Hair Melanins and Hair Color: Ultrastructural and Biochemical Aspects

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The color variants of mammalian hair, including spotting and albinism, are the result of melanocyte activity and have been shown to be determined by the action of multiple genes, some of which operate through the milieu in which the pigment cell resides; others appear to act intracellularly to control the type of melanogenesis. Although there has been much descriptive work on the mode of action of these genes, it has only been with the recent advances in the chemistry and molecular biology of melanin pigmentation that some progress is being made in understanding the nature and origin of hair color. It is the purpose of this article to provide an integrated overview of the major advances so made and to draw attention to certain peculiarities of the melanization processes of hair with respect to those underlying skin pigmentation. Key words: melanins, melanocytes, melanogenesis, hair. J Invest Dermatol 101:825–89S, 1993
ulation of the new bulb during the early anagen phase involves a proliferation and a differentiation of the surviving melanocytes. Radiation studies on murine follicular melanocytes also suggest the existence of this melanocyte reservoir, and it has been estimated that there are about four clonogenic melanocytes per hair follicle [11].

Very little is known about the factors controlling the melanocyte repopulation of hair follicles at each hair cycle. Recent investigations suggest that the c-kit gene encoding a transmembrane tyrosine kinase receptor for the mast cell–stem cell growth factor plays a critical role in this process. Injection of an anti-c-kit monoclonal antibody, an antagonist of c-kit function, in the normal or shaved post-natal mouse skin results in the growth of unpigmented hair [4]. These results indicate that c-kit is required for melanocyte activation that occurs concomitantly with the hair cycle and continues throughout life after neonatal development of the first hair [4]. However, how the activation of hair cycle can trigger c-kit-dependent proliferation of hair melanocytes remains to be elucidated.

Distinctive Features Between the Follicular and Epidermal Melanin Units

Hair bulb melanocytes differ from those in the epidermis only in some respects. They synthesize larger melanosomes than the epidermal melanocytes [12]. Follicular melanocytes are active only during a specific phase of hair production, namely anagen stages III through VI. During the hair growth induced by plucking in mice, tyrosinase visualized on polyacrylamide gels by Detection of tyrosinase is demonstrated in its activity during telogen or during 1–3 d after plucking (anagen stages 1 to III) [13]. By radioimmunoprecipitation using specific anti-tyrosinase antibodies, it has been demonstrated that tyrosinase synthesis occurs during the early anagen phase of the hair cycle, with reduced levels of the protein, until late anagen. No synthesis of tyrosinase is detected during the catagen and telogen stages. During the development of murine anagen hair follicle, the two melanogenic proteins, tyrosinase and Gp75, are regulated in a time-restricted frame [14,15]. The signal-transduction mechanisms implied in the control of melanogenic activities during the hair cycle are largely unknown. Alpha melanocyte-stimulating hormone (αMSH) failed to increase tyrosinase synthesis in human hair follicle. On the other hand, a cyclic AMP analogue stimulated melanogenesis by increasing tyrosinase synthesis in human dark hair follicles [16]. How melanogenesis is linked to the hair cycle is a mystery. When epithelial mesenchymal interactions during mammalian hair follicle development are better understood, it is likely that many questions related to hair melanin pigmentation will be solved [17]. Ultrastructural changes in melanocytes are related to the hair cycle, and in hair follicles of C57 black mice, early anagen is associated with several modifications [10]: increase in volume of the cytoplasm, increase in dendricity, development of the Golgi complex and rough endoplasmic reticulum, and, finally, increase in size and number of melanosomes.

During catagen and telogen, the melanocytes contain only a few small premelanosomes. They exhibit scant cytoplasm with less well developed Golgi complex and rough endoplasmic reticulum, as well as nuclei with prominent heterochromatin patterns. It has also been observed that the shape and internal structural modification of the melanosomes can change during the black hair cycle [10]. Hair melanocytes transfer melanosomes to follicular epithelial cells. Medullary cells receive their melanin from melanocytes in the upper part of the hair bulb, in a pattern similar to that described for the epidermis. Melanosomes are also transferred to immature cortical cells. Due to their larger size, melanosomes are usually distributed singly, whatever the ethnic background [12]. The hair melanosomes are 2 to 4 X larger than the epidermal melanosomes. As the process of keratinization continues, melanin granules, along with other cytoplasmic remnants, become embedded in keratin. A small number of melanosomes are found associated with the spongy-appearing keratin of the hair medulla. Melanin granules are mainly in the corner cells of their long axis being parallel to the hair surface. The cortex cells contain few or no melanosomes. Usually, no melanin is seen in the cells of the inner root sheath. The distribution of melanosomes in the upper part of the outer root sheet resembles that in epidermis. In the lower part of the sheath, the majority of cells contain only a small amount of melanin pigment.

It is likely that these distinctive features between the follicular and epidermal melanin units are largely due to environmental influences. The chemical signals arising from the dermal papilla and the follicular keratinocytes are probably quite different from those coming from the epidermal keratinocytes or from the upper dermis.

The Melanocyte Population of the Skin as a Bicompartamental System

Several clinical situations suggest that the epidermal and follicular compartment of the melanocyte population of the skin are relatively independent. Senile white hair often occurs on a scalp epidermis with a normal melanin pigmentation. On the other hand, body hairs often keep their normal color in a fully depigmented lesion of vitiligo. It is clear, however, that exchanges may occur between these two compartments, which are not closed systems. This has been demonstrated under certain circumstances in which one of the two melanocyte compartments is altered or destroyed. After dermabrasion (removal of the epidermis and the infundibulum follicle), amelanotic melanocytes divide in the middle portion of the hair follicle, become active (dopa-positive), and migrate upward from the outer root sheath to the infundibulum and later into the basal layer of the healing surrounding epidermis [8]. A similar process occurs during epidermal wound healing after pure epidermal destruction by suction (Ortonne et al., personal observation). Evidence for such exchanges has also been obtained from the study of repigmentation of vitiligo skin during oral phototherapy [9]. Experiments performed in the white skin of trichrome guinea pigs suggest that melanocytes injected intradermally are incorporated into the hair bulbs and migrate upwards into the outer root sheath. It is not yet clear, however, if there are other melanocyte precursors distinct from the amelanotic melanocytes present in the outer root sheath of hair follicles. It has been suggested that a reservoir of melanocytes exists in hair follicles of C57 black mice, but its existence has not yet been demonstrated [10].

Less evidence exists to suggest migration of melanocytes from the epidermis into the hair follicles. In most cases, after destruction of hair melanocytes by various physical agents (X-ray, freezing, etc.), regenerated hair follicles remain depigmented, giving rise to white hair [18,19]. Few experiments suggest that such exchanges exist. In the guinea pig, an autograft of full-thickness black skin for 7 d in white skin, later removed, is followed by the appearance of pigmentation due to active melanocytes in the healing wound. Within a few months, white hair grows as well as black hair [20]. This may be due either to the persistence in the wounds of pigmented hair bulbs from the graft, or to migration of isolated melanocytes from the pigment grafts that colonize the regrowing white hair bulbs. In humans, after induction of a pure dermal wound, removing the middle and lowest portion of hair follicles, regrowing hair is still pigmented. It is possible that the melanocytes present in this hair originate from the overlying epidermis [21]. Repigmentation of leukotrichia by epidermal grafting and systemic psoralen plus UV-A has been recently reported [22]. In three vitiligo patients, clinical follow-up suggested a possibility of activation and migration of epidermal melanocytes to the hair follicles. Although elegant, these experiments do not give conclusive evidence for the occurrence of movement of melanocytes from the epidermis towards the hair bulb.

**MELANOCYTES IN HAIR**

**Ultrastructural Features**

Many studies have been carried out on the ultrastructural and biochemical aspects of hair melanin pigmentation. The results will be discussed in relationship to visual hair color.

**Human Red Hair**

In human red hair, specified as pheomelanin by chemical analysis, melanocytes contain spherical melanosomes with...
microvascular (vesiculoglomerular) and proteinaceous matrices on which melanin deposition is spotty and granular [23, 24]. The sequences of melanization of these organelles are identical to those of pheomelanin in yellow mice and tortoise-shell guinea pigs (yellow areas) [25].

In other human red hair specified as "mixed" type melanogenesis by chemical analysis [23], a majority of melanocytes produce spherical melanosomes of pheomelanin form. They also contain "mosaic" melanosomes with features of both eumelanosomes (ellipsoidal shape, regular striation) and pheomelanosomes (spotty and microgranular melanization, lack of electron-lucent bodies in mature melanosomes). The nature of these "mosaic" melanosomes, whether they are eumelanin, pheomelanin, or mixed, remains to be clarified. In addition, there was a second type of melanocyte, producing ellipsoidal melanosomes of typical eumelanin form.

**Human Blond Hair:** Since the identification of pheomelanosomes, few detailed electronmicroscopic studies of human blond hair follicle have been reported. Melanin granules are smaller and less numerous in blond than in dark-haired subjects [24–26]. Melanosomes are not fully melanized even in the dendritic processes of melanocytes. This suggests that the light color in blond hair may be due to a quantitative decrease in the production and melanization of melanosomes.

**Human Black and Brown Hairs:** Typical ellipsoidal melanosomes, at various stages of melanization, are observed in follicular melanocytes of black hair. Their ultrastructural characteristics are identical to those seen in the epidermis of caucasoids and negroids [26, 27]. Melanosomes transferred to neighboring keratinocytes are singly distributed [23]. In brown hair, the follicular melanocytes also contain all the developmental stages of eumelanosomes. Lighter brown hairs have smaller melanosomes [26]. Similar aspects are observed, whatever the racial background [24].

**Senile Gray and White Hairs:** In the melanocytic zone of the senile gray hair bulb, the number of melanosomes appears normal or reduced [28]. These cells show little melanocytic activity and contain very few melanosomes. In senile white hair, there are no dopa-positive melanosomes. By electronmicroscopy, melanocytes are scarce or entirely absent and there is no melanin in the matrix and cortex. Similar findings have been observed in white hair from vitiligo macules. The senile white hairbulbs do not contain immunoreactive tyrosinase antigen [29]. More recently, tyrosinase mRNA or its protein have been detected in senile white hairs, suggesting the presence of amelanotic melanocytes within the outer root sheat [30].

**Biochemical Aspect**

**Main Types of Melanins:** Hair melanins can be roughly classified into those giving dark colors, namely, black and brown and their derivatives, and those giving light ones, with a wide range from the bright yellow coat of some mice to the carrot-like red color of certain human hair. The dark colors are usually regarded as deriving from a fairly homogeneous group of polymeric pigments, the eumelansins, and consisting mainly of 5,6-dihydroxyindole (DHI) and to a lesser extent of 5,6-dihydroxyindole-2-carboxylic acid (DHI-2CA). Some of these units appear to be in the oxidized quinone form, as evidenced by the ability of melanosomal enzymes to undergo reversible reduction. Minor structural contributions include the presence of 5,6-dihydroxyindole semiquinone units and carboxylated pyrrole units; the latter probably arise by the partial fission of the indole units by the hydrogen peroxide formed during melanogenesis [31–33].

The light colors are usually described under the omnibus term of "mixtures" of melanosomes, but these include pigments with different chemical and physical properties. The most extensively investigated are found in certain types of red hair, as well as in the feathers of domestic fowl, e.g., New Hampshire and Rhode Island hens. Analytical and degradative studies have shown that these pheomelansins are polymers or mixtures of polymers that contain a 1,4-benzo triazine unit [6]. The same type of unit is also found in the trichochromes, which are smaller molecules of well defined structure and composition [34]. Examples are the isomeric trichochromes B and C (Fig 1); the latter is the predominant and most representative member of the series.

**Figure 1. Chemical structure of trichochrome B and C.**

From the structural anatomy of these molecules, one can easily recognize their biogenetic origin from dopa and cysteine through formation and subsequent oxidation of 5-S-cysteinyl-dopa and 2-S-cysteinyl-dopa [35]. There is evidence that the same intermediates are involved in the biosynthesis of the polymeric sulfur-containing pheomelansins, but little is definitely known about the reaction pathway beyond the benzothiazine stage. Yet, despite this and other doubts, it is noteworthy that pheomelansins and trichochromes are the end products of the same metabolic pathways that diverge after the formation of a common intermediate. Such a biogenetic relationship explains why the two types of pigments are often found together in pheomelanin hair.

As indicated earlier, in both humans and in some animals yellow or reddish pigments exist that are chemically different from those derived from cysteine and dopa. Currently, little is definitely known about the structure of these varieties of pheomelansins except that they behave like bleached eumelansins. These and other observations [31] have led to speculation that these pheomelanin-"looking" pigments may in fact be structural variants of eumelansins, arising from partial oxidative cleavage of 5,6-dihydroxyindole units. Such a view is not unlikely, considering the high susceptibility of the eumelanin polymer to hydrogen peroxide, which is involved in the later stages of melanogenesis. Thus, in vivo, modulation of eumelanin color by hydrogen peroxide would provide an alternative mechanism to explain the polychromy of epidermal melanin pigmentation, which is otherwise difficult to explain on the basis of only two basic types of pigments, i.e., eumelansins and the sulfur-containing pheomelansins.

Most natural melansins including hair melansins contain a certain amount of sulfur. As an example, as high as 3% sulfur has been found in human black hair [6]. Another study demonstrates rather high sulfur content whatever the hair color: 5.3% in Italian brown hair, 4.9% in Japanese black hair, 8.8% in Irish red hair, and 2.3% in Scandinavian blond hair [36].

Degradation experiments aimed at characterizing the main structural units and the biosynthetic origins of the pigment are also useful tools for the study of natural melansins. A simple and rapid method for the quantitative estimation of eu- and pheomelansins in tissues based on the analysis of degradation products without isolation of melansins made these studies possible [37]. The rationale of this analytical method is that permanganate oxidation of eumelanin gives pyrrole-2,3,5-tricarboxylic acid (PTCA) as a major pyrrole product, whereas hydriodic acid hydrolysis of pheomelansins yields amino-hydroxyphenylalanine (AHP) as a major phenolic aminoacid [37].

In hair of mice and guinea pigs, there is a good correlation between the melanogenesis type defined by ultrastructural analysis and by content of melansins [37]. It was demonstrated that black mouse hairs contain a high level of eumelanin, but a negligible level on pheomelanin. On the other hand, yellow hair contained the lowest level of eumelanins and an intermediate level of pheomelanin, and albino mouse hair contained a medium level of pheomelanin. As follicular melanocytes in black mice produce ellipsoidal-lamelellar melanosomes, these observations suggest that the pigments
yellow, and white hair of tortoise-shell guinea pigs [37]. This also demonstrated that visual differentiation of hair engaged in typical pheomelanogenesis [23]. From this study, it was also concluded that chemical analysis, i.e., the ratio of eumelanin to pheomelanin, corresponded well to the fine structural differentiation of eumelanogenesis and pheomelanogenesis.

All these results demonstrate that human hair follicles, whatever the color, contain various proportions of both eumelansins and pheomelansins. These observations point towards the key role of the switching mechanisms that determine whether pheomelansins or eumelansins are synthesized by follicular melanocytes. The availability of sulfhydryls in melanocytes, more specifically within melanosomes, is probably an important factor in this process. Cysteine and/or glutathione may sidetrack part or all of the generated dopa-quinone to form additional intermediates, namely cystydopas and glutathionedopas [6]. It has been proposed that the role of cysteine/glutathione as a regulatory factor in switching melanogenesis type is not tied to its absolute presence or absence, but rather to the effective concentration within the melanocyte at a given time [39]. Because the addition of cysteine or glutathione to quinones is a rapid reaction, the metabolic fate of dopaquinone is mainly dependent upon the activity of the enzymes of the glutathione system that affect the tissues' sulfhydryl content. Direct support for this view is provided by comparative analysis of the levels of sulfhydryls and sulfhydryl-related enzymatic activities in tortoise-shell guinea pig skin of different colors (black, yellow, red, and white), as well as in the skin of pure black (A/a) and yellow (Ay/a) mice [40].

The lowest levels of glutathione reductase activity were found to be associated with eumelanin-type pigmentation, whereas the highest ones were found in the skin with light pheomelanin-type pigmentation. Moreover, analysis of non-protein thiol pools revealed that GSH levels are lower in black skin than in yellow skin of the agouti mice [41].

Topical application of catecholic antioxidants, e.g., 4,8-tetrahydroxy butyl catechol, inhibits eumelanogenesis but stimulates pheomelanogenesis in melanosomes of the guinea pig skin. This biologic process is associated with elevated activities of glutathione reductase and gammaglutamyltranspeptidase, further suggesting that sulfhydryls and sulfhydryl-related enzymes play a key role in the switch of melanogenesis type [42]. The melanosomal membrane may play an important regulatory role by controlling the uptake of melanogenic substrates and sulfhydryls.

**Hair Melanin, Hair Color, and Tyrosinase Activity:** The possibility that hair color could be related to the melanogenic activity of follicular melanocytes has been raised. Furthermore, the finding that, in mouse hair follicles, similar tyrosinase activities are associated with eumelanogenesis than with pheomelanogenesis [43] suggested that tyrosinase expression in human hair follicular melanocytes could depend upon the type of melanin produced.

Using a micromethod allowing the evaluation of tyrosinase activity (tyrosine hydroxylase) by the Pomerantz' method in single or pooled hairbulbs, red and, to a lesser extent, blond hair follicles were shown to have the highest tyrosinase activities compared to black and brown hair follicles [44]. A second study using a similar method [29] also demonstrated that red hairbulbs have significantly higher tyrosinase activity than the other colors. Except for red, the melanin hairbulb tyrosinase levels were similar in all other hair colors. More recently [16], tyrosinase activity in hair follicles was measured in 23 red- and dark-haired individuals. Tyrosinase activity was also found to be greater in the hair follicles of red-haired subjects than in those from dark-haired individuals. Evaluation of tyrosinase environmental factors by immunoprecipitation of tyrosinase with a specific antibody after metabolic labeling of the protein suggested that red hair follicles presumably resulted from an increased synthesis of this enzyme.

These three studies have focused on the tyrosine hydroxylase activity of tyrosinase. A method for measuring the dopa oxidase activity of human hairbulb tyrosinase based on the measurement of the formation of 5-S-cysteinyldopa has also been developed [45]. With this technique it has been demonstrated that tyrosine hydroxylase and dopa oxidase activities are coordinate functions of hair-bulb tyrosinase over a broad range of hair color.

These results from three different groups suggest that in red hair bulbs, follicular melanocytes have a high melanogenic activity. They contrast with the results obtained in mice, in which pheomelanogenesis is clearly associated with lower tyrosinase activity [43], and illustrate that striking species differences may exist in the control of melanogenesis. It would be of great interest to know if the recently identified regulatory proteins of melanogenesis TRP-1 and TRP-2 [46] are also increased in these circumstances. The finding of a high tyrosinase activity in blond hair follicles suggests that the light color is not due to a low melanogenic activity of follicular melanocytes, but is rather due to the chemical structure of the melamins produced or to a post-tyrosinase block in the melanogenesis pathway.

**Regulation of Melanogenesis in Hair Follicles** The response of epidermal and follicular melanocytes to exogenous or endogenous stimuli differs slightly. Due to their anatomical localization, follicular melanocytes are less exposed to environmental factors than are epidermal melanocytes. This is the case for ultraviolet (UV) radiation. Although psoralen plus UVA is known to affect hair growth and hair pigmentation, it is likely that in normal conditions, UV rays have little or no effect on the determination of constitutive hair pigmentation. Intense solar radiation lightens hair. Brown hairs lighten more than red hair. This suggests that pheomelansins are more resistant to photodegradation than eumelanins [47].

**Genetic Control** Hair pigmentation is under genetic control. Very little is known about this control in humans. In the dark races, there is intense selection for dark hair, but in Caucasians there is no strong selection for any particular color [48]. It is generally agreed that the factors responsible for black hair color and largely also those for brown are epistatic to those that determine red hair color [49]. It is also agreed that red hair color is dominant to blond hair [50]. A strong evidence for a major locus controlling brown hair color being linked to a locus for green eye color and located on chromosome 19 has been obtained. From linkage to the MNS blood group system, a major gene for red human hair has been assigned to chromosome 4 [51]. In mice, more than 50 genetic loci have been identified [1,2]. They act either directly on the melanocyte or indirectly through the follicular environment. These loci can be grouped into four major classes: those affecting the migration, proliferation, and survival of melanocytes; those controlling the amount of melanin produced; those that determine the kind of melanin synthesized; and those that reflected in the shape and ultrastructure of melanocytes [52]. In recent years, the molecular bases of several of these mutations have been identified. As examples, only a few of them will be discussed in detail.

In the first group, the c-kit gene at the W locus affects the proliferation of melanoblasts as well as hemopoietic stem and primordial germ cells during embryogenesis. Mutations at this locus give rise to white patches or spots in the fur due to a failure of melanoblasts to migrate, survive, or proliferate in these regions [8]. Another mutation, the steel locus, was found to affect red hair color, another hair color factor, causes defects in the same three cell lineages as do mutations in the W locus and causes similar phenotypes [51,53]. Mutations of the kit protooncogene have been described recently in human piebald-
<table>
<thead>
<tr>
<th>Hair Color</th>
<th>Blonde(^a)</th>
<th>Brown(^a)</th>
<th>Black(^a)</th>
<th>Red(^a)</th>
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<tr>
<td>Tyrosinase Activity</td>
<td>2.423 ± 0.525</td>
<td>1.141 ± 0.223</td>
<td>1.680 ± 0.482</td>
<td>4.310 ± 1.150</td>
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<tr>
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<td>Light Medium Dark</td>
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<td>(mean ± SD)(^b)</td>
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<td>Lloyd et al [29]</td>
<td>49.9 ± 19</td>
<td>88.0 ± 18</td>
<td>94.1 ± 90</td>
<td>201.5 ± 45(^c)</td>
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<td>(mean ± SEM)(^b)</td>
<td>(n = 5)</td>
<td>(n = 7)</td>
<td>(n = 9)</td>
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<tr>
<td>Townsend et al [45]</td>
<td>TH: 8.03 ± 0.31</td>
<td>From 1.26 ± 0.71 to 7.25 ± 0.49</td>
<td>5.6 ± 0.06</td>
<td>6.1 ± 0.45</td>
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<td>(mean ± SD)(^b)</td>
<td>DO: 9.9 ± 1.2</td>
<td>From 0.5 ± 0.1 to 3.5 ± 9</td>
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<tr>
<td>Burchill et al [16]</td>
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<td>Dark 8.88 ± 0.06(^d)</td>
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<tr>
<td>(mean ± SEM)(^b)</td>
<td>(n = 1)</td>
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<tr>
<th>Ratio of Eumelanin/ Pheomelanin</th>
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<th>Medium dark-brown</th>
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<td>Jinbow et al(^e)</td>
<td>1.63 ± 0.80</td>
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<td>Thody et al [38]</td>
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<th>Type of melanogenesis</th>
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<td>Jinbow et al(^f)</td>
<td>(n = 2)</td>
<td>(n = 6)</td>
<td>(n = 3)</td>
<td>(n = 2)</td>
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\(^a\) Tyrosinase activity expressed as pmol tyrosine oxidized/120 min.
\(^b\) Tyrosinase activity expressed as pmol DOPA formed/3 bulbs/30 min.
\(^c\) TH: Tyrosine hydroxylase activity of tyrosinase expressed as pmol of tyrosine oxidized per h; DO: Dopa oxidase activity of tyrosinase expressed as nmol of 5-S-cysteinyldopa formed per h.
\(^d\) Tyrosinase activity expressed as pmol tyrosine converted/h.
\(^e\) Based on the content of PTCA (eumelanin indicator multiplied by a factor of 50) and AHP (pheomelanin indicator multiplied by a factor of 5) in the follicles.
\(^f\) Based on the ratio/PTCA/AHP content in the follicles.
\(^g\) n within brackets = number of individuals.
\(^h\) Statistically significant.

ism, suggesting that this disorder may be the human homologue of dominant white spotting in the mouse [54].

In the second group, different alleles at the c locus encoding tyrosinase have been analyzed in detail and the molecular basis of mutations identified. This is the case for the albino mutant and for the Malayman mutant allele, due to a temperature sensitivity of the enzyme resulting in a very peculiar phenotype, the mouse having dark ears, feet, and nose against a pale body [52]. Similar mutations have also been described in humans with tyrosinase-negative oculocutaneous albinism and temperature-sensitive albinism [57].

In the third group, the agouti and extension loci have been extensively studied, as the corresponding mutations provide a good system to study the control of melanogenesis in hair follicles, particularly the switching mechanisms for pheo- or eumelanogenesis. The agouti locus is an excellent system to study the control of melanogenesis in hair follicles, although it is not present in humans. The murine agouti locus in chromosome 2 encodes a gene products that determine whether eumelanin, pheomelanin, or both of these pigments are synthesized in hair bulb melanocytes [2]. The wild-type mouse has two types of pigment: eumelanin that colors the tip and base of the dorsal hair, and pheomelanin that colors a stripe across the middle of the hair. This is a striking demonstration that individual melanocytes can switch between the two types of pigment synthesis. Hair follicle melanocytes in transition from black to yellow postac both eu- and pheomelanosomes, demonstrating that the shift from eumelanogenesis to pheomelanogenesis or vice versa occurs within a single cell [55]. Even so, melanosomes with features of both pheomelanosomes and eumelanosomes have been observed in such circumstances [56].

Mutations at the "a" locus disrupt this switching between eu- and pheomelanogenesis. Lethal yellow (Ay) is dominant to all other alleles, resulting in a uniformly yellow coat. At the opposite end of the spectrum, extreme non-agouti (a'f'), recessive to all other alleles, produces a totally black coat [2]. Despite considerable effort, the specific effect of the agouti locus remains unknown. It is established, by grafting and cell culture experiments, that the gene at this locus produces their effect by altering the follicular milieu (i.e., outside the melanocyte) [2]. Several experiments support the notion that agouti genes either directly or indirectly modulate the activity of tyrosinase. Whether this modulation occurs at the transcriptional, translational, or post translational level remains to be established. Recent data [57] suggest that the agouti locus modulates tyrosinase activity at least in part by regulating the total number of tyrosinase molecules. This regulation could operate at the level of transcription or post-translational modification of tyrosinase [58].

The role of sulphydryl compounds in the expression of agouti locus alleles has also been discussed in the case of albinism [59]. From a large set of melanocytes, it was concluded that all melanocytes from "pheomelanin" mice of different agouti locus constitutions synthesize eumelanin under standard in vitro conditions. However, if sulphydryl compounds such as glutathione are added to the nutrient medium, all melanocytes, regardless of their age or agouti locus genotype, could be induced to produce pheomelanin. MSH treatment converts pheomelanogenesis to eumelanogenesis when the former results from the action of agouti locus alleles [60]. The mouse agouti gene has recently been cloned and characterized. It encodes a 131 amino-acid polypeptide.

Another locus called extension also controls the type of melanin pigment produced by follicular melanocytes [2]. The extension series of alleles is located on chromosome 8 and acts autonomously within the melanocytes. The dominant alleles are black whereas the recessive mutation "e" produces yellow mice only when homozygous. In contrast, pheomelanogenesis of the coat of recessive yellow (e/e) mice, and eumelanogenesis was not modified by these treatments [63]. From these observations, it was suggested that the product of the "a" locus probably interacts with alpha-MSH at the alpha-MSH receptor. When alpha-MSH binds to its membrane receptor, the signal is transduced to adenylyl cyclase, which results in an increase in cyclic AMP level in the cytoplasm, resulting in increased tyrosinase activity leading to eumelanogenesis. On the other hand, when Ay/a or A/A follicular cells secrete their product, it competitively interacts with alpha-MSH, blocking the alpha-MSH receptor, resulting in low intracytoplasmic cAMP levels, low tyrosinase activity, and, finally, pheomelanogenesis. The action of the "e" locus could be through the control of the function of adenylyl cyclase in the membrane of mouse melanocytes [63].

In the fourth group, the pallid mutation produces defects in at least three subcellular organelles: platelet-dense granules, kidney lysosomes, and melanosomes. These mice have dilute pigmenta
tion due to abnormally small melanosomes. A recent report suggests that pallid is a mutation in the Epb 4.2 gene, the murine gene for protein 4.2. This protein interacts with band 3 on the erythrocyte membrane and ankyrin in solution. By immunofluorescence, protein 4.2 has been localized in melanosomes in human melanoma cells. Although the function of protein 4.2, renamed pallidin, is unknown, it may be that it plays a role in membrane stabilization. These mouse mutations resemble the human disorders Hermansky-Pudlak syndrome and Chediak-Higashi disease. The pallidin gene is certainly a candidate gene for these disorders [64].

Many alleles of the pink-eyed dilution on mouse chromosome 7 are defined by reduced pigmentation of both coat and eyes. Mutant p melanosomes are structurally abnormal and have a reduced capacity to bind or accumulate melanin. A recent characterization of the human homologue of the mouse pink-eyed dilution gene revealed that it may be associated with Prader-Willi and Angelman syndromes. From these results, it has been suggested that altered expression of this gene may be responsible for the hypopigmentation phenotype exhibited by certain individuals with these disorders [65].

The dilute mutation is associated with a reduction or dilution of pigmentation [2]. This is due to a failure of the melanosome transfer from melanocytes to keratinocytes due to an inability of the pig- ment cell to extend dendrites [2]. Recently, the sequence of the dilute product has been identified. It is a novel myosin heavy chain that probably plays a major role in the extension of the melanocyte dendrites [66].

Other Factors Normal hair color is altered in many disorders of different causes. Among these, hormonal, nutritional, and metabolic disorders are largely represented (for review see [67]), demonstrating the complexity of the regulatory controls of melanogenesis in hair follicles.

Aging of the Follicular Melanin Unit During human life, natural hair color changes may be observed. Many fair-haired children gradually become darker and by middle age have brown hair, whereas other children become brown, sandy, or auburn-haired adults [68]. Graying of hair is the most role in the melanin synthesis [67].

This phenomenon is very common and occurs to various extents in almost all persons. The onset of physiologic canities seems largely hereditary, but other factors are probably involved. It is variable but usually occurs in the late fourth or early fifth decade. Neither sex is affected because the female sexual hormone levels are higher than in light-haired sub-jects, but more fair- than dark-haired subjects are affected slightly grey. The possible explanation for this apparent contradiction is that the first signs of greying are seen more directly against a dark background than against a fair background. Greying is less common in
blacks. Greying of the hair is usually irreversible, but return of pigment has been known to occur [69].

The cellular and subcellular bases of greying and whitening of hair have already been discussed. The precise cause of senile greying of hair has not been established. Alpha-MSH binding sites, noradrenergic innervation, and immune tolerance have already been discussed. The possibility that melanocytes are susceptible to virus infection at a critical stage of differentiation and that virus expression could interfere with cellular functions at some later stage of melanocyte development was also raised [73].

Several experimental data suggest that some of the intermediate metabolites of the melanin pathway have a cytotoxic activity. It may be hypothesized that a progressive loss of some natural protective mechanisms of senile hair follicle melanocytes results in destruction of pigment cells. Recent studies suggest that such mechanisms could be responsible for premature greying in light (B6) mice. In these animals, the hair is pigmented at the tip but very highly or not at all pigmented at the base due to clumping, irregular distribution, and reduced number of melanosomes followed by premature death of follicular melanocytes. This phenotype occurs only in pigmented mice, suggesting that it may be mediated through the inherent cytotoxicity of pigment production [74]. It is the result of a single base alteration at the mouse brown locus coding for the TRP-1. The function of TRP-1 is not yet known, but this observation suggests that it plays a critical role in the stabilization of melanosomal structure. Whether the light mouse is a valuable model for the study of senile and premature greying of hair in man remains to be established.

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