The Pigmentary System: Physiology and Pathophysiology, Second Edition
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17 Photobiology of Melanins

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Summary

Exposure to ultraviolet radiation (UVR) results in long-term deleterious effects such as skin cancer. A well-recognized short-term consequence of UVR is increased skin pigmentation. Skin color is one of most conspicuous ways in which humans vary, yet many aspects regarding the function of melanin remain controversial. Pigmentation, whether constitutive or facultative, has been widely viewed as photoprotective, largely because darkly pigmented skin is at a lower risk of photocarcinogenesis than fair skin. Research is increasingly suggesting that the relationship between pigmentation and photoprotection may be far more complex than previously assumed. For example, photoprotection against erythema and DNA damage has been shown to be independent of the level of induced pigmentation in human white skin types. Growing evidence now suggests that UVR-induced DNA photodamage, and its repair, is one of the signals that stimulates melanogenesis. These findings suggest that tanning may be a measure of inducible DNA repair capacity, and it is this rather than pigment *per se* that results in the lower incidence of skin cancer observed in darker skinned individuals. This is supported by some studies that suggest that repeated UVR exposure in skin types IV, who tan well, results in faster DNA repair in comparison with skin types II, who tan poorly. It has been suggested that epidermal pigmentation may in fact be the mammalian equivalent of a bacterial SOS response.

Introduction

The color of human skin is largely determined by its epidermal melanin content, whether this melanin is constitutively expressed or induced by UVR from the sun or a tanning device. Melanin production, or melanogenesis, occurs in highly specialized dendritic melanocytes that account for about 1% of epidermal cells. Each basal layer melanocyte is associated with about 36 keratinocytes and one Langerhans cell, and this is known as the epidermal melanin unit (EMU) (Fitzpatrick and Breathnach, 1963). Melanogenesis is described in detail in Chapter 14. The composition of melanin, the end-product of melanogenesis that is transferred to adjacent keratinocytes, is still incompletely understood but is a variable mixture of lighter/reddish/yellowish alkali-soluble, sulfur-containing pheomelanin and darker brownish/blackish insoluble eumelanin. In both cases, the rate-limiting step is the oxidation of tyrosine by tyrosinase in a series of reactions known as the Raper–Mason pathway. Eumelanogenesis leads to the formation of indole derivatives that include 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA), formed from the oxidation of the 1,4 addition product of tyrosinase-generated dopaquinone. Intermediates in pheomelanogenesis include cysteinyldopas. Indole and cysteinyldopa precursors and their related *o*-methyl derivatives are released into the epidermis during periods of melanogenic activity induced by UVR or psoralen plus UVA (PUVA). Their lipophilic nature suggests a long half-life in lipophilic dermal and epidermal tissue.

Skin color is a consequence of the mix of melanin types and possibly also the way that melanin is packaged in melanosomes, highly specialized melanocyte-derived organelles that are used for the transfer of melanin to keratinocytes via the melanocyte dendrites. Caucasian melanosomes have a long axis of about 400 nm and tend to occur in groups of 3–8, whereas Negroid melanosomes are much longer (800 nm length) and exist singly (Kollias *et al.*, 1991). Melanocyte density is independent of race (Halaban *et al.*, 2003) but varies with body site, with densities ranging from 2000/mm^2 in the head or forearm to 1000/mm^2 elsewhere. Recent studies indicate that the distribution of melanosomes within keratinocytes (Thong *et al.*, 2003) and melanosome size (Alaluf *et al.*, 2003) play a role in skin color. Melanocyte numbers in non-sun-exposed skin show an age-related decline with an approximately 8–10% reduction per decade (Halaban *et al.*, 2003).

There is considerable intercellular chemical communication between melanocytes and keratinocytes and, to lesser extent, fibroblasts, neurons, mast cells, and other skin cells. Keratinocytes produce a wide range of mitogenic [e.g. basic fibroblast growth factor (bFGF), transforming growth factor (TGF)α] and inhibitory [e.g. interleukin (IL)1, IL6, TGFβ] factors for melanocytes. In addition, the proliferation of melanocytes, melanogenesis, and the transfer of pigment also rely on hormonal controls [α-melanocyte-stimulating hormone (MSH), sex hormones), agouti signal protein, and inflammatory mediators in skin (Chu *et al.*, 2003; Gilchrest *et al.*, 1996). The melanocyte plasma membrane is also thought to be a primary UVR target with the release of membrane-bound factors, such as arachidonic acid and diacylglycerol (DAG), leading to activation of tyrosinase via a protein kinase C (PKC)-mediated pathway (Gilchrest *et al.*, 1996). The behavior of isolated melanocytes *in vitro* is different from their behavior as part of an EMU. For example, recent studies have shown that the pheomelanin/eumelanin ratio is regulated by keratinocytes (Duval *et al.*, 2002). However, it has also been reported that melanosomes from different skin types maintain their melanin type preferences in

melanocytes stimulated with tyrosine *in vitro* (van Nieuwpoort *et al.*, 2004).

The Effects of Solar UVR on Human Skin

The detrimental effects of solar UVR (~295–400 nm) on the skin are well established and are usually categorized as either acute or chronic. Acute effects include DNA (Bykov *et al.*, 1999) and oxidative damage (Sander *et al.*, 2004), mutation (Ziegler *et al.,* 1994), immunosuppression (Ullrich, 2000), and erythema (sunburn) (Harrison and Young, 2002). Molecular biology is increasingly showing that UVR induces the expression of a large number of genes, the physiological consequence of which is poorly understood. Tanning is also an acute effect, and it is debatable whether this is a detrimental or beneficial effect. The chronic effects include skin cancers, which are thought to be a consequence of mutation and immunosuppression (Ullrich, 2002; Ziegler *et al.*, 1994), and photoaging, which is thought to be a consequence of the induction of matrix metalloproteinases (MMPs) (Fisher *et al.*, 2002).

Skin darkening in response to solar UVR occurs via two distinct mechanisms: immediate pigment darkening (IPD) and delayed tanning (DT). Both processes are influenced by genetic factors and are more pronounced with darker constitutive pigmentation. The action spectrum for IPD shows a broad peak in the UVA region (Irwin *et al.*, 1993) and is completely different from the action spectrum for DT (Parrish *et al.*, 1982), which indicates that they are mechanistically different processes. There is a range of non-invasive optical techniques to measure skin pigmentation *in vivo*, but all have disadvantages of various types (Stamatas *et al.*, 2004) and none is in routine use.

IPD starts during UV irradiation as a grayish coloration that gradually fades to a brown color over a period of minutes to days depending on UVR dose and individual complexion. These changes are not due to new melanin synthesis but rather oxidation of pre-existing melanin and redistribution of melanosomes from a perinuclear to a peripheral dendritic location (Routaboul *et al.*, 1999). The color change may be so subtle as to be almost undetectable in fair-skinned individuals but is easily observed in skin types IV (or darker). The transient nature of IPD has made understanding of this phenomenon difficult. *In vivo* reflectance spectroscopy showed a UVA (365 nm) dose-dependent induction of IPD with increased absorbance between 620 and 720 nm (Rosen *et al.*, 1990), similar to that expected from increased native melanin. However, at shorter wavelengths (410–610 nm), the absorbance is less than expected from increased native melanin. Significantly, no photoprotective effect for IPD has been established; hence, its biological function remains unknown.

DT, which results from melanogenesis, is associated with increased melanocyte activity and proliferation. It is evident 3–4 days after UVR exposure and is maximal from 10 days to 3–4 weeks depending on complexion and UVR dose. It may take several weeks for the skin to return to its base constitutive color. UVA-induced DT is two or three orders of magnitude less efficient per unit dose than UVB and has an earlier onset, often directly after IPD. Furthermore, it has a different pathophysiology (Eller and Gilchrest, 2000; Lavker and Kaidbey, 1982) that, unlike UVB, is oxygen dependent.

The only widely recognized beneficial effect of solar UVR exposure is epidermal vitamin D photosynthesis. Sunlight is believed to be the body's major source of vitamin D as few people consume enough food that is naturally rich in vitamin D to meet their dietary requirements. Solar UVB (295–310 nm) converts 7-dehydrocholesterol in the skin to vitamin D_3 (cholecalciferol), a prohormone with no intrinsic biological activity. Vitamin D_3 is metabolized in the liver to 25-hydroxyvitamin D (25(OH)D). Plasma levels of this metabolite are the hallmark for determining vitamin D status as it is used as the substrate for producing the biologically active steroid hormone 1,25-dihydroxyvitamin D. In temperate regions, there is insufficient UVB in the winter months to synthesize vitamin D, and therefore there is a seasonal variation in plasma 25-(OH)D concentrations, with winter levels dependent upon the amount of vitamin D stored in adipose tissue during the previous summer (Devgun *et al.*, 1981; Vieth *et al.*, 2001; Webb *et al.*, 1988). Maintenance of optimal levels of 25(OH)D are essential for bone health, but some epidemiological and intervention trials suggest that vitamin D deficiency may increase the risk of some internal malignancies and autoimmune disorders (Zittermann, 2003).

Skin Chromophores and Their Relationship to Photobiology

The UVR-absorbing properties of skin depend on its natural chromophores, which include DNA, amino acids, urocanic acid, and melanins and their precursors (Young, 1997). Absorption of UVR energy by chromophores may initiate photochemical events that are the basis of all skin photobiology. Chromophores, especially those in the upper epidermis, may also attenuate UVR and thus protect the structure beneath from photodamage. For example, nonsolar UVC radiation (100–280 nm) causes very little damage to DNA in cells of the basal layer because of its attenuation by DNA in the cells above and urocanic acid in the stratum corneum (Chadwick *et al.*, 1995). In contrast, solar-range UVB and UVA radiation readily results in damage to DNA of keratinocytes and melanocytes in the basal layer of human skin *in vivo* (Young *et al.*, 1998a).

The emission spectrum of the sun is rich in UVA (315–400 nm) radiation with UVB (280–315 nm) radiation accounting for less than 5% of total UVR content under most conditions. However, because most skin chromophores are primarily UVB absorbers, it is that part of the solar spectrum

that causes most of the biological effects described above. For example, action spectrum (wavelength dependence) studies have shown that UVB is three to four orders of magnitude more effective per unit physical dose (J/cm^2) than UVA for DNA photodamage (Young *et al.*, 1998b), erythema (Parrish *et al.*, 1982; Young *et al.*, 1998b), tanning (Parrish *et al.*, 1982), and skin cancer in mice (de Gruijl, 1995). However, UVA may be more important for indirect damage to cells caused by oxidative stress. This is caused by reactive oxygen species (ROS) that are generated when UVA (and also UVB) (Sander *et al.*, 2004) is absorbed by as yet poorly defined chromophores that may include flavins and porphyrins.

The chromophore(s) for melanogenesis have not been established with certainty, but there is an increasing body of indirect and direct evidence that supports a major role for DNA (Eller and Gilchrest, 2000). Human studies have shown that the action spectra for DNA photodamage, as assessed by cyclobutane pyrimidine dimers (CPD), and tanning are very similar (Parrish *et al.*, 1982; Young *et al.*, 1998b) as shown in Figure 17.1. This suggests that the photochemical event that initiates tanning may be a consequence of DNA photodamage. Figure 17.2 summarizes some of the photobiological events that initiate melanogenesis.

Skin Type and Melanin

The skin's constitutive melanin content and its melanogenic response to solar UVR form part of the basis of the skin type clinical classification shown in Table 17.1. This scheme was originally devised to optimize UVR doses in phototherapy. Sensitivity to sunburn is routinely evaluated by minimal erythema dose (MED) determination, the MED being the lowest dose (J/cm^2) of UVR that will cause erythema assessed at 24 h. In general, tanning capacity is inversely related to MED, but there is a considerable degree of MED overlap within white skin types I–IV (Harrison and Young, 2002). In other words, MED is not a reliable index for predicting skin type.

Investigators have determined the quantitative and qualitative melanin content in human hair and skin *in vivo* and, in some cases, after skin exposure to UVR (for a review, see Ito and Wakamatsu, 2003). The first study of epidermis *in vivo* was by Thody *et al.* (1991) in skin types I, II, and III, in which there was a positive correlation between skin type and eumelanin but not pheomelanin. However, the ratio of eumelanin/pheomelanin shows considerable variation, especially in skin types II and III. The level of tanning by PUVA

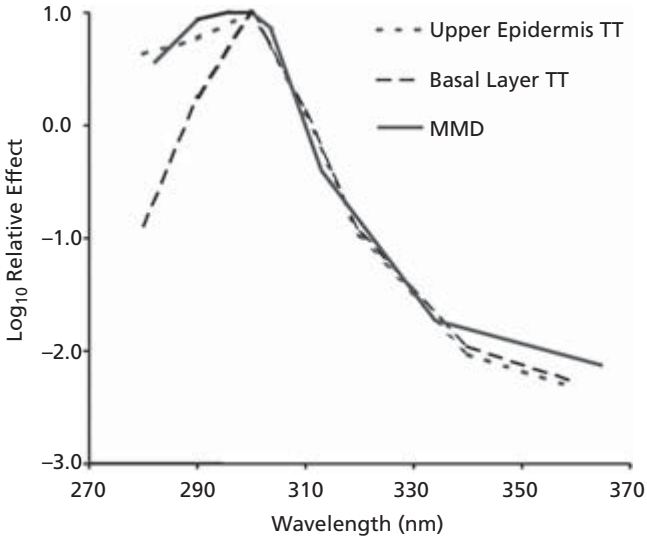

Fig. 17.1. Human action spectra for epidermal DNA photodamage (TT = thymine dimers; data from Young *et al.*, 1998b) and tanning expressed as minimal melanogenic dose (MMD) assessed at 7–14 days (data from Parrish *et al.*, 1982). The TT action spectra show epidermal layer dependence at wavelengths less than 300 nm, probably because of marked UVR attenuation by epidermal chromophores at these wavelengths. These TT and MMD spectra are very similar, which suggests that DNA is an important chromophore for melanogenesis and in particular DNA from the upper epidermis. This suggests the possibility that damage to keratinocytes, with the release of melanogenic factors (see Fig. 17.2), may be more important than direct damage to melanocytes that reside in the basal layer region.

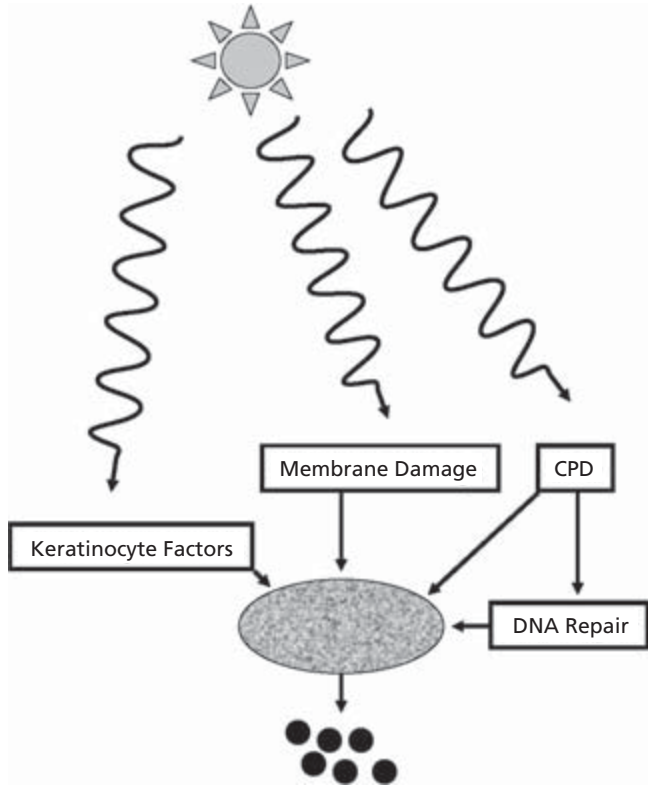

Fig. 17.2. Multiple photobiological events initiate melanin synthesis. These include DNA photodamage (CPD) and its repair, membrane damage, and factors from other epidermal cells, especially keratinocytes.

Table 17.1. Classification of human skin types with respect to relative response to acute and long-term solar exposure.

Skin type	Susceptibility to sunburn	Constitutive skin color	Facultative tanning ability	Susceptibility to skin cancer
I	High	White	Very poor	High
II	High	White	Poor	High
III	Moderate	White	Good	Moderate
IV	Low	Olive	Very good	Low
V	Very low	Brown	Very good	Very low
VI	Very low	Black	Very good	Very low

was correlated with eumelanin but not pheomelanin. Alaluf *et al.* (2001) evaluated melanin content in sun-protected and -exposed sites of skin types V and IV. In comparison with DHICA and DHI eumelanins, pheomelanin content was very low. Solar exposure enhanced eumelanin content, in particular predominant DHI eumelanin, but had no effect on pheomelanin levels. In further studies, these authors (Alaluf *et al.*, 2002) assessed epidermal melanin content in five different ethnic groups using alkali solubility as a means of separating the lighter pheomelanin and DHICA eumelanin from the darker DHI eumelanin on sun-protected and -exposed skins. The difference in total melanin content between the lightest European skin and the darkest African skins was only about twofold in both skin sites, which is consistent with previously reported skin type-dependent differences in tyrosinase activity (Iwata *et al.*, 1990; Pomerantz and Ances, 1975). The data also showed that darker skin was associated with greater quantities of alkali-insoluble melanins, and lighter skins were associated with greater quantities of alkali-soluble melanins. The level of melanin, however assessed, was always higher ($P < 0.01$) in all study populations on sun-exposed sites by factors ranging from 1.3 to 1.9, with the differences in alkali-soluble melanin (1.3–1.5) being smaller than those of alkali-insoluble melanin (1.5–1.9). The 1.8-fold difference in total melanin in sun-exposed and -protected African skin is about the same as the differences in African and European skin on their respective sun-protected and -exposed sites. The darker skins have the lowest values when the data are expressed as % alkali-soluble melanin, and this is reduced slightly (1.2–1.3) by sun exposure in African and Indian skins only. Overall, these data support a role for eumelanin in sun tolerance as indicated by skin type. However, they also show that skin type differences in skin color and the degree of photoprotection are unlikely to be accounted for by differences in melanin alone, and Alaluf *et al.* (2002) have suggested that this may also be related to melanosome size.

Photoprotection by Melanin

The primary function of skin melanin has not yet been established. A number of roles have been proposed that include photoprotection, thermoregulation, antibiotic, cation chelator, free radical sink, and by-product of the scavenging of the superoxide radical in the skin by tyrosinase (Giacomoni, 1995; Hill and Hill, 2000; Morison, 1985). It is often stated that melanin is photoprotective because people with constitutively pigmented skins [e.g. types V (brown) or VI (black)] or skin that tans well (e.g. Mediterranean type IV) have much lower incidences of skin cancer that white-skinned people who tan poorly if at all (e.g. types I and II). Indeed, epidemiological studies confirm an inverse correlation between skin cancer incidence and pigmentation with age-adjusted male cancer incidence at 3.4 per 100 000 for blacks and 232.6 per 100 000 for Caucasian whites in the USA (Scotto and Fraummeni, 1982). At its simplest, this argument assumes that melanin or melanogenesis is the only factor that determines the level of a given photobiological response, e.g. skin cancer, to a given physical dose of UVR. However, there is clear evidence that this is not the case. For example, skin types I/II are more readily immunosuppressed than skin types III/IV when compared on the basis of physical or erythemal dose (Kelly *et al.*, 2000). These studies showed that suberythemal doses of solar simulated radiation (SSR) suppressed the sun-sensitive skin types I/II but erythemal doses were necessary to suppress the sun-tolerant skin types III/IV. Furthermore, there is evidence for skin type-dependent differences in responses to oxidative stress (Kerb *et al.*, 1997), which is thought to play a role in cancer (Sander *et al.*, 2004).

A photoprotective role for melanin, particularly against skin cancer, was first proposed by Home (1820). The Darwinian argument to support this hypothesis would require skin cancer to interfere with reproduction and child-rearing. Blum (1961) dismissed this argument and noted that nonmelanoma skin cancers generally occur after the reproductive age and are rarely fatal. Malignant melanomas are more fatal but account for only 4% of skin cancers and also usually occur after reproduction. Studies in Nigerian albinos show that, even in an extreme solar environment, skin cancer did not result in death below the age of 25 years (Okoro, 1975).

In 2000, a landmark study examined the relationship between UVR levels, skin reflectance (a marker of melanin), vitamin D, and folate levels at different latitudes (Jablonski and Chaplin, 2000). Predicted skin reflectance based on UVR levels was noted to correlate closely, and a strong correlation between skin reflectance, absolute latitude, and UVR was

demonstrated. It was concluded that the gradient between UVR and constitutive pigmentation was a compromise between the beneficial effect of vitamin D photosynthesis and the deleterious effect of folate photolysis that would interfere with reproduction.

In reality, little is in fact known about the relationship between solar UVR and vitamin D status. Population surveys have shown that vitamin D deficiency is more common among institutionalized, elderly individuals with pigmented skin and those who habitually cover the skin with clothing (Bischoff-Ferrari *et al.*, 2004; Devgun *et al.*, 1981; Vieth *et al.*, 2001).

Suberythemal UVB is a potent stimulus of vitamin D synthesis in white-skinned subjects *in vivo*, who show a ninefold increase in circulating vitamin D_3 after a single whole-body exposure of about 0.75 MED (Matsuoka *et al.*, 1989, 1990, 1991). Skin pigmentation is believed to greatly reduce the UVR-mediated synthesis of vitamin D_3, with black subjects ($n = 1$) requiring at least a sixfold greater UVR dose to increase circulating levels of vitamin D_3 than whites ($n = 2$) (Clemens *et al.*, 1982). This finding, based on a very small study, was not confirmed in a later study. Matsuoka and colleagues (1991) found a significant association between skin color and vitamin D_3 synthesis in different ethnic groups ($n = 8$) with white > Oriental > Asian > black, but reported only a twofold difference in vitamin D_3 synthesis between white and black skin. The same skin type trend was seen for serum 25(OH)D levels, but it did not reach significance and no skin type effect was seen for serum 1,25 dihydroxyvitamin D. It should be noted that the single UVR challenge dose used in this study could readily be achieved after 10-min exposure to noonday UK summer sunlight. Therefore, it is unlikely that differences in cutaneous vitamin D_3 synthesis would result in vitamin D deficiency in pigmented individuals. This conclusion is supported by Stamp *et al.* (1975), who showed that increases in serum 25(OH)D were similar in white ($n = 4$), Asian ($n = 2$), and black subjects ($n = 1$) after three daily exposures to the same physical UVR dose, and by Brazerol *et al.* (1988), who also found similar increases in 25(OH)D in white ($n = 13$) and black ($n = 7$) subjects exposed to suberythemal whole-body UVR twice a week for 6 weeks.

It is possible that a higher incidence of vitamin D deficiency in subjects with pigmented skin results from other factors such as behavior or diet. For example, in the UK, low vitamin D status is relatively more common among Asians, especially children, adolescents, and women. A combination of factors, including the type of vegetarian diet, low calcium intake, and limited solar exposure, appears to underlie the risk (Hamson *et al.*, 2003; Stamp, 1975).

Some studies have attempted to determine the photoprotective properties of melanin against specific endpoints in human skin *in vivo*, and these are reviewed below. It must also be noted that melanogenesis and stratum corneum thickening occur concurrently during the normal tanning response, and this must be borne in mind when considering photoprotection by melanin alone. Although photoprotection may be considered to be a passive physical process, e.g. the attenuation of UVR by melanin and/or stratum corneum thickening, as is the case with conventional sunscreens, it may also be considered as an active enzymatic process, e.g. as a means by which DNA repair is enhanced or ROS are inactivated. For example, chimeric epidermal reconstructs with melanocytes from one skin type added to keratinocyte cultures of a different skin type suggest keratinocyte/melanocyte interaction with both cell types regulating antioxidant defense in a skin type-dependent way (Bessou-Touya *et al.*, 1998).

Optical Properties of Melanin and Associated Molecules

The absorption spectra of monocysteinyldopas (5-*S*-cysteinyldopa, 2-*S*-cysteinyldopa, 6-*S*-cysteinyldopa) show a maximum at 292 nm, whereas indoles, their methyl derivatives, and 2,5-*S*-*S*′-dicysteinyldopa show maxima at 302–330 nm (Kollias *et al.*, 1991). Diffuse spectroscopy has been used to calculate melanin absorption *in vivo* by comparing normal with melanin-depleted skin in the same vitiligo patient. This approach reveals a maximum at 335 nm, with a steep decline at shorter wavelengths. Absorption is also noted within the visible range (400–800 nm). Differences in pigmentation induced by various bands of UVR (UVB, UVA, and PUVA) have also been analyzed using *in vivo* reflectance spectroscopy by comparing the results of tanned and untanned skin in the same individuals (Kollias *et al.*, 1994). UVB- and PUVA-induced melanin showed an absorption peak at 305 nm whereas UVA resulted in a 360-nm-centered loss in UVR absorption with a concomitant relative increase in the absorption of visible light. These data suggest that different stimuli induce different pathways of melanogenesis. Interestingly, derivatives in the eumelanogenesis pathway such as DHI and DHICA also showed significant absorption in the solar UVR range (the latter in particular showing strong UVA absorption), and hence could possibly be classified as photoprotective.

The particulate nature of melanin results in scattering as well as absorption of UVR. Studies on the cuttle fish melanin particles (size range 20–300 nm) found that the reported level of scatter in the wavelengths 580–633 nm did in fact correspond to the levels predicted (Vitkin *et al.*, 1994). Melanosomes measuring ≥ 300-nm diameter mainly cause forward scatter of UVR in contrast to the smaller particles such as melanin dust found in keratinocytes (< 30 nm), which display symmetrical scattering profiles (Chedekel *et al.*, 1995).

Erythema

In the field, Cripps (1981) assessed the protection factor afforded by a Wisconsin (~45°N) summer tan (obtained over 3.5 months) in skin types II, III, and IV by comparing the SSR MED on tanned and untanned buttock skin. Protection factors were 2.4 ± 0.65 (SD), 2.45 ± 0.5, and 2.1 ± 0.22, respectively, with a mean of 2.33 ± 0.05. Although not stated, it is presumed that tanning was according to skin type, and it seems that better tans did not afford any better protection against erythema. In laboratory studies, Sheehan *et al.* (1998)

induced tanning in skin types II and III with repeated suberythemal exposure to SSR. Photoprotection was evaluated by assessing the MED on untanned and tanned sites, included tanned sites that had had the stratum corneum removed by tape stripping. As expected, the tanning response in skin type III was greater than that in skin type II, but there was no significant difference in the level of photoprotection with a protection factor of about 2 in both skin types. Thus, the degree of photoprotection could not be correlated with the level of the tan. Removal of the stratum corneum generally reduced the level of photoprotection by about 20%, which suggested that the stratum corneum was relatively unimportant in the tanning response. In a comparable later study but without the tape-stripping component, Sheehan *et al.* (2002) used a similar protocol in skin types II and IV. Despite the difference in the tanning response, the protection factor against erythema, assessed by MED determination, was again of the order of 2 with no difference between the skin types. Overall, the data of Sheehan *et al.* (1998, 2002) in skin types II, III, and IV show that the degree of photoprotection against erythema was very similar but could not be correlated with the level of tan, as was presumed to be the case in the field study by Cripps (1981). This suggests that other, as yet undefined, inducible forms of photoprotection may be in operation and that the level of melanin may be of less importance.

Ha *et al.* (2003) used reflectance spectroscopy to assess the relationship between acute UVB-induced erythema and constitutive pigmentation. Regression analyses for a given UVB dose (119–300 mJ/cm^2) were done on a panel of individuals and showed an inverse relationship, but that slope depends on the dose used. Although these data show that constitutive melanin is photoprotective, they also suggest that this protection is less effective at higher UVB doses.

Gniadecka *et al.* (1996) specifically assessed the photoprotective role of the stratum corneum in vitiliginous and normal adjacent skin (skin type not specified) that had had no UVR exposure for 3 months. Sensitivity to erythema was compared with the thickness of the stratum corneum and the viable epidermis as well as pigmentation assessed by a reflectance device. Overall, the authors concluded that the stratum corneum accounted for almost two-thirds of the photoprotection of normal skin and was therefore more important than pigmentation, and that the thickness of the viable epidermis was not important in photoprotection.

DNA Photodamage

UVR induces structural changes in DNA that include the formation of potentially mutagenic CPD and pyrimidine (6–4) pyrimidone photoproducts ((6–4) pp). Human studies have shown that epidermal CPD and ((6–4) pp) are readily induced with suberythemal exposure of SSR, UVB, and UVA (Chadwick *et al.*, 1995; Young *et al.*, 1998a, b). There is increasing evidence that epidermal DNA is a major chromophore for many of the acute and long-term effects of solar exposure (Young, 1996). Photodamage to DNA may result in highly characteristic gene mutations, e.g. p53 which is thought to be the initial step in nonmelanoma skin cancer (Brash *et al.*, 1996). Furthermore, there is also considerable evidence that DNA photodamage, especially CPD, initiates many of the immunological effects of UVR (Yarosh, 2004). Given the significance of DNA photodamage, it is not surprising that living organisms have developed highly effective DNA repair mechanisms. Studies in our laboratory and by others have shown that, after a single exposure of SSR, ((6–4) pp) repair in human skin *in situ* is relatively rapid and that CPD repair is much slower with many lesions persisting for at least 24 h (Bykov *et al.*, 1999; Young *et al.*, 1996). Such slow repair also means that lesion induction may be cumulative if skin is exposed to UVR the following day, as is the case in "real life." There is also evidence that cytosine-containing lesions (C = C, C = T) are repaired more rapidly than those with thymine only (T = T) (Bykov *et al.*, 1999; Xu *et al.*, 2000). Apart from the DNA repair response, it might also be expected that photoprotection by melanin would include protection against DNA photodamage *in situ*.

One study compared the SSR induction of T = T in white and black skin *ex vivo* and came to the conclusion that constitutive pigmentation afforded DNA protection factors of 2–4 (Strickland *et al.*, 1988). Bykov *et al.* (2000) reported an inverse relationship between constitutive pigmentation in white-skinned people and UVB-induced epidermal DNA photodamage. Tadokoro *et al.* (2003) reported fewer CPD in skins with high levels of constitutive melanin after a dose of about 1 MED. Some studies have assessed the ability of UVR-induced pigmentation in white skin to protect epidermal cells from CPD by a subsequent challenge dose of UVR. Gange *et al.* (1985) reported that UVB- and UVA-induced tans, in people who tanned well, resulted in protection factors of about 2–3 against the induction of epidermal CPD by UVB. Sheehan *et al.* (2002) treated skin types II and IV with 0.65 MED SSR for 2 weeks and exposed SSR-treated and untreated skin to 2 MED SSR 1 week after the last tanning treatment. An analysis of the data, taking into account CPD caused by the tanning treatment, showed that pigmentation was associated with a DNA protection factor of about 2–3. Surprisingly, despite the superior tan in skin type IV, the level of photoprotection was not significantly higher than in skin type II. Assessment of photoprotection against erythema was also made, and the protection factors for erythema and CPD were virtually identical, as might be expected in that DNA is the chromophore for erythema. These data are in contrast to those of Gange *et al.* (1985) who, as stated above, reported that comparable UVB- and UVA-induced tans gave comparable protection against UVB-induced CPD, but that only the UVB tan resulted in protection against UVB-induced erythema with a protection factor of 3. These data, as do those of Tadokoro *et al.* (2003) described above, suggest a lack of relationship between DNA photodamage and erythema.

In a study of skin explants taken from habitually sun-exposed skin from two people of skin type III, Kobayashi *et al.* (1998) showed an inverse relationship between the level of melanin in supranuclear caps and UVB-induced DNA photodamage in the corresponding keratinocytes. The authors

showed DNA protection factors ranging from just over 1 to about 5. Barker *et al.* (1995) compared the UVB dose–responses for CPD *in vitro* in human melanocytes of different skin types. Melanocytes from skin types IV–VI showed much more tyrosinase activity and melanin than those from skin types I and II. However, the dose–response data show a relatively modest difference in CPD from a single UVB exposure that suggests a protection factor of less than 2. Melanocytes and adjacent basal layer keratinocytes in untanned human skin *in situ* show similar sensitivity to the induction of CPD by UVB and UVA, suggesting no inherent differences in sensitivity to DNA photodamage (Young *et al.*, 1998a). In combination, the data from these two studies would suggest that melanocytes have no great advantage over keratinocytes in terms of photoprotection from DNA damage.

Studies on reconstructed human skin with and without melanocytes from skin types II and III showed that the presence of melanocytes had no effect on the induction of CPD or ((6–4) pp) (Cario-Andre *et al.*, 2000). This is perhaps not surprising, as melanin was not detected in the preparations. However, the presence of melanocytes inhibited the formation of apoptotic sunburn cells (SBC) that are considered to be a means of eliminating keratinocytes with mutagenic and therefore carcinogenic potential (Ziegler *et al.*, 1994). In theory, a reduction in SBC is likely to enhance skin cancer risk. These data suggest that melanocytes might influence the effects of UVR in ways that are unrelated to melanin production.

Rijken *et al.* (2004) compared the effects of a single 2-MED exposure of fluorescent SSR on white buttock skin (types I, II, and III) with comparable physical (i.e. suberythemal) doses on black buttock skin (type VI) *in vivo*. Subjective assessment of CPD by immunostaining showed comparable levels of CPD in all volunteers in the suprabasal epidermis when sampled immediately after irradiation. CPD was also seen in the basal epidermis and the dermis in the white skin types but not in skin type VI. Fisher *et al.* (2002) also reported similar differential of epidermal CPD in skin types I/III and V/VI after exposure to UVB plus UVA II (approximately fourfold higher doses in darker skin types) and UVA I (110 J/cm^2 in both skin type groups) when samples were taken 24 h after irradiation, which allows time for repair. Both these studies also showed a reduction in other indicators of photodamage (e.g. induction of matrix metalloproteinases, infiltrating neutrophils) in black skin compared with white. Overall, these data suggest that melanin is photoprotective of basal layer DNA, but the experimental design does not provide any indication of the level of protection. However, the data of Fisher *et al.* (2002) could also be explained by possible skin type differential repair.

Overall, several human studies suggest that the acute photoprotection afforded by constitutive and induced pigmentation against DNA photodamage in keratinocytes and melanocytes and erythema is equivalent to wearing a sunscreen with a sun protection factor (SPF) of 2–3. The generally comparable results with DNA photodamage and erythema provide additional evidence that DNA is an important chromophore for erythema. Protection factors of 2–3 should result in a 50–60% reduction in biologically effective dose. Such a reduction, if maintained over long periods, would be significant in terms of long-term risk of skin cancer. However, the maintenance of a tan requires repeated exposure to UVR, from either the sun or an artificial source, and it is likely that this is associated with the accumulation of epidermal DNA photodamage (Sheehan *et al.*, 2002) that may minimize the benefits of the protection. There are conflicting data about the role of a tan in affording protection against malignant melanoma in women, with Weinstock *et al.* (1991) suggesting that a tan may afford protection and Holly *et al.* (1995) reporting no benefit.

Is UVR-induced Melanogenesis an Indicator of DNA Repair?

Extensive data suggest that DNA damage *per se* or DNA repair intermediates initiate melanogenesis, as summarized in Figure 17.2. Several studies have examined the effect of thymine dinucleotides (pTpT), as a model for thymine dimers, on pigmentation. Cloudman S91 mice melanoma cell lines show increased pigmentation in response to UVR. A sevenfold increase in melanin content was also observed after treatment with 50 μm of pTpT for 4 days compared with diluent-treated controls (Eller *et al.*, 1994). Significantly, treatment of cells with pdApdA (a dinucleotide rarely seen as a photoproduct) showed only modest increases in melanin content of 20–30%. These results suggest that the response is specific to UVR-induced DNA damage products. However, agents that induce single-strand DNA breaks are also able to stimulate melanogenesis *in vitro* (Eller *et al.*, 1996). Cells treated with pTpT displayed not only an increase in melanin content, but also a two- to threefold rise in mRNA for tyrosinase. Interestingly, a rise in tyrosinase levels was noted within 4 h of the addition of pTpT, which is well before mRNA changes were detectable. It would thus appear that pTpT influences gene expression at both the mRNA and the protein level (Eller *et al.*, 1994).

In addition to these *in vitro* studies, *in vivo* experiments on shaved guinea-pig skin have demonstrated that the topical application of pTpT twice daily for 5 days was able to induce a visible tanning after 1 week, reaching a maximum 1–2 weeks later. When skin sections were examined histologically, they demonstrated the presence of melanin, primarily in the basal epithelial layers, but also in suprabasal caps over nuclei (Eller *et al.*, 1994). This is analogous to the picture seen in the human tanning response.

The significance of the dipyrimidine form in inducing a tanning response was verified by Pedeux *et al.* (1998). Induction of pigmentation in human melanocyte and melanoma cells was demonstrable in response to the dinucleotide pTpT but not the monomer pT alone. Addition of pTpT in concentrations capable of inducing a pigmentary response was not cytotoxic to the cells, and no increase in apoptosis was observed. Treated melanoma cell lines did however show a

temporary arrest in the S phase of the cell cycle 24 h after the addition of pTpT, with resumption to normal cycling by 48 h. The mechanism and significance of this phenomenon are yet to be understood.

The capacity of DNA photoproducts to induce pigmentation varies with oligonucleotide length and base composition. Although initial studies were largely carried out on pTpT, other DNA fragments are also able to stimulate pigmentation. For example, the p9-mer oligonucleotide pGpApGpTp ApTpGpApG and the p7-mer pApGpTpApTpGpA stimulated melanogenesis in Cloudman S91 murine melanoma cells by up to 800% compared with controls, whereas the p5-mer pCpApTpApC had no effect (Hadshiew *et al.*, 2001).

T4 endonuclease V (T4N5) is a bacterial phage enzyme which, apart from catalyzing the rate-limiting step in excision of CPDs, has no other recognized function (Grossman *et al.*, 1988). T4N5 has been shown to accelerate repair of CPD in both cultured cells (Ceccoli *et al.*, 1989; Yarosh *et al.*, 1992) and intact skin (Yarosh, 1990). The effects of this enzyme on pigmentation following UVR have been studied *in vitro*. Both murine S91 melanoma cells and human melanocytes demonstrated greater melanogenesis (with an almost doubling of melanin content) when treated with T4N5 after irradiation compared with treatment with either diluent alone or heat-inactivated enzyme (Gilchrest *et al.*, 1993). These results suggest that accelerated and extensive excision of CPD enhances tanning.

A photoprotective effect of DNA fragments has been shown in animal studies. Guinea pigs were treated with topical application of pTpT for 1 month to induce tanning (Gilchrest and Eller, 1999). Their shaved skin was then exposed to a previously determined 6 MED of UVB, and biopsies were taken from both UV-irradiated treated and untreated skin at the height of sunburn reaction (24 h after irradiation). Fontana Mason staining revealed higher melanin content in pTpT treated compared with untreated skin. Routine hematoxylin and eosin staining revealed extensive epidermal necrosis with intraepidermal blistering in UV-irradiated untreated skin. In contrast, pTpT-treated irradiated skin showed no histological damage and was almost indistinguishable from unirradiated skin apart from slight increases in basilar and suprabasilar melanin. Thus, it was concluded that pTpT was fully protective to 6 MED. This capacity of small DNA fragments, especially pTpT, to induce protective tanning responses in the absence of DNA damage has far-reaching therapeutic implications. Topical application of these products may potentially be used to provide a photoprotective tan without the harmful effects of UVR.

Sheehan *et al.* (2002) reported that 10 repeated weekday doses of 0.65 MED SSR resulted in more cumulative CPD in skin type IV than in skin type II, almost certainly because skin types IV have higher MEDs than skin types II. When skin biopsies were examined 1 week after treatment, there was a nonsignificant reduction in lesions in skin type II but significant loss in skin type IV. These data suggest better DNA repair in skin type IV, which is in accordance with the hypothesis that tanning is associated with DNA repair (Gilchrest and Eller, 1999). Such a hypothesis would suggest that the inverse relationship between skin type and skin cancer, as indicated in Table 17.1, is a consequence of DNA capacity rather than, or as well as, the ability of melanin to act as a photoprotective agent. Recent data suggest that αMSH, associated with the tanning process especially eumelanogenesis (Thody and Graham, 1998), may enhance repair of UVR-induced DNA damage (Böhm *et al.*, 2004).

Mechanism of pTpT-induced Tanning

Repair of DNA photolesions requires cell cycle arrest prior to replication and mitosis (Murray, 1992). The tumor suppressor gene p53, known as guardian of the genome, plays a vital role in the repair of DNA and apoptosis. pTpT directly activates p53, with treated cells demonstrating reduced proliferative rates and upregulation of p53-mediated p21, a protein known to mediate cell cycle arrest (Eller *et al.*, 1997). Nuclear translocation of p53 was also observed, a known indicator of p53 activation. Thus, the data unequivocally suggest that pTpT acts at least partially via induction of p53, which regulates tyrosinase gene expression (Khlgation *et al.*, 2002). It has been hypothesized that the DNA photodamage to the telomere 3′ overhang (TTAGGG) may be a specific trigger for the cellular defense responses to UVR (Eller *et al.*, 2003) and that this is the reason why oligonucleotides with homology (i.e. TT) to this sequence are able to induce such responses as p53 activation.

Melanin Synthesis may be a Damage Response System

Although the major DNA photoproducts CPD and 6–4PP are excised by a well-recognized family of DNA repair proteins, the metabolic fate of the excised photoproduct containing a single-stranded DNA (ssDNA) fragment is relatively understudied. In bacterial studies, however, it has been demonstrated that ssDNA plays an important role in photoprotection. Single-strand DNA in prokaryote systems generated from DNA damage/repair interacts and activates a protease, the Rec A protein. Rec A is then responsible for the lifting of repression of over 40 genes important in DNA repair, replication, and cell survival (Courcelle and Hanawalt, 2003). This phenomenon, known as the SOS response, not only increases bacterial survival after irradiation, but also enhances resistance to subsequent UVR-induced DNA damage. Thus, repeat exposure to a sublethal dose of UVR in these bacteria would result in more efficient repair of DNA damage and enhanced survival (Crowley and Hanawalt, 1998). It has been postulated that tanning may form part of a mammalian SOS response (Eller and Gilchrest, 2000).

Immunosuppression by UVR

There is considerable evidence that UVR-induced immunosuppression may play a role in skin cancer, and animal studies

also suggest a role for susceptibility to infectious disease (Sleijffers *et al.*, 2002). There have been two human studies on the photoprotective effects of melanin on UVR-induced immunosuppression (Selgrade *et al.*, 2001; Vermeer *et al.*, 1991), and both came to the conclusion that pigmentation, even in dark-skinned people, had no effect on the ability of UVR to suppress the induction phase of the contact hypersensitivity (CHS) response, which is regarded as a model for some of the photoimmunological events that are important in skin cancer. The reasons for this are not known, but one explanation would be that a superficial chromophore such as stratum corneum urocanic acid (UCA) is more important than epidermal DNA. Mouse studies have shown that photoimmunosuppression is initiated via either DNA or UCA depending on the viability of the antigen (Kim *et al.*, 2003).

Photosensitization by Molecules Associated with Melanogenesis

It has been reported that albinos, who have tyrosinase-deficient melanocytes (i.e. not producing melanin), are prone to nonmelanoma skin cancers but not malignant melanoma (Streutker *et al.*, 2000). This suggests the possibility of melanogenesis-related photosensitization in malignant melanoma. Several *in vitro* studies have identified photosensitizing properties of melanins or their intermediates (Hill, 1992; Kvam and Dahle, 2004). 5-SCD photobinds to native DNA after exposure to 300-nm radiation and also induced single-strand breaks (SSB) in DNA (Koch and Chedekel, 1986). More recently, it has been reported that 5-SCD is photochemically unstable in the presence of UVA radiation and oxygen (Costantini *et al.*, 1994) and that the eumelanin-soluble precursor DHICA sensitizes DNA SSB with 313-nm exposure, especially in the presence of oxygen (Routaboul *et al.*, 1995). Kipp and Young (1999) showed that the addition of DHICA to human keratinocytes increased their sensitivity to UVA-induced SSB by the generation of ROS. Kvam and Tyrrell (1999) concluded that melanogenesis, but not melanin itself, was associated with oxidative base damage in human melanoma cells. Wenczl *et al.* (1998) compared the UVA sensitivity of melanocytes from skin type I and skin type IV that had been induced to synthesize melanins by a high concentration of L-tyrosine in the culture medium. The ratio of pheomelanin to total melanin remained the same in skin type IV, but relatively more pheomelanin was induced in skin type I. This was associated with an increase in UVA-induced SSB in DNA. In recent *in vivo* studies in different mouse strains, Takeuchi *et al.* (2004) have reported that melanins, in particular pheomelanin, are UVB and UVA photosensitizers in mammalian skin *in vivo*. It should be stressed that the clinical significance, if any, of these reactions is unknown, but they clearly demonstrate the photobiological potential of melanogenesis intermediates, and it is therefore possible that pheomelanin plays a role in the susceptibility of skin types to skin cancer and other types of photodamage.

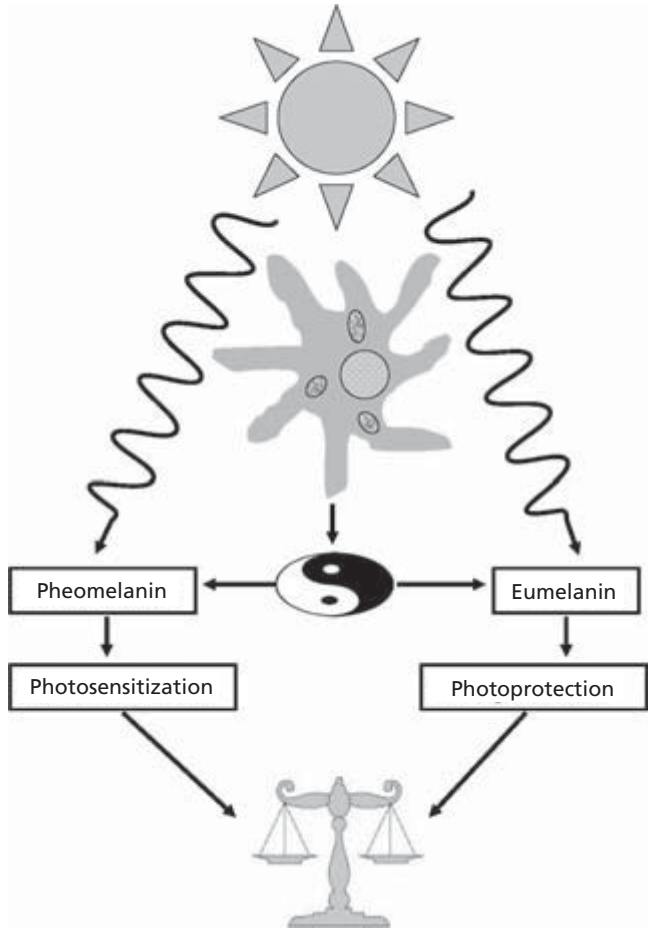

Fig. 17.3. Solar UVR activates melanocytes to initiate the synthesis of a mix of pheomelanin or eumelanin in specialized organelles called melanosomes (represented by the oval structures). The balance of melanin type depends on genetic factors such as skin type. Pheomelanin and eumelanin and their precursors interact in different ways with solar UVR. Pheomelanogenesis may favor photosensitization via ROS, whereas eumelanin is more likely to favor photoprotection.

Perspectives

Exposure to solar UVR, especially UVB, results in epidermal DNA photodamage such as CPD that, if unrepaired, can result in mutation, immunosuppression, and consequent skin cancer, especially in people who do not tan readily. Despite concerted public health campaigns and increased awareness of the hazards of UVR, the incidence of skin cancer continues to rise in white-skinned populations. Indeed, a tan is still widely regarded as a desirable sign of health and well-being, especially by the young, and is often justified by its photoprotective properties, which are relatively modest even in those who tan well. The level of photoprotection may ultimately depend on the ratio of pheomelanin to eumelanin as indicated in Figure 17.3, as there is experimental evidence to suggest that pheomelanin may be associated with photosensitization.

There is increasing evidence that melanogenesis is initiated by CPD and its repair and may therefore be seen as a biomarker for DNA repair capacity which, in white-skinned people at least, may be more important in skin cancer prevention than in optical photoprotection. The relationship between DNA damage/repair potentially has major therapeutic implications given that topical application of small DNA fragments may induce melanogenesis and a measure of photoprotection, without the harmful effects of UVR. In effect, this may make a skin type II respond more like a skin type IV, and this is an area for further research from which there may be significant public health benefit.

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