Enzymatic control of pigmentation in mammals

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ABSTRACT **Visible pigmentation in mammals results from the synthesis and distribution of melanin in the skin, hair bulbs, and eyes. The melanins are produced in melanocytes and can be of two basic types: eumelanins, which are brown or black, and phaeomelanins, which are red or yellow. In mammals typically there are mixtures of both types. The most essential enzyme in this melanin biosynthetic pathway is tyrosinae and it is the only enzyme absolutely required for melanin production. However, recent studies have shown that mammalian melanogenesis is not regulated solely by tyrosinase at the enzymatic level, and have identified additional melanogenic factors that can modulate pigmentation in either a positive or negative fashion. In addition, other pigment-specific genes that are related to tyrosinase have been cloned which encode proteins that apparently work together at the catalytic level to specify the quantity and quality of the melanins synthesized. Future research should provide a greater understanding of the enzymatic interactions, processing, and tissue specificity that are important to pigmentation in mammals. — Hearing, V. J.; Tsukamoto, K. Enzymatic control of pigmentation in mammals. *FASEB J.* 5: 2902-2909; 1991.**

Key Words: *melanin* • *pigmentation* • *melanogenesis*

PIGMENTATION IN ANIMALS IS VARIED, and often strikingly beautiful. In birds, most feather coloration is due to the presence of carotenoid pigments (recently reviewed, see ref 1), whereas in mammals most visible pigmentation results from the synthesis and distribution of melanins. Melanins are heterogeneous biopolymers produced by specialized dendritic cells, termed melanocytes, which are located primarily in the skin, hair bulbs, and eyes (reviewed in refs 2, 3). Besides their function in protective coloration and sexual attraction within species, melanins play a crucial role in the absorption of free radicals generated within the cytoplasm and in shielding the host from various types of ionizing radiations, including UV light. That latter role unfortunately seems destined to become even more critical due to the alarming predicted increases in incident UV at the Earth's surface.

The regulation of pigmentation in mammals is controlled at many different levels, and is quite complex at each level. In the mouse, for example, more than 150 different mutations have been identified which affect pigmentation, and these occur at more than 50 distinct genetic loci. Melanocytes are initially derived from the neural crest and migrate throughout the embryo during development; these migration patterns are under strict genetic control and can lead to some interesting patterns when the final distribution of melanocytes in the skin is not uniform, such as in zebras, giraffes, piebald animals, and so on. Pigmentation is also regulated at the cellular level: melanocytes synthesize melanin within discrete organelles, termed melanosomes, which can be produced in varying sizes, numbers, and densities. The melanosomes are then passed on, in skin to keratinocytes and in hair bulbs to the hair shaft, where the final distribution patterns of the pigment are determined. This distribution plays an important role in determining color; note for example the variety of colors in the skin, hair, and eyes of humans. Last, melanogenesis is regulated at the subcellular level where the synthesis and expression of various melanogenic enzymes and inhibitors play a critical role.

Melanocytes are influenced by a variety of extracellular factors which determine not only whether melanin is synthesized, but what type of melanin is made. Perhaps the most commonly known melanogenic stimulus is melanocyte-stimulating hormone (MSH),[3] a peptide produced by the posterior pituitary. After MSH binds to melanocyte surface receptors, dramatic (up to 100-fold) increases in melanogenesis follow, at least in murine systems and lower vertebrates (cf. Discussion below, and reviewed in refs 4, 5). Melanocyte-stimulating hormone elicits the production of eumelanins rather than phaeomelanins (6–9) and a mechanism (or mechanisms) involved in those responses are still under study. Melanocytes work in close harmony with their neighboring cells in the epidermis by producing the melanosomes to be phagocytosed and distributed within keratinocytes, and by responding to various cytokines the keratinocytes produce, such as growth factors, prostaglandins, interleukins, and interferons (10–14). Presumably these provide the complex signals that stimulate pigmentation after trauma, UV exposure, or other environmental stimuli that induce alterations in levels of pigment production. Although it is not the topic of this review, stimulation of mammalian melanogenesis may be commonly mediated through increased expression of surface MSH receptors; such a mechanism has been implicated in response to interferons (13), UV light (15), and substrates (16).

It is the purpose of this review to summarize recent studies that have elaborated the complexity of the regulation of melanin synthesis at the enzymatic level, and to integrate those results with the cloning and sequencing of a family of tyrosinase-related, pigment-specific genes that work together at the catalytic level to specify the quantity and quality of melanins produced by mammalian melanocytes.

MELANIN BIOSYNTHESIS AND REGULATORY FACTORS

It has been known for some time that the copper-containing enzyme tyrosinase (E.C. 1.14.18.1) is essential to melanin for-

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[3]Abbreviations: DOPA, 3,4-dihydroxyphenylalanine; DHI, 5,6-dihydroxyindole; DHICA, 5,6-dihydroxyindole-2-carboxylic acid; MSH, melanocyte stimulating hormone; IBMX, isobutylmethylxanthine.

0892-6638/91/0005-2902/$01.50.

mation (reviewed in refs 17–19). Tyrosinase is an unusual enzyme that can catalyze three different reactions in the biosynthetic pathway of melanin (**Fig. 1**): *1*) the hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA); *2*) the oxidation of DOPA to DOPAquinone; and *3*) the oxidation of 5,6-dihydroxyindole (DHI) to indole-quinone. The first of these activities is the most critical biologically because the remainder of the reaction sequence can proceed spontaneously at physiological pH. It has recently been found that many other reactions in the biosynthetic pathway of melanin formation are under active regulatory control (cf. below), but there is no doubt that the activity of tyrosinase is the most essential, i.e., rate-limiting, to the process. The melanins produced can be of two basic types: *1*) eumelanins derived from the metabolites of DOPAchrome; and *2*) phaeomelanins derived from metabolites of cysteinylDOPA (reviewed in refs 20, 21). The mixed-type melanins usually found in humans are mixtures of the eu- and phaeomelanins in different proportions (3, 22). Although it was originally postulated that the eumelanins consisted of a homogeneous polymer of indole-quinone, this has since been disproved. It is now known that melanins are heterogeneous polymers with subunits consisting of all of the cyclized derivatives of DOPAquinone (Fig. 1), perhaps including DOPAquinone itself.

It is thought that the primary determinant of whether phaeomelanins rather than eumelanins will be synthesized is the availability of sulfhydryls; when present, glutamine or cysteine will be quantitatively bound to DOPAquinone as quickly as it can be generated (23). By implication, enzymes that influence the metabolism of these sulfhydryls play a role in regulation of melanogenesis (24). Because the melanosome is a membrane-bound intracellular organelle, the intramelanosomal concentration of melanogenic substrates may be regulated in part by the melanosomal membrane, although tyrosine uptake through that membrane is not so regulated (25). One can see the effects of various switches in the pathway on the hair color of mice (cf. Fig. 1). Wild-type mice are termed agouti, and bands of eu- and phaeomelanin alternate in their hair. Some mutations in mice elicit the production of phaeomelanin only, such as *lethal yellow* (A^y/-) or *recessive yellow* (e/e, not shown). Nonagouti (black) animals (a/a) produce only eumelanin which typically appears uniformly black, unless other mutations also occur, such as *brown* (b/b) or *albino* (c/c), where the quality and quantity of melanin produced is further modified.

In addition, other critical regulatory steps operate in eumelanin synthesis after the activity of tyrosinase. These include catalytic activity which results in the production of DHICA rather than DHI, which will be spontaneously generated from DOPAchrome in the absence of such an enzyme (reviewed in ref 26). This catalytic activity was originally termed DOPAchrome conversion factor (27), later named DOPAchrome oxidoreductase (28, 29), then DOPAchrome isomerase (30), and finally DOPAchrome tautomerase (E.C. 5.3.2.3) (31). In light of the fact that this latter name is the most accurate and specific, it is the one we will use in this review, but readers might want to remember that it has aliases in the literature. To further complicate this aspect of the discussion, the same catalytic activity toward DOPAchrome can be elicited by the action of certain divalent transition metal cations, such as Co^{2+}, Cu^{2+}, or Ni^{2+} (32,33). Which activity is actually relevant biologically (enzyme vs. metal) is still in dispute, although it is only fair to mention that they are not mutually exclusive. It is not yet clear what structural or functional differences exist between DHI-derived melanins and DHICA-derived melanins, but they must be significant because DHI-melanin is black and flocculent whereas DHICA-melanin is yellowish-brown and finely dispersed (31). DOPAchrome tautomerase occurs in melanosomes complexed with tyrosinase, and perhaps other melanosomal membrane proteins (34, 35). It has also been reported recently that peroxidase can utilize DHI as a substrate (36), and its potential role in melanogenesis is under active study.

The final type of activity that significantly influences mammalian pigmentation are the melanogenic inhibitors. Tyrosinase uses the ubiquitous amino acid tyrosine as a substrate, and virtually any peptide or protein with an exposed tyrosine or phenylalanine residue can function as a competitive inhibitor. There is also a variety of exogenous inhibitors of pigment production that act by inhibiting tyrosinase or interfering with the normal formation of the polymer (reviewed in ref 37). Of more interest in the metabolic regulation of melanogenesis are the naturally occurring inhibitors endogenous to melanocytes. Several have been purified

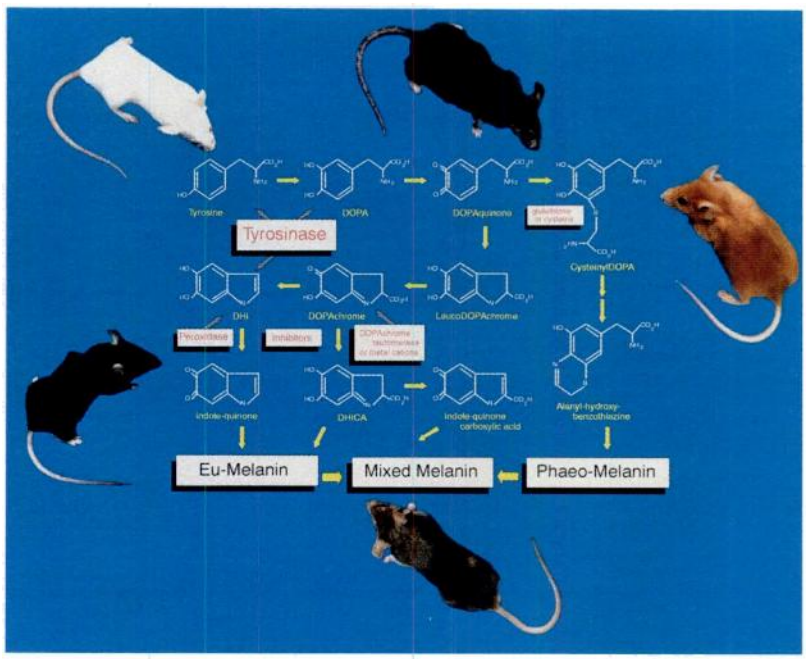

Figure 1. The melanin synthetic pathway in mammals. The chemical pathway for the production of eu- and phaeomelanins in mammalian melanocytes (as cited in the text). Note that tyrosinase can utilize three different substrates (tyrosine, DOPA, DHI) to catalyze three different reactions. The conversion of DOPAchrome into DHI (which occurs spontaneously) can be diverted to DHICA in the presence of DOPAchrome tautomerase or certain metal cations. Melanogenic inhibitors can interfere with melanin production at numerous points in the pathway, and there is some evidence that peroxidase can also utilize DHI as a substrate to produce melanin. Examples of mice with different coat colors have been strategically arranged on the chart to indicate the point of the pathway most relevant to their phenotype - *albino* (top left), *lethal yellow* (top right), *agouti* (bottom right), *black* (middle left), *brown* (bottom left).

and partially characterized (38–40). These inhibitors interfere with the production of melanin at various stages, although their exact mechanisms of inhibition have not been elucidated fully. There is no doubt that such inhibitors play a critical role in pigmentary disorders of hypopigmentation where active tyrosinase is present although no melanin is synthesized, as for example in tyrosinase-positive albinism (41). The role of these inhibitors in regulating basal levels of melanin synthesis is also under intense study.

PHYSICAL CHARACTERISTICS AND MELANOGENIC REGULATION

The intracellular pathway for synthesis and processing of tyrosinase in melanocytes has been well characterized (reviewed in refs 17, 18). After synthesis, tyrosinase is glycosylated en route to and within the Golgi and subsequently delivered to melanosomes via coated vesicles. Tyrosinase is functionally active within the smooth endoplasmic reticulum, Golgi, and coated vesicles, but the mechanism whereby it is inactivated until delivery to the melanosome has always been a puzzle. Another missing piece of that puzzle is that DOPA, in addition to being the product of the initial reaction (tyrosine hydroxylation), is a necessary cofactor for the reaction. DOPA is not a natural amino acid; it is not clear how tyrosinase is initially activated. The first part of the puzzle has been solved, although the mechanism has not yet been completely clarified. We now know that an inhibitor is present in the nonmelanosomal organelles of the melanocyte which precludes melanin formation there (38, 39). The question regarding the mechanism of initial activation of tyrosinase in the absence of exogenous DOPA may arise because mammalian tyrosinase can be activated by ferrous ions to hydroxylate tyrosine in the absence of DOPA (42). This important observation regarding the potential role of iron in the catalytic activity and function of tyrosinase will be discussed further.

The known physical characteristics of tyrosinase and of DOPAchrome tautomerase are summarized in **Table 1.** These two enzymes have very different characteristics with the obvious exception of their melanocyte specificity. The gene for mammalian tyrosinase has now been cloned as has that for DOPAchrome tautomerase (cf. later discussion of various pigment-related genes).

Melanocytes can be stimulated to differentiate and synthesize pigment by several mechanisms. Consequently, a natural area for study has been to examine how such responses are mediated at the catalytic level. Many studies have shown that melanin production can be increased dramatically by MSH, UV light, or agents that mimic those responses (4, 5, 43–45). The primary manner of activation of melanogenesis is by stimulation of preexisting tyrosinase activity within the melanocyte (46–51). Expression of tyrosinase mRNA and synthesis of the protein increase two- to threefold within several days of melanogenic stimulation, whereas catalytic activities typically increase 20- to 100-fold during the same period. The presence of melanogenic inhibitors are consistent with this inactive pool of tyrosinase. If levels of the inhibitor are reduced after melanogenic stimulation, with continued or slightly increased levels of tyrosinase synthesis, this would soon lead to the dramatic increases seen in pigment synthesis. Melanocyte-stimulating hormone will increase the activity of DOPAchrome tautomerase (26, 52). The lack of direct correlation between tyrosinase synthesis and expression and pigment production has also been noticed in other studies where tyrosinase is often expressed (typically in normal quantities) in amelanotic melanocytes (40, 53–55).

Although our intent is not to detail the mechanisms involved in melanogenic responses to stimulation by MSH, it may represent the only major difference detailed between murine and human pigmentation. It is well documented that MSH stimulates melanogenesis dramatically in murine melanocytes both in vivo and in vitro (reviewed in refs 4, 5). This is mediated through binding of MSH to surface receptors, internalization of the complex, and activation of protein kinase A activity in the classical manner. Immediate increases in cAMP occur, and alternate inducers of this pathway (e.g., isobutylmethylxanthine [IBMX] or cholera toxin) have similar effects. In human melanocytes, which express surface MSH receptors and respond to MSH by increases in cAMP (56, 57), there is not accompanying increase in pigmentation (although one has been reported recently; ref 58). It has recently been shown that human melanocytes can be induced to produce pigment by stimulation of the protein kinase C pathway (59), mediated through the diacylglycerol messenger, thus the mechanism of melanogenic stimulation may be significantly different between human and murine melanocytes. This point is under intense scrutiny now as it

TABLE 1. *Physical characteristics of melanogenic enzymes*

Property	Tyrosinase	DOPAchrome tautomerase
Specificity	Melanocyte	Melanocyte
Molecular weight	65,000 (*de novo*) 75,000 (glycosylated)	46,000 - 80,000 (in dispute)
Isoelectric point	4.3	Unknown
Catalytic activity	Tyrosine → DOPA DOPA → DOPAquinone DHI → Indole-quinone	DOPAchrome → DHICA
Half-life	4–10 h (*in vivo*)	Unknown
Miscellaneous	Heat-stable Protease-stable Chelator-sensitive Glycosylated Membrane-bound Metal chelator-sensitive DOPA cofactor-dependent	Heat-sensitive Protease-sensitive Chelator-insensitive Glycosylated Membrane-bound Metal chelator-insensitive DOPA cofactor-independent

represents the known major difference between murine and human melanogenic systems.

MOLECULAR BIOLOGY OF TYROSINASE

Tyrosinase represents the critical regulatory point in the pathway of melanin formation (as outlined previously) and is the key lesion in many types of albinism. Because of its prominent role in the modulation of visible pigmentation and its potential application as a genetic marker, there has been tremendous interest in the cloning of its gene. The genes for tyrosinases from nonmammalian species (reviewed in ref 60) had been available for some time, and have served as useful tools for structural and functional studies. Because primary sequence data for mammalian tyrosinase were not available, the direct approach of synthesizing probes by which the mammalian gene could be identified and isolated was not useful. Various laboratories used alternative and indirect approaches to clone that gene, and several candidate clones were obtained from murine and human systems (61–65). Unexpectedly, several putative but distinct clones were identified which shared significant sequence homology. More important, each clone encoded a protein that had many properties predicted for tyrosinase, including, for example, melanocyte specificity, correct size, transmembrane region, two potential copper binding domains, and multiple glycosylation sites (cf. Table 1, and reviewed in ref 60). A model consistent with those features which shows the conserved areas of the primary strucutres of proteins encoded by three of those genes is shown in **Fig. 2.**

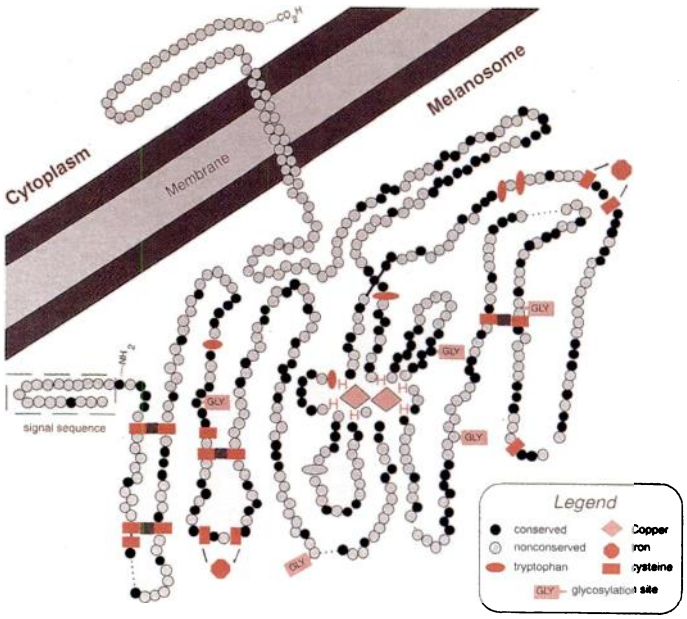

Figure 2. Structural model of tyrosinase-related proteins. A model consistent with computer predictions of secondary folding has been generated from the primary structure conserved in murine *albino*, *brown* and *slaty* locus encoded proteins (as cited in the text); the major constraint placed on the model has been the structure of the copper binding site, which has been determined by crystallography for lower forms of tyrosinase (100); this binding depends on 3 histidines in highly conserved regions to bind to each copper atom - the importance of histidines in such binding has also been shown for the mammalian enzyme (101). 15 of the 16 cysteine residues have been conserved in all three proteins, as have 7 of the 8 tryptophans; these residues are thought to be important for enzyme structure and stability, as well as for catalytic function. Little homology is present at the termini, including the transmembrane region and cytoplasmic determinant. The two potential iron-binding domains (77) are indicated, and the two cysteines critical to enzyme function that are altered in the *albino* and *brown* mutations (83, 98) are in the iron-binding domain at the bottom left. The three areas where there are minor sequence deletions (1–5 residues) between the proteins are indicated by the dotted lines (·····).

The *Albino* locus

The *albino* locus has historically been proposed as the structural locus for tyrosinase, because of the dramatic lack of pigment produced by mutations at that locus (cf. Fig. 1). cDNAs which mapped to the *albino* locus were originally cloned from murine (64) and human (63) melanocytes. The original sequence reported for the murine gene was a prematurely truncated version of the full-length transcript, and the full sequence has been subsequently published (53, 66, 67). The *albino* gene in mice (and its human homolog) is composed of five exons and four introns, and is predominantly untranslated material. In mice the gene is ~70 kb (68) and in humans it is >35 kb (69). The procesed mRNAs are ~2.4 kb in length. Thus, the precursor mRNA must be processed to delete the introns. This splicing is not always accurate, and misspliced mRNAs (10–40% of the total) are generated which translate into altered proteins (68, 70, 71). This may represent an important regulatory step. The altered proteins are not competent catalytically but whether they fulfill some other role in the melanocyte, such as a competitive inhibitor, has not yet been determined. Although the *albino* gene is present as a single copy (on chromosome 7 in mice and on chromosome 11 in humans), in situ hybridization shows (72) that there is a second site (also on human chromosomes 11) that is partially homologous. This second site has now been shown to be a pseudogene (69) of exons 4 and 5 of the authentic gene. The fact that the albino protein is expressed in melanocytes has been confirmed with antibodies generated against synthetic peptides that correspond to the amino and carboxyl termini predicted by the nucleic acid sequence (73).

The ability of the protein encoded by the *albino* gene to function as tyrosinase has now been shown by several approaches. Initially, transfection of the gene into tyrosinase-deficient cells produced tyrosinase activity (54, 68). It was shown that both the full-length and correctly spliced mRNA encoded active tyrosinase, and that several aberrantly spliced mRNAs encoded proteins that were catalytically incompetent (66). Subsequently, minigenes (with the tyrosinase coding sequence and a competent regulatory sequence) were used to produce transgenic mice (74–76). Although the transgenic animals were not fully and normally pigmented, there were dramatic increases in pigmentation and the pigmentation was specifically restricted to melanocytes. As the regulatory sequence used to construct the minigene was derived from the authentic 5' flanking region of the tyrosinase gene, that regulatory sequence is probably critical and sufficient for the tissue-specific expression of tyrosinase. The ability of the *albino* locus-encoded protein to function catalytically as tyrosinase has also been shown by immunopurification (73, 77). There is no doubt that the *albino* locus encoded protein is tyrosinase, and that its function is crucial to pigment production. Whether it is the sole protein that can perform as a tyrosinase, and exactly how it interacts with other melanogenic proteins, are still unclear. Although expression of *albino* locus-encoded tyrosinase is absolutely necessary for pigment production, it is not the only regulatory deter-

minant, as some melanocyte lines with normal expression of either the tyrosinase mRNA or protein are unpigmented (53-55). Similarly, although melanogenic agents that stimulate pigmentation typically increase the transcription and translation of the *albino* gene two to threefold (51, 66), such stimulations cannot explain the much greater increases in tyrosinase activities. This implies that other control points in the pathway are also critical to the regulation of melanogenesis (as described previously).

The effects of mutations, either natural or induced, on the structure and function of proteins encoded by the mutated gene can provide important insights into the mechanisms of biologic processes. Mutations at the *albino* locus can lead to dramatic changes in pigmentation. Initial studies in this field depended heavily on animals with mutations at pigment-related loci, but recently, immortalized melanocyte cell lines derived from coat color mutants have become available which have made more definitive studies possible (78, 79). Studies of several mutations at the *albino* locus, including Himalayan and chinchilla, have shown that these mutant melanocytes produce tyrosinases with significantly altered properties. In the *albino* mutation, catalytic activity is virtually lost. In the *Himalayan* (c^h), there is an alteration in glycosylation which results in a temperature-sensitive phenotype. In *chinchilla* (c^{ch}), there is an increased sensitivity to proteolytic inactivation, and thus a decrease in enzyme function (78). The mutations that elicit these changes are now being detailed (74, 80-82) and should be invaluable for understanding the functional properties of the enzyme.

Perhaps the most dramatic recent revelation is a point mutation which changes a conserved cysteine to a serine in the first cysteine-rich domain of tyrosinase (83). This is the exact amino acid change responsible for the albino phenotype in mice. That single change in primary structure causes the virtually complete loss of catalytic function. Whether this results from an alteration in secondary structure, loss of metal binding activity, or some other factor, has not yet been determined. However, in light of the virtually identical nature of the point mutation which causes the brown phenotype (cf. later), there can be no doubt that future studies will be directed at this domain which appears to be so critical to its activity. All strains of albino mice examined had this same mutation; presumably all were derived from the same initial mutation (83).

Naturally there has been tremendous interest in defining mutations critical to tyrosinase activity in humans. To date, many different mutations in oculocutaneous albinos have been described, all of which appear to result in loss of tyrosinase activity and thus in melanin production. The mutations now number in the dozens, occur at various areas on the enzyme (i.e., they are not confined to a single region or domain), and result from a variety of mechanisms, including point mutations and insertions, which occur in either the structural or the promoter region of the gene (84-88). In humans and mice, nondestructive polymorphisms have been described also.

OTHER PIGMENTATION-SPECIFIC GENES AND PROTEINS

The *Brown* locus

The first cloned pigment-related gene proposed to be the structural locus for tyrosinase (61) was quickly mapped to the *brown* locus on chromosome 4 in the mice (65, 89). The structure and organization of this gene is remarkably similar to the *albino* gene, and the predicted protein has all of the features characteristic of tyrosinase (reviewed in ref 60). There is only a single copy of this gene in the genome, and the homologous gene has now also been identified and cloned in humans (90, 91). In general, there is about 90% sequence identity between the *brown* protein expressed by murine and human melanocytes (this is similar to the conservation found between murine and human tyrosinase), and there is about 55% nucleic acid identity overall between the *brown* and *albino* genes (and about 43% primary sequence identity). The conservation of residues is especially high in several areas of these melanogenic proteins thought to be important to their structure and function (such as the copper binding and cysteine rich domains). The high conservation of sequence in putative copper binding regions is noted not only in mammalian tyrosinases, but also in those from lower species (see ref 60 for review). Similarity is almost completely absent elsewhere, such as in the transmembrane region and cytoplasmic determinant (Fig. 2). The *brown* locus gene (~18 kb in mice) is broken into 8 exons and 7 introns, an alternative processing occurs with this gene, as it does with the *albino* gene (92, 93).

The specific function of the *brown* locus encoded protein is not altogether clear. Phenotypically, we can see that a mutation at this locus causes the production of brown rather than black melanin, but what this means chemically or enzymatically is undefined. Nevertheless, it is obvious that whatever the role of the brown protein, it must somehow elicit the production of black (vs. brown) melanin in animals (cf. Fig. 1). The brown protein has been postulated to be: *1*) DOPAchrome tautomerase (65), *2*) DHI conversion factor (53); *3*) a melanosomal specific catalase (77); or *4*) another tyrosinase (73, 94). The substrates and products of reactions catalyzed by the brown protein have not yet been identified conclusively, and the question remains open. The brown protein is present in a higher quantity (typically ~10-fold) in melanocytes compared with the albino protein. These proteins are expressed in similar relative quantities by human melanocytes (91, 95). No human mutation of the *brown* locus has been identified. But now that the human gene has been cloned and sequenced, one may appear soon. It is also an indication of the important function of this protein that there is typically a better correlation of its expression with visible pigmentation than there is with the expression of the albino protein, which suggests that it might serve to stabilize or activate synergistically the albino protein (96; K. Tsukamoto, K. Urabe, and V. J. Hearing, unpublished results).

There are multiple mutations at the *brown* locus in mice, and several are actively being studied, including *cordovan* (b^c) and *light* (B^{lt}) (97). As with the *albino* mutation, the critical molecular lesion resulting in the brown phenotype has been identified (98). Brown results from a point mutation that leads to the replacement of a conserved cysteine residue with a tyrosine. The critical substitution occurs in the first cysteine-rich domain of the protein, only three residues away from the mutation site in albino mice (cf. legend to Fig. 2). This could be coincidence, but in light of the recent proposals that these cysteines may be involved in an iron binding site (77), and that iron may be critical to tyrosinase activation (42), the importance of this domain to the structure and function of these pigment-related proteins is sure to draw much interest in the coming years. As with the basic *albino* mutation, all brown mice from numerous different strains have the same sequence mutation. Presumably, all brown mice are derived from the same original mutation (65).

The *Slaty* locus

The clone identified by Jackson (originally termed 5A (65), but now called TRP2) which was initially thought to be identical to the *brown* locus gene, has now been identified as a distinct gene, but one that shares significant sequence homology with tyrosinase (I. J. Jackson, D. M. Chambers, K. Tsukamoto, N. G. Copeland, D. J. Gilbert, N. A. Jenkins, and V. J. Hearing, unpublished results). This gene has been definitively mapped to chromosome 14 and assigned to the *slaty* locus. It has features in common with the *brown* and *albino* genes, including a transmembrane region, highly conserved putative copper binding sites, two conserved cysteine-rich domains, potential glycosylation sites, and a signal peptide. The size of the protein encoded by this gene (~75–80 kDa) is somewhat larger than the products of the *albino* and *brown* loci. This gene has now been shown to function as DOPAchrome tautomerase (K. Tsukamoto, I. Jackson, K. Urabe, P. M. Montague, and V. J. Hearing, unpublished results).

The *Silver* locus

Another clone with significant homology to the tyrosinase gene was originally termed Pmel17-1 (63). The expression of its mRNA was specific for melanocytes, it could be induced with MSH (again ~twofold) or IBMX, and its abundance correlated well with pigmentation (62). The protein encoded by this gene is homologous to that encoded by the *albino* locus. The protein has a predicted molecular weight similar to tyrosinase (~70 kDa), putative glycosylation sites and a transmembrane region (99). There is approximately 95% identity between the sequence of the human and the murine protein. This gene has been definitively mapped to chromosome 12 in humans and to chromosome 10 in mice, and has been tentatively assigned to the *silver* locus of mice. The function of this protein is unknown, but it is also a candidate for melanogenic enzyme activity.

FINAL COMMENTS

The molecular biology of pigmentation has become both interesting and complex. The plethora of genes that constitute a tyrosinase family was totally unexpected and has underscored the complexity of the regulation of mammalian melanogenesis—a process once thought to be a simple one enzyme:one product system. The next several years should see the characterization of even more pigment-related genes and more information should soon be forthcoming from those gene sequences. Questions currently being addressed to elucidate the regulatory controls of mammalian melanogenesis include:

- Identification of the critical regulatory elements within the tyrosinase gene which control the tissue specific and timely expression of these pigment related genes. Recent studies have begun to describe the promoters and enhancer-like elements that may be critical to the regulation of the expression of these tyrosinase-related genes.

- Does alternative splicing play a role in the regulation of tyrosinase activity? We now know melanocyte responses to environmental stimuli involve increased levels of transcription and translation of the *albino* and *brown* loci, although not enough to explain the dramatic increases in melanin production by those cells. We suggest that either posttyrosinase factors are important in controlling melanin production, that the accuracy of processing of precursor mRNA that leads to increases in functionally competent enzyme (or enzymes) might improve, or both.

- The catalytic functions of the products of the cloned pigment-related genes must be described. More important, their interactions that determine melanogenic function must be explained.

- What are the intracellular processing and delivery pathways involved in the delivery of these gene products to the melanosome? Are all of these melanogenic proteins transported to the melanosome en masse within the same vesicles, or are they segregated in different vesicles and combined only after their arrival at the melanosome? The latter pathway would provide a potential mechanism whereby melanogenesis might be delayed until all melanogenic factors are in place in the melanin granule.

- What are the roles of melanogenic inhibitors and post-tyrosinase factors? These additional points of melanogenic regulation gain added significance in light of what we now know about the regulation of gene expression. FJ

The authors wish to express their gratitude to Drs. Zalfa Abdel Malek, Raymond Boissy, Ruth Halaban, Alan Houghton, Richard King, Ian Jackson, Byoung Se Kwon, Lloyd Law, Paul Montague, Seth Orlow, John Pawelek, Anna Palumbo, Giuseppe Prota, Shigeki Shibahara, Andrzej Slominski, Richard Spritz, Takuji Takeuchi, Anthony Thody, and Wilfred Vieira for numerous helpful discussions, comments, and critiques, and for the use of unpublished information.

REFERENCES

1. Brush, A. H. (1990) Metabolism of carotenoid pigments in birds. *FASEB J.* **4**, 2969–2977
2. Quevedo, W. C., Fitzpatrick, T. B., Szabo, G., and Jimbow, K. (1987) Biology of melanocytes. In *Dermatology in General Medicine* (Fitzpatrick, T. B., Eisen, A. Z., Wolff, K., Freedberg, I. M., and Austen, K. F., eds) Vol. 1, pp. 224–251, McGraw-Hill, New York
3. Wick, M. M., Hearing, V. J., and Rorsman, H. (1987) Biochemistry of melanization. In *Dermatology in General Medicine* (Fitzpatrick, T. B., Eisen, A. Z., Wolff, K., Freedberg, I. M., and Austen, K. F., eds) Vol. 1, pp. 251–258, McGraw-Hill, New York
4. Pawelek, J. M. (1976) Factors regulating growth and pigmentation of melanoma cells. *J. Invest. Dermatol.* **66**, 201–209
5. Pawelek, J. M. (1985) Studies on the Cloudman melanoma cell line as a model for the action of MSH. *Yale J. Biol. Med.* **58**, 571–578
6. Tamate, H. B., and Takeuchi, T. (1981) Induction of the shift in melanin synthesis in lethal yellow (A^y/a) mice in vitro. *Devel. Gen.* **2**, 349–356
7. Burchill, S. A., Thody, A. J., and Ito, S. (1986) Melanocyte-stimulating hormone, tyrosinase activity and the regulation of eumelanogenesis and pheomelanogenesis in the hair follicular melanocytes of the mouse. *J. Endocrinol.* **109**, 15–21
8. Burchill, S. A., Virden, R., and Thody, A. J. (1989) Regulation of tyrosinase synthesis and its processing in the hair follicular melanocytes of the mouse during eumelanogenesis and phaeomelanogenesis. *J. Invest. Dermatol.* **93**, 236–240
9. Granholm, N. H., and Van Amerongen, A. W. (1991) Effects of exogenous MSH on the transformation from phaeo- to eumelanogenesis within C57Bl/6J-A^y/a hairbulb melanocytes. *J. Invest. Dermatol.* **96**, 78–84
10. Abdel-Malek, Z., Swope, V. B., Amornsiripanitsch, N., and Nordlund, J. J. (1987) In vitro modulation of proliferation and

melanization of S91 melanoma cells by prostaglandins. *Cancer Res.* **47**, 3141-3146
11. Halaban, R., Langdon, R., Birchall, N., Cuono, C., Baird, A., Scott, G., Moellmann, G., and McGuire, J. (1988) Basic fibroblast growth factor from human keratinocytes is a natural mitogen for melanocytes. *J. Cell Biol.* **107**, 1611-1619
12. Gordon, P. R., Mansur, C. P., and Gilchrest, B. A. (1989) Regulation of human melanocyte growth dendricity, and melanization by keratinocyte derived factors. *J. Invest. Dermatol.* **92**, 565-572
13. Kameyama, K., Tanaka, S., Ishida, Y., and Hearing, V. J. (1989) Murine α, β and γ-interferons stimulate melanogenesis by increasing the number of α-melanocyte stimulating hormone receptors on JB/MS murine melanoma cells. *J. Clin. Invest.* **83**, 213-221
14. Swope, V. B., Abdel-Malek, Z., Kassem, L. M., and Nordlund, J. J. (1991) Interleukins 1α and 6 and tumor necrosis factor-α are paracrine inhibitors of human melanocyte proliferation and melanogenesis. *J. Invest. Dermatol.* **96**, 180-185
15. Bolognia, J., Murray, M., and Pawelek, J. (1989) UVB-induced melanogenesis may be mediated through the MSH-receptor system. *J. Invest. Dermatol.* **92**, 651-656
16. Slominski, A., and Pawelek, J. (1987) MSH binding in Bomirski amelanotic hamster melanoma cells is stimulated by l-tyrosine. *Biosci. Rep.* **7**, 949-954
17. Hearing, V. J. (1987) Mammalian monophenol monooxygenase (tyrosinase): purification, properties and reactions catalyzed. In *Methods in Enzymology—Metabolism of Aromatic Amino Acids and Amines* (Kaufman, S., ed) vol. 142, pp. 154-165, Academic, New York
18. Hearing, V. J., and Jiménez, M. (1987) Mammalian tyrosinase: the critical regulatory control point in melanocyte pigmentation. *Int. J. Biochem.* **19**, 1141-1147
19. Körner, A. M., and Pawelek, J. (1982) Mammalian tyrosinase catalyzes three reactions in the biosynthesis of melanin. *Science* **217**, 1163-1165
20. Prota, G. (1988) Progress in the chemistry of melanins and related metabolites. *Med. Res. Rev.* **8**, 525-556
21. Prota, G. (1988) Some new aspects of eumelanin chemistry. In *Progress in Clinical and Biological Research* (Bagnara, J., ed) vol. 256, pp. 101-124, A. R. Liss, New York
22. Rorsman, H., and Pavel, S. (1990) Metabolic markers and melanoma. In *Cutaneous Melanoma Biology and Management* (Cascinelli, N., Santinami, M., and Veronesi, U., eds) pp. 79-82, Masson, Milan, Italy
23. Jara, J. R., Aroca, P., Solano, F., Martinez, J. H., and Lozano, J. A. (1988) The role of sulfhydryl compounds in mammalian melanogenesis: the effect of cysteine and glutathione upon tyrosinase and the intermediates of the pathway. *Biochim. Biophys. Acta* **967**, 296-303
24. Prota, G. (1980) Cysteine and glutathione in mammalian pigmentation. In *Natural Sulfur Compounds* (Cavallini, D., Gaull, G., and Zappia, V., eds) pp. 391-398, Plenum, New York
25. Jara, J. R., Martinez-Liarte, J. H., Solano, F., and Peñafiel, R. (1990) Transport of L-tyrosine by B16 F10 melanoma cells: the effect of the intracellular content of other amino acids. *J. Cell Sci.* **97**, 479-485
26. Pawelek, J. M. (1991) After DOPAchrome? *Pigment Cell Res.* **4**, 53-62
27. Körner, A. M., and Pawelek, J. (1980) DOPAchrome conversion: a possible control point in melanin biosynthesis. *J. Invest. Dermatol.* **75**, 192-195
28. Barber, J. I., Townsend, D., Olds, D. P., and King, R. A. (1984) DOPAchrome oxidoreductase: a new enzyme in the pigment pathway. *J. Invest. Dermatol.* **83**, 145-149
29. Leonard, L. J., Townsend, D., and King, R. A. (1988) Function of DOPAchrome oxidoreductase and metal ions in DOPAchrome conversion in the eumelanin pathway. *Biochemistry* **27**, 6156-6159
30. Pawelek, J. M. (1990) DOPAchrome conversion factor functions as an isomerase. *Biochem. Biophys. Res. Commun.* **166**, 1328-1333
31. Aroca, P., Garcia-Borron, J. C., Solano, F., and Lozano, J. A. (1990) Regulation of distal mammalian melanogenesis. I. Partial purification and characterization of a DOPAchrome converting factor: DOPAchrome tautomerase. *Biochim. Biophys. Acta* **1035**, 266-275
32. Palumbo, A., d'Ischia, M., Misuraca, G., and Prota, G. (1987) Effect of metal ions on the rearrangement of DOPAchrome. *Biochim. Biophys. Acta* **925**, 203-209
33. Palumbo, A., d'Ischia, M., Misuraca, G., Prota, G., and Schultz, T. M. (1988) Structural modifications in biosynthetic melanins induced by metal ions. *Biochim. Biophys. Acta* **964**, 193-199
34. Hearing, V. J., Körner, A. M., and Pawelek, J. (1982) New regulators of melanogenesis are associated with purified tyrosinase isozymes. *J. Invest. Dermatol.* **79**, 16-18
35. Pawelek, J., Chakraborty, A. K., and Orlow, S. (1991) Purification of a high molecular weight melanogenic complex containing MSH receptors, dopachrome isomerase, gp75 and tyrosinase. *J. Clin. Invest.* In press
36. d'Ischia, M., Napolitano, A., and Prota, G. (1991) Peroxidase as an alternative to tyrosinase in the oxidative polymerization of 5,6-dihydroxyindoles to melanin(s). *Biochim. Biophys. Acta* **1073**, 423-430
37. Mishima, Y., Hatta, S., Ohyama, Y., and Inazu, M. (1988) Induction of melanogenesis suppression; cellular pharmacology and mode of differential action. *Pigment Cell Res.* **1**, 367-374
38. Hatta, S., Mishima, Y., Ichihashi, M., and Ito, S. (1988) Melanin monomers within coated vesicles and premelanosomes in melanin synthesizing cells. *J. Invest. Dermatol.* **91**, 181-184
39. Chakraborty, A. K., Mishima, Y., Inazu, M., Hatta, S., and Ichihashi, M. (1989) Melanogenic regulatory factors in coated vesicles from melanoma cells. *J. Invest. Dermatol.* **93**, 616-620
40. Kameyama, K., Jiménez, M., Muller, J., Ishida, Y., and Hearing, V. J. (1989) Regulation of mammalian melanogenesis by tyrosinase inhibition. *Differentiation* **42**, 28-36
41. King, R. A., and Olds, D. P. (1985) Hairbulb tyrosinase activity in oculocutaneous albinism: suggestions for pathway control and block location. *Am. J. Med. Gen.* **20**, 49-55
42. Palumbo, A., d'Ischia, M., Misuraca, G., Carratu, L., and Prota, G. (1990) Activation of mammalian tyrosinase by ferrous ions. *Biochim. Biophys. Acta* **1033**, 256-260
43. Burchill, S. A., and Thody, A. J. (1986) Melanocyte-stimulating hormone and the regulation of tyrosinase activity in hair follicular melanocytes of the mouse. *J. Endocrinol.* **11**, 225-232
44. Friedmann, P. S., and Gilchrest, B. A. (1987) Ultraviolet radiation directly induces pigment production by cultured human melanocytes. *J. Cell Physiol.* **133**, 88-94
45. Burchill, S. A., Marks, J. M., and Thody, A. J. (1990) Tyrosinase synthesis in different skin types and the effects of α-melanocyte stimulating hormone and cyclic AMP. *J. Invest. Dermatol.* **95**, 558-561
46. Wong, G., and Pawelek, J. (1975) Melanocyte stimulating hormone promotes activation of preexisting tyrosinase molecules in Cloudman S91 melanoma cells. *Nature (London)* **255**, 644-646
47. Fuller, B. B., Lunsford, J. B., and Iman, D. S. (1987) α-Melanocyte stimulating hormone regulation of tyrosinase in Cloudman S91 mouse melanoma cell cultures. *J. Biol. Chem.* **262**, 4024-4033
48. Burchill, S. A., Virden, R., Fuller, B. B., and Thody, A. J. (1988) Regulation of tyrosinase synthesis by α-melanocyte stimulating hormone in hair follicular melanocytes of the mouse. *J. Endocrinol.* **116**, 17-23
49. Jiménez, M., Kameyama, K., Maloy, W. L., Tomita, Y., and Hearing, V. J. (1988) Mammalian tyrosinase: biosynthesis, processing and modulation by melanocyte stimulating hormone. *Proc. Natl. Acad. Sci. USA* **85**, 3830-3834
50. Hoganson, G. E., Ledwitz-Rigby, F., Davidson, R. L., and Fuller, B. B. (1989) Regulation of tyrosinase mRNA levels in mouse melanoma cell clones by melanocyte-stimulating hormone and cyclic AMP. *Som. Cell Mol. Gen.* **15**, 255-263
51. Eller, M., Naeyaert, J. M., Gordon, P. R., and Gilchrest, B. A. (1990) Rate of melanogenesis in cultured human melanocytes is not regulated by tyrosinase message level. *Br. J. Der-*

matol. **94**, 521 (abstr.)
52. Barber, J. I., Townsend, D., Olds, D. P., and King, R. A. (1985) Decreased DOPAchrome oxidoreductase activity in yellow mice. *J. Hered.* **76**, 59-60
53. Kwon, B. S., Wakulchik, M., Haq, A. K., Halaban, R., and Kestler, D. (1988) Sequence analysis of mouse tyrosinase cDNA and the effect of melanotropin on its gene expression. *Biochem. Biophys. Res. Commun.* **153**, 1301-1309
54. Bouchard, B., Fuller, B. B., Vijayasaradhi, S., and Houghton, A. N. (1989) Induction of pigmentation in mouse fibroblasts by expression of human tyrosinase cDNA. *J. Exp. Med.* **169**, 2029-2042
55. Tamate, H. B., Hirobe, T., Wakamatsu, K., Ito, S., Shibahara, S., and Ishikawa, K. (1989) Levels of tyrosinase and its mRNA in coat-color mutants of C57BL/10J congenic mice: effects of genic substitution at the agouti, brown, albino, dilute and pink-eyed dilution loci. *J. Exp. Zool.* **250**, 304-311
56. Ghanem, G. E., Communale, G., Libert A., Vercammen-Grandjean, A., and Lejeune, F. L. (1988) Evidence for alpha-melanocyte stimulating hormone (α-MSH) receptors on human malignant melanoma cells. *Int. J. Cancer* **41**, 248-255
57. Friedmann, P. S., Wren, F., Buffey, J., and MacNeil, S. (1990) α-MSH causes a small rise in cAMP but has no effect on basal or ultraviolet-stimulated melanogenesis in human melanocytes. *Br. J. Dermatol.* **123**, 145-151
58. Iwata, M., Iwata, S., Everett, M. A., and Fuller, B. B. (1990) Hormonal stimulation of tyrosinase activity in human foreskin organ cultures. *In Vitro Cell. Dev. Biol.* **26**, 554-560
59. Gordon, P. R., and Gilchrest, B. A. (1989) Human melanogenesis is stimulated by diacylglycerol. *J. Invest. Dermatol.* **93**, 700-702
60. Hearing, V. J., and Jiménez, M. (1989) Analysis of mammalian pigmentation at the molecular level. *Pigment Cell Res.* **2**, 75-85
61. Shibahara, S., Tomita, Y., Sakakura, T., Nager, C., Chaudhuri, B., and Muller, R. (1986) Cloning and expression of cDNA encoding mouse tyrosinase. *Nucl. Acids Res.* **14**, 2413-2427
62. Kwon, B. S., Halaban, R., Kim, G. S., Usack, L., Pomerantz, S. H., and Haq, A. K. (1987) A melanocyte-specific complementary DNA clone whose expression is inducible by melanotropin and isobutylmethyl xanthine. *Mol. Biol. Med.* **4**, 339-355
63. Kwon, B. S., Haq, A. K., Pomerantz, S. H., and Halaban, R. (1987) Isolation and sequence of a cDNA locus for human tyrosinase that maps at the mouse c-albino locus. *Proc. Natl. Acad. Sci. USA* **84**, 7473-7477
64. Yamamoto, H., Takeuchi, S., Kudo, T., Makino, K., Nakata, A., Shinoda, T., and Takeuchi, T. (1987) Cloning and sequencing of mouse tyrosinase cDNA. *Jpn. J. Gen.* **62**, 271-274
65. Jackson, I. J. (1988) A cDNA encoding tyrosinase-related protein maps to the brown locus in mice. *Proc. Natl. Acad. Sci. USA* **85**, 4392-4396
66. Müller, G., Ruppert, S., Schmid, E., and Schütz, G. (1988) Functional analysis of alternatively spliced tyrosinase gene transcripts. *EMBO J.* **7**, 2723-2730
67. Terao, M., Tabe, L., Garattini, S., Sartori, D., and Mintz, B. (1989) Isolation and characterization of variant cDNAs encoding mouse tyrosinase. *Biochem. Biophys. Res. Commun.* **159**, 848-853
68. Ruppert, S., Müller, G., Kwon, B. S., and Schütz, G. (1988) Multiple transcripts of the mouse tyrosinase gene are generated by alternative splicing. *EMBO J.* **7**, 2715-2722
69. Takeda, A., Matsunaga, J., Tomita, Y., Tagami, H., and Shibahara, S. (1989) Molecular analysis of the DNA segments cross-hybridizable to the tyrosinase gene in patients afflicted with oculocutaneous albinism. *Tohoku J. Exp. Med.* **159**, 333-340
70. Shibahara, S., Tomita, Y., Tagami, H., Muller, R. M., and Cohen, T. (1988) Molecular basis for the heterogeneity of human tyrosinase. *Tohoku J. Exp. Med.* **156**, 403-414
71. Porter, S., and Mintz, B. (1991) Multiple alternatively spliced transcripts of the mouse tyrosinase gene. *Gene* **79**, 277-282
72. Barton, D. E., Kwon, B. S., and Francke, U. (1988) Human tyrosinase gene, mapped to chromosome 11 (q14- >q21), defines second region of homology with mouse chromosome 7. *Genomics* **3**, 17-24
73. Jiménez, M., Tsukamoto, K., and Hearing, V. J. (1991) Tyrosinases from two different loci are expressed by normal and by transformed melanocytes. *J. Biol. Chem.* **266**, 1147-1156
74. Yamamoto, H., Takeuchi, S., Kudo, T., Sato, C., and Takeuchi, T. (1989) Melanin production in cultured albino melanocytes transfected with mouse tyrosinase cDNA. *Jpn. J. Gen.* **64**, 121-135
75. Beermann, F., Ruppert, S., Hummler, E., Bosch, F. X., Müller, G., Ruther, U., and Schütz, G. (1990) Rescue of the albino phenotype by introduction of a functional tyrosinase gene into mice. *EMBO J.* **9**, 2819-2826
76. Tanaka, S., Yamamoto, H., Takeuchi, S., and Takeuchi, T. (1990) Melanization in albino mice transformed by introducing cloned mouse tyrosinase gene. *Development* **108**, 223-227
77. Halaban, R., and Moellmann, G. (1990) Murine and human b locus pigmentation genes encode a glycoprotein (gp75) with catalase activity. *Proc. Natl. Acad. Sci. USA* **87**, 4809-4813
78. Halaban, R., Moellman, G., Tamura, A., Kwon, B. S., Kuklinska, E., Pomerantz, S. H., and Lerner, A. B. (1988) Tyrosinases of murine melanocytes with mutations at the albino locus. *Proc. Natl. Acad. Sci. USA* **85**, 7241-7245
79. Bennett, D. C., Cooper, P. J., Dexter, T. H., Devlin, L. M., Heasman, J., and Nester, B. (1989) Cloned mouse melanocyte lines carrying germline mutations albino and brown: complementation in culture. *Development* **105**, 379-385
80. Takeuchi, T., Yamamoto, H., and Takeuchi, T. (1988) Expression of tyrosinase gene in amelanotic mutant mice. *Biochem. Biophys. Res. Commun.* **155**, 470-475
81. Kwon, B. S., Halaban, R., and Chintamaneni, S. (1989) Molecular basis of mouse Himalayan mutation. *Biochem. Biophys. Res. Commun.* **161**, 252-260
82. Shibahara, S., Okinaga, S., Tomita, Y., Takeda, A., Yamamoto, H., Sato, M., and Takeuchi, T. (1990) A point mutation in the tyrosinase gene of BALB/c albino mouse causing the cysteine to serine substitution at position 85. *Eur. J. Biochem.* **189**, 455-461
83. Jackson, I. J., and Bennett, D. C. (1990) Identification of the albino mutation of mouse tyrosinase by analysis of an in vitro revertant. *Proc. Natl. Acad. Sci. USA* **87**, 7010-7014
84. Tomita, Y., Takeda, A., Okinaga, S., Tagami, H., and Shibahara, S. (1989) Human oculocutaneous albinism caused by a single base insertion in the tyrosinase gene. *Biochem. Biophys. Res. Commun.* **164**, 990-996
85. Giebel, L. B., Strunk, K. M., King, R. A., Hanifin, J. M., and Spritz, R. A. (1990) A frequent tyrosinase gene mutation in classic, tyrosinase-negative (Type IA) oculocutaneous albinism. *Proc. Natl. Acad. Sci. USA* **87**, 3255-3258
86. Spritz, R. A., Strunk, K. M., Giebel, L. B., and King, R. A. (1990) Detection of mutations in the tyrosinase gene in a patient with Type IA oculocutaneous albinism. *New Engl. J. Med.* **322**, 1724-1728
87. Takeda, A., Tomita, Y., Matsunaga, J., Tagami, H., and Shibahara, S. (1990) Molecular basis of tyrosinase-negative oculocutaneous albinism. A single base mutation in the tyrosinase gene causing arginine to glutamine substitution at position 59. *J. Biol. Chem.* **265**, 17792-17797
88. King, R. A., Mentink, M. M., and Oetting, W. S. (1991) Non-random distribution of missense mutations within the human tyrosinase gene in type 1 (tyrosinase-related) oculocutaneous albinism. *Mol. Biol. Med.* **8**, 19-29
89. Bennett, D. C., Huszar, D., Laipis, P. J., Jaenisch, R., and Jackson, I. J. (1990) Phenotypic rescue of mutant brown melanocytes by a retrovirus carrying a wild-type tyrosinase-related protein gene. *Development* **110**, 471-476
90. Cohen, T., Muller, R. M., Tomita, Y., and Shibahara, S. (1990) Nucleotide sequence of the cDNA encoding human tyrosinase-related protein. *Nucl. Acids Res.* **18**, 2807-2808
91. Vijayasaradhi, S., Bouchard, B., and Houghton, A. (1990) The melanoma antigen gp75 is the human homologue of the mouse b locus gene product. *J. Expl. Med.* **171**, 1375-1380
92. Shibahara, S. (1988) A study of murine albinism using tyrosinase cDNA. *Prog. Clin. Biol. Res.* **256**, 263-271
93. Shibahara, S., Taguchi, H., Müller, R. M., Shibata, K., Cohen, T., Tomita, Y., and Tagami, H. (1991) Structural organization of the pigment cell-specific gene located at the brown locus in mouse. Its promoter activity and alternatively spliced transcripts. *J. Biol. Chem.* **266**, 15895-15901
94. Jiménez, M., Maloy, W. L., and Hearing, V. J. (1989) Specific identification of an authentic tyrosinase clone. *J. Biol. Chem.* **264**, 3397-3403
95. Vijayasaradhi, S., and Houghton, A. N. (1991) Purification of an autoantigenic 75-kDa human melanosomal glycoprotein. *Int. J. Cancer* **47**, 298-303
96. Tsukamoto, K., Jiménez, M., and Hearing, V. J. (1991) The nature of tyrosinase isozymes. In *Proc. XIV Intl. Pigment Cell Conf.* (Mishima, Y., ed), Munksgaard, Copenhagen. In press
97. Jackson, I. J., Chambers, D., Rinchik, E. M., and Bennett, D. C. (1990) Characterization of TRP-1 mRNA levels in dominant and recessive mutations at the mouse brown (b) locus. *Genetics* **126**, 451-460
98. Zdarsky, E., Favor, J., and Jackson, I. J. (1990) The molecular basis of brown, an old mouse mutation, and of an induced revertant to wild-type. *Genetics* **126**, 443-450
99. Kwon, B. S., Chintameneni, C., Kozak, C. A., Copeland, N. G., Gilbert, D. J., Jenkins, N., Barton, D., Francke, U., Kobayashi, Y., and Kim, K. K. (1991) A melanocyte-specific gene, Pmel 17, maps near the silver coat color locus on mouse chromosome 10 and is a syntenic region on human chromosome 12. *Proc. Natl. Acad. Sci. USA* **88**, 9228-9232
100. Lerch, K. (1988) Protein and active-site structure of tyrosinase. *Prog. Clin. Biol. Res.* **256**, 85-98
101. Martinez, J. H., Solano, F., Garcia-Borron, J. C., Iborra, J. L., and Lozano, J. A. (1985) The involvement of histidine at the active site of Harding-Passey mouse melanoma tyrosinase. *Biochem. Int.* **11**, 729-738