Current Biology 21, 1906–1911, November 22, 2011 ©2011 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2011.09.047

Report

UVA Phototransduction Drives Early Melanin Synthesis in Human Melanocytes

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Summary

Exposure of human skin to solar ultraviolet radiation (UVR), a powerful carcinogen [1] comprising ~95% ultraviolet A (UVA) and ~5% ultraviolet B (UVB) at the Earth's surface, promotes melanin synthesis in epidermal melanocytes [2, 3], which protects skin from DNA damage [4, 5]. UVB causes DNA lesions [6] that lead to transcriptional activation of melanin-producing enzymes, resulting in delayed skin pigmentation within days [7]. In contrast, UVA causes primarily oxidative damage [8] and leads to immediate pigment darkening (IPD) within minutes, via an unknown mechanism [9, 10]. No receptor protein directly mediating phototransduction in skin has been identified. Here we demonstrate that exposure of primary human epidermal melanocytes (HEMs) to UVA causes calcium mobilization and early melanin synthesis. Calcium responses were abolished by treatment with G protein or phospholipase C (PLC) inhibitors or by depletion of intracellular calcium stores. We show that the visual photopigment rhodopsin [11] is expressed in HEMs and contributes to UVR phototransduction. Upon UVR exposure, significant melanin production was measured within one hour; cellular melanin continued to increase in a retinal- and calcium-dependent manner up to 5-fold after 24 hr. Our findings identify a novel UVA-sensitive signaling pathway in melanocytes that leads to calcium mobilization and melanin synthesis and may underlie the mechanism of IPD in human skin.

Results and Discussion

Ultraviolet Radiation Evokes Retinal-Dependent Calcium Flux in Human Epidermal Melanocytes

To investigate ultraviolet radiation (UVR)-activated signaling pathways, we designed a system that permits real-time imaging of cultured cells and simultaneous exposure to irradiances comparable to solar UVR. Our light source comprises ~90% ultraviolet A (UVA) (320–400 nm) and ~10% ultraviolet B (UVB) (280–320 nm) and each 10 mJ/cm^2 exposure equates to 10 s of solar UVR exposure on a day with a UV index ~10 (see Figure S1 and Supplemental Information available online). We tested the effect of physiological UVR doses on intracellular calcium (Ca^{2+}) levels in primary human epidermal melanocytes (HEMs) using the fluorometric Ca^{2+} indicator Fluo-4 AM. We found that UVR (100 mJ/cm^2) evoked rapid Ca^{2+} transients in HEMs preincubated with the 11-*cis* retinal analog 9-*cis* retinal (10 μM) [12] but failed to elicit such responses in the absence of retinal (Figures 1A and 1B), suggesting that the effect is mediated by an opsin-like photopigment. To characterize the irradiance dependence of Ca^{2+} responses, we measured the amplitude of transients elicited by increasing UVR doses (20–150 mJ/cm^2) and found that it increased as a function of stimulus irradiance (Figures 1C and 1D).

We investigated the spectral sensitivity of Ca^{2+} transients by stimulating HEMs with 200 mJ/cm^2 of UVR (280–400 nm), blue light (435–460 nm), or green light (500–550 nm) (Figures 1E and 1F). Only UVR elicited significant Ca^{2+} transients (normalized peak fluorescence: $F_{norm,\ max}$ = 0.48 ± 0.01, n = 107; $p < 0.001$). We then exposed HEMs separately to the constituent UVA (180 mJ/cm^2) and UVB (20 mJ/cm^2) doses comprising a 200 mJ/cm^2 UVR pulse and found that UVA elicited significantly larger Ca^{2+} transients than UVB ($F_{norm,\ max}$ = 0.32 ± 0.01, n = 101 for UVA versus 0.03 ± 0.01, n = 72 for UVB; $p < 0.001$) (Figures 1G and 1H). Thus, retinal-dependent Ca^{2+} responses are maximally photosensitive to wavelengths between 320–400 nm.

A GPCR Drives Calcium Mobilization from Intracellular Stores in Melanocytes

To assess the contributions of extracellular and intracellular Ca^{2+} to this signaling pathway, we measured UVR-induced responses of HEMs in Ca^{2+}-free extracellular buffer and found that they did not significantly differ from those measured in the presence of extracellular Ca^{2+} (Figures 2A and 2B). However, depletion of intracellular Ca^{2+} stores with thapsigargin (1 μM) [13] abolished UVR-induced transients ($F_{norm,\ max}$ = 0.07 ± 0.03, n = 11 with thapsigargin versus 0.50 ± 0.02, n = 17 without thapsigargin; $p < 0.001$) (Figures 2A and 2B), indicating that in HEMs, UVR exposure induces Ca^{2+} mobilization from intracellular stores.

To test whether Ca^{2+} mobilization is initiated downstream of G protein-coupled receptor (GPCR) activation, we measured UVR-induced responses in HEMs treated with the G protein inhibitor suramin (50 μM) [14, 15] and found that preincubation significantly reduced Ca^{2+} responses ($F_{norm,\ max}$ = 0.14 ± 0.01, n = 70 with suramin versus 0.49 ± 0.02, n = 67 without suramin; $p < 0.0001$) (Figures 2C and 2D). Because G proteins can cause Ca^{2+} release via phospholipase Cβ (PLCβ) activation, we tested the effect of the PLC antagonist U73122 [16] on UVR-induced Ca^{2+} responses. Treatment of HEMs with U73122 (9 μM), but not its inactive analog U73343 (9 μM), significantly inhibited UVR-induced Ca^{2+} transients ($F_{norm,\ max}$ = 0.17 ± 0.01, n = 55 for U73122 versus 0.53 ± 0.02, n = 53 for U73343; $p < 0.001$) (Figures 2E and 2F) suggesting that UVR-induced Ca^{2+} responses require PLC activation.

We next evaluated the effects of retinoid substitution. Surprisingly, when we substituted 9-*cis* with all-*trans* retinal, UVR-induced Ca^{2+} transients in HEMs were essentially unchanged (Figures 2G and 2H), suggesting that melanocytes may have an intrinsic isomerization mechanism to generate *cis* from *trans* retinal.

The retinal dependence of UVR-evoked Ca^{2+} transients raises the question of an endogenous source in skin. In principle, retinal could be derived from serum retinoids [17] taken up by epidermal cells [18] or from vitamin A (all-*trans* retinol), which is present at significant concentrations in skin (>1 nmol/g) [19, 20] and mediates a wide range of cellular processes [21].

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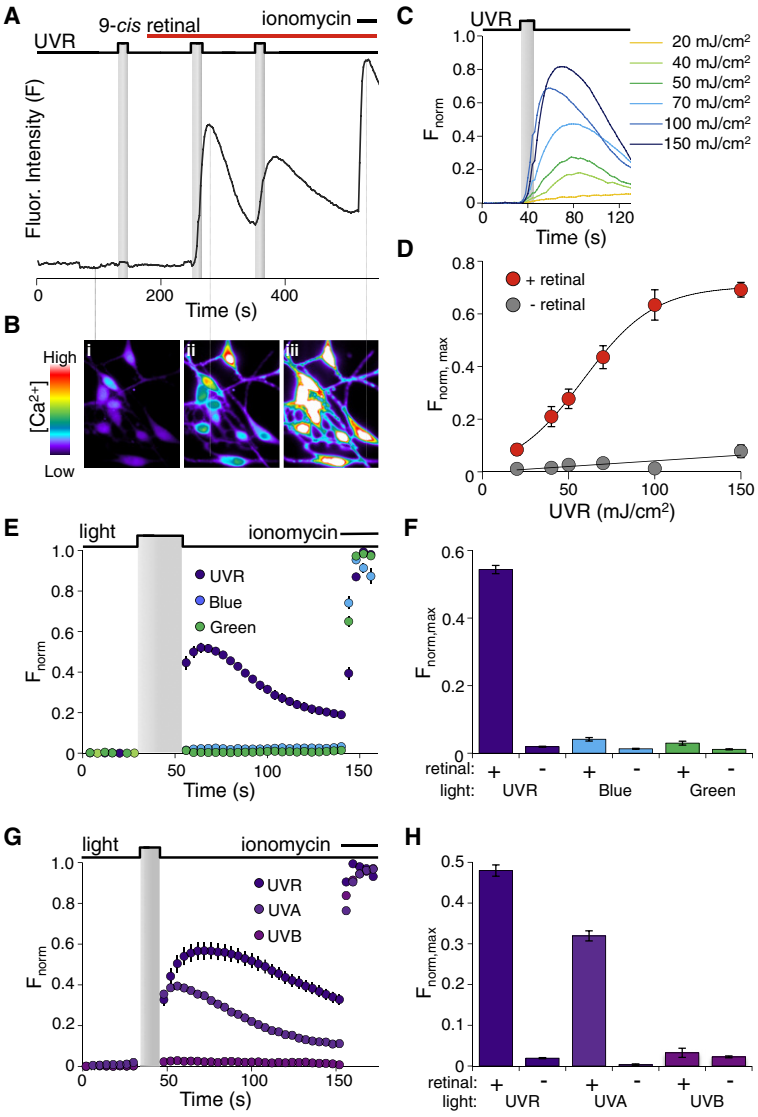

Figure 1. Ultraviolet Radiation Induces Retinal-Dependent Calcium Responses in Human Epidermal Melanocytes

(A) Fluorometric Ca^{2+} imaging trace of a representative human epidermal melanocyte (HEM) stimulated with two 100 mJ/cm^2 (10 mW/cm^2 for 10 s) ultraviolet radiation (UVR) pulses shows a measurable increase in fluorescence intensity (F) only after incubation with 9-*cis* retinal (10 μM). Ionomycin (1 μM) was added for maximal fluorescence intensity.

(B) Pseudochrome fluorescence images of HEMs at three time points during the Ca^{2+} imaging protocol shown in (A) indicate (i) no Ca^{2+} increase with UVR stimulation in the absence of 9-*cis* retinal, (ii) increased Ca^{2+} in response to UVR applied after incubation with 9-*cis* retinal, and (iii) maximal Ca^{2+} increase with ionomycin.

(C) Normalized fluorescence intensity (F_{norm}) of representative HEMs preincubated with 9-*cis* retinal (10 μM) and stimulated with 20–150 mJ/cm^2 of UVR (10 mW/cm^2 for 2–15 s) as a function of time.

(D) Mean amplitudes of Ca^{2+} responses ($F_{norm,\ max}$) measured in HEMs exposed to the indicated UVR dose, either with (red) or without (gray) 9-*cis* retinal represented as a function of dose and fit with a sigmoid function. n ≥ 13 cells for each data point; ± standard error of the mean (SEM).

(E) Normalized fluorescence intensity (F_{norm}) of HEMs preincubated with 9-*cis* retinal (10 μM) and stimulated with 200 mJ/cm^2 (10 mW/cm^2 for 20 s) UVR (280–400 nm), blue light (435–460 nm), or green light (500–550 nm) as a function of time. Ionomycin (1 μM) was used for normalization. n ≥ 8 cells for each trace; ± SEM.

(F) Wavelength profile of retinal-dependent Ca^{2+} responses in HEMs. Peak fluorescence responses ($F_{norm,\ max}$) of HEMs exposed to 200 mJ/cm^2 (20 s of 10 mW/cm^2) of UVR (purple), blue light (blue), or green light (green), in the absence (−) or presence (+) of 9-*cis* retinal (10 μM). Ionomycin (1 μM) was used for normalization. n = 16–32 cells, from ≥3 independent experiments; ± SEM, $p \leq 0.001$ for UVR versus either blue or green light.

(G) Normalized fluorescence intensity (F_{norm}) of HEMs preincubated with 9-*cis* retinal (10 μM) and stimulated with 200 mJ/cm^2 (20 mW/cm^2 for 10 s) UVR (280–400 nm; ~90% UVA, ~10% UVB), 180 mJ/cm^2 (18 mW/cm^2 for 10 s) UVA, or 20 mJ/cm^2 (2 mW/cm^2 for 10 s) UVB, as a function of time. Ionomycin (1 μM) was used for normalization. n ≥ 8 cells for each condition; ± SEM.

(H) Mean amplitudes ($F_{norm,\ max}$) of Ca^{2+} increases of HEMs exposed to either UVR, ultraviolet A (UVA), or ultraviolet B (UVB) as in G, in the absence (−) or presence (+) of 9-*cis* retinal (10 μM). Ionomycin (1 μM) was used for normalization. n = 16–32 cells from ≥3 independent experiments; ± SEM, $p < 0.001$ for UVR or UVA versus UVB. See also Figure S1.

Serum retinoids could be converted by the RPE65 isomerase that regenerates *cis*-retinal in the retina [22, 23] and is expressed in epidermal keratinocytes [24]. Alternatively, vitamin A could be converted to *cis*-retinal in skin via a pathway similar to the cone visual cycle [25, 26].

The dependence of light-evoked Ca^{2+} transients on retinal and UVR dose, together with their pharmacological profile, suggests that UVR activates an endogenous opsin receptor in HEMs to initiate Ca^{2+} mobilization from intracellular stores.

Rhodopsin Is Expressed in Human Melanocytes

To identify photopigments that might mediate retinal-dependent Ca^{2+} signaling in HEMs, we sought to determine opsin expression in these cells. We performed RT-PCR on HEM RNA using degenerate primers corresponding to homologous regions of human opsins (see Supplemental Information) and amplified a ~700 bp transcript corresponding primarily to rhodopsin sequence (Figure 3A). Subsequent amplifications using rhodopsin-specific primers yielded a ~1 kb transcript corresponding to full-length human rhodopsin (NM_000539) (Figure 3A). We did not detect expression of any other opsin using primers specific for full-length melanopsin (OPN4) [27, 28], neuropsin (OPN5) [29], or panopsin (OPN3) [30]. We next assessed rhodopsin expression in HEMs by western blot. Analysis using an anti-rhodopsin antibody revealed a ~37 kDa band in extracts from HEMs and HEK293 cells expressing HA-tagged rhodopsin, but not extracts from untransfected HEK293 cells (Figure 3B).

To investigate whether rhodopsin contributes to UVR-induced Ca^{2+} signaling, we reduced endogenous rhodopsin levels using lentivirally transduced microRNAs (miRNAs) and tested the ability of UVR to induce Ca^{2+} transients in HEMs expressing either rhodopsin-targeted or control miRNA. Treatment with targeted miRNA produced a ~75% reduction in rhodopsin messenger RNA (mRNA) (mRNA relative to control = 0.21–0.33, n = 3; $p < 0.001$) (Figure 3C) and markedly reduced

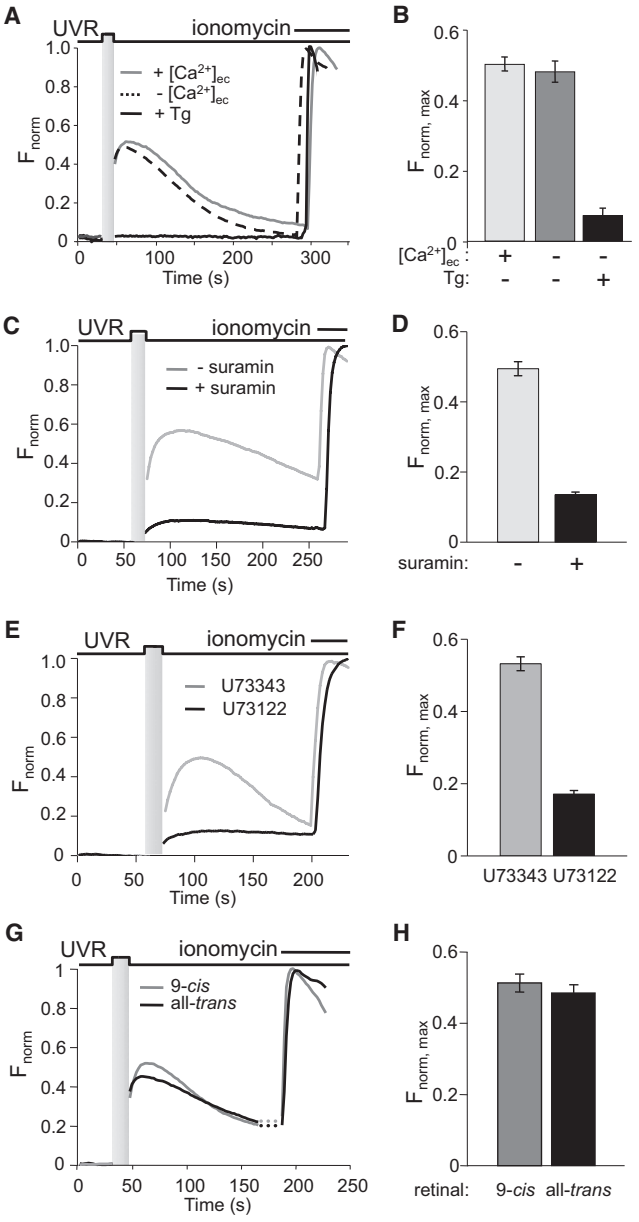

Figure 2. A G Protein-Coupled Receptor Mediates Retinal-Dependent UVR-Induced Calcium Mobilization in HEMs

(A) Dependence of responses on extracellular and intracellular Ca^{2+}. Normalized fluorescence intensity of HEMs preincubated with 9-*cis* retinal (10 μM) and stimulated with UVR (200 mJ/cm^2; 20 mW/cm^2 for 10 s) with extracellular Ca^{2+} (1.5 mM, gray), without extracellular Ca^{2+} (0 mM Ca^{2+}, 1.5 mM ethylene glycol tetraacetic acid [EGTA], dashed), or after treatment with thapsigargin (1 μM, black). n = 2–6 cells for each condition.

(B) Mean peak fluorescence responses ($F_{norm,\ max}$) of HEMs preincubated with 9-*cis* retinal (10 μM) and stimulated with UVR (200 mJ/cm^2; 10 s of 20 mW/cm^2) were measured in the presence of extracellular Ca^{2+} (1.5 mM, light gray), or in the absence of extracellular Ca^{2+} (0 mM Ca^{2+}, 1.5 mM EGTA, dark gray), or after treatment with thapsigargin (1 μM, black). n = 11–17 cells for each condition, from ≥3 independent experiments; ± SEM, p < 0.001 for Ca^{2+} versus thapsigargin.

(C) Effect of G protein inhibition. Normalized fluorescence intensity of HEMs preincubated with 9-*cis* retinal (10 μM) and stimulated with 250 mJ/cm^2 (10 s of 25 mW/cm^2) UVR in the absence of (gray) or after treatment with 50 μM suramin (black). n = 10 cells for each condition.

(D) Suramin treatment (50 μM, black) reduced the mean amplitude of fluorescence responses ($F_{norm,\ max}$) of HEMs preincubated with 9-*cis* retinal (10 μM) and stimulated with 250 mJ/cm^2 (10 s of 25 mW/cm^2), compared to control untreated cells (gray) n ≥ 67 cells for each condition, from ≥3 independent experiments; ± SEM, p < 0.001.

(E) Effects of phospholipase C inhibition. Normalized fluorescence intensity of HEMs preincubated with 9-*cis* retinal (10 μM), stimulated with 250 mJ/cm^2 UVR (25 mW/cm^2 for 10 s), and treated with U73122 (9 μM, black) or its inactive analog U73343 (9 μM, gray). n = 5–8 cells for each condition.

(F) Mean amplitude of fluorescence responses ($F_{norm,\ max}$) of HEMs preincubated with 9-*cis* retinal (10 μM) and stimulated as in (E) in the presence of U73343 (9 μM, gray) or U73122 (9 μM, black). n ≥ 53 cells for each condition, from ≥3 independent experiments; ± SEM, p < 0.001.

(G) Effects of substituting 9-*cis* with all-*trans* retinal on UVR-induced Ca^{2+} transients. Normalized fluorescence intensity in response to 200 mJ/cm^2 (20 mW/cm^2 for 10 s) UVR of HEMs preincubated with all-*trans* retinal (10 μM, black) or 9-*cis* retinal (10 μM, gray). Ionomycin was used for normalization. n = 8 cells for each condition.

(H) Mean peak fluorescence responses ($F_{norm,\ max}$) of HEMs preincubated with all-*trans* retinal (10 μM, black) or 9-*cis* retinal (10 μM, gray) and exposed to 200 mJ/cm^2 UVR (10 s of 20 mW/cm^2). n = 16 cells for each condition, from ≥3 independent experiments; ± SEM, p > 0.42.

Ca^{2+} transients in response to UVR ($F_{norm,\ max}$ = 0.23 ± 0.02, n = 23 for targeted versus 0.53 ± 0.02, n = 24 for control miRNA; p < 0.001) (Figures 3D and 3E), suggesting that rhodopsin contributes to UVR-induced Ca^{2+} signaling in HEMs.

These results demonstrate that rhodopsin is expressed in skin [31, 32] and suggest that it may contribute to nonvisual phototransduction. However, the spectral profile of the light-evoked Ca^{2+} responses presented here (UVA >> UVB > blue ≥ green) is surprisingly different from the spectral sensitivity of rhodopsin (11-*cis* bound; λ_{max} ~500 nm) or isorhodopsin (9-*cis* bound; λ_{max} ~478 nm) [33], measured spectrophotometrically [34], electrophysiologically [35], or in a heterologous system [36]. Direct measurement of photopigment absorption in HEMs is not feasible as a result of the interfering absorptive properties of melanin and low rhodopsin expression levels. Photon absorption by rhodopsin generates the active metarhodopsin II (meta II) intermediate [37], which absorbs maximally at ~380 nm and can regenerate functional pigment upon photon absorption [38]. It is therefore plausible that the cellular environment of HEMs permits stabilization of a meta II-like state that can trigger G protein activation and Ca^{2+} signaling in response to UVR. Alternatively, the shifted absorption spectrum might result from an endogenous chemical modifier like vitamin A, which increases the sensitivity of rhodopsin to UVR wavelengths [39] or from additional UVR-sensitive molecular components. Recent studies have shown that GPCRs expressed in roundworm neurons [40, 41] and fly larvae [42] mediate retinal-independent UVR-sensing by an unknown mechanism. It is thus conceivable that in skin, rhodopsin, similar to other GPCRs [43], functions as a heterodimer with another opsin or alternate light-sensitive receptor.

UVR Causes Retinal- and Calcium-Dependent Early Melanin Synthesis

Opsin-mediated phototransduction in melanocytes might regulate melanogenesis. To test this hypothesis, we irradiated cells with physiological UVR doses (1–5 J/cm^2; equivalent to ~20–80 min of UVR exposure on a day with a UV index ~10) and quantified cellular melanin concentration by measuring absorption of purified cellular extracts containing melanin at 405 nm [44]. These exposures did not appreciably alter cellular morphology (Figure S2A) and resulted in a sustained Ca^{2+} response (Figures S2B–S2E).

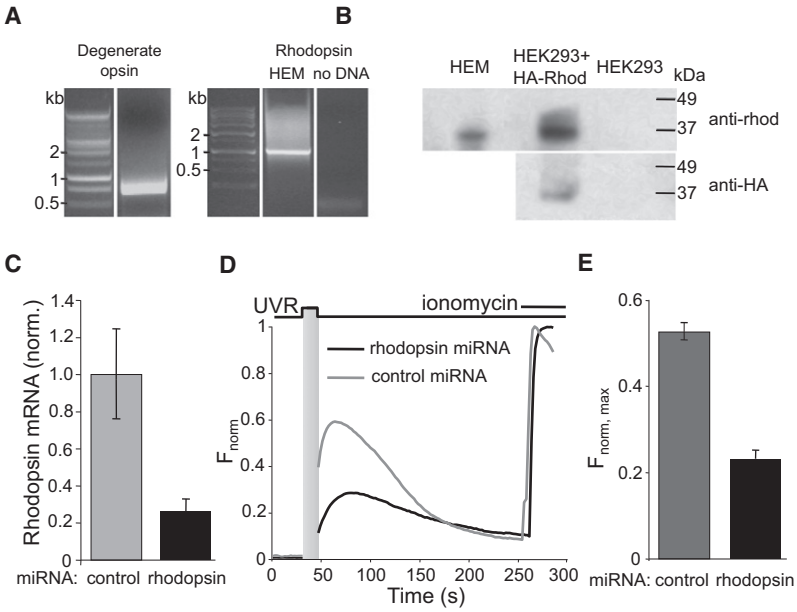

Figure 3. Rhodopsin Contributes to UVR-Induced Calcium Mobilization in HEMs

(A) RT-PCR using HEM RNA and either degenerate opsin primers (left) or rhodopsin-specific primers (right) identified a band corresponding to rhodopsin complementary DNA.

(B) Western blot analysis of HEM and HEK293 cell extracts probed with anti-rhodopsin antibody (anti-rhod, top) shows a ~37 kDa band in HEM and HEK293 cells expressing hemagglutinin (HA)-tagged rhodopsin. Anti-HA antibody (anti-HA, bottom) detected a band of similar size in HEK293 cells expressing HA-rhodopsin. Representative of three independent experiments.

(C) Quantitative PCR analysis of rhodopsin messenger RNA (mRNA) transcript levels in control and rhodopsin-targeted microRNA (miRNA)-treated HEMs. n = 3; ± SEM, $p < 0.001$.

(D) Normalized fluorescence intensity of HEMs preincubated with 9-*cis* retinal (10 μM) and expressing rhodopsin-targeted (black) or control (gray) miRNA. n = 2–3 cells for each condition. See also Figure S2.

(E) Mean amplitude of fluorescence responses ($F_{norm,\ max}$) of HEMs expressing control (gray) or rhodopsin-targeted (black) miRNA, preincubated with 9-*cis* retinal (10 μM) and stimulated with 200 mJ/cm^2 (10 s of 20 mW/cm^2) UVR. n = 23–24 cells for each condition, from ≥3 independent experiments; ± SEM, $p < 0.002$.

To test whether rhodopsin-mediated phototransduction regulates melanogenesis, we measured melanin production in HEMs expressing rhodopsin-targeted or control miRNA and exposed to 4 J/cm^2 UVR. We failed to detect significant differences, likely as a result of the sustained Ca^{2+} responses caused by residual rhodopsin expression (Figure 3C; Figures S2B and S2C). Instead, we mimicked receptor knockdown by excluding retinal. We compared the melanin concentrations of HEMs at 1, 4, 8, and 24 hr after UVR exposure (4 J/cm^2) in the presence or absence of retinal and found that HEMs treated with 9-*cis* retinal exhibited significantly higher UVR-induced melanin increases compared with cells stimulated in the absence of retinal (relative increase with retinal: 1.51 ± 0.05, n = 9, at 4 hr; 2.67 ± 0.22, n = 12, at 8 hr; 5.03 ± 0.54, n = 12, at 24 hr; $p \leq 0.002$) (Figure 4A). Notably, we measured a significant increase in cellular melanin concentration as early as 1 hr after UVR exposure only in the presence of retinal (1.46 ± 0.10, n = 9 with retinal versus 1.15 ± 0.08, n = 9 without retinal; $p < 0.001$) (Figure 4B). No significant changes in cellular protein concentrations were measured after UVR exposure (two-tailed $p > 0.16$ for UVR irradiated versus nonirradiated cells at 1 hr, n = 51). Additionally, exposure to increasing irradiances (1–5 J/cm^2) resulted in proportionally larger retinal-dependent melanin concentrations (Figure 4C), further evincing a receptor-mediated mechanism.

We next investigated whether Ca^{2+} mobilization is required for retinal-dependent early melanin synthesis by measuring the melanin concentration of HEMs stimulated with UVR and incubated under Ca^{2+}-free conditions. Depletion of intracellular stores with thapsigargin (2 μM), combined with intracellular BAPTA (BAPTA-AM, 20 μM) and extracellular EGTA (1.5 mM), abolished sustained UVR-induced Ca^{2+} transients (Figure S2E) and markedly reduced retinal-dependent melanin increases at 4 hr after exposure to 4 J/cm^2 (1.11 ± 0.10, n = 2 for Ca^{2+}-free versus 1.40 ± 0.01, n = 2 with Ca^{2+}; $p < 0.04$) (Figure 4D). Thus, we conclude that in HEMs, UVR-induced Ca^{2+} release directly contributes to early melanin synthesis within hours of exposure.

Early Melanin Synthesis Is Driven by UVA

We reasoned that if retinal-dependent Ca^{2+} release and early melanin synthesis are part of the same pathway, they should exhibit similar action spectra. To test the wavelength dependence of early melanin synthesis, we exposed HEMs to UVA (2.25 J/cm^2), UVB (0.25 J/cm^2), or the equivalent dose of UVR (2.5 J/cm^2), and measured melanin concentration at 8 hr after exposure (Figure 4E). UVA elicited retinal-dependent melanin increases similar to those measured in response to UVR (1.45 ± 0.13, n = 6 for UVA versus 1.49 ± 0.18, n = 4 for UVR), whereas UVB did not significantly alter melanin concentrations under our conditions. The similar spectral sensitivities of early melanogenesis and light-evoked Ca^{2+} release suggest that both events participate in the same phototransduction pathway.

The spectral sensitivity and timescale of the early melanogenesis described here suggest that UVA phototransduction in melanocytes might underlie the elusive mechanism of immediate pigment darkening (IPD). It is widely accepted that UVA causes IPD, but whether UVA exposure results in melanin synthesis [9, 45] or in photooxidation of existing melanin [46, 47] remains controversial. To distinguish between these possibilities, we exploited the different absorption profiles of oxidized and nonoxidized melanin [48, 49]. We compared the absorption spectra (300–500 nm) of our melanin extracts with those of synthetic (nonoxidized) melanin and found that all samples displayed the same characteristic linear absorption profile of synthetic melanin (Figure S3). We thus conclude that changes in absorption at 405 nm are not due to melanin oxidation or absorption by other cellular components but instead reflect melanin synthesis.

Melanin synthesis via the UVB-mediated DNA damage pathway occurs >12 hr after exposure [7, 50] and requires de novo generation of tyrosinase, the key enzyme necessary for synthesis. In contrast, the mechanism underling the UVA- and retinal-dependent melanogenesis reported here causes synthesis within 1–4 hr of exposure. This relatively rapid time course suggests that early synthesis occurs through a novel

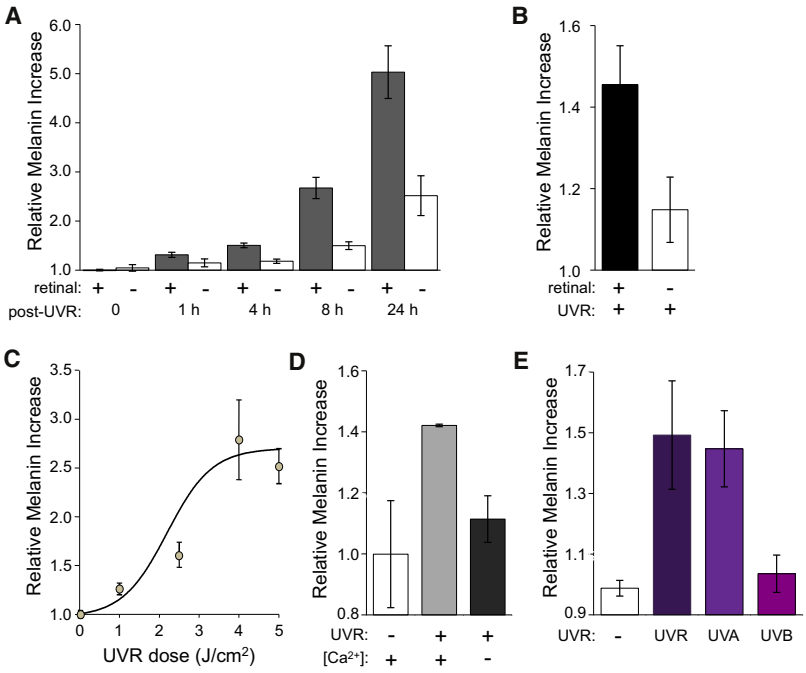

Figure 4. UVA Induces Retinal-Dependent Melanin Increases in HEMs

(A) Time-dependent changes in intracellular melanin concentration of HEMs preincubated with (gray) or without (white) 9-*cis* retinal (10 μM) and irradiated with 4 J/cm^2 (17.5 mW/cm^2 for 228 s) UVR, normalized to melanin concentrations of nonirradiated cells. n = 9–30 for each time point; ± SEM, $p < 0.002$ for all time points.

(B) Melanin concentration of HEMs preincubated with 9-*cis* retinal (10 μM, black) quantified 1 hr after UVR exposure (4 J/cm^2), in parallel with HEMs not treated with retinal (white). n = 9; ± SEM, $p < 0.001$.

(C) UVR-dose dependence of intracellular melanin concentration of HEMs preincubated with 9-*cis* retinal (10 μM) and quantified 8 hr after exposure to the indicated doses (normalized as in A) and fitted with a sigmoid function. n = 6; ± SEM.

(D) Intracellular melanin concentration of HEMs preincubated with 9-*cis* retinal (10 μM) quantified at 4 hr after exposure to 4 J/cm^2 (17.5 mW/cm^2 for 228 s) either in the presence of Ca^{2+} (1.5 mM) or in the absence of Ca^{2+} (0 mM Ca^{2+}, 1.5 mM EGTA, 2 μM thapsigargin, and 20 μM BAPTA-AM). n = 2; ± SEM, $p < 0.04$ for Ca^{2+}-free versus with Ca^{2+}.

(E) Melanin concentration of HEMs preincubated with 9-*cis* retinal (10 μM) quantified at 8 hr after exposure to UVA (2.15 J/cm^2; 15 mW/cm^2 for 143 s), UVB (0.215 J/cm^2; 1.5 mW/cm^2 for 143 s), or the equivalent dose of UVR containing both UVA and UVB (2.5 J/cm^2; 17.5 mW/cm^2 for 143 s). n = 4–6; ± SEM, $p < 0.02$ for UVA versus UVB and $p < 0.043$ for UVR versus UVB. See also Figures S2 and S3.

mechanism, which may use existing tyrosinase that becomes enzymatically active downstream of receptor activation. Such a mechanism could involve phosphorylation of the cytosolic domain of tyrosinase by protein kinase Cβ [51, 52], a model consistent with the Ca^{2+}-dependence of UVA-induced melanin production in HEMs.

In conclusion, our results demonstrate that human melanocytes use a novel mechanism to sense and respond to ultraviolet light, in which UVA activates endogenous opsin receptors to cause calcium mobilization via a G protein- and PLC-mediated pathway. Our finding that the visual photopigment rhodopsin is expressed in melanocytes and contributes to UVR phototransduction suggests that human opsin receptors function outside the eye. Moreover, in our system, UVA exposure leads to calcium-dependent melanin synthesis on a timescale remarkably faster than that previously reported [50]. This novel UVR phototransduction mechanism has implications for understanding nonocular opsin signaling pathways and their function in skin physiology and pathology.

Supplemental Information

Supplemental Information includes three figures and Supplemental Experimental Procedures and can be found with this article online at doi:10.1016/j.cub.2011.09.047.

Acknowledgments

Primary support came from grants from Brown University (E.O.). Additional support came from the Natural Sciences and Engineering Research Council Postgraduate Scholarship to N.L.W. and the National Institutes of Health National Institute of Arthritis and Musculoskeletal and Skin Diseases Ruth L. Kirschstein National Research Service Award to J.A.N. We thank members of the Oancea laboratory for technical assistance, C.L. Makino and A.L. Zimmerman for helpful discussions, and D.M. Berson, J.A. Kauer, A.L. Zimmerman, and J. Marshall for critical readings of the manuscript.

Received: July 11, 2011
Revised: August 2, 2011
Accepted: September 29, 2011
Published online: November 3, 2011

References

1. U.S. Environmental Protection Agency. (2010). Health Effects of Overexposure to the Sun (http://www.epa.gov/sunwise/doc/healtheffects.pdf).
2. Gilchrest, B.A., Park, H.Y., Eller, M.S., and Yaar, M. (1996). Mechanisms of ultraviolet light-induced pigmentation. Photochem. Photobiol. *63*, 1–10.
3. Lin, J.Y., and Fisher, D.E. (2007). Melanocyte biology and skin pigmentation. Nature *445*, 843–850.
4. Pathak, M. (1995). Functions of melanin and protection by melanin. Melanin: Its Role in Human Photoprotection (Overland Park, KS: Valdenmar Publishing Co.), pp. 125–134.
5. Riley, P.A. (1997). Melanin. Int. J. Biochem. Cell Biol. *29*, 1235–1239.
6. Clingen, P.H., Arlett, C.F., Roza, L., Mori, T., Nikaido, O., and Green, M.H. (1995). Induction of cyclobutane pyrimidine dimers, pyrimidine(6-4)pyrimidone photoproducts, and Dewar valence isomers by natural sunlight in normal human mononuclear cells. Cancer Res. *55*, 2245–2248.
7. Eller, M.S., Ostrom, K., and Gilchrest, B.A. (1996). DNA damage enhances melanogenesis. Proc. Natl. Acad. Sci. USA *93*, 1087–1092.
8. Marrot, L., and Meunier, J.R. (2008). Skin DNA photodamage and its biological consequences. J. Am. Acad. Dermatol. *58*, S139–S148.
9. Pathak, M.A., Riley, F.J., Fitzpatrick, T.B., and Curwen, W.L. (1962). Melanin formation in human skin induced by long-wave ultra-violet and visible light. Nature *193*, 148–150.
10. Routaboul, C., Denis, A., and Vinche, A. (1999). Immediate pigment darkening: description, kinetic and biological function. Eur. J. Dermatol. *9*, 95–99.
11. Palczewski, K. (2006). G protein-coupled receptor rhodopsin. Annu. Rev. Biochem. *75*, 743–767.
12. Fan, J., Rohrer, B., Moiseyev, G., Ma, J.X., and Crouch, R.K. (2003). Isorhodopsin rather than rhodopsin mediates rod function in RPE65 knock-out mice. Proc. Natl. Acad. Sci. USA *100*, 13662–13667.

13. Thastrup, O., Dawson, A.P., Scharff, O., Foder, B., Cullen, P.J., Drøbak, B.K., Bjerrum, P.J., Christensen, S.B., and Hanley, M.R. (1989). Thapsigargin, a novel molecular probe for studying intracellular calcium release and storage. Agents Actions *27*, 17–23.
14. Beindl, W., Mitterauer, T., Hohenegger, M., Ijzerman, A.P., Nanoff, C., and Freissmuth, M. (1996). Inhibition of receptor/G protein coupling by suramin analogues. Mol. Pharmacol. *50*, 415–423.
15. Melyan, Z., Tarttelin, E.E., Bellingham, J., Lucas, R.J., and Hankins, M.W. (2005). Addition of human melanopsin renders mammalian cells photoresponsive. Nature *433*, 741–745.
16. Bleasdale, J.E., Bundy, G.L., Bunting, S., Fitzpatrick, F.A., Huff, R.M., Sun, F.F., and Pike, J.E. (1989). Inhibition of phospholipase C dependent processes by U-73, 122. Adv. Prostaglandin Thromboxane Leukot. Res. *19*, 590–593.
17. Roos, T.C., Jugert, F.K., Merk, H.F., and Bickers, D.R. (1998). Retinoid metabolism in the skin. Pharmacol. Rev. *50*, 315–333.
18. Huang, J., and Vieira, A. (2006). Evidence for a specific cell membrane retinol-binding protein transport mechanism in a human keratinocyte line. Int. J. Mol. Med. *17*, 627–631.
19. Vahlquist, A. (1982). Vitamin A in human skin: I. detection and identification of retinoids in normal epidermis. J. Invest. Dermatol. *79*, 89–93.
20. Vahlquist, A., Lee, J.B., Michaëlsson, G., and Rollman, O. (1982). Vitamin A in human skin: II Concentrations of carotene, retinol and dehydroretinol in various components of normal skin. J. Invest. Dermatol. *79*, 94–97.
21. Fu, P.P., Xia, Q., Boudreau, M.D., Howard, P.C., Tolleson, W.H., and Wamer, W.G. (2007). Physiological role of retinyl palmitate in the skin. Vitam. Horm. *75*, 223–256.
22. Moiseyev, G., Chen, Y., Takahashi, Y., Wu, B.X., and Ma, J.X. (2005). RPE65 is the isomerohydrolase in the retinoid visual cycle. Proc. Natl. Acad. Sci. USA *102*, 12413–12418.
23. Jin, M., Li, S., Moghrabi, W.N., Sun, H., and Travis, G.H. (2005). Rpe65 is the retinoid isomerase in bovine retinal pigment epithelium. Cell *122*, 449–459.
24. Hinterhuber, G., Cauza, K., Brugger, K., Dingelmaier-Hovorka, R., Horvat, R., Wolff, K., and Foedinger, D. (2004). RPE65 of retinal pigment epithelium, a putative receptor molecule for plasma retinol-binding protein, is expressed in human keratinocytes. J. Invest. Dermatol. *122*, 406–413.
25. Mata, N.L., Ruiz, A., Radu, R.A., Bui, T.V., and Travis, G.H. (2005). Chicken retinas contain a retinoid isomerase activity that catalyzes the direct conversion of all-trans-retinol to 11-cis-retinol. Biochemistry *44*, 11715–11721.
26. Mata, N.L., Radu, R.A., Clemmons, R.C., and Travis, G.H. (2002). Isomerization and oxidation of vitamin a in cone-dominant retinas: a novel pathway for visual-pigment regeneration in daylight. Neuron *36*, 69–80.
27. Berson, D.M. (2007). Phototransduction in ganglion-cell photoreceptors. Pflugers Arch. *454*, 849–855.
28. Provencio, I., Rodriguez, I.R., Jiang, G., Hayes, W.P., Moreira, E.F., and Rollag, M.D. (2000). A novel human opsin in the inner retina. J. Neurosci. *20*, 600–605.
29. Tarttelin, E.E., Bellingham, J., Hankins, M.W., Foster, R.G., and Lucas, R.J. (2003). Neuropsin (Opn5): a novel opsin identified in mammalian neural tissue. FEBS Lett. *554*, 410–416.
30. Halford, S., Freedman, M.S., Bellingham, J., Inglis, S.L., Poopalasundaram, S., Soni, B.G., Foster, R.G., and Hunt, D.M. (2001). Characterization of a novel human opsin gene with wide tissue expression and identification of embedded and flanking genes on chromosome 1q43. Genomics *72*, 203–208.
31. Tsutsumi, M., Ikeyama, K., Denda, S., Nakanishi, J., Fuziwara, S., Aoki, H., and Denda, M. (2009). Expressions of rod and cone photoreceptor-like proteins in human epidermis. Exp. Dermatol. *18*, 567–570.
32. Miyashita, Y., Moriya, T., Kubota, T., Yamada, K., and Asami, K. (2001). Expression of opsin molecule in cultured murine melanocyte. J Investig Dermatol Symp Proc *6*, 54–57.
33. Makino, C.L., Groesbeek, M., Lugtenburg, J., and Baylor, D.A. (1999). Spectral tuning in salamander visual pigments studied with dihydroretinal chromophores. Biophys. J. *77*, 1024–1035.
34. Wald, G., and Brown, P.K. (1958). Human rhodopsin. Science *127*, 222–226.
35. Baylor, D.A., Nunn, B.J., and Schnapf, J.L. (1984). The photocurrent, noise and spectral sensitivity of rods of the monkey Macaca fascicularis. J. Physiol. *357*, 575–607.
36. Shi, W., Osawa, S., Dickerson, C.D., and Weiss, E.R. (1995). Rhodopsin mutants discriminate sites important for the activation of rhodopsin kinase and Gt. J. Biol. Chem. *270*, 2112–2119.
37. Okada, T., Ernst, O.P., Palczewski, K., and Hofmann, K.P. (2001). Activation of rhodopsin: new insights from structural and biochemical studies. Trends Biochem. Sci. *26*, 318–324.
38. Williams, T.P. (1964). Photoreversal of Rhodopsin Bleaching. J. Gen. Physiol. *47*, 679–689.
39. Miyazono, S., Isayama, T., and Makino, C.L. (2011). Vitamin A as an activator and sensitizing chromophore for rhodopsin. Biophys. J. *100*, 22a.
40. Edwards, S.L., Charlie, N.K., Milfort, M.C., Brown, B.S., Gravlin, C.N., Knecht, J.E., and Miller, K.G. (2008). A novel molecular solution for ultraviolet light detection in Caenorhabditis elegans. PLoS Biol. *6*, e198.
41. Liu, J., Ward, A., Gao, J., Dong, Y., Nishio, N., Inada, H., Kang, L., Yu, Y., Ma, D., Xu, T., et al. (2010). C. elegans phototransduction requires a G protein-dependent cGMP pathway and a taste receptor homolog. Nat. Neurosci. *13*, 715–722.
42. Xiang, Y., Yuan, Q., Vogt, N., Looger, L.L., Jan, L.Y., and Jan, Y.N. (2010). Light-avoidance-mediating photoreceptors tile the Drosophila larval body wall. Nature *468*, 921–926.
43. Rozenfeld, R., and Devi, L.A. (2011). Exploring a role for heteromerization in GPCR signalling specificity. Biochem. J. *433*, 11–18.
44. Oancea, E., Vriens, J., Brauchi, S., Jun, J., Splawski, I., and Clapham, D.E. (2009). TRPM1 forms ion channels associated with melanin content in melanocytes. Sci. Signal. *2*, ra21.
45. Parrish, J.A., Jaenicke, K.F., and Anderson, R.R. (1982). Erythema and melanogenesis action spectra of normal human skin. Photochem. Photobiol. *36*, 187–191.
46. Beitner, H. (1988). Immediate pigment-darkening reaction. Photodermatol. *5*, 96–100.
47. Wolber, R., Schlenz, K., Wakamatsu, K., Smuda, C., Nakanishi, Y., Hearing, V.J., and Ito, S. (2008). Pigmentation effects of solar-simulated radiation as compared with UVA and UVB radiation. Pigment Cell Melanoma Res *21*, 487–491.
48. Ou-Yang, H., Stamatas, G., and Kollias, N. (2004). Spectral responses of melanin to ultraviolet A irradiation. J. Invest. Dermatol. *122*, 492–496.
49. Kayatz, P., Thumann, G., Luther, T.T., Jordan, J.F., Bartz-Schmidt, K.U., Esser, P.J., and Schraermeyer, U. (2001). Oxidation causes melanin fluorescence. Invest. Ophthalmol. Vis. Sci. *42*, 241–246.
50. Cui, R., Widlund, H.R., Feige, E., Lin, J.Y., Wilensky, D.L., Igras, V.E., D'Orazio, J., Fung, C.Y., Schanbacher, C.F., Granter, S.R., and Fisher, D.E. (2007). Central role of p53 in the suntan response and pathologic hyperpigmentation. Cell *128*, 853–864.
51. Park, H.Y., Russakovsky, V., Ohno, S., and Gilchrest, B.A. (1993). The beta isoform of protein kinase C stimulates human melanogenesis by activating tyrosinase in pigment cells. J. Biol. Chem. *268*, 11742–11749.
52. Park, H.Y., Perez, J.M., Laursen, R., Hara, M., and Gilchrest, B.A. (1999). Protein kinase C-beta activates tyrosinase by phosphorylating serine residues in its cytoplasmic domain. J. Biol. Chem. *274*, 16470–16478.