The genetics of pigmentation: from fancy genes to complex traits

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Genes that control mammalian pigmentation interact with each other in intricate networks that have been studied for decades using mouse coat color mutations. Molecular isolation of the affected genes and the ability to study their effects in a defined genetic background have led to surprising new insights into the potential interaction between tyrosine kinase and G-protein-coupled signaling pathways. Recent developments show that homologous genes in humans are responsible not only for rare diseases, such as albinism and piebaldism, but also for common phenotypic variations, such as red hair and fair skin.

Some of the most obvious traits that distinguish different individuals of our species are the colors of eyes, hair and skin. Identifying and understanding the genes that control these traits could shed light on recent events in human evolution, and might provide biological explanations for differences that underlie social perceptions of historical and current significance. The genetics of pigmentation, however, are complex and quantitative: more than a few genes are responsible for the continuous range of pigmentation phenotypes apparent among different ethnic and racial groups.

Of the several genes known to affect human pigmentation, most have been recognized by severe loss-of-function mutations that produce albinism, biochemical abnormalities that affect the ability of melanocytes to make normal pigment[1], or piebaldism, developmental abnormalities that result in the absence of mature melanocytes from discrete areas of the body[2] (Table 1). While one might postulate that polymorphism in these genes can explain the observed differences in the hues of our hair and the shades of our skin, the genetics of mouse pigmentation predicts a more complicated situation.

In mice, nearly 100 genes have been identified that affect coat color, many of which exhibit complex and fascinating interactions described by Silvers almost 30 years ago[3]. A large number of coat color variants, such as brown, silver, pink-eyed dilution and yellow, have roots in the European and Asian mouse fancy communities of the 18th and 19th century, wherein animals with unusual and/or striking variation in their pelage were prized for their beauty and singularity[4]. Fancy mice provided some of the initial tools used to create inbred strains, whose development in the early part of this century laid the foundation for much of mammalian genetics[5]. Though the classical genetics of mouse coat color has a long history, it is only in the last several years that a significant number of coat color genes have been isolated and their protein products characterized.

Comprehensive reviews of human and mouse pigmentation genetics have been published recently by Spritz and Hearing[6], and Jackson[7], respectively. This review describes several recent advances that illustrate how mouse coat color genetics is a useful starting point to study variation in human pigmentation phenotypes (Fig. 1). Each of these advances is based on the study of previously existing mutations in mice and, in some cases, humans. However, modern techniques of transgenesis and gene targeting now allow questions raised by the study of cells and molecules to be examined in the context of an organismal phenotype, and ways in which mouse genetics can be used to study human pigmentation are also suggested. Finally, lest we become too anthropocentric, it is important to remember that mice do not exist solely for the purpose of providing experimental models, and I hope to show that mouse coat color is not only a model for human skin, eye and hair color, but also can provide insight into fundamental aspects of cell and developmental biology relevant to many different organisms.

A hormone receptor for red hair and fair skin

Hormonal control of pigmentation is apparent in a variety of situations, two diverse examples being the seasonal changes of fur color observed in certain arctic mammals, and the skin hyperpigmentation that serves as a cardinal sign of primary adrenal gland insufficiency in humans. The latter example is caused by increased pituitary production of adrenocorticotrophic hormone (ACTH), one of three melanocortin peptides, the others being α- and γ-melanocyte-stimulating hormone (α- and γ-MSH), derived from a single polyprotein precursor, pro-opiomelanocortin (POMC; reviewed in Ref. 8). The potent effects of these molecules on pigmentation are mediated by the melanocortin 1 receptor (MC1R), a seven transmembrane G-protein-coupled receptor expressed at high levels in melanocytes, whose activation produces elevated levels of intracellular cAMP (Ref. 9).

As reported recently by Valverde *et al.*[10], molecular variants of the human *MC1R* gene are more likely to be found among individuals with red hair, fair skin and poor tanning ability than in those of us with a lesser need for a high SPF sunblock. Among 135 Irish or British Caucasians with differing shades of hair color, whose *MC1R* gene was analyzed by PCR-based sequencing, nine different alleles predicted to give rise to altered proteins were found in 20 of 38 red-haired individuals, but only in 11 of 76 brown- or black-haired individuals. The significance of this observation is clearer when broken down according to genotype: the 'relative risk' for red hair is 15-fold among individuals that carry one variant allele, but rises to 170-fold among individuals that carry two variant alleles (homozygotes or compound heterozygotes).

Interest in the MC1R as a possible determinant of human pigmentation was presaged by the discovery of a loss-of-function mutation in the mouse *Mc1r* gene as the cause of *recessive yellow* (Ref. 11). Formerly designated as *e*, *recessive yellow* is one of several alleles at the *extension* locus, in which dominant mutations extend the amount of black pigment and diminish the amount of yellow pigment (as in *sombre*, see below), while recessive alleles diminish the amount of black pigment and extend the amount of yellow pigment (as in *e*). At first glance, these observations provide a good example of what mouse genetics can provide for human

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TABLE 1. Functions and mutant phenotypes of selected pigmentation genes[a]

Mutation[b]	Gene product[b]	Phenotype in mice and humans
albino (*c*)	Tyrosinase; catalyzes oxidation of dopaquinone; required for tyrosine to eumelanin and pheomelanin synthesis	Complete absence of all pigmentation (snow-white hair and red eyes); abnormal connections of visual and auditory neurons; oculocutaneous albinism type 1 (OCA1) in humans
Agouti (*A*)	Secreted protein; antagonist of MC1R	In mice, loss of function produces black hair while gain of function produces yellow hair; no effect on retinal pigmentation; defects not yet described in humans
brown	Tyrosinase-related protein 1 (*Tyrp1*); catalyzes oxidation of dihydroxyindole-carboxylic acid (DHICA), a distal step in eumelanin synthesis	Loss of function produces brown, instead of black, hair in mice; rare form of mild albinism ('brown oculocutaneous albinism' or OCA3) in humans
lethal spotting	Endothelin 3 (*Edn3*); 21 amino acid peptide produced from larger precursor	Piebald spotting, deafness and intestinal aganglionosis (leading to death) in mice; no effect on retinal pigmentation; defects not yet described in humans
piebald spotting	Endothelin receptor type B (*Ednrb*); seven-transmembrane-domain receptor coupled to G_i and/or G_q	In mice, identical to the phenotype produced by *lethal spotting*; human mutations produce a similar phenotype including intestinal aganglionosis (Hirschsprung disease), but with variable expressivity
pink-eyed dilution (*p*)	Integral membrane protein of eumelanosomes	Loss of function produces pink eyes and grey/yellow hair in mice; OCA2 in humans (blond to white hair, pale skin, visual abnormalities)
recessive yellow	Melanocortin 1 receptor (*Mc1r*); seven-transmembrane-domain receptor coupled to G_s	In mice, loss of function produces yellow hair while gain of function produces black hair; no effect on retinal pigmentation; red hair and fair skin in humans
slaty	Tyrosinase-related protein 2 (*Tyrp2*); catalyzes production of DHICA from dopachrome, proximal step to TYRP1 in synthesis of eumelanin	Partial loss of function produces slight dilution of black pigment in mice; defects not yet described in humans
Steel	Mast-cell growth factor (*Mgf*); paracrine growth factor with soluble and membrane-bound isoforms; ligand for Kit	White hair with black eyes in mice (accompanied by sterility and multiple hematopoietic deficiencies); defects not yet described in humans
White spotting	Receptor tyrosine kinase encoded by proto-oncogene *Kit*; used by developing germ cells,hematopoietic cells and melanoblasts	Similar to *Steel* in mice, but partial loss of function produces piebald spotting whereas hypomorphic *Mgf* alleles produce overall dilution of pigment (i.e. steel colored); in humans produces the piebald trait

[a]See text and reviews in Refs 6 and 7 for additional details.
[b]Current gene symbols (in parantheses) can refer either to the mutation or to the gene product as indicated by their placement under these respective headings. The albino mutation is designated *c* for historical reasons.

genetics and vice versa: the functional significance of the human variants rests largely on furry shoulders, and it will no doubt be easier to find additional *MC1R* mutations among red-haired humans than yellow mice. On closer inspection, however, the analogy between these two situations has some problems. What does red hair in humans have to do with yellow hair in mice? Why are *MC1R* alleles apparently semidominant in humans, yet recessive in mice? And, finally, what causes red hair in humans with a normal MC1R?

The answer to the first question is found in the annals of pigment biochemistry and cell biology: there are two basic types of tyrosine-derived melanin in mammals, phaeomelanin (red/yellow) and eumelanin (brown/black), that differ in their biochemical composition and ultrastructural appearance in intracellular organelles (reviewed in Ref. 12). Pheomelanosomes are spherical, lack internal structure and contain a relatively soluble cysteine-rich material; eumelanosomes are elliptical and contain a highly organized matrix with an insoluble cysteine-poor material (Fig. 2). In humans, the highest amounts of pheomelanin are found in 'fire red' hair, while black, grey and blond hair is mostly eumelanin[13–15]. By contrast, fire red hair is not found in laboratory mice, and while the color of animals homozygous for *recessive yellow* is superficially similar to blond hair in humans, hair pigment in these animals is composed almost entirely of pheomelanin[16,17].

Given the biochemical and ultrastructural similarities between human red hair and mouse yellow hair, some or all of the human *MC1R* variants are likely to impair receptor signaling. Eight of the nine variants lie within the N-terminal portion of the protein. However, our current knowledge of the three dimensional structure of melanocortin receptors is limited and functional studies will be required to determine whether the altered MC1R proteins affect membrane insertion, ligand binding, or interaction with intracellular effectors.

Similar to other G-protein-coupled receptors with peptide ligands, allelic variation of the *MC1R* can produce increased as well as decreased signaling. Indeed, Robbins *et al.*[11] found that alterations of the mouse *Mc1r* that produced hyperactive or constitutively active receptors were responsible for graded degrees of hyperpigmentation in mice that carried the *sombre*, *sombre 3-J* and *tobacco* mutations. Whether analogous alterations in the human *MC1R* might contribute to racial differences in skin color might be difficult or impossible to answer by association studies. The 'British and Irish Caucasian' sample studied by Valverde *et al.*[10] is likely to represent a population with relatively little admixture, but comparisons across racial groups would need to take into account founder effects and/or genetic drift, which could produce associations with no functional significance.

Is heterozygosity for an *MC1R* variant sufficient to produce a phenotype in humans but not in mice? Valverde *et al.*[10] are appropriately cautious in stating that red-haired individuals apparently homozygous or heterozygous for the 'normal' *MC1R* allele (7 or 11 of 38, respectively) might actually have alterations in receptor expression that were undetected by DNA-based mutational analysis. However, a logical corollary of this explanation – that red hair should segregate as a simple mendelian recessive trait – has not been supported by population genetic studies of the past. Instead, a more likely alternative, also acknowledged by the authors, is the potential for interaction with other loci such that *MC1R* expressivity and dominance (or lack thereof) would depend on genetic background. The genetics of mouse coat color would be the logical system in which to test this hypothesis but, to date, *E/e* animals seem to be indistinguishable from *E/E* animals regardless of genetic background.

FIGURE 1. Phenotypes of homologous pigmentation genes in humans and in mice. Defects of the *Kit* gene produce the phenotype piebald spotting (originally named *White spotting*) in mice (a) and dominantly inherited piebald trait in humans (b). The animal shown in (a) is heterozygous for the Kit^{3J} allele. (c) Defects of the *Mc1r* gene produces *recessive yellow* in mice and are likely to cause red hair in humans (d). See text and Ref. 10 for further discussion.

More than one way to make red hair?

By contrast, the study of mouse coat color genetics allows some clear predictions about causes of red hair other than *MC1R* allelic variation. The key player here is the Agouti protein, a novel paracrine signaling molecule that inhibits the effects of melanocortin signaling. Transient expression of *Agouti* during the midphase of hair growth is responsible for the subterminal band of pheomelanic pigment present in individual hairs of the animals, *Agouti paca* and *Agouti taczanowskii*, for which the gene is named (reviewed in Ref. 18).

The effects of many mouse *Agouti* alleles on coat color are identical to those produced by mutations of *Extension* (i.e. *Mc1r*), in a manner reminiscent of the *Steel* (*Mgf*)–*White spotting* (*Kit*) and the *piebald spotting* (*Ednrb*)–*lethal spotting* (*Edn3*) relationships (see below). However, in the case of *Mgf* and *Kit*, or of *Ednrb* and *Edn3*, a loss-of-function in the ligand mimics a loss-of-function in the receptor. By contrast, recessive *Mc1r* alleles mimic the pigmentary effect of dominant *Agouti* alleles. This observation and others[18] provided the genetic groundwork for the work of Lu *et al.*[19], who demonstrated recently that recombinant Agouti protein inhibits the ability of melanocortins to bind to and activate the MC1R expressed in heterologous cells. Synthetic receptor antagonists are widely used in pharmacologic research and as therapeutic agents, but the Agouti protein is the only known natural antagonist of a G-protein-coupled receptor. This unique situation – modulation of MC1R signaling by altering the levels of antagonist against a constant background level of melanocortin ligand – avoids the necessity for mechanisms that adjust α-MSH levels independently of other POMC-derived products, such as ACTH and β-endorphin. It also allows local control, because α-MSH circulates actively through the blood (it can also be produced by keratinocytes[20]), but Agouti protein has a very small sphere of action[21]. Given this background, a logical hypothesis is that red hair in humans might be caused not only by loss-of-function *MC1R* alleles, but also by gain-of-function alleles in the human homolog of *Agouti*, *ASIP* (Refs 22, 23). Unlike receptors, however, gain-of-function mutations in ligands are nearly always regulatory rather than structural, and identification of putative variants in *ASIP* might require expression and/or linkage studies.

The search for causes of human red hair other than variants in the *MC1R* and possibly *ASIP* is not solely the domain of the geneticist, and could be guided by a biochemical approach towards proteins that act specifically on the MC1R signaling pathway. As alluded to above, abnormalities that affect availability of α-MSH or related

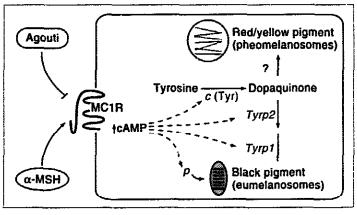

FIGURE 2. Regulatory and biochemical control of melanogenesis. Agouti and α-MSH are extracellular proteins that regulate MC1R signaling at the cell membrane to depress and elevate, respectively, intracellular levels of cyclic AMP (cAMP) as described in the text. Tyrosine is oxidized by the enzyme tyrosinase (Tyr), encoded by the *c* locus, to produce dopaquinone, a common precursor of pheomelanin and eumelanin. Intermediate enzymatic steps from dopaquinone to pheomelanin have not been identified, but the tyrosinase-related enzymes encoded by the *Tyrp2* and *Tyrp1* genes catalyze oxidative steps in the pathway from dopaquinone to eumelanin[12]. All three enzymes and their products are localized within melanosomes because the oxidative intermediates of melanin synthesis are cytotoxic. The product of the *pink-eyed dilution* gene (*p*) is an integral membrane protein specific to eumelanosomes and appears to help stabilize tyrosinase and the two tyrosinase-related enzymes as part of a multiprotein complex[28]. Increased levels of intracellular cAMP stimulate expression of the *c*, *Tyrp1*, *Tyrp2* and *p* genes (indicated by the dashed arrows), which provides a mechanism whereby MC1R signaling, regulated positively or negatively at the extracellular level by α-MSH or Agouti protein, respectively, determines whether dopaquinone is diverted into the eumelanin or the pheomelanin pathway.

ligands are likely to have pleiotropic effects, but some intracellular pathways for MC1R signaling might well have components that are tissue specific. For example, a receptor kinase or arrestin, or even a G protein subunit expressed only in melanocytes, might prove to be a likely candidate for explaining red hair in individuals with a normal *MC1R* gene. Conversely, the MC1R is not the only seven-transmembrane-domain receptor expressed in melanocytes, and intracellular abnormalities in G-protein signaling might lead to a combination of piebaldism and red hair (see below).

Shedding light on fair skin

How does MC1R signaling get translated into instructions to make red hair or black hair? Studies of cultured melanoma cells show a relationship between the concentration of extracellular α-MSH and the accumulation of intracellular cAMP, in which the levels of hormone and second messenger can both vary over a wide range (reviewed in Ref. 24). However, the abrupt change from black to yellow pigment that occurs in agouti hairs points to qualitative, as well as quantitative, changes in gene expression at the end of the pigmentation circuit. Here, the predictions made by the genetics of mouse coat color identify some of these downstream genes and also provide insight into why fair skin is associated with red hair.

The *Tyrp1* and *p* genes encode a melanosomal enzyme[25] and an integral membrane protein[26], respectively, whose absence is responsible for the *brown* (*Tyrp1*^{b}) and *pink-eyed dilution* (*p*) mutations (reviewed in Ref. 7). However, the effects of *Tyrp1*^{b} or of *p* become apparent only on a eumelanotic background; mice that carry the *brown* and *recessive yellow* mutations (i.e. *Tyrp1*^{b}/*Tyrp1*^{b} *Mc1r*^{e}/*Mc1r*^{e}), for example, have pheomelanic coats that are nearly indistinguishable from those that carry only *recessive yellow* (*Tyrp1*$^{+}$/*Tyrp1*$^{+}$ *Mc1r*^{e}/*Mc1r*^{e}). Recent RNA and protein expression studies from Kobayashi *et al.*[27] and Lamoreux *et al.*[28] show high-level expression of *P*, *Tyrp1* and *Tyrp2* as long as hair follicles synthesize eumelanin, but during the nadir of MC1R signaling that accompanies pheomelanogenesis, expression ceases completely. Thus, these genes seem to function as downstream components of pigment-type switching in an on/off mode (Fig. 3).

The same cannot be said, however, for tyrosinase, a melanosomal enzyme encoded by the *c* gene which catalyzes the formation of dopaquinone from tyrosine, and in which loss-of-function mutations produce type I oculocutaneous albinism (OCA1) in humans and the *albino* mutation in mice[6,7]. Unlike *Tyrp1* and *P*, tyrosinase is rate-limiting for synthesis of pheomelanin as well as eumelanin, but lower rates of dopaquinone formation are required for pheomelanin than for eumelanin synthesis (discussed in Refs 29, 30). This point is dramatically illustrated by the coat color phenotype of the *chinchilla* (*c*^{ch}) allele, in which an alteration in the tyrosinase protein causes reduced enzymatic activity. In *c*^{ch}/*c*^{ch} mice, high levels of MC1R signaling cause depostion of eumelanin granules that are gray instead of black, but low levels of MC1R signaling (which would normally induce pheomelanogenesis) produce a level of tyrosinase activity too low to support any pigment synthesis, altering the agouti phenotype from black–yellow–black to grey–white–grey (Fig. 3).

Viewed from this perspective, fair skin in humans could be a consequence not only of an increased pheomelanin:eumelanin ratio, but also a reduced level of tyrosinase and, therefore, would be influenced not only by factors that impinge on MC1R signaling, but also by genetic variation at the human homolog of *c*, the *TYR* gene. Many *TYR* alleles that severely impair protein function have been identified in individuals with OCA1 (Ref. 1). Unlike the *MC1R*, however, variation in *TYR* coding sequence is rare, and putative *TYR* polymorphisms that influence skin color might lie at a considerable distance from transcribed regions[31].

Fair skin in humans, of course, is also found in the absence of red hair where, presumably, the level of MC1R signaling is relatively robust. In this case a logical place to search for an underlying cause begins with the human homolog of the *P* gene, where loss-of-function alleles produce OCA2, but many structural variants have been identified in the absence of an obvious phenotype[32,33]. In contrast to *TYR* mutations, where complete loss-of-function always causes snow-white hair, the phenotype of OCA2 varies according to racial background, and most affected individuals have some minimal pigmentation of their hair and skin (reviewed in Ref. 1). Partial loss-of-function mutations of *TYR* also produce minimal pigmentation, but, intriguingly, these patients can tan while those with severe OCA2 cannot. This observation and others[34] suggest that UV-irradiation stimulates

eumelanogenesis specifically, which is intact albeit curtailed in mild OCA1, but abrogated in OCA2.

From pathways to networks: modifiers of piebaldism in mice and in humans

Pigment cells hold interest for the developmental as well as the cell biologist: programmed alterations in gene expression act together with environmental cues to bring about the changes in cell fate, migration and differentiation that allow the journey from neural crest to melanocyte (Fig. 4, reviewed in Refs 35, 36). Shortly after the closure of the neural tube, melanoblasts are recognized as a subset of nascent neural crest cells that express *Tyrp2* (Ref. 37; Fig. 4; Table 1). Transition from melanoblast to melanocyte is defined operationally by the expression of tyrosinase and the appearance of pigment granules, and coincides with increasing dendritic differentiation at the dermal–epidermal junction, or the base of developing hair follicles for intrafollicular or epidermal melanocytes, respectively[38,39].

Pigmentary manifestations of mutations that compromise neural crest development are usually manifest as the localized absence of melanocytes at birth, otherwise known as piebaldism (and distinguished from vitiligo: the loss of melanocytes from localized areas after birth). For example, defects of the mouse *Pax3* and *mi* genes give rise to *Splotch* and *White* (*Mi^{wh}*) mice, respectively, while defects of their human homologs *PAX3* and *MITF* give rise to Waardenburg syndrome I and II, respectively (Fig. 4; reviewed in Refs 40, 41). The recent molecular identification of four additional genes, *Steel, White spotting, piebald spotting* and *lethal spotting*, has drawn attention to the interplay between tyrosine kinase and G-protein-coupled signaling systems. Several years ago, defects in a receptor tyrosine kinase encoded by the *Kit* gene were recognized as the cause of *White spotting* (now renamed *Kit^{W}*), and defects in its ligand mast cell growth factor were recognized as the cause of *Steel* (now renamed *Mgf^{Sl}*, reviewed in Ref. 7). More recently, defects in the G-protein-coupled type B endothelin receptor were recognized as the cause of *piebald spotting* (now renamed *Ednrb^{s}*) and defects in its ligand endothelin 3 were recognized as the cause of *lethal spotting* (now renamed *Edn3^{ls}*)[42,43].

While *Mgf–Kit* or *Edn3–Ednrb* ligand–receptor defects produce different sorts of pleiotropic abnormalities (Table 1), their effects on pigmentation are quite similar and, consequently, double mutant studies cannot be used to order the *Mgf–Kit* and *Edn3–Ednrb* signaling systems in a genetic pathway. However, *Edn3* and *Ednrb* abnormalities affect multiple neural crest derivatives while *Mgf* and *Kit* abnormalities do not, suggesting that endothelin signaling acts before Kit signaling during pigment cell development. This hypothesis is supported by recent studies from Pavan and Tilghman[44], and Wehrle-Haller and Weston[45] of melanoblast development in *Ednrb* or *Mgf* mutant backgrounds, respectively. Midgestation embryos homozygous for a severe *Ednrb* allele, *piebald-lethal*, exhibited a complete absence of neural-crest-derived cells that express *Tyrp2*. By contrast, in embryos that lack *Kit* or *Mgf* function, *Tyrp2*-positive cells were found next to the neural tube, the so-called migration staging area, but failed to reach lateral mesenchyme.

These considerations suggest a directional view of pigment cell development wherein *Ednrb* signaling precedes *Kit* signaling, which precedes *Mc1r* signaling in a serial pathway (Fig. 4). However, recent work by Pavan *et al.*[46] suggests a somewhat different view. More than 60 years ago, work by Dunn and Charles indicated that the severity of white spotting in piebald (i.e. *Ednrb*-defective) mice was very sensitive to genetic background effects[47]. The application of quantitative trait loci (QTL) analysis[48] to this problem has identified several

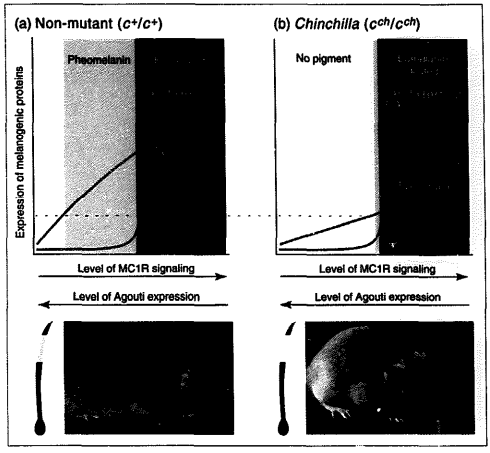

FIGURE 3. Interaction of genes that control melanogenesis. (a) In animals with a non-mutant *c* allele (*c*$^{+}$), low to intermediate levels of MC1R signaling cause low to intermediate expression of tyrosinase but expression of the P, TYRP1 and TYRP2 proteins is not detectable, and consequently pheomelanin is synthesized. Above a certain threshold level of MC1R signaling, expression of P, TYRP1 and TYRP2 begins, and eumelanin is synthesized instead of pheomelanin[27]. There is a lower limit of tyrosinase expression, indicated by the dashed line, below which no pigment is synthesized. This limit, and the phenotypic interaction between MC1R signaling and tyrosinase expression, is most readily detected in mice when the maximal level of tyrosinase expression is reduced by structural alteration of the protein as in the *chinchilla* (*c^{ch}*) allele as shown in (b). In humans, low levels of MC1R signaling caused by structural alterations in the MC1R protein might also result in fair skin due to depression of tyrosinase activity.

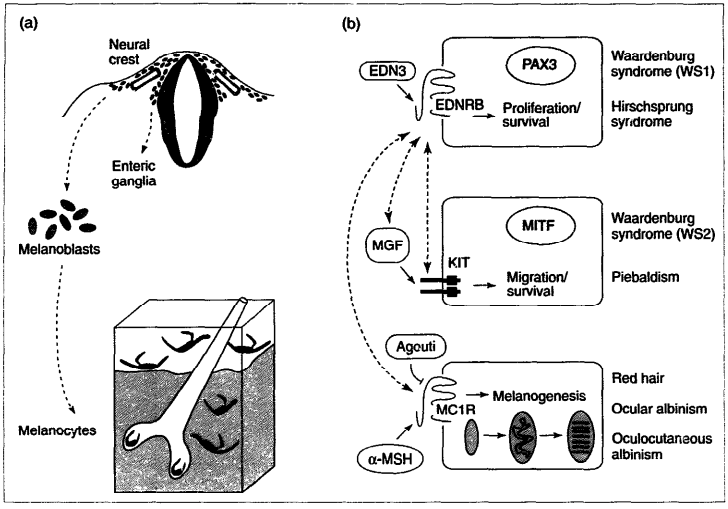

FIGURE 4. Gene action and interaction during pigment cell development. (a) Shows pigment cell development during embryogenesis. A subpopulation of neural crest cells overlying the somite expresses the *Kit* and *Tyrp2* genes; these cells subsequently enter the dorsolateral migration pathway and eventually infiltrate developing skin and hair[38,39]. (b) Shows how defects in the *Edn3* or *Ednrb* genes act relatively early to affect the proliferation and/or survival of neural crest cells destined for the pigment and the enteric ganglia lineage. Defects in the *Mgf* or *Kit* genes act somewhat later to affect melanoblast migration and/or survival (see text), and defects in the *Agouti* or *Mc1r* genes principally affect melanogenic control as shown in Figure 2. Also shown are two genes, *Pax3* and *Mitf*, which encode transcription factors required for melanoblast development and whose absence is responsible for Waardenburg syndrome types I and II, respectively (reviewed in Refs 6, 7, 40, 41). The double-headed gray arrows illustrate potential interactions between *Ednrb* and the *Mgf*, *Kit* or *Mc1r* genes as discussed in the text[46].

loci that modify piebaldism caused by *s*, an old *Ednrb* allele derived from mouse fanciers. Remarkably, candidates for three of these modifier loci suggest that pigment cell development can be viewed as a network, in which the effect of *Ednrb* signaling is just one of several steps whose outcomes intersect to control the likelihood of postnatal melanocyte survival.

Similar to many other attributes of interest to mammalian geneticists, piebaldism is variably expressed, even in a so-called congenic line in which different animals are genetically identical or nearly so (discussed in Ref. 49). Among the C3H *s/s* mouse strain, the level of melanocyte deficiency, quantitated by measuring the proportion of the body surface area covered by spots, is normally distributed about a mean of 14.96 ± 3.25%. By contrast, a strain of *s/s* mice bred selectively for extensive spotting by Thomas Mayer[50] exhibits melanocyte deficiency from 53.5 ± 6.24% of their body surface. To identify genes responsible for the difference between the C3H and Mayer strains, Pavan *et al.*[46] genotyped backcross progeny between the two strains for 70 microsatellite markers spaced at approximately 30 cM intervals throughout the genome. An F_1 cross between Mayer and C3H strains produced spotting similar to the C3H parent (17.30 ± 5.85%), and a cross of the F_1 animals to the parental Mayer strain then produced a distribution of spotting that overlapped each of the parental strains (32.69 ± 11.88%).

Pavan *et al.*[46] followed an intuitive principle of QTL mapping: backcross progeny that lie at the 'dark' end of the spotting spectrum carry an excess of C3H-derived alleles that decrease spotting, while backcross progeny that lie at the 'light' end of the spotting spectrum carry an excess of Mayer-derived alleles that increase spotting. This approach and the subsequent testing of additional markers and animals led to the identification of six modifier loci, four of which, on chromosomes 2, 5, 8 and 10 could account for most of the difference between the C3H and Mayer strains. It is satisfying and intriguing that excellent candidates for the loci on chromosomes 5 and 10 are none other than the *Kit* and *Mgf* genes. Satisfying, because *White spotting* (Kit^W) and *piebald spotting* ($Ednrb^s$) were previously known to have synergistic effects on pigmentation, and intriguing, because intracellular proteins have been identified recently that link signals from G-protein-coupled receptors (i.e. *Ednrb*) to those from receptor tyrosine kinases (i.e. *Kit*)[51,52]. Finally, an interesting candidate for the

locus on chromosome 8 is none other than the aforementioned *Mc1r*, which suggests that melanocortins and the agouti protein could affect melanoblasts as well as melanocytes[3].

Identification and analysis of the piebald genes and their modifiers in mice has important implications for human pigmentation. Mutations of the *KIT* gene are a well recognized cause of isolated human piebaldism[2] and, recently, a mutation of the *EDNRB* gene has been identified in a Mennonite kindred as a cause of intestinal aganglionosis (Hirschsprung disease), piebaldism, heterochromia irides and deafness[53]. While a deficiency of neural crest cells is the underlying defect, the phenotype is variably expressed and the presence of pigmentary abnormalities in this disorder, and others, might well depend on variation at the *KIT*, *MGF* or *MC1R* loci.

Conclusions and future directions

The advances reviewed above can be ascribed directly to the increasing pace of molecular biology over the last decade, but would not have been possible without the foresight of mouse and human geneticists who identified and characterized heritable abnormalities in pigmentation over the last century. There is, of course, much work left to do: commitment of a neural crest cell to the pigment cell lineage is poorly understood, and the subcellular pathways of melanosome biogenesis are largely uncharacterized. The resource of previously existing mouse coat color mutations is far from exhausted, however, and promises to provide insight into these problems in the near future. Finally, common variations in human pigmentation phenotypes are beginning to yield some secrets, and it should not be too long before we know whether the light cast by the lamp-post of previously existing mutations in mice is bright enough to understand human eye, hair and skin color.

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