROS.54,55

MELANIZATION

The major differentiated function of melanocytes is to synthesize melanin in specialized organelles within the melanocytes, the melanosomes, and to transfer melanosomes to neighboring keratinocytes to provide protection from UV irradiation (Fig. 70-8). Pigmentation, the synthesis and distribution of melanin in the epidermis, involves several steps: transcription of proteins required for melanogenesis, melanosomal biogenesis, sorting of melanogenic proteins into the melanosomes, transport of melanosomes to the tips of melanocyte dendrites, and transfer of melanosomes to keratinocytes. Disruption in any of these events results in hypopigmentation.

MELANOSOMES

Melanosomal Biogenesis

The melanosome is a unique membrane-bound organelle in which melanin biosynthesis takes place. Because melanosomes contain enzymes and other proteins also present in lysosomes, they are thought to represent a modified version of the latter. Proteins common to both organelles include the liposomal-associated membrane proteins (LAMPs) that participate in autophagy and regulation of intra-vesicu-

lar pH (reviewed in ref. 56), as well as acid phosphatase, a marker enzyme for lysosomes.56 Also like lysosomes, melanosomes can endocytose receptors that are targeted for degradation.56

Depending on the type of melanin synthesized, melanosomes can be divided into eumelanosomes and pheomelanosomes (Fig. 70-9). Eumelanosomes are large (~0.9 × 0.3 µm), elliptical in shape, and contain a highly structured fibrillar glycoprotein matrix required for eumelanin synthesis.40 Pheomelanosomes are smaller (~0.7 µm in diameter), spherical in shape, and their glycoprotein matrix appears disorganized and loose.40 Although both eumelanosomes and pheomelanosomes may be present within a single melanocyte,57 once committed, they do not change.58

Melanosomes display four maturation stages (see Fig. 70-9). Stage I melanosomes or premelanosomes likely develop from the endoplasmic reticulum (ER).40 They have an amorphous matrix and display internal vesicles that form as a result of membrane invagination. Premelanosomes already contain the glycoprotein Pmel17 (gp100), but it requires further processing to become a component of the final fibrillar matrix.59 Stage II eumelanosomes have organized structured fibrillar matrix, but no active melanin synthesis, whereas in stage II pheomelanosomes, melanin synthesis already takes place. Although no active melanogenesis takes place in stage II eumelanosomes, they already contain the enzyme tyrosinase. Deposition of melanin on the fibrillar matrix is found in stage III eumelanosomes, whereas stage IV eumelanosomes are fully melanized, and their internal matrix is masked by melanin deposits (reviewed in refs. 60 and 61).

Melanogenic Proteins

The timely and organized sorting of melanogenic enzymes and structural proteins to melanosomes is an integral part of melanosomal maturation. Melanosomes express sorting signals at their amino terminus, and these direct them into the ER and eventually into the melanosomes (reviewed in refs. 40, 60, and 61).

ENZYMES Tyrosinase. Tyrosinase is present in plants, insects, amphibians, and mammals. It was initially identified in the early 1900s in mushroom extracts and was subsequently isolated and purified in 1949 from murine melanoma cells.62 Mouse and human tyrosinase genes are 60 to 70 kb, and 50 kb long,
respectively. The murine tyrosinase gene maps to chromosome 7, whereas human tyrosinase gene maps to chromosome 11. The human tyrosinase gene is composed of 5 exons and 4 introns, and tyrosinase messenger RNA is approximately 2-kb long (gene bank access number NM_000372).

Tyrosinase is synthesized in the ER as a precursor protein whose nascent chain is processed in the Golgi complex where sialic acid and neutral sugars are added to the peptide via N- and O-glycosidic linkages through a process called glycosylation (reviewed in ref. 64). At least four forms of tyrosinase, all differing with regard to their degree of glycosylation, have been identified. The glycosylation steps have been shown to be important for proper association of tyrosinase with melanosomes, as well as for its activity. After the glycosylation steps, mature tyrosinase is folded in the ER, a step required for appropriate trafficking/sorting of tyrosinase into the Golgi apparatus and ultimately into endosomes and finally into melanosomes. A strict control mechanism guarantees the elimination of defective tyrosinase.

Within the melanosome, tyrosinase spans the melanosomal outer membrane (Fig. 70-11). It has three domains: an inner melanosomal domain, a melanosomal transmembrane domain, and a cytoplasmic domain. The inner domain that contains the catalytic region is approximately 90 percent of the protein. It is followed by a short transmembrane domain and a cytoplasmic domain composed of approximately 30 amino acids (reviewed in ref. 65). Histidine residues present in the inner (catalytic) portion of tyrosinase bind copper ions, and the latter are required for tyrosinase activity. The biologic function of the tyrosinase cytoplasmic domain was not known for a long time. In a mouse model in which the entire cytoplasmic domain is missing, tyrosinase protein is inserted into the cellular plasma membrane instead of into the melanosomal membrane, suggesting that tyrosinase cytoplasmic domain is required for proper trafficking of tyrosinase into melanosomes. Indeed, it was found that the motif EXXQPLL (glutamic acid-X-X-glutamine-proline-leucine-leucine, where “X” stands for any amino acid) in the cytoplasmic domain is responsible
for tyrosinase trafficking into the melanosomes. In addition, protein kinase C-β (PKC-β) (see Protein Kinase C-β) must phosphorylate two serine residues on this cytoplasmic domain to activate tyrosinase, and in the absence of those phosphorylation events pigmentation does not occur.

Tyrosinase mutations, including missense, nonsense frameshift, and deletion mutations that lead to inactivation of the enzyme, are found in ocukulocutaneous albinism type I (see Chap. 71 and Albinism database: http://albinismdb.med.umn.edu/). Such mutations may affect tyrosinase glycosylation, interfering with enzyme maturation, or may involve copper binding sites, disrupting tyrosinase activity (reviewed in ref. 64).

**Tyrosinase-Related Proteins.** Two TRPs, TRP-1 and TRP-2, play important roles in melanogenesis. They are structurally related to tyrosinase and share ~40 percent amino acid homology. Also, similar to tyrosinase, TRP-1 and TRP-2 are glycoproteins located within the melanosomes and span the melanosomal membrane. The conserved nucleotide and amino acid sequences among these three melanogenic enzymes suggest that they originated from a common ancestral gene.71,72

**TRP-1.** In mice, TRP-1 maps to the brown locus on chromosome 4 and spans ~18 kb on the genomic DNA. The human homolog of TRP-1 maps to chromosome 9 and spreads over 24 kb of the genomic DNA. Like tyrosinase, TRP-1 is synthesized in the ER and undergoes several glycosylation steps (see Fig. 70-10). The exact function of TRP-1 in melanin biosynthesis, especially in human melanogenesis, is not well understood. In mice, TRP-1 displays a wide range of enzymatic activities, including DOPAchrome tautomerase, tyrosine hydroxylase, DOPA oxidase, catalase, and 5,6-dihydroxyindole-2-carboxylic acid (DHICA) oxidase (see Melanin Biosynthesis). Conversely, in humans TRP-1 does not display DHICA oxidase activity, and the additional activities described above have not been reported to date for this enzyme.

Nevertheless, the presence of TRP-1 appears to be required for melanin synthesis in human melanocytes, as TRP-1 absence results in the pigmentary disorder ocukulocutaneous albinism type 3 (see Chap. 71). Because recently TRP-1 was shown to influence tyrosinase activity by forming a complex with tyrosinase, it is possible that TRP-1 plays a role in tyrosinase activation and/or stabilization. A known TRP-1 function in both murine and human melanocytes at least in vitro is to increase the ratio of eumelanin to phaeomelanin. TRP-1 may also play a role in melanosomal biogenesis, as suppression of TRP-1 is associated with structurally abnormal melanosomes.

**TRP-2.** In mice, TRP-2, also known as DOPAchrome tautomerase, maps to the Slty locus on chromosome 14, whereas human TRP-2 maps to chromosome 13. Mouse and human TRP-2 share 84 percent nucleotide homology. Like tyrosinase and TRP-1, TRP-2 is a glycoprotein that is synthesized in the ER and undergoes several maturation steps in the Golgi and trans-Golgi network (see Fig. 70-10). Like tyrosinase and TRP-1, it eventually localizes to melanosomes, and it spans the melanosomal membrane. Within the melanosome, TRP-2 is complexed with tyrosinase and TRP-1. During melanin synthesis, TRP-2 converts DOPAchrome to the carboxylated derivative DHICA. As is the case for tyrosinase, TRP-2 also requires metal ions for its enzymatic activity, but it is zinc rather than copper that is required for TRP-2 function.

**Protein Kinase C-β.** PKC constitutes a family of at least 12 isoforms (reviewed in ref. 86) among which PKC-β has been shown to be involved in regulating tyrosinase activity. The mechanisms through which PKC mediates a wide range of membrane-generated signals and their relevance to melanocyte biology are further discussed below (see Signaling Pathways Regulating Melanocyte Functions. PKC-β phosphorylates serine residues on the cytoplasmic domain of tyrosinase, thus activating tyrosinase. Still, the means by which PKC-β-mediated phosphorylation of tyrosinase leads to the enzyme activation is not well elucidated. It has been suggested that phosphorylation of tyrosinase causes a complex to form between tyrosinase and TRP-1, an event known to stabilize tyrosinase and increase its enzymatic activity.

In melanocytes, activated PKC-β is associated with melanosomes, and the enzyme is found in close proximity to the melanosomal membrane. Although structural differences among PKC isoforms may contribute to their association with particular subcellular fractions, receptors for activated C-kinase (RACK), unique for each PKC isoform, primarily determine the translocation of specific PKC isoforms to specific cellular compartments to activate its target on the membrane (Fig. 70-12). RACK-I is the PKC-β partner, and in human melanocytes, the activated PKC-β/RACK-I complex translocates to the melanosome membrane to allow tyrosinase phosphorylation (see Fig. 70-12).

**Structural Proteins.** Fibrillar matrix proteins within the melanosomes are required for proper deposition of melanin. Pmel17 and MART-1 are two such melanosomal structural matrix proteins.

Pmel17. Pmel17, also known as gp100 and the silver locus product, is a glycoprotein recognized by the antibodies
MART-1/Melan A. MART-1, also known as Melan A, is a membrane-associated protein that is present in stage I and II melanomas and forms a complex with Pmel17 (see Fig. 70-10). MART-1 affects the expression, stability, trafficking, and processing of Pmel17 within the melanosomes. To date, no hypopigmented phenotypes associated with nonfunctional MART-1 have been identified.

ADDITIONAL MELANOCYTIC PROTEINS P Protein. The p protein (pink-eyed dilution) is a transmembrane protein with 12 membranes spanning domains whose sequence is homologous to that of other transmembrane transport proteins, including anion transporters thought to function as a transport protein. Studies have identified the protein as an adenosine triphosphate–associated proton pump responsible for maintaining acidic environment within the melanosomes (reviewed in ref. 102). Other proposed functions of p protein include stabilizing the tyrosinase/ TRP-1/TRP-2 complex and/or transporting tyrosine into the melanosomes. Individuals lacking functional p protein display ocoulcuteaneous albinism type 2, largely due to improper melanosomal pH. Also, Angelman and Prader-Willi syndromes display deletion mutations that include the p locus on chromosome 15.

Heterotetrameric Adaptor Protein Complexes. Sorting of membrane-associated proteins, including tyrosinase, TRP-1, TRP-2, and Pmel17, and directing them to the appropriate cytosolic organelles are facilitated by heterodimeric adaptor protein complexes (APs). AP-3 and possibly also AP-1 facilitate tyrosinase transport from endosomes to melanosomes (see Fig. 70-10). Patients with Hermansky-Pudlak syndrome, an autosomal recessive disorder of ocoulcuteaneous albinism, platelet dysfunction, and pulmonary disease (see Chap. 71), have defects in specific subunits of the AP-3 adaptor protein complex and as a result display several anomalies associated with cellular transport of molecules.

SLC24A5. SLC24A5 is a melanosomal protein whose structure and homology to cation exchange proteins suggests that it is a melanosome-associated cation exchanger. Mutations in slc24a5 in zebrafish lead to hypopigmentation of the organism. The ancestral human homolog is expressed by darker complexioned individuals, including Africans and Asians, whereas lighter-complexioned Europeans tend to express a variant allele.

LYSOSOMAL-ASSOCIATED MEMBRANE PROTEINS. LAMPs are linked to melanososome membranes and/or matrix. They are thought to protect melanosomal integrity by acting as scavengers of free radicals that are produced during melanin biosynthesis. Because LAMPs are also present in lysosomes, it is thought that melanosomes and lysosomes share a common ancestral origin.

REGULATORY PROTEINS Microphthalmia-Associated Transcription Factor. A major melanogenic proteins, tyrosinase, the master gene for melanocyte survival and is a key factor regulating the transcription of the major melanogenic proteins, tyrosinase, TRP-1, TRP-2, PKC-β, and MART-1. Mitf binds to conserved consensus elements in gene promoters, specifically the M- (AGTCTAGTGC) and E- (CATGTC) boxes. It can bind as a homodimer or a heterodimer with another related family member (reviewed in ref. 114). Mitf appears to be a key regulator determining cell fate, as transfection of human Mitf complementary DNA into mouse fibroblasts converts these cells into dendritic cells expressing melanocyte-specific genes.

Mitf comprises a family of nine isoforms, Mitf-M, -A, -B, -H, -C, -D, -E, -J, and -Mc. Mitf-M expression is highly specific for melanocytic cells. Melanocytes express in addition other Mitf isoforms, specifically, Mitf-A, -B, and -E. In melanocytes, it is the Mitf-M isoform that stimulates transcription of tyrosinase and PKC-β. The biologic role of other Mitf isoforms in normal melanocytes is not known.

REGULATION OF MICROPHTHALMIA-ASSOCIATED TRANSCRIPTION FACTOR ACTIVITY AND EXPRESSION. The activity and stability of Mitf are modulated by phosphorylation of the protein. Mitf activity is increased on its phosphorylation by the mitogen-activated protein kinase-2 (MAP kinase-2), whose activity is in turn induced by binding of SF/c-Kit to c-Kit receptor (see Fig. 70-12). Phosphorylated Mitf binds to another protein, p500/CBP, that belongs to a coactivator family of proteins and acts to enhance Mitf transcriptional activity. Another kinase that is activated by SF/c-Kit interaction is p90RSK, which also phosphorlates Mitf, but at a different site from that phosphorylated by MAP kinase-2. These phosphorylation events both activate Mitf and at the same time decrease the stability of the protein, as phosphorylated Mitf is targeted for degradation by proteosomes (Fig. 70-13A). Mitf expression is under the control of several transcription factors, including Sox10 (mutated in Waardenburg syndrome type 4, see Chap. 71) and Fas. Mitf expression is also controlled by the cyclic adenosine monophosphate (cAMP)-responsive element binding protein (CREB) and Lef1 transcription factor, which participate in Wnt signaling. These transcription factors bind to specific sites within MITF promoter regions to induce Mitf transcription (reviewed in ref. 114). The promoter region of the MITF gene contains a cAMP-response element (CRE) that interacts with CREB when the cAMP-dependent pathway is activated. Therefore, cAMP-elevating agents such as α-MSH induce the expression of Mitf (see Fig. 70-13B).
proposed, as under certain conditions, Mitf induces the expression of the cell cycle–associated kinase Cdk2 that is involved in the progression of cells from G1 into S phase of the cell cycle. Mitf also suppresses the expression of p21, a protein that inhibits Cdk2 activation. Conversely, under different conditions, Mitf can induce p21 expression, and it can also stimulate the expression of p16INK4a, a protein that inhibits the activation of kinases required for progression through the cell cycle, thus promoting cell cycle arrest. Because Mitf cooperates with other transcription factors to induce its effects, it is to be expected that these transcription factors would influence Mitf activity, resulting in either stimulation or inhibition of melanocyte proliferation.

Mice bearing null mutations of MITF display loss of melanocytes, deafness, and failure of differentiation of retinal pigment epithelium. Mutations in MITF are found in the pigmentary disorder Waardenburg syndrome type 2 (see Chap. 71).

**Melanocortin 1 Receptor.** Melanocortin receptors (MCRs) comprise a family of five related receptors (MC1R, MC2R, MC3R, MC4R, and MC5R). Each has seven transmembrane domains, and they belong to the G-protein–coupled receptor superfamily. MC3R and MC4R are mainly found in the central nervous system, and they are absent in melanocytes, and are thought to control energy intake. MC2R is expressed in the adrenal cortex, and MC5R is expressed in peripheral adipocytes. MC1R is expressed in a number of cells, such as endothelial cells, fibroblasts, and keratinocytes, but the highest expression is found in melanocytes. α-MSH and adrenocorticotropic hormone (ACTH), a 39 amino acid propio-

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**FIGURE 70-13** Microphthalmia-associated transcription factor (Mitf) regulation. A. Mitf is activated when steel factor (SF) binds to its cell surface receptor c-Kit. SF/c-Kit interaction activates mitogen-activated protein (MAP) kinases, which then phosphorylate Mitf. Phosphorylated Mitf then recruits the co-transcription activator CBP/p300, and the complex binds to the M- or E-boxes (M/E box) within the promoter of target genes to regulate their transcription. Mitf can also be phosphorylated at a different site by p90/RSK kinase. Both phosphorylation events lead to Mitf ubiquitination and subsequent proteasome-mediated degradation. B. On binding to melanocortin receptor-1 (MC1R), α-melanocyte-stimulating hormone (α-MSH)/adrenocorticotropic hormone (ACTH) activate the enzyme adenylyl cyclase that upregulates the cyclic adenosine monophosphate (cAMP) level, leading to cAMP-response element binding protein (CREB) activation and binding its DNA consensus sequence CRE in the Mitf promoter to induce Mitf transcription.
different skin/hair color among different ethnic groups. At least 30 MC1R variants have been identified, and nine of them display loss of function (see Fig. 70-14B), not being able to induce intracellular cAMP production in response to \(\alpha\)-MSH despite adequate receptor/ligand binding. Other MC1R variants have reduced affinity for \(\alpha\)-MSH. Three MC1R variants, each with only a single amino acid substitution, have been associated with red/yellow hair and fair skin of Northern Europeans and Australians. Mice expressing a loss-of-function MC1R variant receptor also fail to respond to UV irradiation with increased pigmentation despite an increased level of epidermal \(\alpha\)-MSH, do tan if provided forskolin, a chemical enhancer of pigmentation that bypasses the receptor to directly increase cAMP, demonstrating that the intracellular melanogenic pathway is functional in such individuals.

**MELANIN BIOSYNTHESIS**

Two types of melamins are synthesized within melanosomes: eumelanin and pheomelanin. Eumelanin is dark, brown-black, and insoluble, whereas pheomelanin is light, red-yellow, sulfur-containing, and soluble. Melanins are indole derivatives of DOPA, and they are formed in melanosomes through a series of oxidative steps (reviewed in ref. 157) (Fig. 70-16). Melanosomal pH affects the activity of the melanogenic enzymes and influences melanin polymerization.

The synthesis of both types of melanin involves the rate-limiting catalytic step in which the amino acid tyrosine is oxidized by the enzyme tyrosinase (also called \(\alpha\)-melanocyte-stimulating hormone (\(\alpha\)-MSH) analogue [Nle\textsuperscript{4} D-Phe\textsuperscript{7}]-\(\alpha\)-MSH is modified by the exchange of methionine (M) with norleucine and \(L\)-phenylalanine (F) with D-phenylalanine. In red are critical amino acids required for binding to the MCR. Branches structures represent \(N\)-linked glycosylation sites. Reduced function mutants (red circles), variants common in red- or blond-haired and fair skinned individuals (orange circles), and the conserved C-terminal cysteine (green circle), the possible site for fatty acid acylation and anchoring to the plasma membrane, are indicated. Ac = acetylated; ACTH = adrenocorticotropic hormone; NH\textsubscript{2} = amidated; TM = transmembrane domain.

**FIGURE 70-15** Eumelanin and pheomelanin presentation in mice. A. Two mice with different coat colors are shown. The one on the left displays brown/black coat color due to eumelanin, and the one on the right displays red/yellow coat color due to pheomelanin. B. Representative hair shafts of these mice. (From Sharov et al: Bone morphogenic protein (BMP) signaling controls hair pigmentation by means of cross-talk with the melanocortin receptor 1 pathway. PNAS 102:93, 2004, with permission.)
in tyrosinase, resulting in increased affinity for both tyrosine and L-DOPA. L-DOPA is oxidized into DOPAquinone.\textsuperscript{160} DOPAquinone is further converted to DOPAchrome, and DOPAchrome can be converted to 5,6-dihydroxyindole (DHI) or to 5,6-dihydroxyindole-2-carboxylic acid (DHICA). The latter reaction is catalyzed by the enzyme DOPAchrome tautomeras or TRP-2. The level of brown versus black eumelanin appears to correlate with the DHI/DHICA ratio, with a higher ratio leading to the formation of black eumelanin and a lower ratio to brown eumelanin.\textsuperscript{161} DOPAquinone can also combine with glutathione or cysteine to form pheomelanin.\textsuperscript{161} Interestingly, tyrosinase also catalyzes a more distant step in melanin biosynthesis, namely DHI conversion to indole-5,6-quinone.

In mice, the enzyme TRP-1 (also called DHICA oxidase) converts DHICA to indole-5,6-quinone carboxylic acid. However, the TRP-1 role in human melanin biosynthesis is not well established.

The main function of melanin is to provide protection against UV-induced DNA damage by absorbing and scattering UV radiation (280 to 400 nm). Accordingly, energy absorption by melanin is maximal in this portion of the electromagnetic spectrum and decreases gradually across the visible light spectrum. UV absorbed by melanin is converted into heat, a less toxic form of energy (reviewed in ref. 162). Still, in vitro studies conducted by several investigators suggest that melanin’s capacity to act as a sunscreen is limited, and that melanin, when incorporated into a cream and spread over the skin, absorbs only 50 percent to 75 percent of incident sunlight. Naturally, it is possible that in vivo, by virtue of localizing above the nucleus, melanin in melanosomes achieves a higher level of protection.

Melanin intermediates as well as melanin itself can also be harmful to the cell because, depending on their molecular weight and polymerization state, they can promote UVA (320 to 400 nm)-induced DNA damage, most likely through the generation of ROS.\textsuperscript{163} It has been suggested that the increased incidence of UV-induced melanomas in light-skinned, red-haired individuals is not only due to decreased ability of pheomelanin to protect against UV-induced DNA damage, but may also be due to mutagenic capacity of pheomelanin and possibly other melanin intermediates as a result of their pro-oxidant capacity.\textsuperscript{164}

**FIGURE 70-16** Melanin biosynthesis. Melanin biosynthesis begins with the amino acid tyrosine that is converted to L-DOPA (3,4 dihydroxyphenylalanine) in the rate-limiting step of melanin biosynthesis catalyzed by tyrosinase. L-DOPA is subsequently converted to DOPAquinone by the same enzyme. DHI (5,6-dihydroxyindole) and DHICA (5,6-dihydroxyindole-2-carboxylic acid) are then formed to produce either black or brown eumelanin. Alternatively, through incorporation of glutathione or cysteine, DOPAquinone can form pheomelanin. MW = molecular weight; TRP = tyrosinase-related protein.
ratinocytes. Actin is a major structural component of melanocyte dendrites, and actin filament disruption leads to dendrite loss. Co-cultures of keratinocytes and melanocytes demonstrate that keratinocyte-derived factors play a role in melanocyte dendricity (reviewed in ref. 166). These factors include ET1, nerve growth factor (NGF), α-MSH, ACTH, prostaglandins E2 (PGE2) and F2α (PGF2α), and β-endorphin. Integrins, receptors that mediate actin-extracellular matrix contact, are likely to play a role in dendrite formation as well.

Another group of proteins, the Rho family, also plays a role in melanocyte dendrite formation. Rho proteins become active when they bind guanosine triphosphate and inactive when binding guanosine diphosphate. It appears that when Rho is activated, dendrites retract; whereas when its family member Rac is activated, dendrites form. Indeed, it is currently assumed that by increasing cAMP levels, α-MSH inhibits Rho, enhancing melanocyte dendricity. Thus, the equilibrium between Rho and Rac appears to be an important factor influencing melanocyte dendricity.

### MELANOSOME TRANSPORT

#### Within Melanocytes

Melanosomes are transferred from their site of origin in melanocyte perikaryon to the tips of melanocyte dendrites. Melanosome transport takes place on microtubules that are arranged parallel to the long axis of the dendrite and is controlled by two classes of microtubule-associated motor proteins: kinesin (anterograde) and dynein (retrograde). At the tip of the dendrite, melanosomes are captured in the actin-rich periphery. Myosin-Va (MyoVa) mediates melanosome binding to actin through the linker proteins Rab27a and melanophilin (Mlph).

Mutations in any of the above gene products results in decreased cutaneous pigmentation. Griscelli syndrome, a rare autosomal recessive disorder in which individuals display dilute skin and hair color, is the result of mutations of myosin-Va, Rab27α or melanophilin (see Chap. 71). Myosin-Va and Rab27α are closely located on chromosome 15. Because myosin-Va is also expressed in the brain, mutations of this gene may also cause neurologic abnormalities. Rab27α also plays a role in immunoregulation, and individuals with mutations of this gene display abnormalities of the immune system. Mutations of melanophilin result only in the distinctive hypopigmentation that characterizes the syndrome.

#### To Keratinocytes

Transfer of melanosomes from melanocytes to neighboring keratinocytes is a critical step in normal pigmentation. Studies suggest several ways for melanosomal transfer, including exocytosis, cytophagocytosis, fusion of plasma membranes, and transfer by membrane vesicles (reviewed in ref. 185).

The exocytosis pathway of melanosomal transfer involves fusion of the melanosomal membrane with the melanocyte plasma membrane, melanosome release into the intercellular space, and phagocytosis by surrounding keratinocytes. Cytophagocytosis is a term indicating the phagocytosis of a live cell or a portion of it. With regard to keratinocytes, they cytophagocytose the tip of a melanocyte dendrite, which then fuses with lysosomes inside the keratinocyte, which is transported to a supranuclear location where the phagolysosomal membranes break up, releasing the melanosomes. Fusion of keratinocyte and melanocyte plasma membranes creates a space through which melanosomes are transferred from the melanocyte to the keratinocyte. Indeed, high-resolution photography shows the presence of filopodia, slender, filiform, pointed, cytoplasmic projections at the tip of melanocyte dendrites. These filopodia...
adhere and fuse with keratinocyte plasma membrane before melanosome transfer. The fourth way of melanosome transfer involves shedding of melanosome-filled vesicles followed by phagocytosis of these vesicles by keratinocytes or their fusion with keratinocyte plasma membrane.

The molecular and cellular mechanisms involved in melanosome phagocytosis have been partially elucidated. It appears that keratinocytes express a seven transmembrane G-protein–coupled receptor called protease-activated receptor-2 (PAR-2). PAR-2 is activated when serine proteases cleave the extracellular portion of the receptor, exposing a new segment that acts as a tethered (attached) ligand. Activation of PAR-2 increases keratinocyte phagocytic activity.

Interestingly, and consistent with its role in melanosome phagocytosis, UV induces the activity and expression of PAR-2. UV effect on PAR-2 activity and expression is more pronounced in individuals with skin phototypes II and III than in those with skin phototype I. Keratinocyte growth factor receptor has also been implicated in enhancing phagocytosis of melanosomes by keratinocytes.

REGULATION OF MELANOCYTE FUNCTION

Melanocyte behavior in skin is largely influenced by signals from neighboring keratinocytes as well as autocrine signals and environmental factors such as UV irradiation (also see Ultraviolet Irradiation and Melanocytes). The synthesis and secretion of most keratinocyte-derived factors is increased by UV irradiation, but it is also evident that UV can directly stimulate melanocyte dendritivity and melanin production. Melanocytes receive both positive and negative paracrine signals that modulate their proliferation and differentiated function.

Melanogenic Stimulators

PROPOIOMELANOCORTIN AND DERIVED PEPTIDES It is well documented that MSH and ACTH are potent stimulators of melanogenesis. They belong to a family of peptides derived from the precursor proopiomelanocortin (POMC) that is synthesized, in addition to the pituitary gland, also by epidermal keratinocytes. Interestingly, POMC expression in keratinocytes is induced by UV, phorbol esters, and interleukins (ILs). In rodents, α-MSH stimulates melanogenesis and favors eumelanin over pheomelanin production, but systemic administration of α-MSH, β-MSH, and ACTH to people increases skin pigmentation predominantly in sun-exposed areas. However, in certain disease conditions characterized by abnormally high levels of ACTH, such as Addison disease or Nelson syndrome (ACTH-secreting pituitary adenoma), more generalized hyperpigmentation of the skin has been observed.

Aside from its effect on melanogenic proteins and eumelanin synthesis, α-MSH was also reported to enhance the repair of UV-induced DNA damage in melanocytes, specifically the repair of pyrimidine dimers, and also to reduce the level of UV-induced hydrogen peroxide in the cell. These data suggest a role for POMC-derived peptides beyond merely stimulating melanogenesis.

ENDOTHELIN-1 ET1 appears to play a role in mature melanocytes, inducing melanogenesis by activating tyrosinase and increasing TRP-1 levels. ET1 also leads to melanocyte proliferation and promotes dendrite formation.

Cultured keratinocytes synthesize and secrete ET1 and UV irradiation stimulates ET1 production by keratinocytes. ET1 can also cooperate synergistically with other growth factors/cytokines to further influence melanocyte function.

ET1 upregulates the MC1R level and increases MC1R affinity for α-MSH. Similar to α-MSH, ET1 displays photoprotective effects on melanocytes, enhancing thymine dimer repair, decreasing the level of UV-induced hydrogen peroxide, and inducing the level of anti-apoptotic proteins.

STEEL FACTOR Like other keratinocyte-derived factors, SF is induced by UV irradiation, and, in guinea pigs, anti-Kit antibodies block UV-induced tanning. SCF can also act synergistically with other cytokines such as IL-3, IL-6, IL-7, IL-9, and granulocyte-macrophage colony-stimulating factor to regulate UV-induced melanogenesis and melanocyte survival.

INFLAMMATORY MEDIATORS Several inflammatory mediators can affect skin pigmentation. PGs (arachidonic acid-derived metabolites) and leukotrienes (lipid compounds related to PGs), both mediators of inflammatory responses, affect melanocyte function. Their level is elevated in sunburned skin and in a variety of inflammatory dermatoses, including atopic dermatitis (see Chap. 14) and psoriasis (see Chap. 18).

Human melanocytes express several PG receptors, including the receptors for PGE 2 and PGF 2α. Indeed, PGF 2α stimulates melanocyte dendrite formation and activates tyrosinase, and UV irradiation upregulates the level of PG receptors on melanocytes. Similarly, leukotrienes B 4 and C 4 increase melanin synthesis and stimulate melanocyte proliferation and motility. Interestingly, melanocytes also contribute to cutaneous inflammatory responses, as they synthesize and release IL-8 when stimulated by the pro-inflammatory cytokines IL-1 and tumor necrosis factor-α.

Melanocytes also respond to histamine released by mast cells during cutaneous inflammation. Histamine binds H 1 and H 2 receptors to induce melanocyte dendriticity and upregulate tyrosinase level. ET1 also leads to melanocyte proliferation and promotes dendrite formation.

NEUROTROPHINS Neurotrophins (NTs) are a family of molecules that enhance neuronal survival in the central and peripheral nervous systems. They include NGF, NT3, NT4, and brain-derived neurotrophic factor. Melanocytes express the low affinity receptor common to all NTs, p75 NTR, as well as the high affinity receptors for NGF (TrkA) and NT3 (TrkC) (reviewed in ref. 226). Keratinocyte-derived NGF, whose expression is upregulated by UV irradiation, is chemotactic for melanocytes and induces their dendriticity. Both NGF and NT3, the latter expressed by dermal fibroblasts, increase melanocyte survival. Specifically, after UV irradiation, NGF supplementation increases the level of the anti-apoptotic Bcl2 protein, reducing melanocyte apoptotic cell death. Thus, in addition to other keratinocyte-derived cytokines, NGF may help preserve the population of cutaneous melanocytes that would otherwise be depleted by UV damage.

BASIC FIBROBLAST GROWTH FACTOR Basic fibroblast growth factor (bFGF), named for its ability to stimulate the growth of fibroblasts, was one of the first identified melanocyte mitogens. It is produced by keratinocytes, but lacks a
secretory signal and hence is presumed to affect melanocytes through cell-cell contact. It binds tyrosine kinase transmembrane receptors to induce its mitogenic effect in the presence of cAMP-elevating factors. Like other keratinocyte-derived cytokines, it is upregulated in response to UV irradiation. Keratinocyte growth factor, another member of the FGF family of proteins, has been shown to promote melanosome transfer from melanocytes to keratinocytes.190

**NITRIC OXIDE**. Nitric oxide (NO) is a diffusible free radical displaying pleiotropic bioregulatory effects in diverse cells and tissues. Melanocytes and keratinocytes produce NO in response to inflammatory cytokines, and NO production in keratinocytes is induced by UV irradiation. NO increases tyrosinase activity and melanogenesis and is thus an autocrine as well as paracrine molecule that affects melanocyte behavior in skin.

**Melanogenic Inhibitors**

Numerous reports have suggested the existence of endogenous melanogenic inhibitors, but only few specific molecules have been identified. One group of inhibitors include sphingolipids, a class of membrane-associated lipids (reviewed in ref. 242) that act as signal molecules have been identified. One group of inhibitors include sphingolipids, a class of membrane-associated lipids (reviewed in ref. 242) that act as signal transducers. Sphingolipids were shown to decrease melanogenesis, at least in part by enhancing Mitf degradation via ubiquitin-mediated pathways. Another melanogenic inhibitor, BMP-4, downregulates tyrosinase expression in melanocytes, also in part via its effects on Mitf. Interestingly, physiologic doses of UV irradiation, a potent melanogenic stimulator, decrease the expression of BMP receptors on melanocytes, presumably eliminating its inhibition during UV-induced tanning. Mice that transgenically overexpress the physiologic BMP antagonist noggin have a darker coat color than wild-type mice, and their hairs have a higher eumelanin-pheomelanin ratio.

**SIGNALING PATHWAYS REGULATING MELANOCYTE FUNCTION**

Growth factors, cytokines, hormones, and other ligands for receptors expressed on melanocytes exert their biologic effect by interacting with their specific cell surface receptors, generating a signaling cascade involving activation or inhibition of protein kinases, and leading to distinct patterns of protein phosphorylation. Two types of kinases participate in cellular signaling: serine/threonine and tyrosine kinases that by definition phosphorylate serine and/or threonine residues and tyrosine residues, respectively, on their specific target proteins. This section reviews the major signaling pathways that affect melanocyte behavior in skin.

**Cyclic Adenosine Monophosphate/PKA–Dependent Pathway**

cAMP, one of the first identified intracellular second messengers, plays a key role in diverse biologic functions such as cellular metabolism, growth, and differentiation. It also mediates α-MSH effect (reviewed in ref. 250) and was one of the first recognized regulators of mammalian pigmentation. The intracellular level of cAMP is upregulated by a membrane-associated enzyme called adenylate cyclase that is activated on receptor/ligand interaction in receptors that are coupled to guanosine triphosphate–binding proteins like MC1R (see Fig. 70-13; Fig. 70-18). cAMP is also elevated by reagents such as choleragen or isobutylmethyl xanthine. Providing melanocytes with dibutyryl cAMP, a cAMP analog, increases the intracellular level of cAMP and induces signaling that leads to melanogenesis. The cAMP-dependent protein kinase (PKA) mediates most of the biologic actions of cAMP. PKA is a serine/threonine kinase consisting of two regulatory subunits and two catalytic subunits. It exists in the cytosol in an inactive form, and binding of cAMP to its regulatory subunits releases the catalytic subunits, activating the enzyme. PKA phosphorylates the CREB that binds its DNA consensus sequence CRE in the Mitf promoter to induce Mitf transcription (see Fig. 70-13). cAMP elevation also affects other target genes, increasing or decreasing their transcription (see Fig. 70-18). In vitro, PKA effect can be antagonized by the protein kinase inhibitor that acts as a pseudo substrate for the catalytic subunit of PKA and thus prevents it from phosphorylating its endogenous substrates.

**Protein Kinase C–Dependent Pathway**

PKC is a serine/threonine kinase involved in diverse cellular functions, including growth, transformation, and differentiation. PKC resides as an inactive enzyme in the cytoplasm, and it is activated by diacylglycerol (DAG), a compo-
phosphate in melanocytes to induce melanogenesis. Inhibition of NO and cyclic guanosine monophosphate signaling impedes UV-induced tanning.

ULTRAVIOLET IRRADIATION AND MELANOCYTES

**Tanning Response**

Melanocyte survival, proliferation, and differentiated function are influenced by environmental factors, the most important of which is UV irradiation. UV irradiation induces tanning, so-called *facultative skin color*, an increase above baseline or constitutive skin pigmentation that provides protection against future UV irradiation (reviewed in ref. 264). Tanning is divided into immediate tanning and delayed tanning.

**IMMEDIATE TANNING** Immediate tanning, or immediate pigment darkening, occurs within 5 to 10 minutes of exposure and fades within minutes to days depending on the UV dose and the complexion of the individual (Fig. 70-19B). As summarized in Table 70-1, immediate tanning does not provide photoprotection and does not increase epidermal melanin level. It is primarily produced by UVA irradiation, although visible light can also induce immediate tanning. Immediate tanning is only visible in darker individuals and is of greyish-brown color, and it is thought to represent melanosome relocation from the perikaryon to melanocyte dendrites.

**DELAYED TANNING** Delayed tanning, summarized in Table 70-1 and shown in Fig. 70-19A, occurs within 3 to 4 days after UV exposure. UV is arbitrarily divided into UVC (100 to 280 nm), UVB (280 to 320 nm), and UVA (320 to 400 nm). The UVC portion of the spectrum is generally not present in terrestrial sunlight because it is absorbed by the atmospheric ozone layer. Delayed tan-
UVB wavelengths far more effective than UVA.264 Especially in darker-skinned individuals, sub-erythemogenic UV doses may be effective as well. Delayed tanning peaks between 10 days and 3 to 4 weeks, depending on the absorbed UV dose and the individual’s skin type, then fades gradually over a few weeks. Histologically, there are increased epidermal melanocytes, melanocyte dendricity, and melanosomal transfer to keratinocytes, with greater melanization of individual melanosomes.265,267 Overall, total epidermal melanin is increased, providing additional photoprotection from UV irradiation.

**Direct and Indirect Effects of Ultraviolet Irradiation**

UV irradiation affects melanization and melanocyte proliferation and survival, both directly and indirectly, through its effect on keratinocytes, inducing the synthesis and secretion of paracrine keratinocyte factors.

**DIRECT EFFECTS** UV irradiation triggers several biologic reactions through its interaction with cellular chromophores that absorb photons. Photochemical reactions affect proliferation, survival, and the differentiated function of melanocytes. Most UVA effects are assumed to be the result of oxidative damage mediated through UVA absorption by cellular chromophores like melanin precursors that act as photosensitizers, leading to the generation of ROS and free radicals.268 UVB irradiation is directly absorbed by cellular DNA, leading to the formation of DNA lesions, mainly cyclobutane dimers and pyrimidine (6-4) pyrimidone photoproducts.269 DNA damage repair systems are activated, at least in part through the tumor suppressor p53 protein. It has been shown that plasma membrane lipids are also affected by UV irradiation to release DAG,270 which then activates PKC-β to stimulate melanogenesis by activating tyrosinase (see Protein Kinase C-β).

**INDIRECT EFFECTS** Key keratinocyte paracrine factors induced by UV irradiation and their effects on melanocytes are summarized in Table 70-2. These factors can act alone and/or synergistically to modulate melanocyte function. Interestingly, UV irradiation also induces the

<table>
<thead>
<tr>
<th>TABLE 70-1</th>
<th>Immediate Tanning vs. Delayed Tanning</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Onset</strong></td>
<td>Immediate: Minutes278,282</td>
</tr>
<tr>
<td></td>
<td>Delayed: 3–4 days278,282</td>
</tr>
<tr>
<td><strong>Peak intensity</strong></td>
<td>Immediate: Minutes to a few hours278,282</td>
</tr>
<tr>
<td></td>
<td>Delayed: 10–28 days278,282</td>
</tr>
<tr>
<td><strong>Fading</strong></td>
<td>Immediate: Within 24 h278,282</td>
</tr>
<tr>
<td></td>
<td>Delayed: Weeks265,278,282</td>
</tr>
<tr>
<td><strong>Mechanism</strong></td>
<td>Immediate: Redistribution of melanosomes278,283</td>
</tr>
<tr>
<td></td>
<td>Delayed: Keratinocyte-derived melanogenic cytokines</td>
</tr>
<tr>
<td></td>
<td>Tyrosinase level and activity</td>
</tr>
<tr>
<td></td>
<td>Melanin synthesis</td>
</tr>
<tr>
<td></td>
<td>Melanocyte dendricity</td>
</tr>
<tr>
<td></td>
<td>Melanosome number</td>
</tr>
<tr>
<td></td>
<td>Melanosome transfer</td>
</tr>
<tr>
<td></td>
<td>Melanocyte proliferation265,278,282–284</td>
</tr>
<tr>
<td><strong>Photoprotection</strong></td>
<td>Immediate: Unchanged278</td>
</tr>
<tr>
<td></td>
<td>Delayed: Increased278,282</td>
</tr>
<tr>
<td><strong>Change in skin color</strong></td>
<td>Immediate: Undetectable in fair skin278,283</td>
</tr>
<tr>
<td></td>
<td>Delayed: Obvious in most light-skinned and all dark-skinned individuals283</td>
</tr>
</tbody>
</table>

**TABLE 70-2**

<table>
<thead>
<tr>
<th>Keratinocyte-Derived Factor</th>
<th>Proliferation</th>
<th>Dendricity</th>
<th>Melanogenesis</th>
<th>Melanosomal Transfer</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basic fibroblast growth factor</td>
<td>↑↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Endothelin-1</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Interleukin-1 α/1 β</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>Adrenocorticotropic hormone</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>α-Melanocyte-stimulating hormone</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Prostaglandin E2/prostaglandin F2α</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Granulocyte-macrophage colony-stimulating factor</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
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<tr>
<td>Tumor necrosis factor-α</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
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<tr>
<td>Nerve growth factor</td>
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level of tumor necrosis factor-α and IL-1, cytokines that inhibit melanogenesis, suggesting a fine-tuned epidermal equilibrium between melanogenic stimulators and inhibitors after UV irradiation, with the final outcome of increased melanogenesis and melanocyte proliferation.

Role of DNA Damage in Melanogenesis

Interestingly, the action spectrum for tanning is virtually the same as that for the formation of thymine dimers, and UV-induced melanogenesis can be augmented in pigment cells by treatment with T4 endonuclease V, an enzyme that acts exclusively to enhance the repair of UV-induced DNA damage. Moreover, treatment of melanocytes with agents that act exclusively by damaging DNA, unlike UV that has multiple cellular targets, also stimulates melanogenesis.

A central role for DNA damage and/or its repair in stimulating melanogenesis is further suggested by the fact that p53, a tumor suppressor protein and transcription factor termed the Guardian of the Genome, when activated, upregulates the level of tyrosinase messenger RNA and protein, enhancing melanogenesis. Thus, tanning may be viewed as part of a p53-mediated DNA damage adaptive response that protects the skin during subsequent exposure to UV irradiation.

Melanocyte Aging and Photoaging

Epidermal melanocyte aging is affected by both genetic and environmental factors. With aging, there is a decrease in the density of epidermal melanocytes (number per unit area of skin surface), approximately 10 percent per decade. However, the number of DOPA-positive melanocytes is greater in chronically sun-exposed skin than in sun-protected skin, possibly due to melanocyte proliferation after sun exposure and/or UV-induced keratinocyte-derived paracrine factors. Melanocyte loss is especially notable in hair follicles with age, with total loss of melanocytes in approximately one-half of all scalp follicles by middle age. Hair graying (depigmentation) occurs over the entire body but is usually first noted on the scalp, perhaps because of the long anagen (growth) cycle and resulting requirement for melanocyte proliferation and sustained high level of melanogenesis.

In vitro melanocytes derived from older individuals show decreased proliferative capacity compared to those derived from younger individuals. Also, with aging in vitro, there is a general increase in the levels of total melanin as well as in the level of differentiation-associated proteins such as Mitf, TRP-1, and TRP-2 and decrease in the level of proliferation-associated proteins such as cyclin D1 and cyclin E.

Key References

The full reference list for all chapters is available at www.digm7.com.