Incorporating Mouse Genome Mammalian

The *Tyr* (*albino*) locus of the laboratory mouse

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Abstract

The albino mouse was already known in ancient times and was apparently selectively bred in Egypt, China, and Japan. Thus, it is not surprising that the *c* or *albino* locus (now the *Tyr* locus) was among the first used to demonstrate Mendelian inheritance in mammals at the dawn of the past century. This locus is now known to encode tyrosinase, the rate-limiting enzyme in the production of melanin pigment, and the molecular basis of the *albino* (Tyr^c) mutation is known. Here we describe the congenic series of *Tyr*-locus alleles, from wild type to null (*albino*). We compare eye and skin pigmentation phenotypes and the genetic lesions that cause each. We suggest that this panel of congenic mutants contains rich, untapped resources for the study of many questions of basic cell biological interest.

The albino mouse was already known in ancient times and, over a century ago, was used to first demonstrate Mendelian inheritance of a genetic trait in mammals (Castle and Allen 1903). Very early on it was suggested that the *albino* gene locus was responsible for a ''factor'' that is necessary for melanin pigment to form in the melanocytes. This ''factor'' has been identified as tyrosinase, the rate-limiting enzyme for melanogenesis (Coleman 1982). Tyrosinase is encoded at the *albino* (*Tyr*) locus of the mouse on Chromosome 7 (Kwon et al. 1989b), where multiple natural mutations and manmade mutations (Fig. 1) have helped to define the functions and interactions of this enzyme with other proteins that together effect normal pigmentation. In human, the defect in tyrosinase is called oculocutaneous albinism type 1 (OCA1) and is often specified as OCA1A or OCA1B to distinguish between no pigment or less pigment, respectively. The genetic defect in the tyrosinase gene affects the quantity of pigment produced within the melanosome; melanin is absent or reduced, but melanocytes are present in the skin and hair follicles and they contain melanosomes. In addition to effects on pigmentation, albino mice have defects in the visual projections at the optic chiasm (Jeffery et al. 1994), decreased numbers of rod photoreceptors (Donatien and Jeffery 2002; Rachel et al. 2002a), and spatiotemporal defects in neuronal production (Rachel et al. 2002a). Furthermore, a role for tyrosinase in the occurrence of glaucoma by a mechanism apparently unlinked to melanin production has recently been suggested (Libby et al. 2003).

Melanin pigment is produced primarily in two different cell types, the neural crest-derived melanocytes found in skin, hair follicle, and the choroid, ciliary body, and iris of the eye, and the retinal pigment epithelium, a cell layer of the retina that is derived from the optic cup. In the pigment cells, melanin is synthesized and deposited within endolysosome-like organelles, termed ''melanosomes,'' by a series of enzymatic reactions beginning with tyrosine as substrate, and involving the copper–glycoenzyme tyrosinase (Garcia–Borron and Solano 2002). The melanin product is deposited within the melanosome as eumelanic (brown or black) or pheomelanic (yellow/red) pigment. It had long been believed that the first two reactions in the melanogenic pathway—the hydroxylation of tyrosine to dopa (3,4-dihydroxyphenylalanine) and the oxidation of dopa to dopaquinone—are catalyzed by the enzyme tyrosinase (del Marmol and Beermann 1996).

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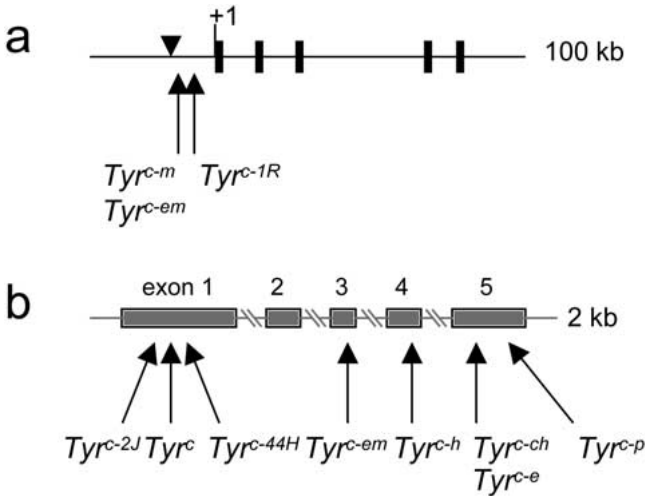

Fig. 1. The mouse *tyrosinase* gene and location of identified mutations. (**a**) The *Tyr* gene locus, showing relative size of introns (Ruppert et al. 1988) and location of the enhancer/dominant control region at −15 kb (black arrowhead). The transcription start site is indicated as +1. (**b**) Exon/intron structure of the *tyrosinase* gene and location of mutations which were identified within coding sequence. Note that $Tyr^{c\text{-}em}$ is listed twice since it appeared on a $Tyr^{c\text{-}m}$ background.

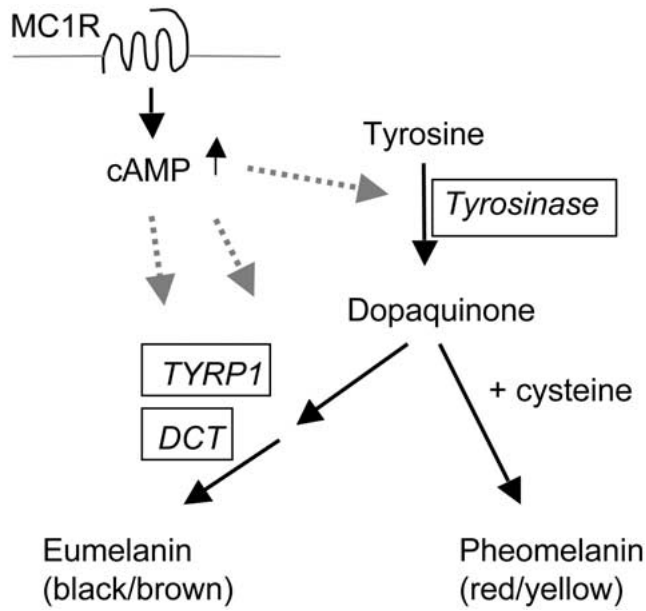

Fig. 2. Scheme of melanin synthesis. Activation of melanocortin receptor 1 (MC1R) increases cAMP levels, thus enhancing tyrosinase activity and favoring eumelanin synthesis (del Marmol and Beermann 1996; Barsh 2003). Tyrosinase-related protein 1 (TYRP1) and dopachrome tautomerase (DCT) are enzymes of eumelanin synthesis only.

More recent chemical analyses have suggested, however, that tyrosinase-catalyzed oxidation of tyrosine leads directly to dopaquinone, which then can lead to eumelanin formation via spontaneous formation of dopachrome (Wakamatsu and Ito 2002). Accordingly, dopa itself can act as a cofactor in tyrosine oxidation and is not derived by tyrosinase enzyme activity, but indirectly by reduction of dopaquinone (Riley 1999). The pheomelanic pathway is thought to be initiated by a reaction between cysteine and dopaquinone, thus leading to cysteinyldopa, and further to benzothiazoles. Accordingly, the balance between pheomelanin and eumelanin may be determined by the availability of cysteine as precursor (Land and Riley 2000).

Besides tyrosinase, two other enzymes function in melanogenesis, dopachrome tautomerase (DCT) and tyrosinase-related protein 1 (TYRP1). DCT is encoded at the *slaty* (now *Dct*) locus of the mouse, and TYRP1 is encoded at the *black/brown* (now *Tyrp1*) locus. When all three of these enzymes function normally, eumelanin pigment is deposited within the melanosome (Fig. 2). Analysis of mice that are mutant at the *Tyr* or *Tyrp1* locus has shown that mutation in one may affect the phenotype associated with the other. Accordingly, TYRP1 may affect stability of tyrosinase (Manga et al. 2000), and both proteins are transported together from the endoplasmic reticulum to the melanosome (Toyofuku et al. 2001). Mice lacking *Tyrp1* [*Tyrp1* deletions (Rinchik et al. 1994)] or *Dct* [*Dct* knockout (Guyonneau et al. 2004)] are only slightly affected in pigmentation and show a rather brown (*Tyrp1* deficiency) or dark gray (*Dct* deficiency) coat color. Several other gene loci function within the melanosome and are necessary for normal pigmentation but have not been shown to be so intimately interactive with tyrosinase protein (Bennett and Lamoreux 2003). These loci include *pink-eyed dilution* (*p*, mutation causing OCA2 in human) (Chiu et al. 1993), *underwhite* (*uw*, now *Matp*, mutation causing OCA4 in human) (Newton et al. 2001; Costin et al. 2003), and MITF (*Mitf*) that modulates the expression of a number of melanocyte-specific genes, including *Tyr* and *Tyrp1*, at the transcriptional level and influences the eumelanin/pheomelanin switch (Goding 2000; Widlund and Fisher 2003).

Eumelanin (which is black or brown depending upon the genotype at the *Tyrp1* locus) is produced in the melanosome as a result of the normal activity of the MSH (melanocyte stimulating hormone) receptor, which regulates levels of cyclic AMP (cAMP) within the cell and is present in melanocyte cell membranes (Fig. 2). The MSH receptor (MC1R) is encoded at the *melanocortin-1 receptor* (*Mc1r*) locus [formerly *extension* (*e*) locus] in the mouse and is

responsive to the environment of the hair follicle in which the melanocyte resides. It is thus capable of switching from an active state that raises cAMP levels and results in the production of eumelanin within the melanosomes of the cell to an inactive state, when pheomelanin is produced (Barsh 2003). In the eumelanic state, elevated cAMP levels are followed by an increase in activity of tyrosinase, DCT, and TYRP1. In the pheomelanic state, the melanosomes produce yellow-colored melanin, cAMP levels are reduced, tyrosinase activity is lower, and DCT and TYRP1 activities are absent (Lamoreux et al. 1995). Mutation at *Mc1r* can result in melanocortin receptors that are not responsive to the environment and are constitutively active, resulting in production of only eumelanin, as in the *sombre* ($Mc1r^{E\text{-}so}$) mutant, or constitutively inactive, resulting primarily in the production of pheomelanin as in the hair follicles of the *yellow* mutant ($Mc1r^{e}$) mouse. Tyrosinase is required for both types of pigment, but activity is reduced in pheomelanic melanocytes. Wild-type MC1R is active unless blocked by the protein encoded at the *agouti* locus, thus switching pigment synthesis from the eumelanin pathway toward the pheomelanin pathway (Barsh 2003). Thus, mice that are yellow because of mutation at the *agouti* locus continue to produce the *agouti*-locus protein inappropriately and do not switch back and forth from the production of eumelanin to the production of pheomelanin as is normal in wild-type agouti mice. In addition, several other pigment loci encode proteins that interact with the Mc1r/agouti protein switch mechanism. These include *mahogany* (*Atrn*) and *mahoganoid*, both of which result in a reduction of pheomelanin, or rather an increase in eumelanin, in the hair coats of mutant mice (He et al. 2003). Interestingly, the *Tyr*-locus mutants (except for platinum) preferentially reduce the amount of pheomelanin compared with the reduction in eumelanin. Hence, the impact of *Tyr*-locus mutations is greater in pheomelanic mice than in eumelanic mice (Lamoreux and Pendergast 1987; Lamoreux et al. 2001) and also in pheomelanic locations on a mouse as for example the belly.

Availability of multiple mouse *Tyr* (*albino*) locus alleles with various sorts of genetic lesions provides an opportunity to evaluate the dynamic interactions in the processes that intervene between the transcription of the tyrosinase gene and the resulting phenotype of the animal. These include transcription, translation, post-translational processing, and transport mechanisms, as well as interactions with the products of other loci. Moreover, many other mutations causing albinism act via tyrosinase by affecting tyrosinase processing [e.g., pink-eyed dilution (Chen et al. 2002)] or tyrosinase trafficking [e.g., forms of Hermansky–Pudlak syndrome (Huizing et al. 2002)]. In this review, we report on the current state of knowledge regarding the molecular bases and phenotypic consequences of mutations at the mouse *Tyr* (*albino*) gene locus (Fig. 3, Table 1).

The allelic series

In the absence of mutations at other loci, mice that are wild type at the *Tyr* locus are fully pigmented and are black (*Tyr*+/*Tyr*+, *a*/*a*). Wild type is dominant to all other alleles at the locus, though one semidominant mutant (albino-strong, $Tyr^{c\text{-}s}$) is reported at the JAX web site (http://www.informatics.jax.org/searches/mlc.cgi?14347). Mice that are lacking the *Tyr* locus as a result of overlapping deletions ($Tyr^{c\text{-}6H}$/$Tyr^{c\text{-}14CoS}$) are unpigmented, although melanosomes are present, confirming the requirement of the *Tyr* locus and a functional tyrosinase for pigment production (Russell et al. 1982; Rinchik et al. 1993). Similarly, two natural mutations have been identified that result in an albino phenotype associated with lack of tyrosinase activity, Tyr^{c} and $Tyr^{c\text{-}2J}$. The classic mouse *albino* mutation, Tyr^{c}, which is present in common albino mouse strains such as BALB/c or FVB, is characterized by the complete absence of pigmentation in both skin and eyes and by aberrant decussation of the optic nerve at the level of the chiasm (Guillery 1974; LaVail et al. 1978). Even though tissue homogenates of BALB/c mice might retain a slight amount of tyrosinase-dependent melanin synthesis *in vitro* (Hearing 1973), Tyr^{c} is considered a null mutation since the mutated protein is not active *in vivo* and is retained in the ER (Halaban et al. 2000; Toyofuku et al. 2001). The molecular change in Tyr^{c} is a G-to-C mutation at position +387, which results in the substitution of a cysteine for a serine residue at position 103 (Kwon et al. 1989b; Shibahara et al. 1990; Yokoyama et al. 1990). The $Tyr^{c\text{-}2J}$ mutation arose spontaneously in C57BL/6J mice, and homozygous $Tyr^{c\text{-}2J}$/$Tyr^{c\text{-}2J}$ mice are phenotypically identical to Tyr^{c}/Tyr^{c} mice. The mutation was identified as a G-to-T change at position +309, resulting in an arginine-to-leucine substitution at codon 77 and furthermore led to increased alternative splicing within exon 1 (Lefur et al. 1996, 1997). Enzymatic activity of tyrosinase is absent in normal melanocytes of these mice, but melanomas occurring on this $Tyr^{c\text{-}2J}$ genetic background can be pigmented and are tyrosinase positive (Cohen–Solal et al. 2002), in contrast to melanomas appearing on BALB/c or FVB mice (Tyr^{c}) (Cohen–Solal et al. 2001). Thus, it is

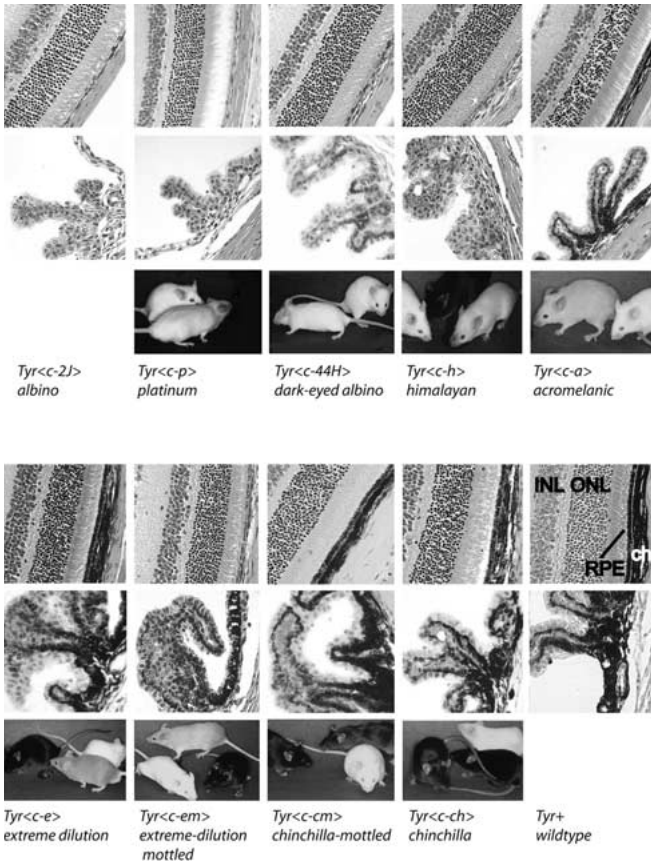

Fig. 3. Phenotype of alleles at the *Tyr* gene locus. Shown are (top row) sections of the retina of mice mutant at the *Tyr* locus, (middle row) sections through the uvea and ciliary body, and (bottom row) pictures of the mutant mice (RPE, retinal pigment epithelium; ch, choroid; ONL, outer nuclear layer; INL, inner nuclear layer). First and last columns contain no mouse photograph because albino ($Tyr^{c\text{-}2J}$/ $Tyr^{c\text{-}2J}$) and wild-type (C57BL/6J–*Tyr*+/*Tyr*+) mice are used as negative and positive controls in several other columns. Each column is labeled at the bottom with the name of the mutant illustrated as follows: *Albino*, C57BL/6J–$Tyr^{c\text{-}2J}$/$Tyr^{c\text{-}2J}$. *Platinum*, C57BL/6J–$Tyr^{c\text{-}p}$/$Tyr^{c\text{-}p}$: mouse in the foreground is *platinum*, just slightly more pigmented than the *albino* mutant mouse in the background. Dark-eyed *albino* C57BL/6J–$Tyr^{c\text{-}44H}$/$Tyr^{c\text{-}44H}$. *Himalayan*, C57BL/6J–$Tyr^{c\text{-}h}$/$Tyr^{c\text{-}h}$: the *himalayan* mutant mouse, on the right, is more darkly pigmented on the extremities than in the warmer areas of its body. Control mice in this picture are *albino* on the left and C57BL/6J in the center. *Acromelanic*, C57BL/6J–$Tyr^{c\text{-}a}$/$Tyr^{c\text{-}a}$: the *acromelanic* mutant mouse on the left is somewhat darker in body pigmentation than the *himalayan* mutant mouse (previous column) but shares the characteristically darker extremities. *Extreme dilution*, C57BL/6J–$Tyr^{c\text{-}e}$/$Tyr^{c\text{-}e}$: the *extreme dilution* mutant mouse, in the foreground, is compared with wild type and *albino* controls. Pigmentation in hair coat of this mutant is uniformly distributed. *Extreme dilution mottled*, C57BL/6J–$Tyr^{c\text{-}em}$/$Tyr^{c\text{-}em}$: in the foreground are the control mice, *albino* ($Tyr^{c\text{-}2J}$), and wild type at the *Tyr* locus. The mouse in the background has intermingled areas of beige, and paler and darker hair. *Chinchilla mottled*, C57BL/6J–$Tyr^{c\text{-}m}$/$Tyr^{c\text{-}m}$: the control mice are on the left and in the foreground. The *chinchilla mottled* mutant mouse has intermingled areas of dark gray and very dark gray hair. *Chinchilla*, C57BL/6J–$Tyr^{c\text{-}ch}$/$Tyr^{c\text{-}ch}$: the bright area on the *chinchilla* mutant mouse, on the left, is an artifact of the reflected light. In fact, *chinchilla* mutant mice that are black are superficially difficult to distinguish from wild-type black mice. Wild type, C57BL/6J–Tyr^{+}/Tyr^{+}.

conceivable that $Tyr^{c\text{-}2J}$ is not a true null mutation and is able to produce an unstable but enzymatically active protein in melanoma cells.

Tyr locus alleles that fall between the two extremes of black and albino can be classified into several groups by phenotype. First, there are alleles affecting ocular and cutaneous pigmentation similarly. The *chinchilla* allele was procured in 1922 by Feldman from a fancier (Feldman 1922). C57BL/6J–$Tyr^{c\text{-}ch}$/$Tyr^{c\text{-}ch}$ (*chinchilla*) mice are phenotypically very similar to mice that are wild type at the *Tyr* locus, with black eyes and very dark gray, almost black, hair coat, though the tyrosinase activities of their skin or eyes is approximately one third that of wild-type mice. Moyer (1966) reported that melanosomes look normal in size and number, at least in the retina. In eumelanic brown ($Tyrp1^{b}$) mice, the effect of *chinchilla* is not evident, in either the intensity of pigmentation or reduced tyrosinase activity or change of melanosome structure (Russell 1948; Grüneberg 1952; Lamoreux et al. 2001). Interestingly, in pheomelanic *chinchilla* ($Tyr^{c\text{-}ch}$/ $Tyr^{c\text{-}ch}$) mice, pigmentation is much reduced compared with that of pheomelanic mice that are wild type at the *Tyr* locus. This dichotomy is typical of the phenotypes of most of the *Tyr*-locus mutations (Silvers 1979; Lamoreux and Pendergast 1987), with the exception of *platinum*. Pheomelanic *chinchilla* melanocytes exhibit a greatly reduced number of melanosomes compared with normal pheomelanic melanocytes. Northern blot data and RT-PCR failed to reveal any difference in expression between $Tyr^{c\text{-}ch}$ and wild type (Halaban et al. 1988; Ganss et al. 1994), and it was suggested that *chinchilla* tyrosinase enzyme is less stable than the wild-type tyrosinase enzyme (Halaban et al. 1988). Sequence analysis of the entire coding region revealed a G-to-A point mutation at nucleotide +1523, resulting in an amino acid substitution of alanine to threonine at position +482, close to the transmembrane region (Beermann et al. 1990).

Platinum occurred as a spontaneous mutation in DBA/2 (Dickie 1966). Homozygous *platinum* (C57BL/6J–$Tyr^{c\text{-}p}$/$Tyr^{c\text{-}p}$) mice are very pale with pink eyes, yet their tyrosinase activity is higher than that of *chinchilla* mice ($Tyr^{c\text{-}ch}$/$Tyr^{c\text{-}ch}$). In skin ex-

Table 1. Phenotype and molecular lesion of selected *Tyr* locus alleles

Pigment phenotype	*Allele*	*Molecular lesion*
Eyes and hair similarly pigmented/affected:		
Fully pigmented	*Tyr*+, wildtype	
No pigment, albino	$Tyr^{c\text{-}6H}/Tyr^{c\text{-}14cos}$	Tyrosinase deletion
	Tyr^{c} (albino)	C103S
	$Tyr^{c\text{-}2J}$ (albino)	R77L
Dark gray, black eye	$Tyr^{c\text{-}ch}$ (chinchilla)	A482T
Pale coat, pink eye	$Tyr^{c\text{-}p}$ (platinum)	K507STOP
Eyes more pigmented than hair:		
Mid gray, dark eye	$Tyr^{c\text{-}e}$ (extreme dilution)	A482T (additional mutation?)
Extremities pigmented, dark eye	$Tyr^{c\text{-}h}$ (himalayan)	H420R
	$Tyr^{c\text{-}a}$ (acromelanic)	No protein detected, RNA low
Albino, dark eye	$Tyr^{c\text{-}44H}$ (dark eyed albino)	S146I
Mottled:		
Mottled coat and eye	$Tyr^{c\text{-}m}$ (chinchilla mottled)	Rearrangement in 5′ region
	$Tyr^{c\text{-}em}$ (extreme dilution mottled)	Rearrangement in 5′ region T373I
	$Tyr^{c\text{-}1R}$	IAP element insertion at promoter

See text for references and further details. Numbering of nucleotides and amino acids varied in the past, depending upon the definition of the start site and upon whether the signal peptide was included. In this article we refer to the published transcriptional start site (+1) (Bentley et al. 1994). For the amino acid sequence, the start codon (ATG) is counted as +1.

tracts of *platinum* mice, a large proportion of tyrosinase is present in soluble form (Townsend et al. 1981). Furthermore, phenotypic differences in intensity of pigmentation between pheomelanic and eumelanic *platinum* mice are not evident, and both appear equally pale. These differences between *platinum* and *chinchilla* mice suggested that tyrosinase is functional in *platinum* mice, as the tyrosinase activities of skin and eyes are higher than those of *chinchilla* mice, which have lower tyrosinase activity but much more intense pigmentation. In addition, the effect of pheomelanogenesis on tyrosinase activity is sidestepped in the case of *platinum* mice. Analysis of the tyrosinase protein suggested a mutation at the carboxy terminal part of the protein (Orlow et al. 1993), and electron microscope studies demonstrated tyrosinase activity in the trans-Golgi network and in nearby vesicles, but missing activity in melanosomes (Beermann et al. 1995). Instead, tyrosinase was found at the cell surface. Since melanogenesis is confined essentially to the melanosome, it is thus reasonable not to detect major differences between pheo- and eumelanogenesis in this specific mutant allele. The mutation in platinum is a G-to-A change at +1523 (Beermann et al. 1995), inferring a replacement of a lysine residue in the cytoplasmic tail by a termination codon. The lack of the cytoplasmic tail, which contains the essential di-leucine sorting motif, is causing misrouting of platinum tyrosinase to the cell surface (Beermann et al. 1995; Simmen et al. 1999).

Second, there are alleles with more effect on coat pigmentation, e.g., *extreme dilution* ($Tyr^{c\text{-}e}$). The original $Tyr^{c\text{-}e}$-mutation was found in the wild (Detlefsen 1921). C57BL/6J mice that are homozygous at this locus can be characterized as ''midgray'' in phenotype, more or less midway in intensity between wild type and *albino* but with eyes that are nearer to black. Both tyrosinase activity and amount of eumelanin are greatly reduced in C57BL/6J–$Tyr^{c\text{-}e}/Tyr^{c\text{-}e}$ mice; melanosomes of $Tyr^{c\text{-}e}/Tyr^{c\text{-}e}$ are reduced in both number and size (Markert and Silvers 1956; Moyer 1966). Northern blot analysis of newborns' skins (not shown) showed no reduction in abundance of tyrosinase mRNA in $Tyr^{c\text{-}e}/Tyr^{c\text{-}e}$ mice that are eumelanic. Sequencing the complete coding region demonstrated a deviation from wild type in exon 5, leading to exchange of an alanine by a threonine, the very same mutation (A482T) that had been identified in the *chinchilla* ($Tyr^{c\text{-}ch}$) mutation (Beermann et al. 1990). A482T is the only mutation found in the coding sequence of the *chinchilla* ($Tyr^{c\text{-}ch}$) tyrosinase gene; it affects tyrosinase *in vivo* (Halaban et al. 1988) and following transfections (unpublished data). Therefore, it is rather unlikely that A482T is a polymorphism, with two unidentifed mutations still existing for both *chinchilla* and *extreme dilution*. It is more likely that the $Tyr^{c\text{-}e}$ mutation may have occurred on a $Tyr^{c\text{-}ch}$ background and might contain a yet unidentified second mutation, for example, in the regulatory region.

Himalayan is a spontaneous mutation which occurred in offspring of a cross between DBA/2 and AKR/J (Green 1961). Mice homozygous for the *himalayan* mutation ($Tyr^{c\text{-}h}$), similar to himalayan cats or rabbits, over time develop more intense pigmentation at the extremities where the body is cooler. Their body color is beige, with darker-beige extremities, and eyes are dark ruby. The mutation is an A-to-G change at nucleotide 1338 that alters

a histidine residue to an arginine residue at amino acid 420 (Kwon et al. 1989a). The activity of tyrosinase isolated from skins of $Tyr^{c\text{-}h}/Tyr^{c\text{-}h}$ mice is heat labile (Coleman 1962), but the protein itself has been reported not to be heat-sensitive (Townsend et al. 1985), and it has been stated that the *himalayan* tyrosinase binds an inhibitor differentially at different temperatures (Kidson and Fabian 1981). A similar mutation in human (Giebel et al. 1991), which is located only two codons away, results in a thermosensitive protein upon transfection into HeLa cells. Thus, the *himalayan* ($Tyr^{c\text{-}h}/Tyr^{c\text{-}h}$) mouse would seem to be an excellent model for the condition in man and deserves further study to understand the cause of this thermosensitivity.

Acromelanic ($Tyr^{c\text{-}a}$) is a spontaneous mutation which occurred on the C3H/HeJ strain (Sweet 1987). *Acromelanic* mice are beige in coat color (similar to *himalayan*) but have dark eyes and pigment appearing on tail, ears, and extremities. We sequenced the complete coding sequence, including about 270 bp upstream of the transcription start site, and no change to the wild-type tyrosinase sequence was detected. This result is in accordance with failure to detect protein and message on Western blots (not shown) and Northern blots (not shown). The message was nevertheless detectable by RT-PCR (not shown), thus suggesting a defect in transcriptional regulation or RNA stability. No major rearrangements were detected by Southern blot analysis covering about 15 kb of tyrosinase 5′ sequence (not shown). Since the mutation affects RNA levels, three unsequenced areas remain to be tested for the presence of mutation: (1) the enhancer region (Ganss et al. 1994; Porter and Meyer 1994), (2) the 3′ noncoding sequences which might be involved in tyrosinase regulation and mRNA stability (Takeuchi et al. 2000), and (3) the exon/intron boundaries, which might affect the correct splicing (Ruppert et al. 1988; Lefur et al. 1997). A defect in the enhancer region might explain the different effect in skin melanocytes versus RPE pigmentation by affecting regulation preferentially in either cell type (Porter et al. 1999; Camacho–Hübner and Beermann 2001). On the other hand, the choroidal layer, which equally consists of neural crest-derived melanocytes, is pigmented in acromelanic mice. Thus, it might rather be the steady accumulation of low levels of melanin within the RPE (and the choroidal layer) that makes the eye pigmented but keeps the skin and hair rather unpigmented. In addition, the presence of pigment at the extremities might point to a certain temperature-sensitive effect. How this is explained without an obvious mutation in the cDNA remains to be discovered.

In homozygous *dark-eyed albino* ($Tyr^{c\text{-}44H}$) mice, overall pigment production is greatly reduced and is obvious only in the eyes (Cattanach and Rasberry 1988). *Dark-eyed-albino* mice are born white with ruby-colored eyes, which darken to become almost black by 3–4 months of age. The hair coat, by contrast, remains essentially unpigmented. Enzymatic activity of tyrosinase and melanin levels in the retina of $Tyr^{c\text{-}44H}/Tyr^{c\text{-}44H}$ newborn mice reached levels of only 2.6% (tyrosinase activity) and 11.8% (melanin) of wild type (Rachel et al. 2002b). By Southern blot, Northern blot, and RT-PCR analyses, it was demonstrated that the basis of the phenotype resides in the coding sequences, with a point mutation (G-to-T) in exon 1, at position +515, inferring a substitution of the amino acid serine by isoleucine (position +146) (Schmidt and Beermann 1994).

Third, alleles exist that depict a mottled or variegated coat color ($Tyr^{c\text{-}m}$, $Tyr^{c\text{-}1R}$, $Tyr^{c\text{-}em}$). Mice carrying the *chinchilla-mottled* mutation ($Tyr^{c\text{-}m}$) were found in the offspring of a neutron-irradiated male (Phillips 1970), and $Tyr^{c\text{-}1R}$ arose spontaneously in 1988 in the Oak Ridge National Laboratory in a C3Hf/RI strain (Wu et al. 1997). Northern blot analyses and RT-PCR data showed that expression of tyrosinase is significantly diminished in homozygous $Tyr^{c\text{-}1R}$ mutant mice when compared with wild-type controls (Wu et al. 1997). Both $Tyr^{c\text{-}m}$ and $Tyr^{c\text{-}1R}$ cause a phenotype of mottled pigmentation resembling a chimerism of chinchilla color and a paler shade in homozygous mice. In $Tyr^{c\text{-}m}$, which exhibit dark and light gray stripes on the coat, the mottled pigmentation is due to differential tyrosinase gene expression and changed chromatin structure of the *Tyr* gene locus in melanocytes within a stripe (Porter et al. 1991). This inherited mottling, as seen also in some tyrosinase-transgenic mice, results from the formation of phenotypically different but genetically identical developmental clones among cells of the same type (Bradl et al. 1991). Eyes of $Tyr^{c\text{-}m}/Tyr^{c\text{-}m}$ mice appear dark, and older findings indicate that they are chimeric, with patches of darker and lighter pigmented cells (Deol and Truslove 1980). Molecular analysis of $Tyr^{c\text{-}m}/Tyr^{c\text{-}m}$ DNA demonstrated a normal coding region but a major rearrangement involving 30 kb of 5′ upstream tyrosinase regulatory sequences, including the locus control region (Porter et al. 1991; Porter and Meyer 1994; Lavado Judez and Montoliu 2002). Molecular analysis of $Tyr^{c\text{-}1R}$ revealed insertion of a 5.4-kb intracisternal A particle (IAP) element at −225 bp upstream of the tyrosinase promoter (Wu et al. 1997). Thus, this IAP element isolates the promoter of the

tyrosinase gene from the upstream tyrosinase locus control region, thereby either increasing the distance between this enhancer and the promoter or directly negatively affecting tyrosinase gene expression. The tyrosinase locus control region, which equally exists in human tyrosinase (Fryer et al. 2003; Regales et al. 2003), has recently been shown to have boundary activity, protecting the tyrosinase gene regulation from negative effects of neighboring chromatin (Giraldo et al. 2003). Thus, in the case of the mottled mutations, it is feasible that (1) the boundary activity cannot be exerted, (2) the new introduced sequences result in novel ''negative'' influences as hypermethylation, and (3) interaction of the enhancer sequences with promoter sequences such as the MITF binding site is affected. A third mottled mutation, *extreme-dilution mottled* ($Tyr^{c\text{-}em}$), arose spontaneously in Harwell (UK) in breeding *chinchilla mottled* mice ($Tyr^{c\text{-}m}$). Homozygotes for this allele possess black eyes and light gray fur that is variegated. The molecular basis of this mutation has been identified, on top of the rearrangements inherent in the mottled stock ($Tyr^{c\text{-}m}$, see above), as a point mutation (C to T) in exon 3 of tyrosinase at position +1197 (+1220 according to the numbering of the authors), implying a substitution of the amino acid threonine by isoleucine (position +373) (Lavado Judez and Montoliu 2002).

Conclusion

We have reviewed and described alleles at the *Tyr* locus in the mouse and have added some new information. Most mice congenic with C57BL/6J should soon be available and thus offer a unique resource for the study of genic action and interactions. Regarding pigmentation of the albino series, it is striking that effects on eye and fur pigmentation seem to differ. This might be due to transfer of melanosomes from neural crest-derived melanocytes in skin and hair follicles, whereas they are retained in the retinal pigment epithelial cells and the choroidal melanocytes. This is exemplified by recent analyses on the $Tyr^{c\text{-}44H}$ (dark-eyed albino) allele, where tyrosinase activities in the retina of homozygotes at birth were much more reduced (2.6%) compared with the melanin levels (11.8% of wild type) (Rachel et al. 2002b). Alternatively, there might exist differences in tyrosinase gene expression between the two cell types. Initial experiments by Porter and Meyer (1994) had indicated that the enhancer region (dominant control region) of the mouse tyrosinase gene could be a candidate for such a differential regulation. The presence of the enhancer increased melanin deposition primarily in the neural crest cells (e.g., iris) but not to the same degree in cells of the retinal pigment epithelium (Porter and Meyer 1994). This observation was confirmed later using transgenic mice and transfection experiments (Porter et al. 1999; Camacho–Hübner and Beermann 2001), suggesting that there might be a differential regulation between optic cup-derived and neural crest-derived pigment cells.

Several of the mutants at the *Tyr* locus indirectly affect phenotypes associated with other loci. For example, brown (TYRP1) protein and tyrosinase protein interact rather closely to produce the pigment phenotype. A *chinchilla* mutant ($Tyr^{c\text{-}ch}$) mouse that is black (*Tyrp1*+) exhibits a slight but visible reduction in pigment intensity. In contrast, the difference between a brown ($Tyrp1^{b}$) mouse and a *brown chinchilla* mutant ($Tyrp1^{b}$, $Tyr^{c\text{-}ch}$) mouse is not obvious. It has been shown that tyrosinase-negative albinism, at least in some instances, is an ER-retention disease, with tyrosinase retained in the ER, which also affects localization of TYRP1 (Toyofuku et al. 2001). The availability of multiple alleles at this *Tyr* gene locus which is essential for pigmentation and retinal development but dispensable for survival, and which contains various genetic lesions, provides an opportunity to evaluate the dynamic interactions in the processes that intervene between the transcription of the *tyrosinase* gene and the resulting phenotype of the animal. This rich source of mutations has allowed and will allow studies to address various cellular mechanisms ranging from defects in transcriptional regulation to protein mislocalization and retinal development/organization.

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