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What are melanocytes *really* doing all day long…?

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Abstract: Everyone knows and seems to agree that melanocytes are there to generate melanin – an intriguing, but underestimated multipurpose molecule that is capable of doing far more than providing pigment and UV protection to skin (1). What about the cell that generates melanin, then? Is this dendritic, neural crest-derived cell still serving useful (or even important) functions when no-one looks at the pigmentation of our skin and its appendages and when there is essentially no UV exposure? In other words, what do epidermal and hair follicle melanocytes do in their spare time – at night, under your bedcover? How much of the full portfolio of physiological melanocyte functions in mammalian skin has really been elucidated already? Does the presence or absence of melanoctyes matter for normal epidermal and/or hair follicle functions (beyond pigmentation and UV protection), and for skin immune responses? Do melanocytes even deserve as much credit for UV protection as conventional wisdom attributes to them? In which interactions do these promiscuous cells engage with their immediate epithelial environment and who is controlling whom? What lessons might be distilled from looking at lower vertebrate melanophores and at extracutaneous melanocytes in the endeavour to reveal the 'secret identity' of melanocytes? The current *Controversies* feature explores these far too infrequently posed, biologically and clinically important questions. Complementing a companion viewpoint essay on malignant melanocytes (2), this critical re-examination of melanocyte biology provides a cornucopia of old, but under-appreciated concepts and novel ideas on the slowly emerging complexity of physiological melanocyte functions, and delineates important, thought-provoking questions that remain to be definitively answered by future research.

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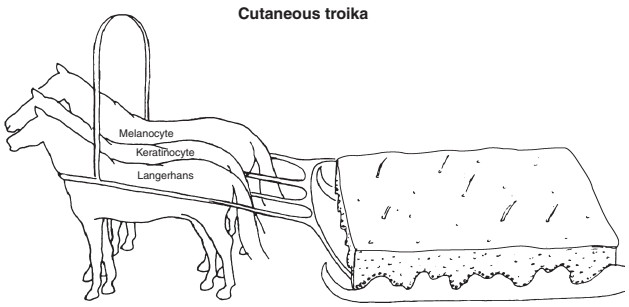
Praeludium pigmentosum

For those uninformed, the skin is an inert plastic wrap nature provides to keep us in and everything else out. How mistaken they are! The skin, in particular the epidermis, is one of the most active of all tissues/organs.

Nature wisely placed the capillary circulation in the dermis. The epidermis has no vascular circulation thereby minimizing the probability that toxic chemicals, bacteria or fungi that penetrate through the stratum corneum can diffuse into the blood stream. That does not leave the epidermis defenseless. The epidermis has proteins called defensins that have anti-microbial properties. There are Toll-like receptors that recognize invading organisms and incite a host response. Even more interesting, it is well known that keratinocytes are avidly phagocytic. They have the capacity to phagocytize the wandering, invasive fungi or bacteria and digest them. It is both interesting and important that α-MSH stimulates the ingestion of candida by keratinocytes. α-MSH has a wide array of activities, only one of which is to stimulate the synthesis of melanin. There are receptors for α-MSH on Langerhans cells and keratinocytes as well as melanocytes. It has the ability to suppress inflammation and alter keratinocyte proliferation. That seems like an odd property for a molecule that enhances pigmentation until it is recalled that melanocytes are also phagocytic cells and are part of the inflammatory response. Responding virtually to all inflammatory events in the epidermis, the pigment system participates usually by producing more melanin (postinflammatory hyperpigmentation) or occasionally by suppressing melanization (i.e. postinflammatory hypopigmentation such as pityriasis alba). One of the most common and desirable forms of postinflammatory hyperpigmentation is tanning following exposure to sunlight. It is mediated in part by α-MSH. Sunlight damages the epidermis and sunburn is the inflammatory response to radiation injury. Production of melanin stimulated by α-MSH is accompanied by all sorts of useful results such as darker skin that is more resistant to subsequent sunburn. α-MSH also stimulates repair of damaged DNA. It also downregulates the immune response of the epidermis, maybe preventing more cases of autoimmune disorders such as lupus erythematosus.

That keratinocytes and melanocytes are involved in host defenses and inflammation seems incongruous. After all why should they usurp the function of Langerhans cells?



But then it is clear that Langerhans cells also affect melanization as well as keratinization. Langerhans cells make a variety of cytokines that affect keratinocytes and melanocytes just as melanocytes make α-MSH and other cytokines such as the interleukins that affect Langerhans cells and keratinocytes. In a recent review, the numerous ways that these cells interact and the response of all of them to common cytokines has been reviewed (3).

Histological examination of the epidermis shows basal keratinocytes as proliferating cells. Melanocytes are not only dendritic but also confined to the basilar layer. Langerhans cells are mid-epidermal. Most of the epidermal cells are keratinocytes, about 90% or more. How can these cells seemingly interact? It is a common misperception that dendrites of melanocytes and Langerhans cells are fixed in a given position. The opposite is true. Dendrites are extended, contracted and moved between different pairs of keratinocytes continuously. Each melanocyte using just three or four dendrites must copulate with at least 30 basilar keratinocytes and probably others in the suprabasilar layers. And it must be in contact with distantly located Langerhans cells. Of course Langerhans cells must provide protection for even larger numbers of keratinocytes and melanocytes lying beneath them. They have just a few dendrites, but must form a network to trap any invading organisms or microorganisms coming from above. They move their dendrites to form a moving network.

The melanocytes inject melanosomes into the cytoplasm of keratinocytes. Coitus with all of the surrounding cells requires melanocytes to move their dendrites around in search of unprotected keratinocytes lacking melanin. Although both Langerhans cells and melanocytes are dendritic cells, they must hold hands so to speak by touching each others dendrites to communicate. In the absence of a vascular circulation, melanocytes and Langerhans cells have dendrites that move constantly to service each other and keratinocytes. They form one type of circulation within the epidermis. It is the promiscuity of melanocytes and Langerhans cells to form a ménage a' trios or as shown below a troika with keratinocytes that makes the epidermis the unique, highly active tissue that alerts the entire body to the environment in which we live.

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Viewpoint #1

As long as we keratinocytes can remember, melanocytes have lived inconspicuously among us at the basal membrane and have been primarily known as meek and obedient pigment producers in the epidermis. However, recent investigations are suggesting that these fellows have several identities, work undercover in many other places in the human body and have functions we can only speculate about (as summarized in Table 1). Let us critically re-consider, then, the well-known properties and the more obscure abilities of our ancient, pigment-producing friends.

The keratinocytes' lament

First of all, how does one recognize a melanocyte if it is encountered somewhere outside the epidermis? They are usually identified by their expression of melanocyte-specific proteins, e.g. tyrosinase (TYR), TYRP1, DCT, Pmel17/gp100, MART-1 and/or MITF (1). However, melanocyte precursors (known as melanoblasts) are more difficult to identify as they do not produce melanin and therefore do not usually express those markers, although occasionally DCT and/or KIT are detectable as specific markers. Therefore, whatever functions melanoblasts may have in skin, these are as easily missed as these cells are difficult to detect.

The favourite habitat of melanocytes is the epidermis, but large numbers of them can be found in hair follicles and in the eyes where they manufacture melanin for hair and eye pigmentation respectively and in other less well-known locations as discussed below. The fact that we keratinocytes control melanocytes in the skin via an armamentarium of growth factors (2) has led to the impression that keratinocytes and melanocytes live in a sort of master–slave relationship. However, the dependency is not unilateral at all; melanocytes transport melanin in membrane-bound organelles (termed melanosomes) via their elongated dendrites and then transfer them to us (3), whereupon we arrange them to form a critical protective barrier (known as supranuclear 'caps') to shield our DNA from UV radiation (4).

Melanocytes (and melanin) also function early during human development; they play critical roles during embryonic development as can be seen in individuals with oculocutaneous albinism type 1 (OCA1). OCA1 results from the dysfunction of TYR, which leads not only to impaired pigmentation of skin, hair and eyes (5) but also to misrouting of the optic nerves at the chiasm (6). Melanocytes express the melanocortin 1 receptor (MC1R) that regulates the quality and quantity of their melanin production. MC1R is controlled by the agonists melanocyte-stimulating hormone (MSH) and adrenocorticotropic hormone (ACTH) (7), which stimulate the melanogenic cascade and thus the synthesis of eumelanin, as well as by the antagonist agouti signalling protein (ASP) (8). It is known that ASP elicits the production of pheomelanin, but it was shown only recently that ASP also modulates the expression of genes involved in morphogenesis (especially in nervous system development) (9).

Besides their existence in the skin, melanin and melanocytes have been reported to appear in the stria vascularis of the cochlea (10), in the leptomeninges (11), substantia nigra and locus coerulus (12) of the brain, in the heart (13,14) and there is evidence that they operate even in such inhospitable territories as adipose tissue (15).

Note in passing that there are two distinct types of melanocytes: differentiated melanocytes that originate from the neural crest and can be found at various locations in the body, and a second type, the retinal pigment epithelium (RPE), specifically present only as a single layer of cells lying behind the retina that develop *in situ* from the optic cup of the brain (16). The RPE plays a critical role in the active phagocytosis and turnover of the rod outer segments of the retina as well as in the uptake, processing and transport of retinoids and consequently has an important function in vision (17).

Melanocytes are also present as intermediate cells in the stria vascularis of the cochlea. Strial intermediate cells are required for the generation of endolymph-mediated action potentials that are necessary for normal hearing (10). Hearing impairment can be associated with inherited pigmentary disorders, e.g. Waardenburg syndrome (18), and studies have shown that the extent of induced temporary hearing loss is inversely related to skin pigment type (19). Melanin granules produced by melanocytes in the inner ear even play important roles in balance (20).

Extracutaneous melanocytes located in the brain may have several neuroendocrine functions. Human melanocytes express lipocalin-type prostaglandin D synthase, which generates prostaglandin D_2 (PGD_2) (21). Besides, in melanocytes, β-endorphin, an endogenous opioid, is generated from proopiomelanocortin together with MSH and ACTH. Does this suggest that melanocytes regulate sleep? PGD_2 is a potent sleep-inducing substance (22) and opioid receptors are located in the nuclei that are active in sleep regulation (20). Also, there are indications that a certain melanocyte-derived factor might be involved in controlling the central chemosensor that generates the respiratory rhythm (20). Pigment in the brain, termed neuromelanin, consists of a large, complex, eumelanin-covered pheomelanin core which may also contain aliphatics and peptides (23). Neuromelanin is primarily localized in dopaminergic neurons of the substantia nigra and in the locus coerulus and accumulates in the human substantia nigra with age (12). A selective loss of dopaminergic neurons containing neuromelanin is associated with Parkinson's disease (12). Various studies support the concept that neuromelanins have a protective function by binding/removal of reactive oxygen species (ROS) and metals that would otherwise be highly toxic to neurons (12,24,25). A recent study showed that virtually all brain tissues contain significant amounts of neuromelanin, which are thought to play important roles in reducing toxicity in those tissues (26).

It has also been brought to our attention that melanocytes are located in the valves and septa of the heart (13,14). Mice presenting with hyper (hypo-) pigmented skin show increased (or decreased) heart pigmentation (14). Cardiac melanocytes may originate from the same precursor population as skin melanocytes as they depend on the same the signalling molecules known to be required for proper skin melanocyte development (13), but their function in this location so far is obscure. It may well turn out that the production of melanin is not always of benefit, either in the heart or in other tissues, such as the lungs, where in a rare disease known as lymphangioleiomyomatosis (27), muscle cells revert towards their developmental origins and express some melanocyte markers, such as

Table 1. Locations and functions of melanocytes (and melanocyte imitators)

Location	Function(s)	References
Skin – epidermis	Constitutive skin pigmentation, and responses to and protection against the environment (primarily UV)	2–4
Skin – hair follicles	Hair pigmentation, removal of toxic byproducts	3
Skin – hair bulge region	Melanocyte stem-cell reservoir for skin	
Eye – choroid	Constitutive eye pigmentation, protection against UV	5,6
Eye – RPE	Vision, metabolism of rod outer segments and retinoids	16,17
Ear – cochlea	Hearing	10,18,19
Inner ear –	Balance	20
Brain – all tissues	Neuroendocrine and detoxification	11,12,21–26
Heart	Anti-inflammation, reduction/binding of ROS	13,14
Adipose tissue	Reduction/binding of ROS, cellular survival	15
Lung	Unwanted? No known function, lethal consequences	27

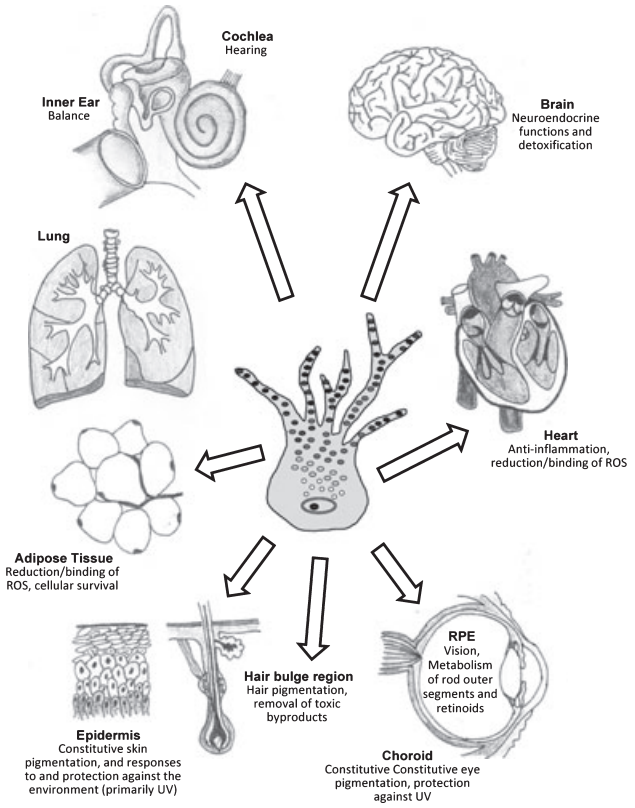

Figure 1. Schematic summarizing the different locations in the body for melanocytes and potential functions in those locations.

tyrosinase, Pmel17, etc. The resulting production and accumulation of melanin in lung tissues is eventually lethal.

Recently, we have learnt that melanin biosynthesis also takes place in the visceral adipose tissue of morbidly obese humans (15). Hypothetically, the ectopic synthesis of melanin in the cytosol of obese adipocytes may serve as a compensatory mechanism to act as an anti-inflammatory factor and to reduce oxidative damage. During increases in cellular fat deposition, adipocytes become more exposed to endogenous apoptotic signals, especially ROS, which could be counteracted by ectopically produced melanin. In addition, adipocytic melanin may suppress the secretion of proinflammatory molecules (15).

In conclusion, we think it unfair that melanocytes reap all the glory for their role in pigmenting the skin and providing it critical protection against UV damage, when in fact it is we as keratinocytes that form the bulk population of that tissue deserve all the credit. It adds insult to injury that melanocytes are now beginning to take more and more glory for their roles in other tissues of the body. Where will it all end?

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Viewpoint #2

'But now here's the third command: Machine, do Nothing!' The machine sat still. Klapaucius rubbed his hands in triumph, but Trurl said:
'Well, what did you expect? You asked it to do nothing, and it's doing nothing'.
'Correction: I asked it to do Nothing, but it's doing nothing'.
'Nothing is nothing!'
'Come, come. It was supposed to do Nothing, but it hasn't done anything and therefore I've won'.
S. Lem (1965) 'How the World Was Saved' (1)

While our task here is to dissect non-pigmentary functions of the melanocyte, the problem of melanin production and transfer is inseparable from this. Let us begin therefore with a simple question: Are melanocytes doing anything at all, besides producing melanin?

The outlines of an answer to this question are hidden in a few, rarely considered facts:

1. *Melanin is not indispensable to dark-colour pigmentation*: e.g. a simple model eukaryote, the myxomycete *Metatrichia vesparium,* reveals dark pigmentation of the plasmodium because of high concentration of manganese (II), but not melanin (2).

2. *Cells are not necessary to produce melanin*. The capacity to produce melanin evolved from the more ancient phenomenon of extracellular autooxidation of phenolic compounds (3,4). Recently, we have shown (3) that if cytoplasm of another myxomycete *Fuligo septica* turns black in the process of sclerotization, the pigmented sclerotium is not able to reproduce viable plasmodium. Moreover, among several hundreds of myxomycetes, only one, *Physarum nudum*, has been so far reported to produce melanin in the living plasmodium in response to light (5). These experiments carried out on simple, aerobic eukaryotes strongly suggest that the first thing which evolutionarily ancient cells must have achieved in the oxidizing atmosphere was clearly how to INHIBIT melanogenesis, i.e. HOW NOT TO PRODUCE melanin. Later on, their descendants 'learned' (in the evolutionary sense of this term) how to control extra- and intracellular melanin production, deposition and how to take advantage of the ability to synthesize melanin (3,4).

3. *Melanocytes are not necessary to produce melanin*. There are numerous coloured organisms producing melanin in other cells (6). Melanocytes appeared earliest in *Deuterostomia* (7,8) because of their unique embryogenesis of the neural system – developing as a tube at the back side of the animal. In this way, the embryonal neural system generates a by-product – the neural crest, the origin of a huge variety of cells, including neurons and skin melanocytes (6–8). Melanocytes must have evolved in advanced *Chordata*, probably in early vertebrates, in which the presence of the neural crest is, for many investigators, the most important feature, if not the only interesting feature really unique for vertebrates (8). *Protostomia* are only in the possession of cells analogical to melanocytes (6). Many of them produce melanin in or outside the cells, let alone plants and lower organisms – fungi and prokaryotes (4,6).

4. Moreover, even in the presence of melanocytes, melanin and melanosomes can be generated *ab ovo* in other types of cells (9–11).

5. *Epithelial cells can produce melanin*. Interestingly and importantly, in the cuttlefish ink sack, an exocrine gland of the holocrine type, the cells responsible for melanin ('ink') production are in fact specialized epidermal cells (12,13). In *Deuterostomia*, epidermal cells can theoretically produce melanin by themselves, as well. In fact, one is even tempted to shift the general question covered by this current Controversies to a different, but related enquiry: Why is it that melanocytes do, and keratinocytes DO NOT produce melanin in mammalian epidermis or hair follicles? Ah, now you see…! The likely answer directly leads to the conclusion that melanocytes must be doing something else, from which the ability to produce melanin has evolved or has become a mere by-product.

6. Among many disorders of pigmentation, including total lack of melanin synthesis, *the more the impaired function is basic for melanocyte biology* (14–17). Among the examples of total lack of melanocytes, all of which are associated with a severe impairment of neural crest embryogenesis (e.g. Waardenburg syndrome), many are lethal or sub-lethal (18,19).

7. *There are plenty of melanocytes which do not normally engage in melanogenesis*, e.g. the amelanotic melanocytes in the outer root sheath of – heavily pigmented! – anagen VI hair follicles (15).

Therefore, when looking for potential evolutionary advantages of possessing melanocytes, it seems that the generation of melanin itself is not necessarily producing the decisive selection advantage, but the presence of melanocytes themselves. Even if melanogenesis is the primary function of present-day mammalian melanocytes, it is certainly not the only one, and perhaps not even the most important one.

In fact, it is reasonable to assume that melanocytes have evolved gradually, within an evolutionary continuum of already coloured animals and have only gradually developed the capability to control melanin production, as a secondary specialization. This probably happened in the earliest vertebrates – *Agnatha* (today represented by the lampreys and the hagfish) (8), while typical higher vertebrate skin with all its adnexa and melanocytes submersed in the basal layers of the epidermis may have evolved as late as in *Therapsida* (i.e. the reptilian evolutionary line leading to the mammals) (20,21). Some related cells apparently opted for differential evolutionary paths, evolving into other cells responsible for colouration – namely xanthophores, iridophores and erythrophores (8).

What did the ancient melanocytes do all day long, then? Or, slightly re-phrased: What did neural crest cells do day and night, before they became melanocytes?

The answer is connected with three cellular properties which, on first thought, do not seem to be connected with pigmentation at all, namely the ability to wander around in the organism (migration) and to then settle down in the skin epithelium (intraepithelial residence) (7,8), as well as the ability to create synapses with other cells (22). During embryogenesis, melanocytes cross the epidermal basement membrane and settle down in the basal layer of the epidermis. The tendency to cross the basal lamina seems preserved in mature amphibian melanophores – cells closely related to melanocytes – which, however, do it the other way round (23). An even more important property of

melanocytes, which may betray their possible primary functions, is a striking phenomenon of intercellular interaction, namely the transfer of melanin-containing melanosomes to keratinocytes (22). Melanocytes can also settle down somewhere in the upper layer of the dermis (e.g. in certain nevi) or in subcutaneously located epithelium (i.e. in the hair follicle pigmentary unit) where melanin production occurs in apparent communication with dermal fibroblasts or the specialized fibroblasts of the follicular dermal papilla (24–26). Such pigment cell-fibroblast interactions are already prominent in amphibian melanophores, which transfer melanin to dermal fibroblasts (27).

Melanin is transferred within melanosomes to target keratinocytes by a mechanism that resembles exocytosis (22,28). Melanosomes, again, seem to be related to other secretory granules as they are produced, e.g. by neurons and immunocytes, while the special connections between melanocytes and keratinocytes ('pigmentation synapses') resemble other types of synapses: neuronal, immunological or phagocytic (22). Next, some neurotransmitters and neuromodulators (dopamine) can autooxidate to neuromelanin, a by-product of their oxidative catabolism in *locus coeruleus* and *substantia nigra* (15,29). [In line with this 'synaptic' line of argumentation, it deserves at least a footnote that pigmented hair emerged during evolution as sensory organs (tactile mechanoreceptors) (20,21).]

Finally, the evolutionary ancestry of POMC-coding gene, whose activity eventually leads to the production of pigment-stimulatory neuropeptides (melanocortins), is at least as ancient as the evolution of the neural crest and much older than the evolutionary history of typical melanocytes (30). Taking into consideration the huge variability of physiological processes that are regulated by POMC-coded peptides [of which the regulation of melanogenesis is only one function among multiple others (15,30,31)], one is almost compelled to conclude that the ancient melanocytes must have possessed some regulatory functions. One wonders, did the ancestral melanocytes initially exocytose those secretory granules in which inhibition of autooxidation of neurotransmitters had gone wrong, thus accidentally creating melanin…?

While the record of endo/paracrine actions of melanocytes is long and well-documented (15,32) (see also the other essays in this feature), an old, apparently long-forgotten hypothesis on melanin as a 'solid hormone' deserves to be recalled here (33,34). This hypothesis is closely related to another one, namely that on extraepithelial melanin transfer, in particular in hyperpigmentation phenotypes (33), and in the context of the transfer of melanin-containing apoptotic bodies in catagen to Langerhans cells (35) and further – to lymph nodes and visceral organs (35,36). This phenomenon may be related to the ability of melanins to adsorb various organic and inorganic compounds in their structure, acting e.g. as cationite (37). Consequently, melanins can serve as long-distance vehicles for such substances, working in the systemic dimension, i.e. in an endocrine manner.

Mechanisms and interactions connected with such a melanocytic export to defined target cells or just to the melanocyte environment may turn out to be quite complicated. One intriguing example, which has not been fully explored so far, but which may well be important for future research, shall suffice here. Nitric oxide synthase-1 (NOS-1) was suggested to participate in potentiation of melanogenesis due to UVB by keratinocytes (38,39) which responded to UVB by increase in NOS-1 activity, and by production of NO. This parahormone activated melanocytic guanyl cyclase and, subsequently, the protein-kinase G (PKG) pathway of melanogenesis control (15). Melanocytes were claimed to react to UVB by a constitutive NOS activation, too (38,39). As NOS-1 localizes in synaptic granules thanks to its PDZ-domain (where PDZ stands for PSD-95 discs large/ZO-1 homology domain, and where PSD-95 stands for post synaptic density protein 95, and ZO-1 for *zonula occludens* scaffolding protein 1) (40), it may well be that the primary signal is perceived by melanocytes, where it stimulates NOS-1 gene transcription, with the gene product being co-transported along with melanosomes to the more superficially located keratinocytes. From its new intraepithelial position, in turn, NOS-1 may be envisioned to then increase melanogenesis in melanocytes via NO. Moreover, it has recently been suggested that NOS-3 protein (i.e. the endothelial NOS) can act in co-operation with oestrogen receptors as regulators of transcription of human telomerase in endothelium (41). Perhaps, given its numerous alternate splicing variants (40), NOS-1 may act in a similar manner? Maybe this is even true for NOS-3, as elevated mRNA levels of the latter have also been found in melanocytes (42)?

Be this as it may, these observations suggest that melanocytes are able to perceive UV light. What could be the corresponding photoreceptor(s)? Well, for starters, there is NOS itself: one molecule of NOS contains many prosthetic groups which may serve as primary photoreceptors, like FAD, FMN, BH_4 or haeme (40). Moreover, numerous other substances can act as primary photoreceptors in melanocytes, including rhodopsin (43) and oligomers of phenolic compounds, i.e. melanin precursors absorbing light in the visible range (32).

Are there any other stimuli that can be perceived by melanocytes? Of course, there are numerous growth factors, hormones and neurotransmitters, for which melanocytes express cognate receptors and the stimulation or antagonization of which profoundly impacts on melanocyte survival, proliferation, migration and differentiated functions

(14–17,26,31,32). While most of these extracellular signals sensed by melanocytes within their respective microenvironment participate in regulation of melanogenesis, in some cases, these stimuli induce reactions that are typical of receptors in sensory organs. Besides their well-appreciated presence in the eye, the presence of ancestral melanocytes in mechanoreceptors of primitive vibrissae of therapsids deserves to be recalled here. Another important property of melanocytes, which is particularly pronounced in their close relatives, amphibian and fish melanophores, is the perception of chemical – not unlike the perception of olfactory stimuli [these 'olfactory' properties of melanocytes are important for sudden movements of melanosomes within the melanophores, and the neurogenic change of pigmentation (44,45), vital survival tools in a predator-rich environment].

All these phenomena and considerations invite one to synthesize the vision that the melanocyte is a cell that is able to receive signals from its biological, physical and chemical environment, that can adjust its inner state according to the signals received, and that can generate physical and biochemical signals to both its immediate tissue milieu, and to distant environments.

This concept of melanocyte biology resembles the behaviour of a classical Turing machine – the theoretical model of calculability (46). There are, indeed, aspects of melanocytic functions where the state of the cell changes rather in the digital than the analogue way and where the cells respond in the digital manner to stimuli which change in the analogue way. This concerns e.g. the phaeo/eumelanogenesis switch (47,48), and the pH switch necessary to initiate melanogenesis in melanosomes (31). Although these phenomena concern pigmentation, they can serve to show the analogue-to-digital converter properties of the melanocyte. This approach to cell biology has been developed by many research groups, which have tried to model intracellular processes employing the methodology of the theory of information (49–53) and which have suggested how even Turing-uncomputable formalisms and functions may be computed (52). The thermodynamic aspect of the cellular computation seems of a much greater importance than of artificial computers in which the source of energy used for computation is usually negligible (50). Thus, the melanocyte with its inner system of membranes, with its complex pathways involving coated particles and organelles, its premelanosome genesis, its trafficking of melanogenesis-related proteins, its capacity to induce qualitative switches in melanogenesis, and, finally, with its transfer of melanin to other target cells is perfectly suited to serve as a model of membrane automata (51–53).

These examples invite one to create mathematical or computational models of a living cell that reflect or mimic more or less what really happens in the cell (49–53). In fact, as nicely exemplified by a macroscopic unicellular, multinuclear myxomycete, *Physarum polycephalum*, available evidence suggests that cells engage in the act of computing. For example, they can calculate graphs (54) or perform complex logical Turing-uncomputable calculations inaccessible for classical, deterministic computers (55). Moreover, cells can even reveal such advanced computing characteristics like 'emerging computing' or 'robustness' (54,55).

The pathways of macroscopically visible pigmentation of animals can be satisfactorily described in the terms of Turing-Child models (56–60) (created by the Turing of the Turing machine), which takes advantage of the system of melanocytes wandering around the organism, initiated by the asymmetrical distribution of some morphogens in the zygote (60). If a single melanocyte can be considered as a cognitive system able to perform computation, the system of melanocytes must be a super-system of computing, performing its own unique abilities. It may e.g. optimize production of melanin in hair follicles: depending on the number of hair follicles per skin area unit, it generates more or less hair shaft pigment so that the total amount of melanin produced by a skin area remains constant (61).

The system of potential or actual melanocytes is able to perceive the morphogen asymmetry, and react accordingly, starting from the input contained in the zygote and ending up in the adult organism of a complicated spatial and temporal pattern of pigmentation (seasonally or sexual activity-dependent changes of colouration). No wonder that the temporal aspect is strongly based on the phenomenon of photoperiodicity: particular sub-populations of skin melanocytes vary not only in their phenotypic ability to produce melanin and dendrites (15) but also in the profile of expression of various NOS synthases (62). Some recent studies on the expression of hair-type alpha keratins in reptiles suggest that the evolution of hair follicle may have been longer and concerned not only Therapsida, but more ancient groups of reptiles (63). This leads to the hypothesis that follicular and epidermal melanocytes are more distant relatives than often assumed or even that the epidermal melanocytes originate from the follicular ones [as hair follicle may have evolved earlier than a dry and scales-free skin of mammals (63)]. This may be further supported by the fact that hair follicle melanocytes can re-populate depigmented epidermis in patients (e.g. in vitiligo) as well as in transgenic mice constitutively expressing stem cell factor (SCF) in their epithelial keratinocytes (64), but not vice versa and that the hair follicle appears to be the major seat of melanocyte stem cells (64). In fact, one wonders whether, during vertebrate evolution, the process of photoperiodic regulation has actually been transferred along with the responsible cells from the nervous system

into the skin, i.e. closer to the surface of the organism, where it then produced secondary functions like photoprotection via skin/hair melanogenesis, or a remote control of selected functions of visceral organs.

Conclusion

Even if melanocytes are not able to think, at least they are extensively computing. So, my answer to the overarching question of these Controversies is: computing is what they do all day and even all night long. As long as what exactly they compute remains unknown, let us assume that melanocytes count the card games that Nature plays with the ever-inquisitive pigment cell researcher…

'–What exactly do you guys do here?
–We're counting cards.
–You're counting cards?
–We're counting cards.
We're counting cards.
–What else do you do?
–We're counting cards.'
Barry Morrow (1988) 'Rainman' – the screenplay (65)

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Viewpoint #3

Epidermal melanocytes are surrounded by keratinocytes in approximately a 1:30 ratio, forming the so-called epidermal melanin unit (1). Skin colour *per se* and the mechanisms behind pigmentation have been the targets of active research over many decades. Indeed, the discovery of the pigment-producing cell in the human epidermis and in the eye by Henle dates back to 1837 (2). One area of particular interest is the mechanism for melanin transfer to keratinocytes, which, in part, involves cytophagocytosis and filopodial transport (3). Generations of researchers have turned their interest towards this fascinating mechanism to find a clearer understanding of this intricate process. Despite all efforts, many controversies and open questions remain (4).

The pigment-producing melanocytes provide important physiological functions in the skin (epidermis, hair follicle, sebaceous gland), eyes (uveal tract with choroid, ciliary body and iris), inner ear (stria vascularis and modulus of the cochlea, dark cells of the vestibular organ) and in the leptomeninges of the entire brain (5,6). Melanocytes have even recently been detected in the heart (7). While those melanocytes are neural crest-derived, the retinal pigment-producing epithelium originates from the ectoderm via the optic cup (8).

However, for a long-time, other functions besides the production of melanin have been proposed, especially for those melanocytes in extracutaneous locations and some of these functions have been more substantiated than others. This view point will focus on other activities of this fascinating mostly senescent cell beyond its role as melanin maker.

Melanocytes are potential accessory immuno-competent and secretory cells

The dendritic and large surface area of melanocytes, coupled with their strategic location in the superficial layers of the skin, suggest a potential role as sentinel and perhaps even antigen-presenting cells. Melanocytes express and react to a panoply of cytokines and growth factors and hence they can be considered immuno-competent and immunomodulatory (9). Moreover, IFN-γ can induce the expression of major histocompatibility complex class II (MHC II) antigens in melanocytes and indeed appears to be unique in its ability to induce MHC II expression in these cells (10). *In vitro* expression of intercellular adhesion molecule-1 (ICAM-1) and vascular CAM-1 is upregulated by IFN-γ. ICAM is an important regulator of immune cell–target interactions. Inflammatory dermatoses are commonly associated with striking changes in melanocyte function including increase of eicosanoids (9). The production of various cytokines including IL-1, IL-3, IL-6, IL-8, TNF-α, TGF-β and GM-CSF by melanocytes has been documented under *in vitro* conditions.

Normal melanocytes can process antigen, including the mycobacterial protein HSP65 to CD4+ T cells in an antigen-specific MHC II-restricted manner (11). The same group showed that melanocytes have an antigen presenting and processing capacity in association with elicitation of a T-cell proliferative response (11). More recognition of this melanocyte function was recently provided, where it was demonstrated that upon stimulation by IFN-γ the cell surface expression of CD40 is upregulated together with T-lymphocyte proliferation and IL-12 secretion. These findings strongly support a role for cutaneous melanocytes as active players in the immune response of the skin immune system. Moreover, melanocyte interaction with Langerhans cells – the main skin antigen-presenting cell type – has been a focus of attention (12,13). This is a particularly interesting finding given the observation that vitiligo is associated with loss of contact hypersensitivity, i.e. loss of cutaneous immune reactivity to contact allergens (14). Phagocytosis has been reported for human melanocytes (11,15). This observation represents the first recognition that melanosomes can perform phagosome lysosome-like fusion. Notably, inner ear melanocytes support a secretory role for these cells because they are responsible for regulation of ionic and electrical gradients (6). Melanin granules themselves serve also as calcium scavengers (6).

Are melanosomes chaperones?

While melanosomes are best known as factories for the melanins, recent proteomic assessments have revealed a huge range of proteins expressed by melanosomes at different stages of their maturation (16). For example, these organelles contain albumin amongst others (17). Why albumin? This protein is an excellent buffer. Does melanosomal albumin exert a regulatory role in control of the pH in this organelle? Albumin binds many endogenous substances including calcium via an EF-hands binding site, bilirubin, long-chain fatty acids, eicosanoids (PGE_1) and it also binds the rare amino acid L-tryptophan to name a few. Albumin binds many drugs (18). However, because of 17 disulphide bridges and 1 Cys residue in position 34, albumin offers great reactive oxygen species (ROS) scavenging capacity. Given that catalase levels are very low (but inducible) in melanocytes, it could be that melanosomal albumin contributes to the intracellular redox balance. As melanosomes are transferred to the surrounding keratinocytes, it would be interesting to know whether calcium release from this organelle contributes to the numerous calcium-dependent processes in keratinocytes. The latter function suggests a chaperone-like function for albumin.

Melanosomes also contain (6R) and (7R, S) – 5,6,7,8 tetrahydrobiopterin ($6BH_4$, $7BH_4$) (19,20). The presence of $7BH_4$ appears to be unique because until recently, this pterin was regarded as a non-physiological pterin, which is only present in certain diseases when 4a-pterin carbinolamine dehydratase (EC 4.2.1.96) activity is perturbed (21–23). Under certain conditions, it can also function as a weak co-factor for phenylalanine hydroxylase (EC 1.14.16.1, PAH) (24). However, in vitiligo, this pterin leads to inhibition of PAH because of high concentrations of this unusual pterin (22).

So what is the function of $7BH_4$ in melanosomes besides regulation of tyrosinase via β-MSH? (19). Given that a high affinity MCR-4 is also present on melanosomes and that β-MSH is the best ligand for this receptor (contributing in turn to the cAMP pool in this organelle), the question arises: what else could be the purpose of $7BH_4$ in melanosomes? (25). It is tempting to speculate that this pterin might control the high levels of β-MSH in keratinocytes by

binding this melanocortin via 1:1 stoichiometry (26). Again, melanosomes would release this pterin in keratinocytes after transfer of the organelle as a chaperone.

A third example for possible chaperone function is the presence of the neurotransmitter acetylcholine (Ach) in melanosomes together with its vesicular transporter (VAChT) (27,28). Ach stimulates dendricity of melanocytes. It could be that after the melanin transfer to keratinocytes, this neurotransmitter is released, consequently contributing to extracellular, inter- or intracellular signalling.

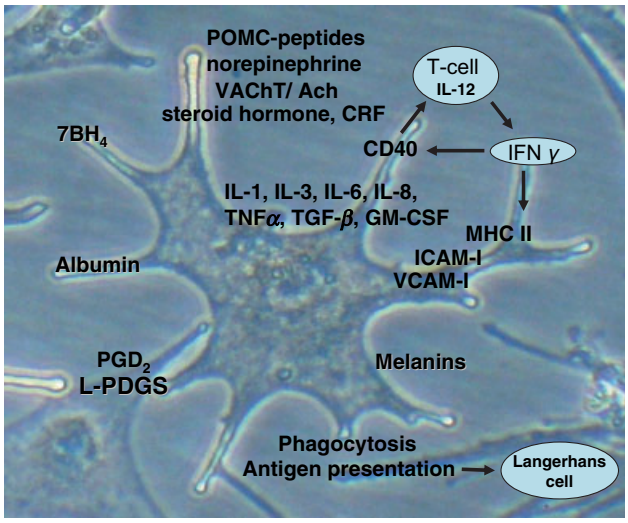

Figure 1. Selected functions of human melanocytes apart from melanogenesis. Melanocytes have the capacity to produce neurotransmitters including acetylcholine, noradrenaline (35), POMC-derived peptides such as ACTH, α-MSH, β-MSH, β-endorphin, CRF and oestrogens (9,25,39). Association of these with melanosomes supports the possibility of their transfer to the extracellular environment. As melanosomes contain the 7-isomer ($7BH_4$) of the natural co-factor $6BH_4$, it could serve other purposes in addition to regulation of melanogenesis (26). This pterin is certainly an effective ROS scavenger. Albumin has been identified in melanosomes (17). This protein binds endogenous and exogenous substances (bilirubin, calcium, long-chain fatty acids, L-tryptophan and other indoles, vitamin B6, many drugs) and also interferons and streptococcal protein G, etc. (18). Moreover, albumin is an effective ROS scavenger. Melanocytes contain L-PDGS producing PGD_2 (32). It binds bilirubin, biliverdin and thyroid hormones. The presence of PGD_2 has been invoked in the sleep/awake behaviour. Melanocytes produce melanin, which is an effective UVR and heat absorbing polymer. It generates quinones/semiquinones, which can exercise *per se* and also because of H_2O_2 generation, an antimicrobial function. Melanin can bind bacterial toxins and many other substances including drugs. Moreover, melanocytes are immuno-competent cells. These cells may phagocytose and serve as antigen-presenting cells (11). Following IFN-γ stimulation, CD40 is expressed, which, in turn, stimulates T-cell proliferation and IL-12 secretion. IFN-γ also stimulates the expression of MHC II, ICAM I and VCAM I. The production of various cytokines in the melanocyte has been shown under *in vitro* conditions. Taken together, melanocytes emerge as a multipotent player with far more capacities than making pigment alone.

L-PDGS in melanocytes

Lipocalin-type prostaglandin D synthase (EC 5.3.99.2, L-PGDS) is expressed in melanocytes, but not in other skin cells (29).

Lipocalin-type prostaglandin D synthase produces prostaglandin D_2 and functions as an intercellular chaperone for lipophilic ligands including thyroid hormones and retinoic acid as well as bilirubin and biliverdin (30). The presence of L-PDGS was also shown in the retinal pigment epithelium (29,31). Interestingly, MITF-M regulates transcription of L-PDGS in melanocytes (32). As PGD_2 is a potent sleep-inducing substance, it has been suggested that melanocytes could be involved in the regulation of sleep/awake behaviour (32).

Endocrine/sensory function of the melanocyte

Melanocytes occupy a uniquely pivotal position in the hypothalamus-pituitary-adrenal axis equivalent in skin and its appendages. The cutaneous pigmentary system can operate as an important stress response element by involving corticotrophin-releasing factor (CRF), catecholamines, Ach, POMC derived peptides and steroid hormones (33–35). Feedback regulation between melanocytes and other skin cells, as well as the entire system in general can be anticipated. Indeed, the POMC-derived peptide-mediated functionality in the skin appears to be organized into symmetrical functional pigmentary units leading to the concept of 'self-similarity' of melanocortin systems based on their expression both at the local (skin) and systemic (CNS) levels, where the only apparent difference appears to be one of scale (36).

Melanocytes and antimicrobial/antifungal properties?

The potent antimicrobial/antifungal properties of the reactive quinone intermediates generated during melanin biosynthesis may have *per se* and/or as H_2O_2 generator selective advantage given the constant exposure of the skin and hair follicle to micro-organisms (37). In this context, it is noteworthy that fungal dermatitis is more prevalent among individuals with fair skin than in those with dark skin (37). Moreover, melanin in the eye can bind bacterial-derived toxins including botulinum A. Here, it is notable that pigmented animals can tolerate this toxin better than albino animals (38).

Concluding remarks

The above selected capabilities indicate that melanocytes are indeed doing something else besides melanin production. The transfer and release of potent neurotransmitters as well as other important regulators place this neuroendocrine-derived dendritic cell in a central position of the skin,

calling for a tight feedback on neuronal and non-neuronal interaction/signalling in the local environment of the skin and also at distant extracutaneous end points (Fig. 1).

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Viewpoint #4

In 1969, Fitzpatrick and Breathnach formulated the concept of the epidermal melanin unit. They proposed that a melanocyte and 36 keratinocytes work together to produce skin colour (1). Later, Nordlund proposed that the epidermal melanin unit needs to be expanded to be called the 'KLM unit' (K = keratinocyte; L = Langerhans cell; M = melanocyte) to emphasize the many ways all three major epidermal cells interact (2). It is now clearly established that melanocytes exert more complex functions than production, transport and transfer of melanin to the surrounding keratinocytes. In 1993, Slominski et al. (3) proposed the hypothesis that melanocytes can be considered as 'sensory' and regulatory cells in human epidermis. They proposed that melanocytes act as intra-epithelial 'stress-sensors', alter keratinocyte functions, have immunomodulatory functions, serve as neuroendocrine cells and amplify and transform signals received from neighbouring cells into chemical messengers within an organized regulatory network for the maintenance of epidermal homeostasis. Further research demonstrated that there is not only an epidermal melanin unit but also a real epidermal–dermal unit that could be called the skin melanin unit. Indeed, in addition to the three 'major' epidermal cells, data now strongly suggest that the fibroblasts, the cutaneous axon terminal and the endothelial cells have key interactions with melanocytes.

Alone a melanocyte is nothing

Melanocytes are very difficult to grow in culture. Indeed, such cultures not only need nutrients but also require several growth factors for their proliferation and their survival (4,5). Such fundamental considerations shed light on the fact that in the skin, melanocytes are surrounded by many kinds of cells that constantly produce cytokines, hormones and growth factors that not only modulate the melanin production but also control the proliferation and the survival of melanocytes.

Human melanocytes *in vitro* respond directly to UV-light with increased melanogenesis (6). One of the main pathways involved in this direct UV response appears to activate a transcriptional factor called USF1 through the stress response kinase p38 (7). However, striking differences are observed in the melanogenic response of normal human melanocytes to UV-irradiation depending on culture conditions and the presence of keratinocytes. Exposure of melanocytes monoculture with strong UVB doses leads to slight melanogenic effects, whereas low UVB doses exposure of melanocytes co-cultured with keratinocytes triggers an increase in melanin synthesis. This experiment demon-

strates that keratinocytes play an important role in mediating UVB-induced pigmentation. These experiments demonstrate that melanocytes alone have a limited power on skin pigmentation and require the effective modulators of melanin synthesis produced by their neighbouring keratinocytes (8).

The key role of the keratinocyte partner

The epidermal melanin unit is a functional unit composed of one melanocyte and several neighbouring keratinocytes. The pigment donors are melanocytes, which synthesize melanin and the pigment recipients are epidermal keratinocytes. Until recently, we did not know how the pigmentary units are working and what determines where melanin is placed. Are keratinocytes acquiring pigment if a melanocyte offers it or do they act proactively by recruiting melanocytes and inducing the transfer of pigments?

Several mechanisms of melanin transfer have been proposed. According to the phagocytosis theory, the keratinocyte receptor protease-activated receptor-2 controls melanosome ingestion and phagocytosis by keratinocytes and exerts a regulatory role in skin pigmentation (9). It was recently shown that Foxn1, a transcription factor first indentifies pigment recipient cells and then recruits pigment donors thereby generating pigmentary units. Foxn1 stimulated epithelial cells (keratinocytes) to emit signals, one of which is FgF2. As a result of these signals, melanocytes recognize the Foxn1-positive cells as targets, connect to these targets via dendrites and transfer pigment (10).

Melanogenic paracrine and autocrine cytokines have been discovered *in vitro* between melanocytes and other types of skin cells. Alpha-melanocyte-stimulating hormone (αMSH), adrenocorticotrophic hormone, basic fibroblast growth factor (bFGF), nerve growth factor (NGF), endothelins (ET), granulocyte-macrophage colony-stimulating factor (GMCSF), stem-cell factor (SCF), leukaemia inhibitory factor and hepatocyte growth factor (HGF) have been reported to be the keratinocyte-derived factors and to regulate the proliferation and/or differentiation of mammalian epidermal melanocytes (11). In fact, numerous factors may be produced in and released from keratinocytes and be involved in regulating the proliferation and differentiation of mammalian epidermal melanocytes through receptor-mediated signalling pathways. ET-1, GMCSF, SCF, HGF and αMSH produced by keratinocytes and their corresponding receptors on melanocytes respectively ETB receptor, GMCSF receptor, c-kit, Cmet and MC1R appear to play a key role in the interactions between keratinocytes and melanocytes (12). Beta-endorphin is an opioid peptide cleaved from the precursor pro-hormone, pro-opiomelanocortin (POMC). Human epidermal melanocytes express a fully functioning beta-endorphin/μ-opiate receptor system. Beta-endorphin has potent melanogenic, mitogenic and dendritogenic effects in cultured epidermal melanocytes (13). These observations suggest that the beta-endorphin/μ-opiate receptor system also participates in the regulation of skin pigmentation.

Thus, this complex network of cytokines, hormones and peptides finely regulates the melanocyte proliferation and the production of melanin. Interestingly, the keratinocytes also appear to control the pheo/eumelanin ratio in cultured normal human melanocytes. Indeed, in melanocytes monocultures, there is a very limited eumelanin production and a very high pheomelanin synthesis leading to a very high pheo/eumelanin ratio. In melanocytes–keratinocytes co-cultures, there is an induction of eumelanin synthesis accompanied by an important reduction in pheomelanin formation. In this situation, the pheo/eumelanin ratio dropped from 1043 (melanocyte monocultures) to about 25 in the presence of keratinocytes–melanocytes co-cultured (14).

Is the fibroblast 'the third man'?

It is commonly accepted that melanocyte and keratinocyte make the necessary couple to product pigmentation within the skin. However, there are growing lines of evidence that a third guy, the fibroblast, may also play a critical role for the regulation of the pigmentation. In 1994, Hori et al., already suggest that fibroblasts might have a role on melanocyte proliferation through the secretion of basic bFGF (15). An increased tyrosinase activity was also reported in melanocytes co-cultured with fibroblasts (16). Human reconstructed skin models with melanocytes, keratinocytes and fibroblasts also showed the importance of the fibroblasts for the melanin production (17). On the other hand, it has been demonstrated in reconstructed skin models that fibroblasts could reduce the pigmentation of the skin (18). However, until recently, the mechanisms of action of fibroblasts on pigmentation remained obscure. One clue came from the observation that pigmented non-palmoplantar epidermis became hypopigmented when they were grafted onto palmoplantar wounds (19). It was then demonstrated that fibroblasts derived from palmoplantar skin expressed high levels of dickkopf 1 (DKK1; an inhibitor of the canonical Wnt signalling pathway), whereas non-palmoplantar fibroblasts expressed higher levels of DKK3. The DKK1 secreted by fibroblasts decreased melanocyte function, through beta-catenin-mediated regulation of microphthalmia-associated transcription factor activity, which in turn modulates the growth and differentiation of melanocytes (20). Thus, DKK1 modulate the melanocyte production of melanin through the Wnt pathway but concomitantly acts on keratinocyte to increase the thickness of the skin and decrease the transfer of melanosomes (21,22). Those results explain, at least in part, the thickness of the skin and the relative hypopigmentation observed in palms and soles.

Moreover, they clearly show the strong impact on melanocyte function of the factors secreted by fibroblasts.

Other lines of evidence suggest the critical role of fibroblasts in regulating skin pigmentation. In vitiligo, it is commonly accepted that extremities are highly difficult to repigment. The most frequently accepted explanation is the lack of hair follicles. However, the recent data on DKK1 and the success of some full skin grafts as compared to epidermal grafts also suggest that dermis' factors play a role in the treatment failures. Recently, a late redepigmentation occurring 7 years after a success of an epidermal graft for treating a nevus depigmentosus was reported (23). This clinical observation additionally suggests that dermal factors might have induced the recurrence of this hypopigmentation. Thus, the study of achromic nevi could help us better understand the dermal influence on pigmentation processes.

The increasing role of the vascularization

Interactions between melanocytes have not yet been studied in depth. However, a recent report suggests that increased vascularity is one of the major findings in melasma (24). Vascular endothelial growth factor (VEGF) may be a major angiogenic factor for altered vessels in melasma. Interestingly, normal human melanocytes *in vitro* express VEGF receptor-1, VEGFR-2 and neuropilin (25). Several data suggest that some of these receptors are functional, and that VEGF may play a role in melanocyte behaviour in skin. Thus, the vascularization of the skin might play a role in the pigmentation processes. This field of research clearly needs to be further studied, but may provide new therapeutic options for pigmentary disorders such as chemical agents with anti-angiogenic properties or physical treatments such as intense pulsed light or lasers that could target the vessels.

Melanocytes and the skin neural system

It has been shown that cutaneous axon terminals and epidermal melanocytes make contact via chemical synapses in human skin (26). The control of melanocytes function by the cutaneous nervous system has been poorly understood. However, NGF is known to contribute to dendrite formation of melanocytes (27). CGRP, a neuropeptide known to be present in intra-epidermal nerve fibres and to induce melanocyte proliferation, upregulates melanogenesis including melanosome maturation, tyrosinase activity and melanin content in melanocytes, via secondary signals from the surrounding CGRP-stimulated keratinocytes. Some CGRP-induced mediators derived from keratinocytes also increase melanocyte dendricity (28).

Human melanocytes are equipped with voltage-dependent Na+ channels a delayed rectifying K+ current and a K+ current in neurones. Whether Na+ channels in melanocytes might be of importance in the functional co-operation between melanocytes and keratinocytes remains to be studied (29). The transient receptor potential (TRP) genes encode for ion-channel proteins that are present on melanocytes. Nevertheless, melanocytes are not considered as excitable cells even if synaptic-like structures and excitable cell-specific ion-channels are present.

Melanocyte: a member of the skin immune system and key-player in an epidermal stress–response system?

The phagocytic capacity of normal human skin melanocytes has been demonstrated by a Dutch group (30). Furthermore, the same group observed that melanocytes can indeed process microorganisms and present antigen in the context of MHC class II molecules to antigen specific T-cell clones. They concluded that melanocytes may be capable of antigen processing and presentation, suggesting their active participation in the skin immune system.

Alpha-melanocyte-stimulating hormone has been identified as a potent anti-inflammatory peptide (by inhibiting the NF-κB activation) effective in a number of cell types including melanocytes and melanoma cells. αMSH is acting via a local paracrine/autocrine mechanism to control the expression of molecules involved in inflammation by attenuation of the pro-inflammatory cytokine pathway (31).

Melanocytes express POMC, corticotrophin-releasing hormone (CRH) and CRH-receptor-1 (CRH-R1) and can produce corticosterone. Normal epidermal melanocytes stimulated by CRH trigger a functional cascade structure hierarchically and arranged along the same algorithm as in the hypothalamic-pituitary-adrenal axis (32). Interestingly, CRH was demonstrated to inhibit NF-κB in melanocytes through the POMC production and thus may serve as a feedback mechanism to self-restrict inflammatory response in the skin (33).

A plea in favour of an epidermal stem-cell reservoir of melanocytes

During embryogenesis, some cells from the neural crest differentiate under the stimulation of several factors into mature melanocytes that colonize the epidermis and the hair follicles. It has been now clearly demonstrated that the lower permanent portion of the hair follicle provides shelter for melanocyte stem-cells that are self-renewing. After stimulation with several factors including the SCF, these cells can migrate in the basal layer of the epidermis and differentiate into mature melanocyte (34). The perifollicular repigmentation of vitiligo patients after phototherapy provides a good clinical example of this recolonization of the epidermis from the hair follicle (35–37).

However, there are growing lines of evidence that the epidermis is also a reservoir of melanocytes. Again, vitiligo

provides very interesting clues that are worth following. Mucosal areas (such as lips) and palms and soles depigmented lesions have been reported to be repigmented after phototherapy (38) (Passeron T & Ortonne JP, unpublished observation). In those cases, the repigmentation did not come from the melanocytes of the borders of the lesions, but was observed in the centre of the depigmented glabrous skin. One indirect clue is the fact that melanocytes can be grown from depigmented vitiligo skin samples that are told to be devoid of melanocytes (39). However, without specific markers of melanocyte progenitors, it remains very difficult to have a direct proof of the existence of an epidermal reservoir of melanocytes. If such an epidermal reservoir exists, it would be of interest from a physiological point of view, but it could also raise new therapeutic options for acquired hypopigmentary disorders including vitiligo. Indeed, repigmentation from areas devoid of follicle is much more difficult to obtain than the follicular repigmentation that can be observed after phototherapy. If such epidermal reservoir exists, it might respond to factors other than the melanocyte progenitors from the follicle niche. Thus, there is a reasonable hope that the determination of those factors could lead us to new treatment options.

Conclusion

Melanocytes are amazing cells that should not be considered anymore as only 'melanin factories'. They are active partners of the skin immune system, they participate in the stress response and have immunomodulatory properties. Their presence, not only in the skin and the hair follicle but also in the central nervous system and in some visceral organs, emphasizes the alternative functions of the melanocytes that still remain poorly understood (40). The melanocytes are in the centre of a very complex and tight regulation that involves mostly all the cell types of the skin. Indeed, keratinocytes, fibroblasts, Langerhans cells and also vascularization and nerve connections actively interact with the melanocytes and influence their functioning. The crucial role of such interactions that are now better understood should stress the importance of studying melanocytes under physiological conditions. Indeed, melanocyte monocultures are still required for some experiments, but the results should be taken with care. The use of reconstructed skin models or studies on skin biopsies by using immunohistochemistry, *in situ* hybridization or transcriptional analysis with arrays using full skin biopsy or laser dissection of specific cells, are part of the tools that should help us further understand those complex regulations. Finally, such tight interactions have to be taken into consideration for the treatment of pigmentary disorders as most of them now only focus their action on melanocytes. Targeting not only the melanocytes but also the surrounding factors might help get better and extended results.

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Commentary #1

Most of what we know about the function of the melanocyte is derived from its well-documented photoprotective role against solar ultraviolet rays (UVR)-induced skin cancers (1,2). The main function of the melanocyte is to synthesize melanin, which confers skin, hair and eye colour. In the skin, melanin is deposited onto specialized organelles, melanosomes which, when mature, are transferred to surrounding keratinocytes (3). Melanosomes form supranuclear caps that protect epidermal cells from damage by limiting the penetration of UVR through the epidermal layers and by scavenging reactive oxygen species that are generated by exposure to UVR (4,5). In this regard, we consider the melanocyte as the 'guardian of the skin'.

In addition to synthesizing melanin, epidermal melanocyte are endowed with the ability to respond to paracrine factors that enable them to repair DNA damage more efficiently and to combat oxidative stress (6–9). We and the laboratory of Bohm et al. reported that melanocytes respond to alpha-melanocyte-stimulating hormone (αMSH) with enhancement of nucleotide excision repair (10,11). Additionally, we showed that α-MSH protects melanocytes from oxidative damage by reducing the generation of hydrogen peroxide following UVR irradiation (10). These effects are expected to diminish UVR-induced genotoxicity, enabling melanocytes to survive with genomic stability. Maintaining the survival of melanocytes in the skin is critical to insure optimal photoprotection and prevention of photocarcinogenesis.

The eye contains two different types of pigment cells, the neural crest-derived uveal (iridal, ciliary and choroidal) melanocytes, and the pigment epithelial cells, primarily retinal pigment epithelium (RPE). *In vivo*, iridal melanocytes and RPE are exposed only to visible light, suggesting that ocular melanin serves a different role than protection against UVR (reviewed in 12). Interestingly, the colour of the iris appears to correlate with the incidence of uveal melanoma and age-related macular degeneration, which is highest in non-Hispanic whites, followed by Hispanics, Asians and blacks(13). The antioxidant effect of melanin seems to be protective against these two diseases. Choroidal melanin is thought to protect the choroid, which is subject to oxidative stress because of its location in the highly vascularized posterior segment of the eye, from oxidative damage. Melanin in the RPE protects the neural retina from reactive oxygen species, thus prevents age-related macular degeneration and uveal melanin reduces the chance for uveal melanoma by protecting melanocytes from oxidative stress.

While melanocytes reside in visible anatomical locations, they also are present in invisible areas, particularly the stria vascularis and cochlea of the inner ear (Figure 1). The absence of these melanocytes, as in Waardenburg syndrome type 4, is associated with sensorineural deafness (14–16). Interestingly, melanocytes in the stria vacularis become hyperpigmented after exposure to a non-continuous impulse-type noise (17) or a broad band white noise (18). Thus, the extent of pigmentation of these melanocytes is thought to determine individual susceptibility to noise-induced temporary threshold shift (15,19). A recent interesting finding is that in the mouse, stria vascularis melanocytes, but not melanocytes in the hair follicles or eyes, express *Gsta4* gene that codes for a cytosolic glutathione *S*-transferase (GST), which participates in detoxification processes in cells (20) reviewed in (21). As loud noises and ototoxic drug-induce oxidative stress (22,23) and Gsta4 prevents lipid peroxidation caused by generation of reactive oxygen species (24), it is conceivable that Gsta4 functions as an antioxidant enzyme in the defense against noise-or drug-induced hearing loss.

Recently, the presence of melanocytes in the mouse heart, mainly in the mitral, tricuspid and aortic valves, was demonstrated (25,26). Interestingly, the number of melanocytes in the heart correlated with the extent of coat pigmentation, being highest in the hyperpigmented Tyr/NRas^{Q61K} mouse and least in the unpigmented Mitf$^{vga9/+}$ (26). In the absence of stress, melanocytes in the heart did not seem to have any obvious function; however, further studies are needed to elucidate the exact role of these cells under pathological conditions.

The pigment melanin is a complex polymer that has the important property of scavenging reactive oxygen radicals.

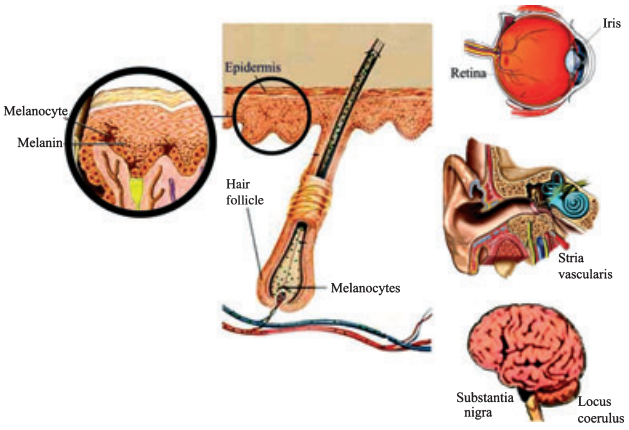

Figure 1. Anatomic locations of melanocytes and melanin**.** In the skin, melanocytes reside in the basal layer and within the hair follicles. Melanocytes are also present in the iris, choroid and RPE of the eye, as well as the stria vascularis of the ear. Melanin, in the form of neuromelanin, is synthesized by dopaminergic neurons in the locus coerulus and substantia nigra of the brain. We hypothesize that reduction in melanocytes or melanin is associated with aberrant function of these various organs and cells and disease processes because of inability to counteract oxidative stress.

In the skin, inflammation results in the generation of reactive oxygen species, as well as in increased synthesis and release of inflammatory mediators that stimulate melanin synthesis by melanocytes (27). In turn, the increased melanin, evident as postinflammatory hyperpigmentation, neutralizes reactive oxygen species more efficiently and feeds back negatively to reduce inflammation. The function of melanin as an antioxidant is also true for neuromelanin, which is synthesized by dopaminergic neurons in the substantia nigra and locus coerulus in the brain and is thought to play a neuroprotective role, preventing neuronal death that occurs in Parkinson's disease (28) reviewed in (29). Given that melanocytes and neurons share common properties, mainly poor proliferative capacity and ability to self renew, melanin synthesis may be a mechanism to preserve their survival by neutralizing oxygen radicals and sequestering toxic metals that kill neurons.

The role of the melanocyte and melanin in various organs and cells suggest that strategies aimed at stimulation of melanin synthesis, retention of melanin and survival of melanocytes might be effective for prevention of many extracutaneous diseases.

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Commentary #2

Melanocytes are well known for their role in melanin production and its transfer to keratinocytes, where it is translocated to the upper pole of the nucleus to protect it from sun exposure and UV damage (1). Accordingly, reduced melanin content in the skin is associated with increased risk of UV-associated malignancies (2,3). The presence of melanin within keratinocytes is also the most important determinant of skin pigmentation. Skin colour is among the most easily discernible phenotype, which is why it was among the first traits to be dissected at the genetic level in mammals (4).

Melanocytes, however, are not restricted to the skin and can be found in the inner ear and the leptomeninges (5,6). The persistence of melanocytes in these non-sun-exposed organs throughout evolution suggests that melanocytes might have a role other than protection from sun radiation. In fact, lack of melanocytes in the inner ear, as seen in Waardenbug syndrome (WS), is associated with sensory-neural hearing loss (7). This is attributed to melanocyte role in normal stria vascularis development (8) and the maintenance of an endocochlear potential (9). Takeda et al. (10) have suggested that melanocytes regulate breathing rate. They showed that microphthalmia-associated transcription factor-deficient (Mitf) mice, lacking melanocytes in their skin, breathe at a lower rate with higher tidal volume than wild type mice. Additionally, both hypercapnea and hypoxia induce an augmented ventilatory response in the Mitf mutant mice (10). Examination of the ventilatory response in human patients with WS associated with *MITF* mutations might demonstrate whether these findings apply to mammals as well.

A growing body of evidence also suggests that melanocytes might have an immunological role. Morphologically, melanocytes display large and long dendritic processes, similar to dendritic and other immunocompetent cells; in addition, their presence among basal keratinocytes suggests a role for these cells in the response to foreign intruders penetrating the skin through the epidermis (11). Additionally, melanocytes have been shown to express a range of immunologically active proteins such as interleukin (IL)-8 (12), monocyte chemotactic and activating factor (12), IL-1α and IL-1β (13), intercellular adhesion molecule 1 and CD40 (11) and even toll-like receptor 4 (14). All this sums

to the concept that melanocytes may be part of the immunological barrier of the skin.

On the other hand, certain genetic disorders, such as piebaldism or WS are associated with complete lack of melanocytes in the skin (15). With over 500 cases reported to date, patients with WS usually present with symptoms including iris pigmentation disturbances, partial hair albinism, sensory-neural hearing loss, congenital partial albinism and other dysmorphic and neurological features (16). Interestingly, however, these patients lack any signs of immunodeficiency or immunological manifestations, arguing against a major role for melanocytes in the maintenance of the epidermal immunological barrier.

It is therefore my opinion that melanocytes do have non-pigmentary role(s) but that their contribution to the regional immune response is not essential.

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Commentary #3

Melanocytes of neural crest origin are distributed in various organs, including the skin, eye, cardiac valves (1–3), inner ear (4) and leptomeninges of the entire brain (5). This distribution already suggests that melanocytes possess non-pigmentary functions (6). Here, we discuss the possible roles of melanocytes in the control of the epidermal homeostasis and the central chemosensor that may regulate the rhythm in respiration and behaviour.

Microphthalmia-associated transcription factor (Mitf) plays an essential role in development and survival of melanocytes (7). Among various Mitf mutant mice, we are particularly interested in homozygous black-eyed white $Mitf^{mi\text{-}bw}/Mitf^{mi\text{-}bw}$ (bw), because bw mice are characterized by the complete white coat colour and deafness, because of lack of melanocytes, with normally pigmented retina (8,9). The bw mouse therefore provides a good model to search for the hitherto unknown functions of melanocytes. By cDNA microarray analysis between wild-type and bw mouse skin, we identified lipocalin-type prostaglandin D synthase (L-PGDS) that is deficient in the bw skin (10). L-PGDS catalyses the isomerization of prostaglandin (PG) H_2 to generate PGD_2 (11) and also functions as an inter-cellular carrier protein for lipophilic ligands, such as bilirubin, biliverdin and retinoic acid (12). PGD_2 exerts pleiotropic actions through its specific G protein-coupled receptors, DP1 and DP2, also known as chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2) (13). Both DP1 and DP2 receptors are expressed in human epidermal melanocytes (14). On the other hand, L-PGDS, which belongs to the lipocalin family of secretory proteins, is actively secreted into cerebrospinal fluid, plasma and seminal plasma (11). It is therefore conceivable that melanocytes may secrete L-PGDS, thereby modulating the signals mediated by lipophilic ligands (Fig. 1). Moreover, L-PGDS is expressed in normal human melanocytes, but not in human melanoma cell lines, as judged by RT-PCR analysis (10), suggesting that L-PGDS may influence the growth potential of melanoma cells.

To explore a hitherto unknown function of melanocytes, we measured breathing frequency, tidal volume and minute ventilation of unanaesthetized and unrestrained bw mice by whole body plethysmography (15,16). Surprisingly, the bw mice show the lowest breathing frequency and the largest tidal volume during air breathing, compared with the wild-type mice (C3H/He) and other eight mouse strains analysed by the same method: A/J, AKR/N, BALB/c, C57BL, DBA/2, NZW, SWR/J and 129Sv (16,17). Subsequently, to assess the functions of the chemoreceptors in the carotid body and the brainstem, we measured the hypoxic and hypercapnic ventilatory responses respectively. Interestingly, only bw mice showed the augmented responses to both hypoxia (10% O_2) and hypercapnea (10% CO_2) (16,17). Moreover, the degrees of the increases were the greatest in bw mice among the mouse strains examined. These results suggest that bw mice are altered in chemosensing probably at the central respiratory controller, including the nucleus

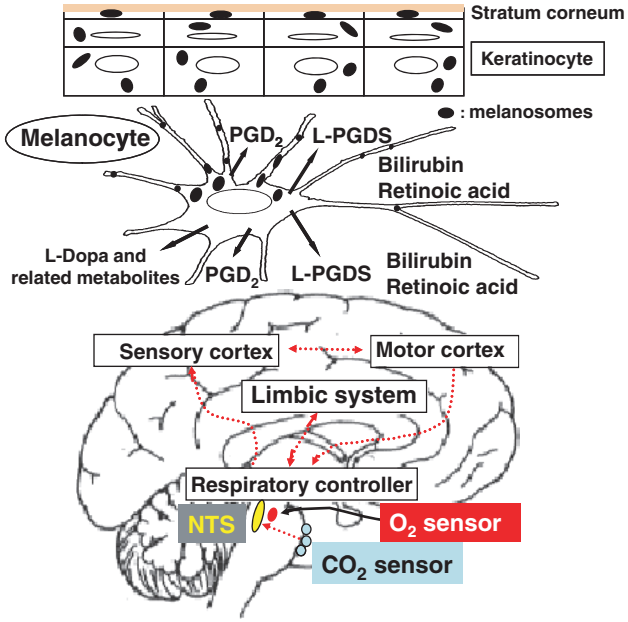

Figure 1. Proposed non-pigmentary functions of the melanocyte. Melanin is produced within melanosomes (closed symbols), which are transported to keratinocytes (nuclei, shown as open ovals). Melanocytes secrete PGD_2 and L-PGDS both of which may regulate the epidermal homeostasis. L-PGDS also functions as a transporter for bilirubin and retinoic acid. Melanocytes, located on the leptomeninges, may influence the function of the nucleus tractus solitarius (NTS) in the medulla, which is an essential centre for augmentation of ventilation during hypoxia and hypercapnia (long oval). Also shown near NTS is central O_2 sensor (small oval). Hypercapnea stimulates ventilation through the central chemoreceptor (CO_2 sensor) in the ventrolaletral medullary area (three circles). Broken lines with arrowhead(s) indicate the direction of neural signalling. The presented cartoon is the modified version of the figures published in the reference (6).

tractus solitarius (Fig. 1). To the best of our knowledge, bw mice represent the first example with the augmented central chemosensitivity. In this connection, the authors have noticed the abnormality in the behaviour of bw mice (Takeda K, Yokoyama S, Han F, Nakai K, Sato H, Yamamoto H, Shibahara S, unpublished observation).

In conclusion, we suggest the possibility that melanocytes may be involved in the complex regulatory network for epidermal homeostasis as well as for the central chemosensing that affects respiration and behaviour.

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Commentary #4

As one who studies the early development and differentiation of melanocytes, I cannot directly answer this question, but I can tell you where melanocytes come from. By the end, perhaps you will be surprised to learn what other functions they evolved to perform. In vertebrates, melanocytes differentiate from the neural crest, a population of cells that emigrate from the neural tube early during development (1). These cells migrate extensively throughout the embryo, where they differentiate into numerous cell types. Some of the neural crest derivatives are melanocytes, neurons and glia of the peripheral nervous system, bone, teeth and connective tissue in the head, smooth muscle in some arteries and the heart and chromaffin cells of the adrenal medulla (1). The neural crest is a cell type exclusive to vertebrates and its evolution has been a subject of considerable study in recent years. Some theorize that the neural crest evolved as cells from the border of the neural plate and ectoderm and developed the ability to migrate and adopt a neural fate (2–5). From there, these migratory neural cells evolved to differentiate into the other neural crest derivatives found in modern vertebrates. However, recent evidence from more basal chordates, such as tunicates and amphioxus, suggests another possibility – that neural crest evolved from pigment cells as they developed the ability to differentiate into other cell types.

To understand this hypothesis requires that we look at pigment cells and gene expression in other chordates. Ascidians are tunicates, and like vertebrates, have a notochord and dorsal hollow neural tube at some time during development. Although tunicate embryos do not have neural crest cells, neural crest-like cells have recently been identified in 12 species of ascidians (6,7). These cells emigrate from the neural tube, migrate throughout the embryo and are positive for the neural crest markers HNK-1 and *Zic* (6–8). In addition, and

of most interest in this context, these cells express tyrosinase and differentiate into pigment cells (6,7).

Jeffery et al. recently analysed expression of 16 genes known to be important for neural crest induction and specification. They discovered that seven of those genes are expressed in these neural crest-like cells in the ascidian *Ciona intestinalis*. One of the genes not expressed, which is key to the discussion here, is *Cs-FoxD*, the Ciona homologue of *FOXD3*, a forkhead-box transcriptional repressor found in neural crest cells in vertebrates. FOXD3 represses melanoblast specification in the neural crest and directs neural crest cells to non-melanogenic fates (9–12). It does so, at least in part, by repressing expression of *MITF* (13–15), a transcription factor that regulates genes necessary for melanin production and is required at the earliest stages of melanoblast specification (9,12). Loss of FOXD3 in chick neural crest cells results in a dramatic increase in the number of melanocytes and, likewise, misexpression of *FOXD3* represses expression of *MITF* and directs neural crest cells to other fates (10,12). In the non-vertebrate chordates amphioxus and *Ciona*, the FOXD3 homologues, *Amphi-FoxD*, and *Cs-FoxD* respectively are expressed in the notochord and other tissues around the neural plate, but not in the neural tube or neural plate (16,17).

Bringing these several pieces of evidence together suggests an evolutionary pathway for development of the neural crest. According to this model, in prevertebrate chordates, melanocyte precursors originated in the neural tube, as they do in tunicates. A *FoxD* transcription factor with expression domains in the notochord and other tissues surrounding the neural plate/tube was co-opted in these pigment cell precursors (likely after a genetic duplication event). This expression of *FoxD* repressed melanogenesis and these cells were then able to differentiate into other cell types. This model is not without precedent; a similar gene co-option event likely led to the expression of *Id* and other transcription factors in the neural plate early in the evolution of the vertebrates, leading to the development of bona fide neural crest (18,19). So, what are melanocytes *really* doing all day long? Well, evolutionarily modified melanocytes may be doing tasks as wide ranging as carrying neural impulses to and from the central nervous system, secreting hormones, maintaining blood pressure and chewing food.

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Commentary #5

The fundamental role of melanocytes in the protection against harmful effects of solar radiation, and as instruments of visual communication or camouflage via production and transfer of melanin pigment to epidermal and hair follicle keratinocytes is well-documented and universally accepted (1). However, the important role of the melanogenic apparatus and pathway in regulation and/or defining additional functions of the skin and its adnexal structures, which we had proposed (2–4), have largely been overlooked by pigment and skin cell researchers alike. Therefore, we take the opportunity of this commentary to re-emphasize the bioregulatory, metabolic and homeostatic functions of melanogenically active melanocytes, which are distinct from their extensive neuroendocrine activity (5–7).

We had proposed that melanocytes regulate epidermal functions by metabolically active melanosomes after transfer to adjacent keratinocytes with subsequent field effects to the respective epithelial skin compartments through alterations in the flow of ions, nutrients and electric current potential (3). This underappreciated, underinvestigated homeostatic and bioregulatory activity of intraepithelial melanocytes in the epidermis, hair follicle and possibly the nail apparatus, is based in part on the intriguing physicochemical properties of melanin. These include melanin's ability to reversibly bind Ca^{2+}, diverse ions and drugs and to serve as free radical scavenger (3,8,9). In addition, the active process of melanogenesis that continuously generates reactive oxygen species (ROS) and quinone/semiquinone intermediates (8–11) forms another basis of the regulatory impact of melanocytes on epithelial cell function.

For example, both melanin and melanogenesis can change the oxido-reduction potential of the cell, the intracellular ROS levels, can consume oxygen and reversibly regulate NAD/NADH and NADP/NADPH ratios (which has powerful metabolic consequences) (reviewed in 6,8–12). These considerations had made it an obvious hypothesis to propose that melanocytes, depending on their melanogenic activity, regulate differentiation and proliferation as well as other functional activities of keratinocytes or macrophages, once melanosomes are injected into or are phagocytized by these cells (2,3). As then, experimental data have accumulated that support our concept directly (13) or indirectly (8,14–16). For instance, active melanogenesis has the potential to stimulate NFκB and AP1 or hypoxia inducible 1α signalling pathways (8,16,17), change cellular metabolism (15), regulate intracellular calcium concentration (13), inhibit immune responses (18) and change cellular responsiveness to chemo- or radiotherapy (19).

Under physiological conditions, the entire process of melanin synthesis has to be tightly regulated starting from the organelle and ending on the organ levels. Such role is played by melanosomal proteins acting as active transporters or ion channels including product of P locus (20), metal (21) or SLC24A5 (22), SLC45A2 (23) transporters or as vacuolar proton ATPases, Na,K-ATPase or ABC transporters (24). Thus, there are many potential mechanisms by which melanosome could regulate cellular and by continuation epidermal homeostasis as proposed previously (3).

In this context, we had also proposed that intermediates of melanogenesis, in particular L-DOPA and products of its intramelanosomal metabolism, can act as intracellular second messengers and as hormone-like bioregulators for intercellular communication (3,25–27). This was based on the capability of L-DOPA (or its metabolites) to induce the synthesis and assembly of the melanogenic apparatus (28), its stimulatory effect on aerobic glycolysis and glucose metabolism (29,30), the inhibition of glycoprotein phosphorylation of L-DOPA (31) and its anti-proliferative effects on lymphocytes (32). This concept, namely, that the melanogenesis-associated production of L-DOPA and L-DOPA metabolites can impact on neighbouring cells, has most recently been supported by our demonstration that L-DOPA not only inhibits lymphocytes DNA incorporation but also abolishes proinflammatory cytokines production (18). In addition, a recent report has suggested that L-DOPA can act as an endogenous ligand for OA1 (33), confirming our original proposition for its hormone-like role and the existence of putative 'DOPA receptors' (26–28,34). Thus, the regulation of epidermal functions by melanocyte-derived L-DOPA or its metabolites should be further explored because of its clinical implications (4,18).

The time has come to appreciate, at long last, that – beyond their neuroendocrine and other secretory functions (2,3,5,6,35) – melanocytes are integral components of epithelial cell biology and regulators of keratinocyte function – just as, vice versa, melanocytes are controlled in multiple ways by the keratinocyte environment they live in (6,36–38). Whether or not, to which extent and under generation of which by-products a melanocyte engages in melanogenesis deeply affect keratinocyte function. One short answer to the central question posed in this Controversies feature, then, is: 'All day long' intraepidermal and intrafollicular melanocytes actively regulate the functions of those keratinocyte into which they inject melanosomes, above and beyond protecting their DNA from UV-induced damage.

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Commentary #6

Melanocytes obviously produce pigment (1) and yes, that has some protective function concerning the ever burning sun (2), although it accounts for just some 10% increased protection against UV-light, much less than we recommend chronically sun damaged skin to protect with. The colour of our hair and skin also has huge social and even economic meaning starting with the grey back of the lead-gorilla and ending with diminishing job-opportunities the greyer we humans get (3). However, all of these functions do not explain why we have so many of them, why they are equipped with receptors for many stress-mediators and inflammatory signals, why they keep close contacts with stress-sensitive peptidergic nerve fibres or why they produce pigment in response to such a wide variety of signalling molecules, which may be viewed as adjuvants in the cutanous stress-response such as acetylcholine, calcitonin gene-related peptide, alpha-melanocyte stimulating hormone, nerve growth factor, interleukin 1, tumour necrosis factor alpha, etc. (4–8) (Fig. 1).

Intriguingly, on one hand, melanocytes produce pigment even better when they are just a little bit stressed, e.g. by UV-light, oxidative stress or even psychoemotional stress (9,10) and they are well equipped to meet oxidative stress (e.g. high catalase, superoxide dismutase, glutathione peroxidise, Bcl-2); on the other hand, they produce their own oxidative stress and die first compared, for example, to their neighbouring keratinocytes or fibroblasts, when the stress-load goes over the top (e.g. extreme cold or heat, oxidative bleachers, repeated excessive psychosocial stress) or when they lose the capacity to deal with oxidative stress during the ageing process (e.g. mitochondrial deletion, oxidized malfunctioning enzymes, decreased levels and function of anti-oxidant enzymes) (3,10–17). And finally, they are found in locations, where endogenous and exogenous oxidative stress is likely, high and quite relevant for organ function such as the skin, the eye or surprisingly, the inner ear and the leptomeninges (18).

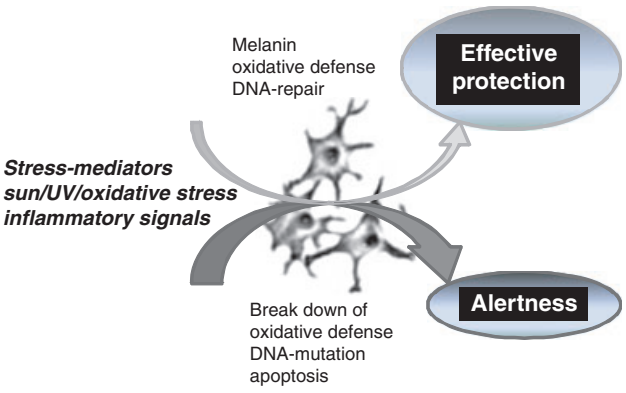

Figure 1. Hypothetical scenario illustrating the two-faced response of melanocytes to stress and its consequences for themselves and the surrounding tissue.

The danger theory suggests that we need a 'danger-signal' to identify stressors that warrantee a neuro-immunological response aiming at elimination of the stressor and moving away from it (19). Antigen-presenting cells are there to detect antigens in a warning milieu; nerve fibres are there to detect mechanical, chemical and even inflammatory disturbances (especially proteases such as tryptase released after innate immune activation by mast cells); melanocytes may be the tip of the iceberg when it comes to sensitivity against oxidative stress (Fig. 1). Especially, the mutagenic power of oxidative stress is sensed by melanocytes as their death is sped by accumulation of mutations damaging the highly sensitive mitochondrial DNA and altering telomeres (11,13,20). However, this sensory function does not only result in enhanced oxidative stress-defense and pigmentation. Through their death and subsequently released danger signals, their job is to warn the surrounding tissue and beyond, when the stress-load is reaching a damaging level (Fig. 1). They also teach us how necessary it is to keep our stress-response mechanisms in good shape as it is possible to train for the encounter and enhance, for example, levels of endogenous anti-oxidants and DNA-repair mechanisms. And last, we learn from that grey backed gorilla that grey hair is a visible sign of experience.

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