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Barrier Requirements as the Evolutionary “Driver” of Epidermal Pigmentation in Humans

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Abstract

Current explanations for the development of epidermal pigmentation during human evolution are not tenable as stand-alone hypotheses. Accordingly, we assessed instead whether xeric- and UV-B-induced stress to the epidermal permeability barrier, critical to survival in a terrestrial environment, could have “driven” the development of epidermal pigmentation. (1) Megadroughts prevailed in central Africa when hominids expanded into open savannahs [≈1.5–0.8 million years ago], resulting in sustained exposure to both extreme aridity and erythemogenic UV-B, correlating with genetic evidence that pigment developed ≈1.2 million years ago. (2) Pigmented skin is endowed with enhanced permeability barrier function, stratum corneum integrity/cohesion, and a reduced susceptibility to infections. The enhanced function of pigmented skin can be attributed to the lower pH of the outer epidermis, likely due to the persistence of (more-acidic) melanosomes into the outer epidermis, as well as the conservation of genes associated with eumelanin synthesis and melanosome acidification (e.g., TYR, OCA2 [p protein], SLC24A5, SLC45A2, MATP) in pigmented populations. Five keratinocyte-derived signals (stem cell factor⇒KIT; FOXn1⇒FGF2; IL-1α, NGF, and p53) are potential candidates to have stimulated the sequential development of epidermal pigmentation in response to stress to the barrier. We summarize evidence here that epidermal interfollicular pigmentation in early hominids likely evolved in response to stress to the permeability barrier.

CURRENT THEORIES ABOUT THE EVOLUTION OF INTERFOLLICULAR PIGMENT IN HOMINIDS

Because the shaved skin of chimpanzees and great apes is pale, it is generally accepted that early hominids likewise would have been pale-skinned initially (Jablonski and Chaplin, 2000). Several theories have been advanced to explain the subsequent latitude-dependent development of pigmentation, including the importance of pigmentation either to protect against nutrient photolysis, and/or to protect against ultraviolet (UV)-induced skin cancer (Table 1). Remarkably, the potential role of cutaneous barrier function in promoting the evolution of epidermal pigmentation has not yet been considered by evolutionary biologists.

The integrity of this barrier is necessary to prevent desiccation of the organism in a terrestrial environment; but it also excludes noxious chemicals, potential allergens, and microbial pathogens (Elias and Choi, 2005; Feingold et al., 2007; Madison, 2003; Steinert, 2000); as well as mediating several other critical protective (= defensive functions) (Table 2). Thus, while it is widely assumed that epidermal pigmentation protects from genotoxic stress from UV-B (Robins, 1991; Yamaguchi et al., 2007), the devastating impact of excess UV-B irradiation on the cutaneous permeability barrier, and the problems posed by xeric stress following the loss of most body hair, remains unacknowledged (There is, however, frequent mention of the “discomfort” of sunburned skin [e.g., *Skin: A Natural History*, Jablonski, 2006, and *Before the Dawn: Recovering the Lost History of Our Ancestors*, Wade, 2006]).

IMPORTANCE OF PERMEABILITY BARRIER FUNCTION

Like most protective functions of the skin (Table 2), the permeability barrier resides in the stratum corneum (SC), a tissue comprised of proteinaceous, anucleate corneocytes embedded in a lipid-enriched extracellular matrix (analogous to the “bricks and mortar” of a masonry wall) [rev in (Elias and Menon, 1991)] (see Fig. 1). The hierarchical importance of permeability barrier function is demonstrated first, by the rapid restoration of skin barrier homeostasis after exogenous perturbations, a sequence of metabolic responses that is orchestrated by a variety of signaling mechanisms [rev. in (Feingold et al., 2007)]. Moreover, molecular genetic studies, which have identified inherited abnormalities of either the cellular or matrix constituents of the SC that result in skin disorders, such as atopic dermatitis (i.e., eczemas) and the inherited ichthyosis [rev. in (Elias et al., 2008; Jung and Stingl, 2008; Schmuth et al., 2008)], further evidence of the critical role of permeability barrier function.

INTERDEPENDENCE OF PERMEABILITY AND ANTIMICROBIAL BARRIER

The consequences of a defective permeability barrier also include a failure of antimicrobial defense, two functions that are both integrated and co-regulated (Aberg et al., 2008; Elias, 2007; Elias and Choi, 2005). The cohesive structure of normal SC, coupled with its low water content and its acidic pH, encourages the growth of the normal flora, while providing a formidable distal layer of the innate immune system that combats the invasion of pathogens (Elias, 2007). The extracellular matrix of the SC is enriched in free fatty acids (FFA) of both epidermal and sebaceous gland origin, which are important not only for the permeability barrier (Fluhr et al., 2001; Man et al., 1995), but also for antimicrobial defense (Drake et al., 2008; Miller et al., 1988; Thormar and Hilmarsson, 2007), and an array of antimicrobial peptides that provide an additional, biochemical shield against invading pathogens (Braff et al., 2006; Elias, 2007; Schaubert and Gallo, 2008; Schroder and Harder, 2006). Conversely, structural defects in the SC extracellular matrix that result in a loss of corneocyte integrity (Cork et al., 2006), and stressors that decrease antimicrobial peptide and lipid content in the matrix likely allow pathogens and allergens to penetrate into the skin (Elias, 2007; Elias et al., 2008). We next explore below correlative evidence consistent with the hypothesis that epidermal (interfollicular) pigmentation developed in response to stress to the barrier.

DEVELOPMENT OF PIGMENTATION CORRELATES WITH CLIMATE CHANGE AND INCREASED UV-B EXPOSURE

Cutaneous adaptations early in hominid evolution

Hominids evolved in central Africa in the late Pliocene over 7 million years ago (mya) (Table 3). These early human ancestors were hairy, and they also likely had pale,

nonpigmented skin, with melanocytes restricted to hair follicles as in modern chimpanzees, great apes, and most other fur-bearing terrestrial mammals (polar bears are an interesting exceptions) (Jablonski and Chaplin, 2000). Hair in these early hominids, like feathers in avians (Menon et al., 1989), would have provided a partial barrier against excess transcutaneous water loss (TEWL). Early hominids evidently became bipedal by 6 mya (Richmond and Jungers, 2008), allowing them to hunt and gather more efficiently. The resulting enhanced nutrition then likely fueled the development of larger brains (Finch, 2007). However, by impairing heat dissipation, a hairy mantle would have severely limited daytime hunting in the open savannahs that developed in sub-Saharan Africa between ≈ 3 and ≈ 1.8 mya (Bobe et al., 2002), providing the impetus for concurrent loss of hair and development of eccrine sweat glands (Jablonski and Chaplin, 2000).

Stress imposed by loss of hair in a drier climate

The loss of hair in early, pale-skinned hominids would risk dehydration due to accelerated transepidermal water loss (TEWL) [TEWL facilitates heat dissipation, independent of eccrine glands (e.g., Moskowitz et al., 2004.)], and the threat of dehydration could have been magnified further by the slow shift towards a much drier climate, resulting in wide-spread replacement of tropical forests by sparsely forested, open savannahs, between ≈ 1.8 and 0.8 mya (Bobe et al., 2002; Chaplin, 2004; DeMenocal, 2004; Lahr and Foley, 1998), which probably drove earlier migrations of *H. erectus* and *H. neanderthalis* into Europe and Asia (Table 3). The period between 800 and 200 kya coincided with widespread droughts, punctuated by even more severe arid episodes, likely greatly restricting the range of hominids (DeMenocal, 2004).

Likely chronology for the development of pigmentation

As early *Homo* extended their range out of tropical forests into open savannahs during the megadrought period, they would have encountered not only a reduced ambient humidity, but also increased exposure to UV-B. Molecular genetic studies suggest that the melanocortin receptor (*MC1R*) gene, a key regulator of eumelanin synthesis, stabilized (i.e., polymorphisms associated with reduced epidermal pigment in chimpanzees were lost ≈ 1.2 mya (Harding et al., 2000; Rana et al., 1999), a chronology that correlates with prior loss of hair and increased movement of some hominids into open savannahs (Table 3).

Climatic pressures on early modern humans

Creatures that looked like *H. sapiens* first appeared in Eastern Africa ≈ 200 kya, quickly displacing pre-existing hominids (Westerhof, 2007). Mitochondrial DNA analyses suggest that at ≈ 100 kya, the original *H. sapiens* split into two lineages: one that migrated downwards towards remaining forested areas of Southern Africa, while the other gave rise to reduced numbers of other humans, who remained in the more hostile environment of Northern and Central Africa [cited in (Wade, 2006)]. This split in *H. sapiens* lineages coincides again with periods of prolonged and severe drought in central Africa (Cohen et al., 2007; DeMenocal, 2004) (Table 3). New matrilineages then began to appear ≈ 90 kya, which coincide temporally with the first radiation of modern humans out of Africa via moist corridors in northern Africa (Osborne et al., 2008). Although these first attempts at settlement in the Levant were unsuccessful, later migrations (≥ 50 kya) from Ethiopia to Southern/Southeast Asia and Australia were successful (Ke et al., 2001). These and still later migrations were facilitated by land bridges and reduced sea levels during the most recent ice age (Peltier and Fairbanks, 2006), which allowed *H. sapiens* to reach Europe ≈ 35 kya.

HYPOTHESIS: EPIDERMAL PIGMENTATION DEVELOPED IN RESPONSE TO XERIC AND UV-B-INDUCED STRESS TO THE BARRIER

We propose that episodic, and often prolonged ambient aridity, coupled with recurrent stress to the barrier from erythemogenic UV-B, would have driven the initial development of interfollicular (epidermal) pigmentation. Our hypothesis is based not only upon paleoclimatologic evidence that drought conditions coincided temporally with the evolution of pigmentation (see above), but also upon the view that loss of hair would have placed early hunter-gatherers at great risk from the dual stress of a lower humidity, and increased exposure to intense UV-B. Accordingly, natural selection would have favored mutations that enhance and protect permeability barrier function. Pigmentation of interfollicular epidermis in response to the dual threats of UV-B and xeric stress would have represented one such adaptation, because pigmented skin displays superior barrier function (see below). Yet, for humans living within heavy forest cover in warm regions, there would have been little adaptive advantage of increased pigmentation, except possibly in improved camouflage, which may have been offset by the fact that darkly pigmented skin absorbs more net heat (Hill, 1992; Rana et al., 1999) (Presumably, the lightly pigmented tribes would be descended from hominids that would have migrated southward to forested areas prior to the emergence of epidermal pigmentation). This would explain the side-by-side residence of lightly pigmented (e.g., San/Bushmen people) with darkly pigmented tribes (e.g., Pygmies) that likely migrated back into forested areas of Africa (Westerhof, 2007).

CRITIQUE OF OTHER HYPOTHESES REGARDING THE EVOLUTION OF EPIDERMAL PIGMENTATION

Skin cancer—pigment link

Melanin-producing cells are widely distributed from fungi to primates, and in extracutaneous tissues of humans, as well, implying that melanin has roles that extend beyond cutaneous UV-B defense (Blois, 1968). Yet, facultative pigmentation of epidermal melanocytes, such as the UV-B-induced increase in eumelanin production above constitutive levels (Quevedo et al., 1975), does not occur in extracutaneous melanocytes (e.g., uveal tract of the eye) (Li et al., 2006). Moreover, increased epidermal pigmentation could provide several potential adaptive advantages to hairless hominids, including protection against UV-L-induced phototoxicity, camouflage, sexual display, and as a free radical absorber (Jablonski and Chaplin, 2000; Parra, 2007; Robins, 1991; Schallreuter et al., 2008). More recent studies suggest additional neuroendocrine functions of epidermal melanocytes (Takeda et al., 2007), and a possible role for melanocytes in cutaneous innate immunity (Mackintosh, 2001; Montefiori and Zhou, 1991).

However, perhaps the most widely held UV-B-based hypothesis proposes that pigmentation evolved from pale skin to protect against genotoxic mutations that favor the development of skin cancer (Table 1) (Goding, 2007; Robins, 1991). Indeed, lightly pigmented skin displays a much higher propensity to develop skin cancers than occurs in darker skin (Harrison, 1973), while conversely darkly pigmented skin transmits 10-fold less UV-B than does fair skin (Yamaguchi et al., 2007). The supranuclear capping of melanin granules over keratinocytes strongly suggest that melanin provides an important shield against UV-B-induced genotoxicity (Gibbs et al., 2000; Kobayashi et al., 1993). Nonetheless, some studies show that melanin is a relatively ineffective UV filter, with a peak action spectrum in the low UV-B to UV-C range (<300 nm) [cited in (Hill and Hill, 2000)]. Moreover, experimental induction of pigmentation reportedly does not decrease UV-B-induced, pyrimidine dimer formation (Niggli, 1990), an initial step in UV-B-induced mutagenesis. Furthermore, hairless mice with interfollicular pigment (*Skh2*) exhibit an increased, rather

than decreased sensitivity to UV-B (Warren et al., 1987). It should be noted that nuclear capping provides only correlative evidence for a role for melanin in protection from genotoxicity (Gibbs et al., 2000; Kobayashi et al., 1993), because supranuclear melanin also would protect keratinocytes from damage to the barrier through UV-B-induced cytotoxicity/apoptosis, providing a link to our hypothesis that pigmentation evolved to enhance barrier function.

Perhaps most pertinently, while some skin cancers (e.g., malignant melanoma) can be lethal, they are relatively uncommon in comparison to the great majority of nonmelanoma skin cancers, which are slow-growing, only locally invasive, and nonlethal. Very lightly pigmented Europeans, living near the equator in northern Queensland in the pre-sunscreen era did not develop skin cancers until late in the third decade, and even albinos living at equatorial latitudes develop skin cancers only in the third decade (Parra, 2007). Since these mostly nonlethal cancers develop relatively late in reproductive life, they might not have reduced reproductive success (Blum, 1961), even when considering the fact that older males remain fertile into the fifth-to-sixth decade, the highly speculative “grandmother effect” (Diamond, 2005). Most importantly, there is no molecular genetic evidence to date for conservation of mutations in DNA repair mechanisms that would support the genotoxic hypothesis. Together, these results suggest that skin cancer prevention, while potentially a co-influence, was not the principal evolutionary “driver” for the development of pigmentation.

UV-nutrient photolysis—pigmentation link

Increased interfollicular pigmentation has been proposed to protect against the photodegradation of serum folic acid (Branda and Eaton, 1978; Chaplin and Jablonski, 2009; Jablonski and Chaplin, 2000). Deficiency of this vitamin during pregnancy can result in both congenital neural tube anomalies (Jablonski and Chaplin, 2000), and reduced spermatogenesis (Mathur et al., 1977). Either or both would provide a strong evolutionary basis for the development of pigmentation. While there is indirect evidence for latitude-dependent photolysis of folic acid, and for reduced folic acid blood levels in lightly pigmented individuals living at equatorial latitudes (Jablonski and Chaplin, 2000), the overall incidence of congenital neural tube defects seems too low to exert evolutionary pressure, even in populations with a high incidence of folic acid-deficiency (Table 1). Thus, nutrient photolysis would not appear to have been the principal evolutionary “driver” of epidermal pigmentation.

SKIN PIGMENT HYPOTHESES: DARK SKIN INTO PALE SKIN (TABLE 1)

Vitamin D—pigmentation link

Although there are several compelling reasons why pigmentation could have evolved in response to intense UVL (see below), two current hypotheses assume the opposite (i.e., that dark skin later devolved into pale skin). The first hypothesis proposes that *H. sapiens* lightened progressively as they radiated out of equatorial Africa, because of a critical requirement to generate vitamin D3 (Loomis, 1967; Murray, 1934; Reichrath, 2007), an intraepidermal process (Holick et al., 1980; Loomis, 1967; Murray, 1934). Fur-bearing mammals generate vitamin D from precursors in sebaceous secretions, but modern humans, a relatively hairless species, instead generate vitamin D3 by UV-B-induced photoconversion of 7-dehydrocholesterol into pre-vitamin D3 within the epidermis, followed by thermal conversion of pre-vitamin D3 into vitamin D3 (cholecalciferol), and delivery of vitamin D3 into the circulation (Holick et al., 1980).

Though attractive in its simplicity, the vitamin D hypothesis is subject to criticism on several grounds (Aoki, 2002; Neer 1975) (Table 1). As noted above, melanin pigments are

distributed widely in the plant and animal kingdom, including several extracutaneous tissues in humans, suggesting that the capacity to synthesize melanin is highly conserved for reasons that predate the cutaneous production of vitamin D in humans (Blois, 1968). Moreover, sufficient vitamin D₃ is formed in pigmented skin, even if sun exposure is restricted, depending upon latitude, time of day, and months of the year (Holick et al., 1981). In fact, substantial UV-B penetrates into the nucleated layers of the epidermis, regardless of pigment type (Hill, 1992; Holick et al., 1980; Loomis, 1967; Thomson, 1955). Instead, blockade of UV-B penetration into the epidermis can be attributed largely to stratum corneum structural proteins (Gambichler et al., 2005; Thomson, 1955) and to endogenous UV filters, such as trans-urocanic acid (Kripke, 1984), which absorb up to 70% of incident UV-B.

As humans moved from Africa to more temperate latitudes, animal pelt/clothing would have replaced hair to allow habituation to colder climates, perhaps restricting UV-B exposure almost as much as epidermal pigmentation would have. Proponents of the vitamin D hypothesis point out that South Asians living in the United Kingdom display higher rates of rickets than do their non-Asian cohabitants [e.g., (Reichrath, 2007)]. Yet, this difference might also reflect cultural practices, like the sequestration of women and girls indoors, or the wearing of burkhas to cover the entire skin surface when outside. In fact, rickets is not common in other darkly pigmented groups (e.g., West Indians), even when living at the latitude of Scotland [cited in (Neer, 1975)]. Furthermore, there is no evidence of rickets in fossils of early *H. sapiens* living at European latitudes. Rickets and its adult variant, osteomalacia, only became common under the relatively recent, atmospheric pall of the Industrial Revolution (Aoki, 2002; Neer, 1975). Finally, none of the recently identified genes that underlie human pigment variations involve the vitamin D endocrine system [e.g., (Lao et al., 2007; Parra, 2007)]. Thus, the vitamin D hypothesis seems untenable as the principal basis for the latitude-dependent loss of pigment in modern humans.

Sexual selection and pigmentation

While Darwin (1871) specifically proposed that modern humans could be preferentially attracted by individuals of different pigment-type, he did not suggest that such selection would be *unidirectional (towards lighter skin)*, as recently proposed by Aoki (2002), and others (Frost, 1988; van den Berghe and Frost., 1986). Based upon studies in diverse cultures, these workers propose that sexual selection in early *Homo* would have favored lighter pigmentation, a process that could have been amplified further by parental selection (Harris, 2006). But the sexual selection hypothesis is inherently susceptible to considerable cultural bias. Indeed, recent studies on the period after the Nubian conquest of Egypt in the 8th century, B.C. have found no evidence that pigmentation influenced social hierarchies (Draper, 2008).

EVIDENCE IN SUPPORT OF BARRIER REQUIREMENTS AS THE DOMINANT STIMULUS FOR THE EVOLUTION OF EPIDERMAL PIGMENTATION

In light of the acknowledged importance of optimal permeability barrier function for life in a terrestrial environment, we propose here that human epidermal pigmentation evolved both in responses to a decline in ambient humidity, and to UV-B-induced stress to the permeability barrier in Africa (Table 3).

Enhanced function of darkly pigmented skin

Although it is widely appreciated that UV-B can produce painful sunburns and damage eccrine glands (Jablonski and Chaplin, 2000), a greater biological threat is the fact that erythemogenic UV-B compromises permeability barrier function (Haratake et al., 1997).

UV-B-induced barrier failure is attributable to premature apoptosis of suprabasal keratinocytes that become unable to synthesize or secrete the extracellular matrix lipids required for permeability barrier function (Holleran et al., 1997). Notably, erythemogenic UV-B exposure, like other exogenous insults, such as wound healing, stimulate the migration of follicular melanocytes to interfollicular epidermis (Funasaka et al., 1998; Staricco and Miller-Milinska, 1962; Walker et al., 2009). While clearly this response provides photoprotection from acute UV-B-induced cytotoxicity, we will discuss below evidence that it also would provide a superior permeability barrier.

Xeric stress improves permeability barrier function

Although a low external humidity increases transepidermal water loss, prolonged xeric stress progressively upregulates cutaneous metabolic processes, including increased epidermal lipid and DNA synthesis, that eventually enhance permeability barrier function (Denda et al., 1998a,b). We propose here that enhanced epidermal pigmentation would have comprised an additional metabolic response to sustained xeric stress.

Superior barrier function in darkly pigmented skin

Development of interfollicular cutaneous pigmentation in response to combined UV-B/xeric stress would, in turn, offer at least four selective advantages: (1) Permeability barrier function is superior in darkly pigmented (Fitzpatrick type IV/V) humans, where it displays much more rapid recovery after acute external perturbations than does lightly pigmented skin (type I/II), a phenomena that is independent of ethnicity or race (Gunathilake et al., 2009; Reed et al., 1995). (2) Pigmentation also determines significant differences in *SC integrity* (the resistance of the SC to repeated sheer forces, such as tape stripping) and *SC cohesion* (the strength of the linkage between adjacent corneocytes, expressed as amount of protein removed per tape stripping) (op. cit). The superior integrity and cohesion of darkly pigmented skin could have provided a substantial advantage over the more fragile SC of lightly pigmented skin. (3) An optimal permeability barrier also presents a formidable antimicrobial barrier [as reviewed in (Elias, 2007)]. (4) Finally, there is substantial evidence that pigmented skin displays superior cutaneous innate immunity [rev. in (Mackintosh, 2001)], which also would have conferred a large adaptive advantage. In the “pathogen soup” of the tropics, darkly pigmented individuals reportedly display fewer infections than occur in light-skinned individuals living at the same latitude (Wassermann, 1965). Together, these functional data provide support for our hypothesis that the development of skin pigmentation would have conferred diverse adaptive advantages in response to a combination of external stressors to the barrier, most notably excess UV-B irradiation, persistent xeric conditions, and repeated microbial assaults that undoubtedly prevailed in a tropical latitude early in human evolution.

Lower pH of stratum corneum accounts for pigment-endowed enhancement of epidermal function

Our studies have begun to delineate the basis for pigment type-determined differences in cutaneous barrier function. Darkly pigmented subjects display a more acidic SC (pH 4.5–5.0 vs. 5.5–6.0), and it is this highly acidic pH that activates or deactivates key SC enzymes that regulate permeability barrier homeostasis and SC integrity and cohesion (Gunathilake et al., 2009). At a lower pH, two acidic-pH optimal enzymes, β -glucocerebrosidase and acidic sphingomyelinase, hydrolyze two key lipid precursors (i.e., glucosylceramides and sphingomyelin, respectively) (Hachem et al., 2003, 2005), into ceramides more efficiently (see Fig. 2). In parallel, the activities of serine proteases (kallikreins) decline at the lower pH of darkly pigmented skin, which in turn enhances SC cohesion (Gunathilake et al., 2009). Conversely, these serine proteases are more active at the higher pH of lightly pigmented SC, causing a host of negative, downstream consequences, including the deactivation and

degradation of the two ceramide-generating enzymes (op. cit.). Moreover, increased kallikrein activity in lightly pigmented skin leads to premature loss of corneodesmosomes, specialized intercellular junctions of the SC. Premature dissolution of corneodesmosomes, in turn, compromises SC integrity and cohesion (Cork et al., 2006; Gunathilake et al., 2009). Finally, the lower pH of darkly pigmented SC would favor the growth of the normal cutaneous microflora (Korting et al., 1990), while conversely, the higher pH of lightly pigmented skin favors colonization by pathogenic microorganisms [rev. in (Elias, 2007)]. Thus, development of pigmentation, by enhancing the acidification of epidermis, would have provided a substantial adaptive advantage for three key functions: permeability barrier homeostasis, SC integrity/cohesion, and antimicrobial defense.

Cellular basis for enhanced stratum corneum acidification by melanosomes

The contents of melanosomes, like other proton pump-containing secretory vesicles of the endocytic pathway (Orlow, 1995), are acidic, as required for the initial steps of melanin synthesis (Schallreuter et al., 2008; Yamaguchi et al., 2007). While the density of melanocytes remains relatively constant in humans of all pigment types, with a ratio of approximately one melanocyte per 30–40 keratinocytes (Yamaguchi et al., 2007), multiple, sparsely melanized melanosomes are collected together in phagocytic vacuoles in keratinocytes of lightly pigmented individuals (Szabo et al., 1969). In contrast, the larger, more heavily melanized melanosomes of darker subjects are taken up within phagolysosomes (also acidic organelles), as single melanin particles (Jimbow et al., 1976; Thong et al., 2003). Because they are smaller, melanin granules in the keratinocytes of lightly pigmented individuals are degraded proteolytically more quickly than are the single melanosome complexes of darker subjects which persist into the outer epidermis and SC (Jimbow et al., 1976). Accordingly, delayed dissolution of melanosomes in the outer epidermis of darkly pigmented subjects could further acidify this already highly acidic milieu (Behne et al., 2002).

Using a pH-sensitive fluorophore (SNARF), applied to freshly obtained, cultured melanocytes from darkly and lightly pigmented individuals, we recently visualized the acidity of the melanocyte cytosol, melanocyte dendrites, and individual melanosomes using dual-channel confocal microscopy (Gunathilake et al., 2009). The melanocyte cytosol from darkly pigmented individuals is significantly more acidic than the cytosol of lightly pigmented melanocytes, and their pigment-transferring dendrites are even more acidic (Gunathilake et al., 2009). As this dendritic acidity localizes to vesicles with the characteristics of melanosomes, darkly pigmented dendrites are therefore equipped to transfer more acidity to adjacent keratinocytes (Gunathilake et al., 2009) (see Fig. 3). Thus, the persistence of acidic melanosomes into the outer epidermis provides a cellular mechanism that explains the enhanced, pH-dependent functions of darkly pigmented skin.

Molecular genetic evidence

Our hypothesis is further supported by population genetic studies that show that a series of melanosomal ion channels and proton pumps (e.g., *OCA2*, *SLC24A5*, *SLC45A2*, and *MATP*) are among the most highly conserved genes in Africans vs. Europeans (see Fig. 3) (Graf et al., 2005; Lamason et al., 2005; Lao et al., 2007; Parra, 2007; Soejima and Koda, 2007; Stokowski et al., 2007). Thus, there is considerable correlative, molecular genetic evidence that epidermal (interfollicular) pigmentation developed in response to stress to the barrier.

KERATINOCYTE SIGNALS THAT COULD REGULATE PIGMENTATION IN RESPONSE TO STRESS TO THE BARRIER

Through transfer of acidity, we have provided a juxtacrine mechanism whereby melanocytes likely regulate epidermal functions (see Fig. 3). Whereas multiple keratinocyte-derived signaling mechanisms regulate the pigmentary response to UV-B exposure [rev. in (Schallreuter et al., 2008; Yamaguchi et al., 2007)], whether these and/or other signals could mediate a barrier-induced increase in epidermal interfollicular pigmentation remains unknown. Most UV-stimulated signaling mechanisms operate either at the level of enhanced pro-opiomelanocortin (POMC) production, or at the melanocyte (MC1R) receptor, a G-protein-coupled plasma membrane receptor that regulates eumelanin synthesis (Lin and Fisher, 2007; Yamaguchi et al., 2007). Increased generation of POMC-derived peptides, such as α -melanocyte-stimulating hormone (α MSH), β MSH, and adrenocorticotrophic hormone (ACTH), in turn, increases pigmentation by activating MC1R (Schallreuter et al., 2008; Yamaguchi et al., 2007). Importantly, these keratinocyte-derived signaling mechanisms regulate pigmentation both under basal conditions (Schauer et al., 1994; Wintzen and Gilchrist, 1996), and also after increased UV-B exposure (Chakraborty et al., 1996; Slominski et al., 1993).

In support of our hypothesis, we have identified five keratinocyte-derived signaling mechanisms that could regulate melanocyte function in response to stress to the barrier (Table 4). Assuming that early hominids were pale-skinned with pigment localized to hair follicles (Jablonski and Chaplin, 2000; Westerhof, 2007), a necessary first step in the evolution of epidermal pigmentation would have been the translocation of melanocytes from follicles to the interfollicular epidermis. The putative signal for this sequence is epidermal stem cell factor (SCF), which is a ligand for the melanocyte KIT tyrosine kinase receptor (KITr) (Longley et al., 1993). KIT-activation stimulates the migration of melanocytes into interfollicular epidermis (Kunisada et al., 1998), as well as melanocyte differentiation and survival in the epidermis (Wehrle-Haller, 2003) (Table 4). The absence of interfollicular melanocytes in early, still-hairy hominids would therefore have reflected the lack of post-natal expression of epidermal SCF, as is known to be the case in rodents (Yoshida et al., 1996). Accordingly, transgenic mice, where different levels of *scf* are targeted for post-natal expression on basal cell membranes, display persistent, dose-dependent increase in interfollicular melanocytes (D'Orazio et al., 2006). Conversely, recent population genetic studies show that *KIT*-gene polymorphisms correlate closely with the loss of pigmentation in Europeans (Miller et al., 2007). Finally, UV-B is well-known to stimulate migration of melanocytes from hair follicles into the interfollicular epidermis (Funasaka et al., 1998; Staricco Miller-Milinska, 1962; Walker et al., 2009). Thus, SCF-KIT-signaling is a candidate to have mediated an early step in the barrier-initiated stimulation of interfollicular pigmentation.

Another signaling mechanism that could have promoted the evolution of cutaneous pigmentation in humans is FOXn1 (Whn, Hfn 11), a transcription factor-like protein that is required for the development of numerous epithelial tissues (Brissette et al., 1996). Recent studies have shown that keratinocytes deploy FOXn1 to recruit melanocytes to the basal layer, thereby inducing their own pigmentation (Weiner et al., 2007). FOXn1 acts through the upregulation of FGF2, which induces pigmentation by promoting melanocyte multiplication, migration, and survival (Halaban et al., 1988; Wu et al., 2006), as well as increased MC1R expression (Scott et al., 2002) (Table 4). Thus, FOXn1, acting through FGF2, may be critical for the establishment and maintenance of the optimal ratio of melanocytes to keratinocytes in epidermis (Weiner et al., 2007).

A third mechanism could be the transcription factor, epidermal p53, a key regulator of the pigmentary response to erythemogenic doses of UV-B (Cui et al., 2007), that notably also abrogate the permeability barrier (Haratake et al., 1997). p53 has been shown recently to regulate expression of the *POMC* gene in human keratinocytes, increasing secretion of α MSH, which stimulates MC1R function in neighboring melanocytes, leading to increased eumelanin synthesis (Cui et al., 2007) (see Fig. 3).

Two additional keratinocyte-derived signals, IL-1 α and nerve growth factor [NGF], potentially regulate pigmentation in response to UV-B, but by largely divergent mechanisms (Table 4). Of particular relevance to our hypothesis, both are rapidly (up)regulated by changes in permeability barrier status (Liou et al., 1997; Wood et al., 1992). In the case of NGF, upregulation is prevented when barrier function is artificially restored in abrogated sites by application of a vapor-impermeable membrane (Liou et al., 1997), demonstrating the specificity of barrier requirements in dictating downstream responses, likely including increased interfollicular pigmentation.

COULD XERIC STRESS HAVE STIMULATED THE DEVELOPMENT OF PIGMENTATION?

Although xeric stress upregulates both DNA and lipid biosynthetic pathways that enhance permeability barrier function (Denda et al., 1998a,b), there is no evidence to date that it regulates epidermal pigmentation. We discuss here one mechanism that could link xeric stress to pigmentation. A member of the transient vanilloid receptor family, TRPV4, has emerged recently as the likely sensor of changes in external humidity (Denda et al., 2007). Very recent studies from our group show further that TRPV4 k.o. mice cannot restore permeability barrier function after acute abrogations, and even minor insults, such as removal of hair, provoke disproportionate abnormalities in barrier function (unpublished observations). Conversely, a topically applied TRPV4 agonist, 4- α -phorbol 12, 13 didecanone, accelerates barrier recovery in normal skin after acute perturbations (Denda et al., 2007). The underlying mechanism for both barrier failure in TRPV4 k.o. mice, and the accelerated recovery in agonist-treated mice appears to be regulation of epidermal lipid synthesis/secretion. Although TRPV4 is not yet known to influence pigmentation, the dominant role of TRPV4 as a sensor of external humidity makes it a candidate to have regulated incremental improvements in barrier function during human evolution, possibly including stimulation of pigmentation by one or more of the above-mentioned signaling responses.

CONCLUDING THOUGHTS

Our hypothesis suggests that hairless hominid predecessors of modern humans likely emerged with their cutaneous melanocytes restricted to hair follicles, and with their relatively hairless skin therefore exposed to sustained stress from environmental aridity and erythemogenic UV-B. Though we provide abundant, correlative evidence in support of our hypothesis, direct experimental evidence for our hypothesis is still lacking. We hope that presentation of this hypothesis article will stimulate new investigations into this putative relationship. Nonetheless, development of epidermal pigmentation clearly would have endowed early humans with an enormous evolutionary advantage in the UV-enriched, arid environment of sub-Saharan Africa. The development of epidermal pigmentation would in turn enhance epidermal permeability barrier function and SC integrity, while simultaneously enhancing cutaneous innate immunity. It should be noted that while these functions alone could provide an evolutionary advantage sufficient to promote the emergence of cutaneous pigmentation, our hypothesis neither excludes nor addresses other potential “drivers,” such as protection from UV-induced genotoxicity, eccrine gland necrosis, or nutrient photolysis

as co-stimuli in the evolution of cutaneous pigmentation. Yet, it seems implausible that environmental conditions in Africa during the radiation of *H. sapiens* out of Africa could be solely responsible for the present geographic sorting of pigmentation (e.g., very light-skinned people in northern Europe and intermediate skin pigmentation in Asia). Perhaps the wide-ranging geographic and temporal changes in African climate during this era prompted substantial genetic variability in early *Homo sapiens*, selecting for different populations of lighter and darker skin, prior to subsequent migrations (Lahr et al., 1998). As these genetically heterogeneous groups subsequently moved out of Africa into Eurasia, drastic climate differences could have continued to exert a selective force on cutaneous pigmentation (Bobe et al., 2002; Chaplin, 2004; Relethford, 1997), differentiating these populations according to where they settled.

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LITERATURE CITED

- Aberg KM, Man MQ, Gallo RL, Ganz T, Crumrine D, Brown BE, Choi EH, Kim DK, Schroder JM, Feingold KR, Elias PM. Co-regulation and interdependence of the mammalian epidermal permeability and antimicrobial barriers. *J Invest Dermatol.* 2008; 128:917–925. [PubMed: 17943185]
- Aoki K. Sexual selection as a cause of human skin colour variation: Darwin's hypothesis revisited. *Ann Hum Biol.* 2002; 29:589–608. [PubMed: 12573076]
- Behne MJ, Meyer JW, Hanson KM, Barry NP, Murata S, Crumrine D, Clegg RW, Gratton E, Holleran WM, Elias PM, Mauro TM. NHE1 regulates the stratum corneum permeability barrier homeostasis. Micro-environment acidification assessed with fluorescence lifetime imaging. *J Biol Chem.* 2002; 277:47399–47406. [PubMed: 12221084]
- Blois MS. Vitamin D, sunlight, and natural selection. *Science.* 1968; 159:652. [PubMed: 5716140]
- Blum HF. Does the melanin pigment of human skin have adaptive value? An essay in human skin have adaptive value? An essay in human ecology and the evolution of race. *Q Rev Biol.* 1961; 36:50–63. [PubMed: 13870200]
- Bobe R, Behrensmeyer AK, Chapman RE. Faunal change, environmental variability and late Pliocene hominin evolution. *J Hum Evol.* 2002; 42:475–497. [PubMed: 11908957]
- Braff MH, Gallo RL. Antimicrobial peptides: an essential component of the skin defensive barrier. *Curr Top Microbiol Immunol.* 2006; 306:91–110. [PubMed: 16909919]
- Branda RF, Eaton JW. Skin color and nutrient photolysis: an evolutionary hypothesis. *Science.* 1978; 201:625–626. [PubMed: 675247]
- Brissette JL, Li J, Kamimura J, Lee D, Dotto GP. The product of the mouse nude locus, Whn, regulates the balance between epithelial cell growth and differentiation. *Genes Dev.* 1996; 10:2212–2221. [PubMed: 8804315]
- Chakraborty AK, Funasaka Y, Slominski A, Ermak G, Hwang J, Pawelek JM, Ichihashi M. Production and release of proopiomelanocortin (POMC) derived peptides by human melanocytes and keratinocytes in culture: regulation by ultraviolet B. *Biochim Biophys Acta.* 1996; 1313:130–138. [PubMed: 8781560]
- Chaplin G. Geographic distribution of environmental factors influencing human skin coloration. *Am J Phys Anthropol.* 2004; 125:292–302. [PubMed: 15386260]
- Chaplin G, Jablonski NG. Vitamin D and the evolution of human depigmentation. *Am J Phys Anthropol.* 2009; 139:451–461. [PubMed: 19425101]

- Cohen AS, Stone JR, Beuning KR, Park LE, Reinthal PN, Dettman D, Scholz CA, Johnson TC, King JW, Talbot MR, Brown ET, Ivory SJ. Ecological consequences of early Late Pleistocene megadroughts in tropical Africa. *Proc Natl Acad Sci USA*. 2007; 104:16422–16427. [PubMed: 17925446]
- Cork MJ, Robinson DA, Vasilopoulos Y, Ferguson A, Moustafa M, MacGowan A, Duff GW, Ward SJ, Tazi-Ahnini R. New perspectives on epidermal barrier dysfunction in atopic dermatitis: gene-environment interactions. *J Allergy Clin Immunol*. 2006; 118:3–21. quiz 22–23. [PubMed: 16815133]
- Cui R, Widlund HR, Feige E, Lin JY, Wilensky DL, Igras VE, D'Orazio J, Fung CY, Schanbacher CF, Granter SR, Fisher DE. Central role of p53 in the suntan response and pathologic hyperpigmentation. *Cell*. 2007; 128:853–864. [PubMed: 17350573]
- D'Orazio JA, Nobuhisa T, Cui R, Arya M, Spry M, Wakamatsu K, Igras V, Kunisada T, Granter SR, Nishimura EK, Ito S, Fisher DE. Topical drug rescue strategy and skin protection based on the role of Mc1r in UV-induced tanning. *Nature*. 2006; 443:340–344. [PubMed: 16988713]
- DeMenocal PB. African climate change and faunal evolution during the Pliocene-Pleistocene. *Earth Planet Sci Lett*. 2004; 220:3–24.
- Denda M, Sato J, Masuda Y, Tsuchiya T, Koyama J, Kuramoto M, Elias PM, Feingold KR. Exposure to a dry environment enhances epidermal permeability barrier function. *J Invest Dermatol*. 1998a; 111:858–863. [PubMed: 9804350]
- Denda M, Sato J, Tsuchiya T, Elias PM, Feingold KR. Low humidity stimulates epidermal DNA synthesis and amplifies the hyper-proliferative response to barrier disruption: implication for seasonal exacerbations of inflammatory dermatoses. *J Invest Dermatol*. 1998b; 111:873–878. [PubMed: 9804352]
- Denda M, Sokabe T, Fukumi-Tominaga T, Tominaga M. Effects of skin surface temperature on epidermal permeability barrier homeostasis. *J Invest Dermatol*. 2007; 127:654–659. [PubMed: 17068482]
- Diamond J. Evolutionary biology: geography and skin colour. *Nature*. 2005; 435:283–284. [PubMed: 15902239]
- Drake DR, Brogden KA, Dawson DV, Wertz PW. Thematic review series: skin lipids. Antimicrobial lipids at the skin surface. *J Lipid Res*. 2008; 49:4–11. [PubMed: 17906220]
- Draper R. Black Pharaohs. *Nat Geogr*. 2008; 213:34–59.
- Elias PM. The skin barrier as an innate immune element. *Semin Immunopathol*. 2007; 29:3–14. [PubMed: 17621950]
- Elias PM, Choi EH. Interactions among stratum corneum defensive functions. *Exp Dermatol*. 2005; 14:719–726. [PubMed: 16176279]
- Elias PM, Hatano Y, Williams ML. Basis for the barrier abnormality in atopic dermatitis: outside-inside-outside pathogenic mechanisms. *J Allergy Clin Immunol*. 2008; 121:1337–1343. [PubMed: 18329087]
- Elias PM, Menon GK. Structural and lipid biochemical correlates of the epidermal permeability barrier. *Adv Lipid Res*. 1991; 24:1–26. [PubMed: 1763710]
- Feingold KR, Schmuth M, Elias PM. The regulation of permeability barrier homeostasis. *J Invest Dermatol*. 2007; 127:1574–1576. [PubMed: 17568800]
- Finch, C. The biology of human longevity: inflammation, nutrition and aging in the evolution of lifespans. Vol. Chapter 6.2. Burlington, MA: Academic Press; 2007. The human life span: present, past, and future; p. 376–402.
- Fluhr JW, Kao J, Jain M, Ahn SK, Feingold KR, Elias PM. Generation of free fatty acids from phospholipids regulates stratum corneum acidification and integrity. *J Invest Dermatol*. 2001; 117:44–51. [PubMed: 11442748]
- Frost P. Human skin color: a possible relationship between its sexual dimorphism and its social perception. *Perspect Biol Med*. 1998; 32:38–58. [PubMed: 3059317]
- Funasaka Y, Chakraborty AK, Hayashi Y, Komoto M, Ohashi A, Nagahama M, Inoue Y, Pawelek J, Ichihashi M. Modulation of melanocyte-stimulating hormone receptor expression on normal human melanocytes: evidence for a regulatory role of ultraviolet B, interleukin-1 α ,

- interleukin-1 β , endothelin-1 and tumour necrosis factor- α . *Br J Dermatol*. 1998; 139:216–224. [PubMed: 9767234]
- Gambichler T, Boms S, Stucker M, Moussa G, Kreuter A, Sand M, Sand D, Altmeyer P, Hoffmann K. Acute skin alterations following ultraviolet radiation investigated by optical coherence tomography and histology. *Arch Dermatol Res*. 2005; 297:218–225. [PubMed: 16215762]
- Gibbs S, Murli S, De Boer G, Mulder A, Mommaas AM, Ponc M. Melanosome capping of keratinocytes in pigmented reconstructed epidermis—effect of ultraviolet radiation and 3-isobutyl-1-methyl-xanthine on melanogenesis. *Pigment Cell Res*. 2000; 13:458–466. [PubMed: 11153698]
- Goding CR. Melanocytes: the new Black. *Int J Biochem Cell Biol*. 2007; 39:275–279. [PubMed: 17095283]
- Graf J, Hodgson R, van Daal A. Single nucleotide polymorphisms in the MATP gene are associated with normal human pigmentation variation. *Hum Mutat*. 2005; 25:278–284. [PubMed: 15714523]
- Gunatilake R, Schurer NY, Shoo BA, Celli A, Hachem JP, Crumrine D, Sirimanna G, Feingold KR, Mauro TM, Elias PM. pH-regulated mechanisms account for pigment-type differences in epidermal barrier function. *J Invest Dermatol*. 2009; 129:1719–1729. [PubMed: 19177137]
- Hachem JP, Crumrine D, Fluhr J, Brown BE, Feingold KR, Elias PM. pH directly regulates epidermal permeability barrier homeostasis, and stratum corneum integrity/cohesion. *J Invest Dermatol*. 2003; 121:345–353. [PubMed: 12880427]
- Hachem JP, Man MQ, Crumrine D, Uchida Y, Brown BE, Rogiers V, Roseeuw D, Feingold KR, Elias PM. Sustained serine proteases activity by prolonged increase in pH leads to degradation of lipid processing enzymes and profound alterations of barrier function and stratum corneum integrity. *J Invest Dermatol*. 2005; 125:510–520. [PubMed: 16117792]
- Halaban R, Langdon R, Birchall N, Cuono C, Baird A, Scott G, Moellmann G, McGuire J. Basic fibroblast growth factor from human keratinocytes is a natural mitogen for melanocytes. *J Cell Biol*. 1988; 107:1611–1619. [PubMed: 2459134]
- Haratake A, Uchida Y, Schmutz M, Tanno O, Yasuda R, Epstein JH, Elias PM, Holleran WM. UVB-induced alterations in permeability barrier function: roles for epidermal hyperproliferation and thymocyte-mediated response. *J Invest Dermatol*. 1997; 108:769–775. [PubMed: 9129231]
- Harding RM, Healy E, Ray AJ, Ellis NS, Flanagan N, Todd C, Dixon C, Sajantila A, Jackson II, Birch-Machin MA, Rees JL. Evidence for variable selective pressures at MC1R. *Am J Hum Genet*. 2000; 66:1351–1361. [PubMed: 10733465]
- Harris JR. Parental selection: a third selection process in the evolution of human hairlessness and skin color. *Med Hypotheses*. 2006; 66:1053–1059. [PubMed: 16527428]
- Harrison GA. Differences in human pigmentation: measurement, geographic variation, and causes. *J Invest Dermatol*. 1973; 60:418–426. [PubMed: 4575896]
- Hill HZ. The function of melanin or six blind people examine an elephant. *Bioessays*. 1992; 14:49–56. [PubMed: 1546980]
- Hill HZ, Hill GJ. UVA, pheomelanin and the carcinogenesis of melanoma. *Pigment Cell Res*. 2000; 13 (Suppl 8):S140–S144.
- Holick MF, MacLaughlin JA, Clark MB, Holick SA, Potts JT Jr, Anderson RR, Blank IH, Parrish JA, Elias P. Photosynthesis of previtamin D3 in human skin and the physiologic consequences. *Science*. 1980; 210:203–205. [PubMed: 6251551]
- Holick MF, MacLaughlin JA, Doppelt SH. Regulation of cutaneous previtamin D3 photosynthesis in man: skin pigment is not an essential regulator. *Science*. 1981; 211:590–593. [PubMed: 6256855]
- Holleran WM, Uchida Y, Halkier-Sorensen L, Haratake A, Hara M, Epstein JH, Elias PM. Structural and biochemical basis for the UVB-induced alterations in epidermal barrier function. *Photodermatol Photoimmunol Photomed*. 1997; 13:117–128. [PubMed: 9453079]
- Jablonski NG, Chaplin G. The evolution of human skin coloration. *J Hum Evol*. 2000; 39:57–106. [PubMed: 10896812]
- Jablonski, NG. *Skin: a natural history*. University of California Press; 2006. p. 281
- Jimbow K, Quevedo WC Jr, Fitzpatrick TB, Szabo G. Some aspects of melanin biology: 1950–1975. *J Invest Dermatol*. 1976; 67:72–89. [PubMed: 819593]

- Jung T, Stingl G. Atopic dermatitis: therapeutic concepts evolving from new pathophysiologic insights. *J Allergy Clin Immunol*. 2008; 122:1074–1081. [PubMed: 18992925]
- Ke Y, Su B, Song X, Lu D, Chen L, Li H, Qi C, Marzuki S, Deka R, Underhill P, Xiao C, Shriver M, Lell J, Wallace D, Wells RS, Seielstad M, Oefner P, Zhu D, Jin J, Huang W, Chakraborty R, Chen Z, Jin L. African origin of modern humans in East Asia: a tale of 12,000 Y chromosomes. *Science*. 2001; 292:1151–1153. [PubMed: 11349147]
- Kobayashi N, Muramatsu T, Yamashina Y, Shirai T, Ohnishi T, Mori T. Melanin reduces ultraviolet-induced DNA damage formation and killing rate in cultured human melanoma cells. *J Invest Dermatol*. 1993; 101:685–689. [PubMed: 8228329]
- Korting HC, Hubner K, Greiner K, Hamm G, Braun-Falco O. Differences in the skin surface pH and bacterial microflora due to the long-term application of synthetic detergent preparations of pH 5.5 and pH 7.0. Results of a crossover trial in healthy volunteers. *Acta Derm Venereol*. 1990; 70:429–431. [PubMed: 1980979]
- Kripke ML. Skin cancer, photoimmunology, and urocanic acid. *Photodermatol*. 1984; 1:161–163. [PubMed: 6531290]
- Kunisada T, Lu SZ, Yoshida H, Nishikawa S, Nishikawa S, Mizoguchi M, Hayashi S, Tyrrell L, Williams DA, Wang X, Longley BJ. Murine cutaneous mastocytosis and epidermal melanocytosis induced by keratinocyte expression of transgenic stem cell factor. *J Exp Med*. 1998; 187:1565–1573. [PubMed: 9584135]
- Lahr MM, Foley RA. Towards a theory of modern human origins: geography, demography, and diversity in recent human evolution. *Am J Phys Anthropol Suppl*. 1998; 27:137–176.
- Lamason RL, Mohideen MA, Mest JR, Wong AC, Norton HL, Aros MC, Jurynec MJ, Mao X, Humphreville VR, Humbert JE, Sinha S, Moore JL, Jagadeeswaran P, Zhao W, Ning G, Makalowska I, McKeigue PM, O'Donnell D, Kittles R, Parra EJ, Mangini NJ, Grunwald DJ, Shriver MD, Canfield VA, Cheng KC. SLC24A5, a putative cation exchanger, affects pigmentation in zebrafish and humans. *Science*. 2005; 310:1782–1786. [PubMed: 16357253]
- Lao O, de Gruijter JM, van Duijn K, Navarro A, Kayser M. Signatures of positive selection in genes associated with human skin pigmentation as revealed from analyses of single nucleotide polymorphisms. *Ann Hum Genet*. 2007; 71(Part 3):354–369. [PubMed: 17233754]
- Li L, Hu DN, Zhao H, McCormick SA, Nordlund JJ, Boissy RE. Uveal melanocytes do not respond to or express receptors for alpha-melanocyte-stimulating hormone. *Invest Ophthalmol Vis Sci*. 2006; 47:4507–4512. [PubMed: 17003446]
- Lin JY, Fisher DE. Melanocyte biology and skin pigmentation. *Nature*. 2007; 445:843–850. [PubMed: 17314970]
- Liou A, Elias PM, Grunfeld C, Feingold KR, Wood LC. Amphiregulin and nerve growth factor expression are regulated by barrier status in murine epidermis. *J Invest Dermatol*. 1997; 108:73–77. [PubMed: 8980291]
- Longley BJ Jr, Morganroth GS, Tyrrell L, Ding TG, Anderson DM, Williams DE, Halaban R. Altered metabolism of mast-cell growth factor (c-kit ligand) in cutaneous mastocytosis. *N Engl J Med*. 1993; 328:1302–1307. [PubMed: 7682288]
- Loomis WF. Skin-pigment regulation of vitamin-D biosynthesis in man. *Science*. 1967; 157:501–506. [PubMed: 6028915]
- Mackintosh JA. The antimicrobial properties of melanocytes, melanosomes and melanin and the evolution of black skin. *J Theor Biol*. 2001; 211:101–113. [PubMed: 11419954]
- Madison KC. Barrier function of the skin: “la raison d’être” of the epidermis. *J Invest Dermatol*. 2003; 121:231–241. [PubMed: 12880413]
- Man MQ, Feingold KR, Jain M, Elias PM. Extracellular processing of phospholipids is required for permeability barrier homeostasis. *J Lipid Res*. 1995; 36:1925–1935. [PubMed: 8558081]
- Mathur U, Datta SL, Mathur BB. The effect of aminopterin-induced folic acid deficiency on spermatogenesis. *Fertil Steril*. 1977; 28:1356–1360. [PubMed: 590546]
- Menon GK, Baptista LF, Brown BE, Elias PM. Avian epidermal differentiation. II. Adaptive response of permeability barrier to water deprivation and replenishment. *Tissue Cell*. 1989; 21:83–92. [PubMed: 2772910]

- Miller CT, Beleza S, Pollen AA, Schluter D, Kittles RA, Shriver MD, Kingsley DM. cis-Regulatory changes in Kit ligand expression and parallel evolution of pigmentation in sticklebacks and humans. *Cell*. 2007; 131:1179–1189. [PubMed: 18083106]
- Miller SJ, Aly R, Shinefeld HR, Elias PM. In vitro and in vivo anti-staphylococcal activity of human stratum corneum lipids. *Arch Dermatol*. 1988; 124:209–215. [PubMed: 3341800]
- Montefiori DC, Zhou JY. Selective antiviral activity of synthetic soluble L-tyrosine and L-dopa melanins against human immunodeficiency virus in vitro. *Antiviral Res*. 1991; 15:11–25. [PubMed: 1709802]
- Moskowitz DG, Fowler AJ, Heyman MB, Cohen SP, Crumrine D, Elias PM, Williams ML. Pathophysiologic basis for growth failure in children with ichthyosis: an evaluation of cutaneous ultrastructure, epidermal permeability barrier function, and energy expenditure. *J Pediatr*. 2004; 145:82–92. [PubMed: 15238912]
- Murray F. Pigmentation, sunlight, and nutritional disease. *Am Anthropol*. 1934; 36:438–445.
- Neer RM. The evolutionary significance of vitamin D, skin pigment, and ultraviolet light. *Am J Phys Anthropol*. 1975; 43:409–416. [PubMed: 1211435]
- Niggli HJ. Comparative studies on the correlation between pyrimidine dimer formation and tyrosinase activity in cloudman S91 melanoma cells after ultraviolet-irradiation. *Photochem Photobiol*. 1990; 52:519–524. [PubMed: 2126628]
- Orlow SJ. Melanosomes are specialized members of the lysosomal lineage of organelles. *J Invest Dermatol*. 1995; 105:3–7. [PubMed: 7615972]
- Osborne AH, Vance D, Rohling EJ, Barton N, Rogerson M, Fello N. A humid corridor across the Sahara for the migration of early modern humans out of Africa 120,000 years ago. *Proc Natl Acad Sci USA*. 2008; 105:16444–16447. [PubMed: 18936490]
- Parra EJ. Human pigmentation variation: evolution, genetic basis, and implications for public health. *Am J Phys Anthropol Suppl*. 2007; 45:85–105.
- Peltier W, Fairbanks R. Global glacial ice volume and last glacial maximum duration from an extended barbados sea level record. *Q Sci Rev*. 2006; 25:3322–3337.
- Quevedo WC, Fitzpatrick TB, Pathak MA, Jimbow K. Role of light in human skin color variation. *Am J Phys Anthropol*. 1975; 43:393–408. [PubMed: 1211434]
- Rana BK, Hewett-Emmett D, Jin L, Chang BH, Sambuughin N, Lin M, Watkins S, Bamshad M, Jorde LB, Ramsay M, Jenkins T, Li WH. High polymorphism at the human melanocortin 1 receptor locus. *Genetics*. 1999; 151:1547–1557. [PubMed: 10101176]
- Reed JT, Ghadially R, Elias PM. Skin type, but neither race nor gender, influence epidermal permeability barrier function. *Arch Dermatol*. 1995; 131:1134–1138. [PubMed: 7574829]
- Reichrath J. Vitamin D and the skin: an ancient friend, revisited. *Exp Dermatol*. 2007; 16:618–625. [PubMed: 17576242]
- Relethford JH. Hemispheric difference in human skin color. *Am J Phys Anthropol*. 1997; 104:449–457. [PubMed: 9453695]
- Richmond BG, Jungers WL. Orrorin tugenensis femoral morphology and the evolution of hominin bipedalism. *Science*. 2008; 319:1662–1665. [PubMed: 18356526]
- Robins, A. Biological perspectives on human pigmentation. Cambridge: Cambridge University Press; 1991.
- Schallreuter KU, Kothari S, Chavan B, Spencer JD. Regulation of melanogenesis—controversies and new concepts. *Exp Dermatol*. 2008; 17:395–404. [PubMed: 18177348]
- Schauber J, Gallo RL. Antimicrobial peptides and the skin immune defense system. *J Allergy Clin Immunol*. 2008; 122:261–266. [PubMed: 18439663]
- Schauer E, Trautinger F, Kock A, Schwarz A, Bhardwaj R, Simon M, Ansel JC, Schwarz T, Luger TA. Proopiomelanocortin-derived peptides are synthesized and released by human keratinocytes. *J Clin Invest*. 1994; 93:2258–2262. [PubMed: 8182158]
- Schmuth M, Jiang YJ, Dubrac S, Elias PM, Feingold KR. Thematic review series: skin lipids. Peroxisome proliferator-activated receptors and liver X receptors in epidermal biology. *J Lipid Res*. 2008; 49:499–509. [PubMed: 18182682]

- Schroder JM, Harder J. Antimicrobial skin peptides and proteins. *Cell Mol Life Sci.* 2006; 63:469–486. [PubMed: 16416029]
- Scott MC, Suzuki I, Abdel-Malek ZA. Regulation of the human melanocortin 1 receptor expression in epidermal melanocytes by paracrine and endocrine factors and by ultraviolet radiation. *Pigment Cell Res.* 2002; 15:433–439. [PubMed: 12453185]
- Slominski A, Paus R, Wortsman J. On the potential role of proopiomelanocortin in skin physiology and pathology. *Mol Cell Endocrinol.* 1993; 93:C1–C6. [PubMed: 8391494]
- Soejima M, Koda Y. Population differences of two coding SNPs in pigmentation-related genes SLC24A5 and SLC45A2. *Int J Legal Med.* 2007; 121:36–39. [PubMed: 16847698]
- Staricco RG, Miller-Milinska A. Activation of the amelanotic melanocytes in the outer root sheath of the hair follicle following ultra violet rays exposure. *J Invest Dermatol.* 1962; 39:163–164. [PubMed: 13983439]
- Steinert PM. The complexity and redundancy of epithelial barrier function. *J Cell Biol.* 2000; 151:F5–F8. [PubMed: 11038193]
- Stokowski RP, Pant PV, Dadd T, Fereday A, Hinds DA, Jarman C, Filsell W, Ginger RS, Green MR, van der Ouderaa FJ, Cox DR. A genome-wide association study of skin pigmentation in a South Asian population. *Am J Hum Genet.* 2007; 81:1119–1132. [PubMed: 17999355]
- Szabo G, Gerald AB, Pathak MA, Fitzpatrick TB. Racial differences in the fate of melanosomes in human epidermis. *Nature.* 1969; 222:1081–1082. [PubMed: 5787098]
- Takeda K, Takahashi NH, Shibahara S. Neuroendocrine functions of melanocytes: beyond the skin-deep melanin maker. *Tohoku J Exp Med.* 2007; 211:201–221. [PubMed: 17347546]
- Thomson ML. Relative efficiency of pigment and horny layer thickness in protecting the skin of Europeans and Africans against solar ultraviolet radiation. *J Physiol.* 1955; 127:236–246. [PubMed: 14354667]
- Thong HY, Jee SH, Sun CC, Boissy RE. The patterns of melanosome distribution in keratinocytes of human skin as one determining factor of skin colour. *Br J Dermatol.* 2003; 149:498–505. [PubMed: 14510981]
- Thormar H, Hilmarsson H. The role of microbicidal lipids in host defense against pathogens and their potential as therapeutic agents. *Chem Phys Lipids.* 2007; 150:1–11. [PubMed: 17686469]
- van den Berghe P, Frost P. Skin color preference, sexual dimorphism and sexual selection: a case of gene culture co-evolution? *Ethni Racial Stud.* 1986; 9:87–113.
- Wade, N. *Before the dawn: recovering the lost history of our ancestors.* New York: Penguin Books; 2006.
- Walker GJ, Kimlin MG, Hacker E, Ravishankar S, Muller HK, Beermann F, Hayward NK. Murine neonatal melanocytes exhibit a heightened proliferative response to ultraviolet radiation and migrate to the epidermal basal layer. *J Invest Dermatol.* 2009; 129:184–193.
- Warren R, Gardner PA, Reed JC. Sensitivity of mouse Skh:HR-2 to ultraviolet radiation: melanocyte inactivation. *J Invest Dermatol.* 1987; 88:266–270.
- Wassermann HP. Human pigmentation and environmental adaptation. *Arch Environ Health.* 1965; 11:691–694. [PubMed: 4284803]
- Wehrle-Haller B. The role of kit-ligand in melanocyte development and epidermal homeostasis. *Pigment Cell Res.* 2003; 16:287–296. [PubMed: 12753403]
- Weiner L, Han R, Scicchitano BM, Li J, Hasegawa K, Grossi M, Lee D, Brissette JL. Dedicated epithelial recipient cells determine pigmentation patterns. *Cell.* 2007; 130:932–942. [PubMed: 17803914]
- Westerhof W. Evolutionary, biologic, and social aspects of skin color. *Dermatol Clin.* 2007; 25:293–302. vii. [PubMed: 17662895]
- Wintzen M, Gilchrist BA. Proopiomelanocortin, its derived peptides, and the skin. *J Invest Dermatol.* 1996; 106:3–10. [PubMed: 8592078]
- Wood LC, Jackson SM, Elias PM, Grunfeld C, Feingold KR. Cutaneous barrier perturbation stimulates cytokine production in the epidermis of mice. *J Clin Invest.* 1992; 90:482–487. [PubMed: 1644919]

- Wu CS, Lan CC, Chiou MH, Yu HS. Basic fibroblast growth factor promotes melanocyte migration via increased expression of p125(FAK) on melanocytes. *Acta Derm Venereol.* 2006; 86:498–502. [PubMed: 17106595]
- Yamaguchi Y, Brenner M, Hearing VJ. The regulation of skin pigmentation. *J Biol Chem.* 2007; 282:27557–27561. [PubMed: 17635904]
- Yoshida H, Hayashi S, Shultz LD, Yamamura K, Nishikawa S, Nishikawa S, Kunisada T. Neural and skin cell-specific expression pattern conferred by steel factor regulatory sequence in transgenic mice. *Dev Dyn.* 1996; 207:222–232. [PubMed: 8906425]

Fig. 1.
Diagram of stratum corneum organization: “Bricks” and “Mortar” subserve different functions.

Fig. 2.

Role of pH in maturation of stratum corneum lamellar membrane. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Fig. 3.

Keratinocyte-melanocyte cross-talk: proposed barrier-initiated signals. Nerve growth factor (NGF) and interleukin 1 α (IL-1 α) signal many downstream pigmentary responses, but both also increase in response to stress to the barrier. Recently recognized keratinocyte signals (stem cell factor [SCF], FOXn1 $\rightarrow$ FGF2 [fibroblast growth factor 2], and p53) could be activated not only by UV-B, but also by stress to the barrier from a decreased relative humidity, which could be “sensed” by the transient vanilloid receptor, type 4 (TRPV4). Stressor-induced ligands also increase the melanocortin receptor type 1 (MC1R), which upregulates eumelanin synthesis (tyrosinase, TYR), perhaps by increasing expression of ion/pH transporters that increase melanosome acidification, and/or the melanosome transporter, myosin VA. Transfer of large, single melanosome complexes from melanocyte dendrites (M) to keratinocytes (K) in the outer epidermis further acidifies the stratum corneum (SC). See text for additional abbreviations.

TABLE 1

Hypotheses commonly advanced to explain latitude-dependent differences in pigmentation and latitude-dependent loss of pigmentation

Latitude-dependent differences in pigmentation	
Pigment evolved	Most cogent arguments
To prevent:	
Vitamin D intoxication	Against: ↑ Photoisomerization to inactive isomers with ↑UV-B; 1,25(OH) ₂ Vit D-generation downregulated as serum Ca ⁺⁺ increases
Photodegradation of folic acid	Against: Congenital neural tube defects too rare to influence reproduction rates, even in population at risk
Skin cancer	Against: Occurs too late to influence reproductive success
To improve:	
Antioxidant defense	Against: Melanin is a free radical absorber, but synthetic intermediates are themselves free radicals
Camouflage	No evidence for or against
Sexual display	No evidence for or against
Innate immunity	For: Consistent with present hypothesis <i>Present hypothesis</i>
<i>Barrier function</i>	
Latitude-dependent loss of pigmentation	
Pigment de-evolved	Most cogent arguments
To Promote:	
Cutaneous vitamin D synthesis	Against: No fossil evidence of rickets in early <i>Homo</i> (Industrial Age phenomenon); clothing blocks more UV than pigment; no sufficient cutaneous vitamin D synthesis occurs in dark skin
Sexual selection	Against: Reflects possible cultural bias
Lack of selective advantage—metabolic cost of melanogenesis, or ‘genetic drift’	For: Nonlatitude-determined polymorphisms in several pigment-related genes in East Asians.

TABLE 2

Defensive functions of mammalian stratum corneum

Function	Localization	Structural basis	Biochemical basis
Permeability barrier + xenobiotic penetration	Matrix	Lamellar bilayer	Cer:Chol:FFA (1:1:1)
Antimicrobial defense	Matrix	Lamellar bilayer	LL-37, hBD2 RNase 7, psoriasin, cathelicidin
Cohesion/desquamation	Matrix	Corneodesmosomes	protease/antiprotease; cholesterol sulfate
Mechanical/rigidity	Corneocyte	Cornified envelope	isopeptide (γ -glutamyl α -linking), Ca^{++}
Hydration	Corneocyte	Corneocyte lipid env	ω -OH-ceramides FLG $\rightarrow$ 'NMF'
	Matrix	Sebaceous glands	Glycerol $\rightarrow$ AQP3
UV defense	Corneocyte cytosol	—	Filaggrin $\rightarrow$ trans-urocanic acid
Antioxidant defense	Surface $\rightarrow$	Sebaceous Glands	Vitamin E, CoQ

TABLE 3

Correlation of climatic changes with evolution of pigmentation (modified from Elias et al, Pigment Cell & Melanoma Res, 2009)

Cutaneous changes	Milestones in human evolution	Time(≈)	Climatic and ecological changes
Hairy	First hominid species	>7 mya	Moist woodlands
	Bipedalism	>6 mya	
	Obligate bipedalism	≈3 mya	First open savannahs; Persistent, moist
Hairlessness → Eccrine glands	<i>H. habilis</i> <i>H. erectus</i> , <i>H. neanderthalis</i> (Hunting → ↑protein uptake)	2.5-1.5 mya	
Pigmentation → (≥1.2 mya)	<i>H. ergaster</i> (sophisticated tools)	1.8 mya	Episodic, climatic drying → large, open savannahs
	Exodus to Europe/Asia		
		0.8-0.2 mya	Megadroughts
	<i>H. sapiens</i>	<0.2 mya	Variable climate
		135-90 kya	Extreme aridity & megadroughts
	Initial exodus of <i>H. sapiens</i> (unsuccessful)	<100 kya	Moist corridors in North Africa (Libya)
		90-70 kya	Climate crossover → Less variability
	Next wave of migrations (successful)	≈50 kya	Recurrent droughts
	Further migrations	35-15 kya	Prolonged drought due to glaciation

TABLE 4

Potential barrier-initiated signals of pigmentation

Keratinocyte signal	UV-B regulated	Barrier-regulated	Mechanism(s) leading to ↑ epidermal pigmentation
SCF → KIT	?	?	Follicular melanocytes → interfollicular epidermis; melanocyte persistence/differentiation
FOXn1→FGF2	yes	?	Melanocytes → basal layer
NGF	yes	yes	↓ Melanocyte apoptosis; ↑ melanocyte growth, ↑ melanocyte differentiation; ↑ melanin synthesis
IL-1 α	yes	yes	↑ MC1R → ↑ POMC → α MSH/ACTH → ↑ melanin synthesis → ↑ fibroblast HGF → melanocyte growth
p53	+	probably	↑ POMC → α MSH/ β MSH/ACTH → melanin synthesis