

Melanocyte biology and skin pigmentation

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Melanocytes are phenotypically prominent but histologically inconspicuous skin cells. They are responsible for the pigmentation of skin and hair, and thereby contribute to the appearance of skin and provide protection from damage by ultraviolet radiation. Pigmentation mutants in various species are highly informative about basic genetic and developmental pathways, and provide important clues to the processes of photoprotection, cancer predisposition and even human evolution. Skin is the most common site of cancer in humans. Continued understanding of melanocyte contributions to skin biology will hopefully provide new opportunities for the prevention and treatment of skin diseases.

Melanocytes can absorb ultraviolet radiation (UVR) and survive considerable genotoxic stress. The skin is the main barrier to the external environment, and relies on melanocytes to provide, among other things, photoprotection and thermoregulation by producing melanin. The degree of pigment production manifests as skin 'phototype' (skin colour and ease of tanning)¹ and is the most useful predictor of human skin cancer risk in the general population.

The colours we see in feathers, fur and skin are largely determined by melanocytes. In addition to carotenoids and haemoglobin, melanin is the main contributor to pigmentation. There are two main types of melanin — red/yellow pheomelanin and brown/black eumelanin. Melanin-containing granules are known as melanosomes and are exported from melanocytes to adjacent keratinocytes, where most pigment is found. As a result, pigmentation differences can arise from variation in the number, size, composition and distribution of melanosomes, whereas melanocyte numbers typically remain relatively constant (Fig. 1a, b).

Mutations affecting pigmentation have been identified in many species because they are easily recognizable. Such mutants can be categorized into four groups: hypopigmentation and hyperpigmentation, with or without altered melanocyte number. These phenotypic distinctions have afforded the opportunity to easily classify genes affecting the melanocyte lineage, with respect to viability or differentiation (or both). Some of these mutants function in non-cell-autonomous manner, thereby further revealing cell-cell communication pathways of physiological importance. Collectively, pigmentation or coat colour mutants have become an invaluable resource for the analysis of melanocyte differentiation and as a model for the broader fields of neural-crest development and mammalian genetics.

There are two discrete melanocytic populations in hair follicles: melanocyte stem cells and their differentiated progeny, which reside in geographically distinct locations to comprise a follicular unit that is tightly linked to the surrounding keratinocyte population. Hair follicle melanocyte stem cells have important roles in both normal hair pigmentation and senile hair greying, and specific genetic defects have shed further light on the survival properties of this cell population.

This review summarizes how pigmentation is regulated at the molecular level and how the tanning response provides protection against damage and skin cancer. We discuss recent advances in our knowledge of the genes involved in these processes and how they affect skin and hair colour. We also cover the developmental origin of melanocytes and how they are maintained by melanoblast stem cells, whose eventual depletion may contribute to hair greying. Finally, we detail some questions that research into melanocyte biology hopes to address in the future.

Regulation of pigmentation

Melanocortin-1 receptor

The contribution of melanocytes to pigmentation is conserved through many species. In certain species such as fish, pigment is provided by other cell types, known as xanthophores and iridiphores² (Fig. 1c). Despite the identification of more than 100 loci involved in vertebrate pigmentation, the melanocortin-1 receptor (MC1R) is consistently a representative locus and major determinant of pigment phenotype³. The extension locus (*Mcl1^{pl/c}*) was first identified in mice on the basis of altered coat colour^{4,5}. The recessive mutants have yellow or pheomelanotic hair, whereas wild-type mice have black/brown eumelanotic hair. This mutation has been conserved in furred animals from mammoths to present-day cats and dogs⁶ (Fig. 1d). Other melanocortin family member receptors are found on various cell lineages — for example, *MC4R* is expressed in the hypothalamus, where it modulates energy metabolism⁷.

MC1R encodes a seven-transmembrane, G-protein-coupled receptor. Agonist-bound MC1R activates adenylyl cyclase, inducing cyclic AMP production⁸, which leads to phosphorylation of cAMP responsive-element-binding protein (CREB) transcription factor family members. CREB, in turn, transcriptionally activates various genes, including that encoding microphthalmia transcription factor (*MITF*), the transcription factor that is pivotal to the expression of numerous pigment enzymes and differentiation factors⁹ (Fig. 2). Agonists of human MC1R include α -melanocyte-stimulating hormone (α -MSH) and adrenocorticotrophic hormone (ACTH), and these cause an increase in eumelanin production through elevated cAMP levels^{10,11}. The agouti (*Asip*) gene encodes an antagonist of MC1R¹², which is responsible for the pheomelanotic banding pattern of wild-type mouse fur. An inactivating mutation (non-agouti) at this locus is responsible for the black fur of the C57BL6 mouse strain. Recently, evidence has been reported for an association between a single nucleotide polymorphism in the 3' untranslated region of the human agouti protein and dark hair and brown eyes¹³.

The role of MC1R in hair pigmentation is striking. The human MC1R coding region is highly polymorphic with at least 30 allelic variants, most of which result in a single amino-acid substitution¹⁴. Certain substitutions, such as R151C, R160W and D294H, are associated with red hair. The 'red-head' phenotype is defined not only by hair colour but also by fair skin, inability to tan and a propensity to freckle. Functional studies suggest that these variants encode hypomorphic mutants that are unable to either bind ligand or activate adenylyl cyclase^{15,16}. Thus, it may be possible to have an additive effect among two variant alleles¹⁷. Two point mutations in the second transmembrane domain have yielded constitutively active

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receptors with a resulting dominant dark coat in mice⁵, although such gain-of-function mutations have not yet been reported in humans¹⁸.

Tracing *MC1R* loci through different skin types and geographic regions has led to different theories on the evolution of human pigmentation. Epidemiological studies suggest that pigmentation is under functional constraint in Africa and that this constraint has been lost in the populations that left the African continent. It is not clear whether the drive for selection was necessitated by UVR-induced vitamin D production over protection from the DNA damage caused by UVR or as a result of an undiscovered critical pathway^{14,18}. *MC1R* might be evolutionarily significant for other biological reasons, such as increased κ -opioid analgesia, which was recently linked to variant *MC1R* alleles in both mice and humans¹⁹. Despite the apparently strong influence of *MC1R* on both hair and skin pigmentation, it is clear that other factors are also involved in the control of skin pigmentation, because there are many fair-skinned but dark-haired individuals in whom *MC1R* alone is unlikely to limit skin pigmentation.

Recently *SLC24A5*, which encodes a putative cation exchanger, was identified as the human homologue of a zebrafish gene that causes the 'golden' phenotype²⁰ (Fig. 1). Although its function is unclear, it is plausible that cation chemistry might modulate melanosomal maturation processes. Humans have two primary *SLC24A5* alleles, which differ by a single amino-acid substitution. In almost all Africans and Asians the substitution is alanine, but in 98% of Europeans the allele encodes threonine. The function of *SLC24A5* in human pigmentation remains to be determined, but the correlation of its variants to human populations is striking and suggests that it is important in the control of cutaneous pigmentation.

Melanosomes and melanogenesis

Melanin production occurs predominantly in a lysosome-like structure known as the melanosome. Pheomelanin and eumelanin differ not only in colour but also in the size, shape and packaging of their granules²¹.

Both melanins derive from a common tyrosinase-dependent pathway with the same precursor, tyrosine. The obligatory step is hydroxylation of tyrosine to dopaquinone, from which L-DOPA can also be derived²². The absence or severe dysfunction of tyrosinase and other key pigment enzymes (including *P* gene, the human homologue of the mouse pink-eyed dilution locus, tyrosinase-related protein 1, TRP1, and membrane-associated transporter protein, MATP) results in oculocutaneous albinism (OCA1–4), which presents with intact melanocytes but inability to make pigment (see ref. 23 for a review).

From dopaquinone, the eumelanin and pheomelanin pathways diverge. Two enzymes crucial to eumelanogenesis are the tyrosinase-related proteins TRP1 (also known as GP75 or b-locus) and TRP2 (also known as dopachrome tautomerase, DCT). TRP1 and 2 share 40–45% identity with tyrosinase and are useful markers of differentiation. Pheomelanin is derived from conjugation by thiol-containing cysteine or glutathione. As a result, pheomelanin is more photolabile and can produce, among its by-products, hydrogen peroxide, superoxide and hydroxyl radicals, all known triggers of oxidative stress, which can cause further DNA damage. Individual melanocytes typically synthesize both eumelanins and pheomelanins, with the ratio of the two being determined by a balance of variables, including pigment enzyme expression and the availability of tyrosine and sulphhydryl-containing reducing agents in the cell²².

Melanin is packaged and delivered to keratinocytes by melanosomes. The formation, maturation and trafficking of melanosomes is crucial to pigmentation, and defects in this process lead to depigmented and dilutionary disorders such as Hermansky–Pudlak Syndrome (HPS) and Chediak–Higashi Syndrome (CHS)²⁴. On the keratinocyte side, the protease-activated receptor-2 (PAR2), a seven-transmembrane receptor on keratinocytes, has a central role in melanosome transfer²⁵. Once in keratinocytes, melanosomes are distributed and, in response to UVR, positioned strategically over the 'sun-exposed' side of nuclei to form cap-like structures resembling umbrellas.

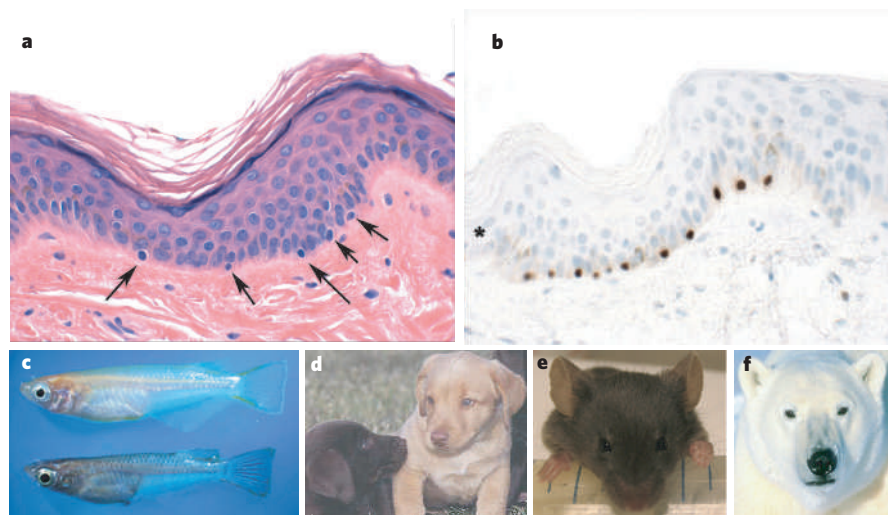
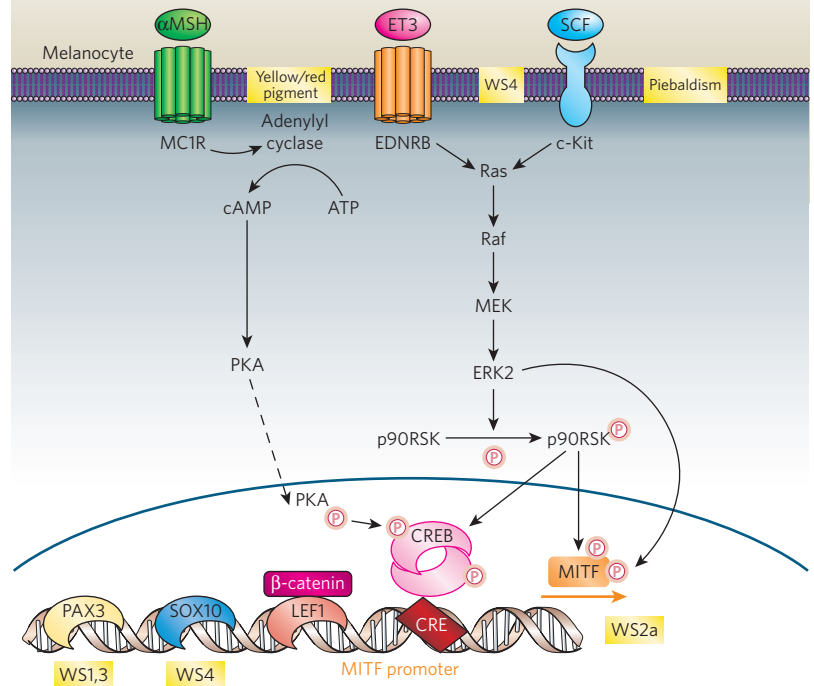


Figure 1 | Vertebrate pigmentation. **a**, Haematoxylin and eosin stain of normal human skin. Cells of the upper layer of the epidermis (keratinocytes) contain large nuclei, which stain blue, and the dermis appears pink as a result of staining of its abundant protein, collagen (muscle and nerve fibres may also stain pink). Normal melanocytes (arrows) have smaller nuclei and inconspicuous cytoplasm compared with the surrounding keratinocytes. Melanocytes are typically located in the basal layer of the epidermis, at the junction with the dermis. Differences in human pigmentation reflect variations in the number of melanosomes in keratinocytes and different melanin granule phenotype (depending on the eumelanin/pheomelanin ratio) rather than variation in the number of melanocytes. (Image courtesy of S. R. Granter, Brigham and Women's Hospital, Massachusetts.) **b**, Immunohistochemical analysis of the same human tissue as shown in **a** identifies melanocytes by using the immunohistochemical marker D5, which stains the MITF transcription factor located in their nuclei (Image courtesy of S. R. Granter.)

c, Medaka goldfish are valuable in pigmentation studies. The wild type, *B/B*, is the lower of the two. Compared with this, *b/b* (top), which is bred for its golden colour, lacks melanin except in the eyes. The *b* locus is highly homologous with the locus for oculocutaneous albinism 4 (OCA-4) in humans, MATP or AIM1. MATP seems to be involved in melanocyte differentiation and melanosome formation. (Image reprinted, with permission, from ref. 82.) **d**, The yellow pigmentation in golden labradors is recessively inherited and results from an amino-acid substitution in *Mc1r* that produces a premature stop codon⁶. The same pigmentation can be seen in mice (*Mc1r*^{pl/c}), horses and cats with hypomorphic *Mc1r* variants. (Image courtesy of Terra Nova.) **e**, Furred animals such as mice lack epidermal melanocytes (except in non-hair-bearing sites such as the ear, nose and paws). **f**, Polar bears have hollow unpigmented fur to blend in with the environment but, unlike other furred animals, have a high density of epidermal melanocytes, which aid in heat retention and produce black skin most notable in non-furred areas. (Image courtesy of First People.)

Figure 2 | The MITF promoter. The MITF promoter is regulated in part by the transcription factors PAX3, SOX10, LEF-1/TCF and CREB during melanocyte development. In humans, mutations affecting the MITF pathway lead to pigmentary and auditory defects that are known collectively as Waardenburg syndrome (the phenotypes of which are highlighted in yellow boxes). The core features are represented by Waardenburg syndrome 2a (WS2a), which results from *MITF* mutations. People with WS1 have craniofacial deformities and those with WS3 can have limb deformities. These are due to different mutations in *PAX3*, a neural-crest-associated transcription factor important for several lineages. WS4 has the additional feature of Hirschsprung's syndrome (megacolon) owing to deficient enteric innervation. Mutations in *SOX10*, endothelin 3 (*ET3*) and its receptor, *EDNRB*, have all been implicated in WS4 and are related to MITF by the pathways illustrated. LEF/TCF is downstream of the WNT/ β -catenin pathway, a key regulator of melanocyte development. MITF transcription is modified by at least two other receptor signalling pathways, those of MC1R and c-Kit, a receptor tyrosine kinase. Hypomorphic/variant alleles of *MC1R* lead to increased pheomelanin/eumelanin ratios, causing blonde/red hair and fair skin. SCF/c-Kit signalling modifies MITF post-translationally through phosphorylation of Ser 73 by the mitogen-activated protein kinase (Ras/Raf/MEK/ERK) pathway, and Ser 409 through the 90 kDa ribosomal S6 protein kinase (p90RSK), thereby altering MITF stability and p300 recruitment (not shown). c-Kit mutations are found in human piebaldism, which presents with extensive skin melanocyte loss and has also been associated with sensorineural deafness, suggesting that piebaldism and WS are in the same spectrum of disorders. PKA, protein kinase A.



Melanocytes and physiological responses

Ultraviolet-induced pigmentation

The most common example of acquired pigmentation is tanning. To the naked eye, the effects of UVR are marked by 'sunburn' and/or 'sun-tan'. Sun sensitivity is the degree of cutaneous inflammation (erythema) and pigmentation that results from exposure to UVR, which can manifest clinically in several ways. Even at low doses of UVR, DNA damage can occur before there is any evidence of change on the skin²⁶.

The tanning response is one of the most striking examples of environmental adaptation in humans. It is well known that α -MSH increases skin darkening in humans, a phenomenon traditionally observed in patients with adrenal insufficiency, whose pituitary glands produce compensatory excesses. Pro-opiomelanocortin (POMC) is the precursor for both α -MSH and ACTH, as well as for other bioactive peptides, including β -endorphin. Although originally identified in the pituitary, POMC production is now known to occur in skin and hair follicles as well^{27,28}. After its production from POMC, α -MSH is secreted by both keratinocytes and melanocytes²⁹. In humans, mutations in *POMC* result in a red-haired phenotype (like that of *MC1R* alleles), as well as metabolic abnormalities such as adrenal insufficiency and obesity³⁰.

There is evidence that DNA damage in itself might be important to the triggering of pigment production. UVR — typically UVB (wavelengths 290–320 nm) — induces thymidine breaks in DNA and the most common signature is cyclobutane dimer formation. Treatment with T4 endonuclease V, an enzyme known to mediate the first and rate-limiting step in repairing thymidine dimers, enhances the tanning response in UV-irradiated melanocytes *in vitro*³¹. Topical application of small DNA fragments such as thymine dinucleotides (pTpT) have been pioneered by Gilchrist and colleagues, and have been shown to induce tyrosinase upregulation and increased pigmentation³¹. The molecular mechanism might be linked to p53, p21 or PCNA (proliferating cell nuclear antigen), levels of which are increased after exposure to pTpT in a time frame similar to that seen after UV irradiation³².

p38 stress-activated kinase has been suggested to participate in UVR-related pigmentation by phosphorylating upstream transcription factor (USF-1), a basic helix–loop–helix leucine zipper transcription factor

that can bind to the tyrosine promoter. In melanocytes derived from *Usf-1*^{-/-} mice, defective UV activation of the POMC and *MC1R* promoters was observed³³. α -MSH has also been shown to activate p38 mitogen-activated protein kinase *in vitro*³⁴. Other pathways implicated include those involving endothelin-1, β -fibroblast growth factor (β -FGF), nitric oxide, p-locus and stem-cell factor (SCF)³⁵. Separate physiological processes — such as X-ray irradiation and DNA-damaging chemotherapeutic agents — can also stimulate a tanning response, which potentially involves pathways that overlap with the UV response³⁶.

Inability to tan highlights several genetic features that might be instructive with regard to the UV-pigmentation response. Variants of *MC1R*, which produce a weakly functioning receptor either through decreased ligand binding or decreased activation of adenylyl cyclase, exhibit relative inability to tan (for example, red-heads cannot tan). Such individuals tend to freckle but do not produce an even, protective pigment. They are also prone to sunburn, although the triggers for this process remain unclear. 'Sunburn cells' — keratinocytes in which apoptosis is triggered by UVR — have been shown by Brash and colleagues to require p53 for their formation³⁷, thus identifying a key role for p53 in modulating a physiological response to a common environmental exposure (UVR), and implicating p53 as a 'guardian of the tissue'³⁸.

Further insight into the UV-tanning response was recently obtained through use of the K14-SCF transgenic mouse¹⁰. By using this transgenic background, fair-skinned mice (*Mcl1*^{pl/e}) containing epidermal melanocytes were obtained, and found to be acutely UVR-sensitive. UVR was observed to trigger a more than 30-fold increase in the induction of POMC/ α -MSH in keratinocytes of both mice and humans, suggesting that keratinocytes might have an important role in 'perceiving' UVR and then synthesizing and secreting α -MSH. Mutant *MC1R* was, not surprisingly, seen to ablate any detectable tanning response. However, a rescue strategy of topically delivered forskolin was used to bypass the *Mcl1* mutation and directly activate adenylyl cyclase, and was observed to induce profound skin darkening in genetically fair-skinned mice¹⁰. The induced pigmentation pattern exhibited normal histological features, such as nuclear 'capping' in keratinocytes, and produced significant protection against sunburn cell formation, pyrimidine dimer formation and

skin cancers after UVR exposure¹⁰. These results suggest that the dark pigmentation machinery remains available if appropriately stimulated, and ongoing studies are examining whether this is the case in humans.

Response to UVR and the risk for skin cancer

How does pigmentation protect skin? Although this is presumed to involve straightforward shielding by melanin, our understanding of the process is far from complete. It is clear that skin pigmentation as well as the capacity to tan portend diminished skin cancer risk¹. When the protective effect of melanin has been calculated using minimal erythemal dose measurements, protection for even the darkest-skinned of individuals is no more than 10–15-fold that seen in the absence of melanin (presumably signifying a relatively weak sun-protection factor, SPF (Box 1)). But the factor of protection in terms of skin cancer risk is 500–1,000 (refs 39, 40), indicating that highly pigmented skin is profoundly protected from carcinogenesis. This discrepancy, although subject to numerous caveats, including quantification estimates and endpoint surrogates, suggests that pigment's protective mechanisms might vary for different endpoints such as sunburn and skin cancer.

One possible explanation for the high cancer protection afforded by dark pigmentation might involve mechanism(s) of increased risk associated with blonde/red pigments. *MC1R* variants have been shown to confer an increased risk of melanoma and non-melanoma skin cancers, independently of skin pigment (including red-haired phenotype)^{41,42}. Pheomelanin might function as a photosensitizer capable of generating reactive oxygen species upon UV irradiation⁴³ and has been associated with higher rates of TUNEL (TdT-mediated dUTP nick end labelling)-positive (apoptotic) cells after UV irradiation⁴⁴. Thus, increased pheomelanin production might be a risk factor for melanoma, although the precise underlying mechanism for this process remains to be fully

elucidated to ensure that it is not merely a marker of melanoma risk but is directly involved in carcinogenesis. It should also be noted that whether and how sunscreens protect against cancer is a complex and controversial issue (Box 1).

Melanocyte development

The migratory pathway of the melanoblast

Melanocyte development from its precursor, neural-crest cells, highlights the unique properties of this cell type. Neural-crest cells are pluripotent cells that arise from the dorsalmost point of the neural tube between the surface ectoderm and the neural plate. In addition to melanocytes, neural-crest cells give rise to neurons and glial cells, adrenal medulla, cardiac cells and craniofacial tissue (see ref. 45 for a review). Melanoblasts, the precursors of melanocytes, migrate, proliferate and differentiate en route to their eventual destinations in the basal epidermis and hair follicles, although precise distribution of melanocytes varies among species (Fig. 1e, f).

Melanocyte development has been well characterized in several species, including the mouse embryo. Melanoblast visualization in mice has been achieved by using a *lacZ* transgene in the *Trp2* promoter developed by Jackson and colleagues⁴⁶ (Fig. 3). In mice, melanoblasts differentiate from pluripotent neural-crest cells at about embryonic day (E)8.5, migrating along the dorsolateral pathway and eventually diving ventrally through the dermis. Defects in melanocyte migration typically appear most prominently on the ventral surface as 'white spotting', as this is at the greatest distance (watershed zone) from the dorsum. By E14.5 in mice, melanocytes exit from the overlying dermis and populate the epidermis and developing hair follicles. Melanocytes also reach the choroid of the back of the eye, the iris, the leptomeninges and the stria vascularis of the cochlea (inner ear).

Box 1 | Questions about the benefits of sunscreen

Many people use sunscreens to protect themselves and their children against the most lethal effects of UVR. Unsettlingly, data so far have failed to show that the use of sunscreen protects against melanoma, the deadliest form of skin cancer (see page 851). This and associated controversies have produced responses ranging from deep epidemiological curiosity to a recently filed class-action lawsuit against the sunscreen industry⁸³.

Sunscreens are defined according to 'sun protection factor' (SPF), which is measured by calculating the minimal dose of UVR necessary to cause confluent redness at 24 h after exposure on protected skin compared with unprotected skin. At present, the SPF measurement is based mainly on protection against UVB radiation (wavelengths 290–320 nm), although newer sunscreens may also shield UVA radiation (wavelengths 320–400 nm). UVB can cause DNA damage, and there is growing evidence that UVA might also have carcinogenic effects⁸⁴. Another question raised is whether sunscreen inhibits vitamin D production⁸⁵. However, there is little evidence to suggest that sunscreen prevents adequate vitamin D production⁸⁶, or that low vitamin D levels are associated with increased melanoma risk⁸⁷.

For keratinocyte-derived skin cancers such as squamous-cell carcinoma, the association between UVR and carcinogenesis has been clearly established^{37,88}. Correspondingly, sunscreens, when applied correctly, are effective at reducing the incidence of squamous-cell

carcinoma and its precursor lesion, actinic keratosis⁸⁹. Rates of basal-cell carcinoma, the most common skin cancer, are not as directly affected by sunscreen use⁹⁰. Both types of tumour can be removed surgically, and, although they rarely metastasize, can cause significant morbidity and occasional mortality.

There is even less compelling evidence for sunscreen protection in melanoma. A randomized controlled trial demonstrated decreased naevi (moles) numbers in children using sunscreen⁹¹. The number of naevi present during childhood is a predictor for melanoma⁹², although only a small minority of melanomas have been seen to arise within naevi^{93,94}. At best, naevi counts serve as an indirect measurement. However, the results of several large studies have suggested that sunscreen use is associated with an increased risk of melanoma^{95,96}. Caveats to this conclusion include potential selection bias (higher-risk patients who burn easily are more likely to use sunscreen), questions of proper sunscreen use, and not accounting for changes that have occurred in sunscreen formulation since the studies were performed, including higher UVB and broader UVA protection. This controversy has prompted large meta-analyses^{97,98}, which have not suggested increased melanoma risk with sunscreen use but have also demonstrated no protective effect of sunscreen against melanoma.

Is it mechanistically plausible that sunscreen might not protect against melanoma? At a simplistic level, if the ability to tan easily

correlates with diminished melanoma risk, then sunblocks might convert easy-tanning individuals from low-risk (darkly tanned) to higher-risk (we do not know whether it is the ability to tan or actually being tanned that confers true protection). A remarkable feature of melanoma risk is the identification of *MC1R* as a melanoma risk factor gene, independently of UVR exposure^{41,99}. Blonde/red pheomelanin pigments have been suggested to enhance intrinsic DNA damage in cells, particularly in response to UVR^{42,100}. Although this observation requires more systematic epidemiological study, it is possible that pheomelanin is weakly carcinogenic and actually contributes to melanoma formation.

We cannot assume that sunscreens protect against melanoma analogously to their protection against sunburn (which is demonstrated by 'missed spots' during application). It remains possible that the kinetics of sunburn, squamous cell carcinoma, and melanoma formation are so different that sunscreen protection from melanoma requires longer follow-up than studies so far have reported. Alternative dosing/applications of sunscreens might offer different protection against these endpoints. Importantly, the data so far do not suggest that sunscreens be abandoned. On the contrary, protection from sunburn and squamous cell carcinoma are important. But protection against melanoma is more complex and an alternative prevention strategy for now is to stay out of the sun.

Various signalling pathways and transcription factors tightly regulate melanocyte migration. These proteins and pathways provide and integrate spatial and temporal signals to create the proper environment for normal development and migration. Mutations in genes affecting this process produce hypopigmentation that arises from lack of melanocytes rather than lack of pigment in viable melanocytes, as occurs in albinism. Key genes in this developmental pathway include *PAX3* (paired-box 3), *SOX10* (sex-determining region Y (SRY)-box 10), *MITF*, endothelin 3 and endothelin receptor B (*EDNRB*). Disruption of these genes has led to clearer understanding of certain human inherited pigmentation disorders, specifically Waardenburg Syndrome (WS), which is characterized by hearing and pigmentary defects. The highly related Tietz syndrome has a similar phenotype but is associated with dominant-negative mutations in *MITF*. Given the defined molecular and phenotypic relationships among the genes responsible for these syndromes, they represent an epistatic tree of interacting melanocytic regulatory factors⁴⁷ (Fig. 2).

Microphthalmia transcription factor

The core phenotype of Waardenburg Syndrome is seen in WS type 2 and is characterized by congenital white forelock, asymmetric iris colour (heterochromia irides) and sensorineural deafness, all resulting from disrupted melanocyte migration. A subset of WS type 2 patients (WS2a) have germline heterozygous mutations in *MITF*. *Mitf* was originally described as a mouse coat colour mutant more than 60 years ago. Homozygous mutant mice have small eyes, white fur and deafness. This is representative of the affected cell lineages, which include retinal pigment epithelial (RPE) cells (*Mitf* mutations result in microphthalmia), osteoclasts (osteopetrosis is seen with certain mutant alleles in homozygotes) and mast cells (for which mutation causes severe dysfunction)⁴⁸. Numerous *Mitf* mutations have been identified in mice as well as other species, and have provided considerable insight into this gene locus and its central role in melanocyte development⁴⁸.

MITF is a member of the Myc-related family of basic helix-loop-helix leucine zipper (bHLH-Zip) transcription factors and is conserved in essentially all vertebrate species⁴⁸. Like other bHLH-Zip factors, it binds to the canonical (CA[T/C]GTG) E box sequence⁴⁸. The MiT subfamily of bHLH-Zip factors includes *MITF*, *TFEB*, *TFE3* and *TFEC*⁴⁸. These MiT factors homodimerize and heterodimerize in all combinations, although restricted tissue expression limits the availability of different partner combinations in specific contexts. In melanocytes, *MITF* alone seems to be the critical family member, whereas *MITF* and *TFE3* heterodimerize and exhibit functional redundancy in osteoclasts⁴⁹. Three MiT factors, including *MITF*, have also been identified as human oncogenes and found to be involved in multiple malignancies, including melanoma⁵⁰ (see page 851).

The WNT/ β -catenin-signalling pathway is also essential for neural crest induction and melanocyte development. Mice lacking wild-type *Wnt1* and *Wnt3a* have pigmentation defects⁵¹. WNT1 and WNT3A trigger a canonical pathway resulting in β -catenin-induced transcription at TCF/LEF (T-cell factor/lymphoid enhancer factor) promoter/enhancer elements (Fig. 2). Numerous targets of TCF/LEF have been identified, including *Myc* and cyclin D1 in non-melanocytes, and *MITF*, *TRP2* and *SOX10* in melanocytes and melanoma cells (see ref. 52 for a review). Overexpression of β -catenin in zebrafish promotes melanoblast formation and reduces the formation of neurons and glia⁵³.

Melanocyte homing to the epidermis and hair follicles

c-Kit is a tyrosine kinase receptor involved in melanoblast expansion, survival and migration⁵⁴. Activation of *c-Kit* by Kit-ligand (KitL, also known as steel factor or SCF) leads to Ras activation and multiple canonical signalling as well as post-translational modification of *MITF*⁵⁵ (Fig. 2). *c-Kit*, *SCF* and *SNAI2* (also known as *SLUG*) mutations have all been identified in human piebaldism, an autosomal dominant ventral depigmentary disorder^{56,57}. Piebaldism is characterized by white forelock but, unlike WS, is not accompanied by deafness and more commonly results in white, depigmented (melanocyte-free) areas of

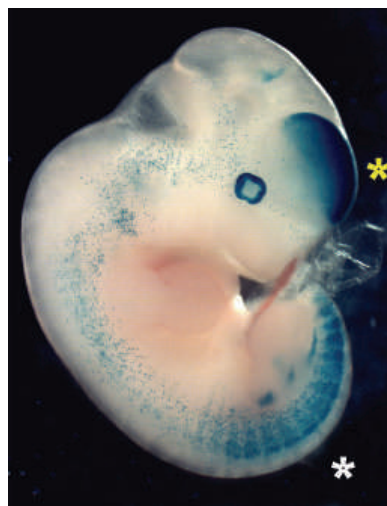


Figure 3 | Labeled melanoblasts in *DCT-lacZ* mouse embryos. The *DCT-lacZ* transgenic mouse labels melanoblasts and melanocytes (blue). On E11.5, labelled melanoblasts can be seen at high density flanking the neural tube (white asterisk) and in the cervical region (yellow asterisk). After dorsolateral migration, the melanoblasts begin to migrate ventrally. (Image reprinted, with permission, from ref. 46.)

skin rather than hair involvement. However, there have been reports of a *c-Kit* mutation carrier with sensorineural deafness and no cutaneous pigmentary changes, suggesting a potential overlap syndrome between piebaldism and WS⁵⁸.

The availability of SCF has a crucial role in permitting melanoblast survival and promoting proliferation, both initially in the dorsolateral migration pathway and later from the dermal mesenchyme to colonize the hair follicles and epidermis^{46,59,60}. Expression of transgenic *Scf* under a keratin promoter (K14), is sufficient to support melanocyte homing to the epidermis in furry animals such as mice, which otherwise have few epidermal melanocytes in fur-bearing regions⁴⁶ (Fig. 1e, f). The use of imatinib mesylate (also known as STI 571 or Gleevec), a potent BCR-ABL tyrosine kinase inhibitor that also inhibits *c-Kit* tyrosine kinase, can result in loss of pigment (loss of melanocytes) in the skin⁶¹.

SLUG encodes a zinc finger transcription factor associated with piebaldism. It was first noted in mice that *Slug* mutations caused a phenotype very similar to piebaldism with anaemia, infertility and white forelock, and depigmentation of the ventral trunk, tail and feet⁶². Humans with piebaldism lacking *c-Kit* mutations were found to have heterozygous deletions encompassing the *SLUG* coding region⁶³. In addition, *SLUG* mutation was also reported in WS2, and a mechanism proposed to account for these effects involves the binding of *MITF* to the *SLUG* promoter⁶³. *SLUG* seems to be required for melanoblast migration and/or survival⁶². In a mouse model of melanoma, the inhibition of *SLUG* by small interfering RNA (siRNA) suppressed metastatic propensity, potentially linking *SLUG* to both migration and metastasis-related behaviours⁶⁴.

In addition to *c-Kit*/SCF, other mechanisms are likely to be involved in the late steps of melanocyte migration from the dermis into the epidermis. These include endothelins 1 and 3, hepatocyte growth factor (HGF) and basic FGF⁶⁵. Evidence for HGF's influence on melanocyte homing has been obtained by Merlino and colleagues, who discovered extensive melanosis at diverse sites in transgenic mice expressing HGF driven by the metallothionein promoter⁶⁶. This model has been particularly interesting because of its high-penetrance melanoma formation after single neonatal doses of UVR⁶⁷. Cadherins are also implicated: as dermal melanoblasts move through the basement membrane they express E-cadherin, which is then downregulated and replaced by P-cadherin during migration into the hair follicles⁶⁸. In mice, a screen of dominant phenotypes by Barsh and colleagues identified two new classes of mutant with dark skin (*Dsk*)⁶⁹. The first group contained activating mutations in G proteins, *Gnal1* and *Gnaq*, producing excess melanoblasts in the dermis even though the correct number reached the epidermis and hair follicles⁷⁰. The *Dsk* mutations were able to rescue pigmentary defects from loss of *PAX3* and *c-Kit*, but did not do so in *Ednrb*^{-/-} mice⁷¹, suggesting a cell-autonomous amplification of normal endothelin signalling through which mutant G α subunits cause an excess number of early melanoblasts.

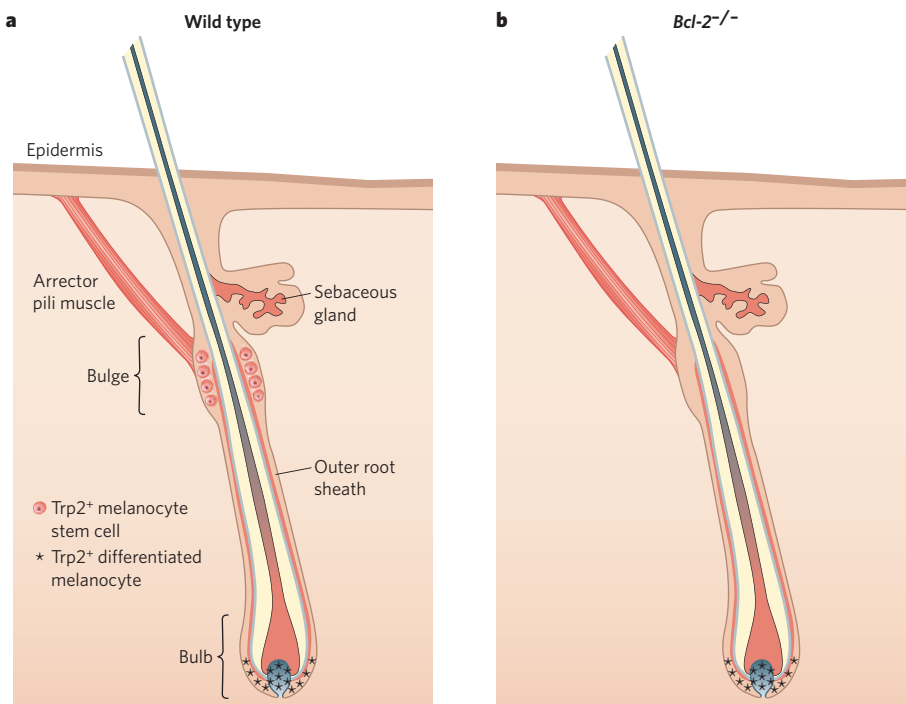


Figure 4 | *Dct-lacZ* melanoblasts in mouse hair follicles. Using *Dct(Trp2)-lacZ* transgenic mice, melanocyte stem cells can be revealed in the bulge region of a hair follicle, geographically distinct from the differentiated melanocytes in the hair bulb, as demonstrated in this diagram. As part of the hair follicle cycle, differentiated melanocytes migrate from the bulge to the bulb, where they export pigment to hair-producing keratinocytes. In *Bcl-2*^{-/-} mice, normal melanocyte stem-cell and differentiated melanocyte populations are seen at birth. At postnatal day 8.5, the bulge melanocytes abruptly disappear, whereas in controls (*Bcl-2*^{+/+}) and bulb melanocytes of the same *Bcl-2*^{-/-} follicles these cells survive until the next catagen/regressive follicle stage. Subsequent hair greying in *Bcl-2*^{-/-} mice is thought to arise from lack of regenerative melanocyte stem cells.

Melanoblast survival

MITF expression is activated early on during the transition from pluripotent neural-crest cells to melanoblasts and is required for the survival of migratin g melanoblasts⁴⁸. Complete deficiency of MITF results in absence of melanocytes, suggesting that MITF is essential for lineage survival, for proliferation or to prevent transdifferentiation towards other neural-crest lineages (such as glia and neurons). Evidence that MITF remains vital for melanocyte survival throughout the life of an organism comes from the hypomorphic mutant allele *Mitf*^{nit}, which exhibits near-normal melanocyte development, but accelerated age-dependent greying of coat colour due to postnatal melanocyte loss⁷².

Bcl-2 (B-cell leukaemia/lymphoma 2), a transcriptional target of MITF⁷³ and known inhibitor of apoptosis, is also required for melanocyte viability⁷⁴. Cyclin-dependent kinase 2 (Cdk2), a cell-cycle regulator, is another MITF target⁷⁵. Melanoma cells have been shown to require Cdk2 expression to maintain their cell cycle and viability⁷⁵. Nevertheless, MITF's interactions with *Bcl-2* and Cdk2 do not fully explain melanocytes' requirement for MITF to remain viable during development, because the *Mitf*-null homozygous phenotype is more severe than either of the single knockouts, and expression of either gene in *Mitf*-knocked-down melanoma cells only partly rescues viability^{75,76}.

The cytokine receptor c-Met has recently been found to be another direct transcriptional target of MITF⁷⁶. Many additional targets of MITF have been identified, including the CDK inhibitors p16^{INK4a} and p21/Cip1 (see ref. 9 for a review). Together, they probably contribute to MITF's dual activities in relation to melanocytic differentiation and survival/proliferation, and some may serve as surrogate drug targets for the MITF oncogenic pathway. The function of MITF-mediated survival should be distinguished from differentiation markers, as MITF is believed to coordinately regulate the expression of pigment genes, although probably in conjunction with other context-dependent factors. In addition to tyrosinase, TRP1 and TRP2, pigment targets of MITF in melanocytes include silver (*PMEL17*, also known as gp100, which encodes the melanoma-diagnostic epitope HMB45), melan-A (also known as MART1), melastatin (TRPM1) and AIM1 (oculocutaneous albinism 4)⁹.

Melanocyte stem cells and grey hair

Melanocytes of the hair follicle are responsible for hair pigment. The life-cycle of the hair follicle melanocyte is closely linked to that of the rest of the hair follicle, which is typically in growth (anagen), but moves through

a brief period of regression (catagen) and finally enters a resting phase (telogen) in which the hair is released and the cycle can begin anew (see page 834). Multipotent epidermal stem cells exist in the bulge region (at the bottom of the permanent portion of the follicle, just below the sebaceous gland)⁷⁷ (Fig. 4). This is also where melanocyte stem cells reside. These are responsible for restoring the pool of differentiated melanocytes to the hair bulb, where the pigment is incorporated while new hair is being synthesized⁴⁶ (Fig. 3). The key features satisfying the definition of 'stemness' include slow cycling, self-maintenance, immaturity and competence to regenerate progeny when appropriately stimulated⁷⁸. Independent validation that the melanocyte stem-cell niche resides in hair follicles comes from patients with vitiligo (absence of epidermal melanocytes) resulting from immunosuppressive treatment, in whom repigmentation is clearly initiated in radial fashion from hair follicles.

The *Dct-lacZ* transgenic mouse⁴⁶ has helped researchers to identify the melanoblast population in the bulge region (Fig. 3). The power of this system was illustrated by the analysis of several mouse models of hair greying⁷⁸. *Bcl-2*^{-/-} mice are born with normal pigment but turn grey at the second hair follicle cycle⁷⁴. At birth, *Bcl-2*^{-/-} mice exhibit normal melanocyte morphology and differentiation, although it has been suggested that melanocyte stem-cell formation could be deficient, at least in certain follicles⁷⁹. Between postnatal days 6 and 8 (ref. 80) there is an abrupt loss of all bulge melanocytes (Fig. 4), whereas bulb melanocytes remain until the end of the first follicle cycle⁷⁸. This striking phenotype has been interpreted to suggest a selective requirement for *Bcl-2* in the maintenance of melanocyte stem cells at a crucial stage in the anagen phase of the follicle cycle⁷⁸.

More gradual greying has been observed in the hypomorphic mouse mutant *Mitf*^{nit} (ref. 78). In this case, bulge melanocytes were lost more gradually, although still in a markedly accelerated manner compared with the wild type. Loss of the stem-cell population was preceded by the appearance of unexpected pigment-producing cells in the bulge region (stem-cell niche). Because differentiation/pigmentation is generally incompatible with stemness it has been proposed that this 'inappropriate' bulge pigmentation might signify aberrant exit from the melanocyte stem-cell pool. Eventual depletion of stem cells in this way would produce grey/white hairs in the subsequent follicle cycle.

Age-related greying in both mouse and human follicles is also accompanied by bulge melanocyte attrition and is preceded by 'inappropriate pigmentation' in that population of (niche) melanocytes⁷⁸. But it remains

to be seen whether other causes of human greying exist, such as in cases that are followed by unexpected darkening (as occasionally reported in patients using imatinib⁸¹), which presumably do not involve depletion of melanocyte stem cells. Such instances might be more amenable to 'rescue' strategies for greyness.

Future prospects

Melanocyte biology remains at the crossroads of the laboratory and the clinic. Numerous pigmentation mutants are phenotypically profound but remain mechanistically uncharacterized. Although pigment mutants are informative with regard to basic genetic and developmental mechanisms, they also provide important clues to processes of photo-protection, cancer predisposition and even human evolution. The elucidation of genetic regulators of pigmentation will probably increase even more markedly with the advent of targeted knockouts that spare embryonic lethality for vital genes.

Certain lineage-specific features of melanocytes have attracted well-deserved attention and leave important questions to be answered. The dispensability of melanocytes to hair follicle viability offers the opportunity to identify and discover genes of particular importance to the melanocyte lineage. The physical separation of melanocyte stem cells from their differentiated progeny also offers distinct experimental advantages. Key questions that remain to be answered include what the signal(s) are that modulate melanocyte stem cells' awakening from and return to quiescence. It will be important to determine the signals modulating stem-cell attrition and 'inappropriate pigmentation' of melanocytes in the bulge that precede stem-cell loss and hair greying. To what degree is the melanocyte stem cell dependent on the specialized niche, and to what degree might it be harnessed for other uses, such as repigmentation of scars or artificial skin? What are the signals modulating melanocyte death during catagen? These questions also stand to provide clues relevant to the biology of epidermal melanocytes, whose intercellular communication is also of enormous importance but only partly understood.

Dysregulation of melanocytic growth or survival is an enormously common occurrence. Almost all people harbour multiple moles, which are benign, senescent neoplasias that only rarely (if ever) transform to invasive melanomas (see page 851). Are BRAF^{V600E}-transformed melanocytes the most common instance of such clonal transformation, or do similar benign neoplasms occur in most tissues, but remain unapparent owing to absence of pigment? Why do melanocytic neoplasms so uncommonly harbour UVR-signature mutations, especially when melanoma incidence is related to the combination of skin phototype and UVR exposure? Could it be that keratinocytes are the primary UV-respondering population, and melanoma formation is largely a consequence of chronic reactive secondary stimulation/proliferation (akin to other chronic-inflammation-associated cancers)?

Skin is the most common site of cancer in man, yet its malignancies are likely to be among the most preventable. A considerable fraction are caused by the known environmental carcinogen, UVR. Behaviours including specific sun-seeking activities abound in many of today's societies. Sun exposure in other cases is casual and unintentional, whereas in the case of sunscreen may be associated with misconceptions involving the types of protection provided. It is likely that opportunities to improve our understanding of melanocytic contributions to cancer will arise increasingly. An ability to exploit melanin and its photoprotective effects might ameliorate cancer risk in vulnerable populations. The availability of ever-improving animal models, high-quality human diagnostic reagents, rich data resources from the post-genomic era and increasing opportunities for targeted therapeutics will hopefully permit new means of safely modulating the melanocyte and its activities for purposes ranging from the development of cosmetics to the prevention of cancer. ■

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