Differential Regulation of Tyrosinase Activity in Skin of White and Black Individuals *In Vivo* by Topical Retinoic Acid

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Tyrosinase activity is a key determinant of melanin production in skin. Because retinoic acid regulates tyrosinase activity in melanoma cells, we analyzed modulation of pigmentation *in vivo* by retinoic acid. Black and white subjects were either not treated, or treated topically for 4 d under occlusion with vehicle, retinoic acid (0.1%), or the irritant sodium lauryl sulfate (2%). In untreated skin, tyrosinase activity and melanin content were significantly greater (2.3 times, and 3.2 times, respectively) in blacks versus whites. Four days of treatment with topical retinoic acid did not alter tyrosinase activity or melanin content in black skin. In contrast, retinoic acid treatment significantly induced (2.7 times, $n = 8$) tyrosinase activity, compared to vehicle treatment, in white skin. Melanin content, however, remained unchanged at 4 d. In separate experiments, tyrosinase activity in white subjects ($n = 25$) was increased 16% ($p = 0.01$) in sodium lauryl sulfate–treated skin, and 77% ($p = 0.0005$) in retinoic acid–treated skin, compared to vehicle-treated skin. The effect of retinoic acid on tyrosinase activity could be differentiated from non-specific irritation, because tyrosinase activity in retinoic acid–treated skin was significantly greater (52%, $p = 0.004$) than sodium lauryl sulfate–treated skin. Similar results were obtained with the dihydroxyphenylalanine reaction done on vehicle, sodium lauryl sulfate–, and retinoic acid–treated white skin. Northern analysis ($n = 6$) and semi-quantitative polymerase chain reaction ($n = 6$) demonstrated that retinoic acid treatment did not alter tyrosinase mRNA levels in white skin. Western analysis revealed that induction of tyrosinase activity by retinoic acid also was not associated with increased tyrosinase protein content ($n = 9$), indicating that regulation of tyrosinase activity by retinoic acid occurs through a post-translational mechanism. These data demonstrate that low tyrosinase activity in white skin *in vivo* is retinoic acid inducible and high tyrosinase activity in black skin *in vivo* is neither further induced nor reduced by retinoic acid. *J Invest Dermatol 100:800–805, 1993*

Tyrosinase is the rate-limiting enzyme in the biosynthesis of the skin pigment melanin [1–4]. Tyrosinase catalyses three different reactions in the biosynthetic pathway of melanin: 1) the hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA); 2)oxidation of DOPA to DOPA-quinone; and 3) the oxidation of 5,6-dihydroxyindole (DHI) to indole-quinone [1]. The rate-limiting steps in melanogenesis occur at the level of the oxidations of tyrosine and dopa, and the quantity of melanin synthesized is proportional to the amount of tyrosinase activity present in the cell [5]. Furthermore, in humans, evidence indicates that human skin color can be correlated with tyrosinase activity [6,7].

Tyrosinase has been purified from mouse and human melanoma cell lines and has been shown to be highly homologous in the two species [8,9]. The cDNA sequences of mouse and human tyrosinase display 80% sequence identity [10,11]. In mouse melanoma cells tyrosinase has been shown to exist in a number of discrete forms ranging in molecular weight from 58,000 to 150,000 [12]. The molecular heterogeneity of tyrosinase is thought to arise through post-translational processing including glycosylation of enzyme [13].

Tyrosinase activity is modulated both positively and negatively by retinoic acid in mouse as well as human melanoma cells [14–16]. We have recently reported that retinoic acid treatment for 6 d does not alter either tyrosinase activity or melanin content in normal cultured melanocytes from white skin [17]. The objectives of the present study were to examine the effect of short-term topical retinoic acid treatment on tyrosinase activity and melanin content in normal non sun-exposed skin of black and white humans.

MATERIALS AND METHODS

Materials L-tyrosine, dihydroxyphenylalanine (L-DOPA), Triton X-100, and citric acid were purchased from Sigma Chemical Co. (St. Louis, MO). Charcoal (Norit A–activated charcoal) was obtained from Aldrich Co. Tyrosine ([ring-3,5-^{3}H]tyrosine, 50 Ci/mmole) and [^{125}I]-goat anti-rabbit IgG (9.8 mCi/mg) were purchased from Dupont New England Nuclear (Boston, MA). Pansorbin (*Staphylococcus aureus* cells) was purchased from Calbiochem (La Jolla, CA). Human tyrosinase cDNA (Pmel34) was a generous gift of Dr. B. S. Kwon, Indiana University, Indiana. Polyclonal anti-tyrosinase antibody was a generous gift of Dr. H. Rorsman, University of Lund, Sweden. All other chemicals were of analytical reagent grade.

Treatment of Subjects and Procurement of Tissue For studies to determine the effects of retinoic acid and sodium lauryl sulfate

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Abbreviations: DHI, 5,6-dihydroxyindole; RA, retinoic acid; SLS, sodium lauryl sulfate.

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(SLS) on tyrosinase activity and melanin content, adult white and black volunteers were treated topically with 0.1% all-*trans* retinoic acid (Retin-A cream), vehicle control (Retin-A vehicle), and the irritant SLS (2% in the retinoic acid vehicle) on the lower hips or buttocks. After application, the areas were covered with plastic wrap, held in place with dermatape, to prevent loss of cream and enhance penetration. Treated areas were biopsied, by keratome, 96 h following treatment. For DOPA reaction, 4-mm punch biopsies were also obtained from skin treated as described above. For studies to compare tyrosinase activity and melanin content in black and white skin, keratome biopsies were obtained from black and white volunteers who received no topical treatment. Tissue was snap frozen in liquid nitrogen and stored at −70°C until used. All procedures involving human subjects were approved by University of Michigan Institutional Review Board.

Preparation of Soluble Tyrosinase Keratome biopsies from treated and untreated skin (approximately 50 mg wet weight) were pulverized by mortar and pestle under liquid nitrogen. The powdered tissue was transferred to a glass homogenizer containing 1 ml of sodium phosphate buffer (0.1 M, pH 6.8) with 1% Triton X-100 and homogenized with 15 strokes. The homogenate was then sonicated three times (5-second bursts with 30-second rests) with a microtip sonicator. The homogenate was left on ice for 1 h prior to centrifugation at 100,000 × *g* for 40 min, and the supernatant served as the source of tyrosinase activity [7].

Tyrosine Hydroxylase Assay The radiometric determination of tyrosinase activity was performed as previously described [15,18]. Briefly, 0.5 ml of a reaction mixture consisting of 0.2 ml skin supernatant, 0.1 mM tyrosine, 0.1 mM L-DOPA, and 2 μCi of [^{3}H]tyrosine in phosphate buffer was incubated at 37°C for 2 h. This assay measures the production of [^{3}H]H_2O during the conversion of [^{3}H]tyrosine to L-DOPA. The reaction was terminated by addition of 500 μl of 10% trichloroacetic acid and centrifuged at 2,000 × *g* for 5 min. Supernatants were transferred to tubes containing 1 ml charcoal (10% w/v in 0.1 M citric acid) and centrifuged at 2,000 × *g* for 10 min. The resultant supernatants (0.3 ml) were counted by liquid-scintillation spectrometry.

Melanin Assay To determine melanin content skin biopsies (50 mg) were homogenized in 1 ml phosphate buffer as described above, and the melanin in 0.35 ml of the resulting homogenate solubilized by treatment with 2 N NaOH at 60°C for 4 d. An equal volume of water was then added followed by the addition of 0.7 ml of chloroform : phenol (1 : 1). The mixture was vortexed and centrifuged at 5000 × *g* for 10 min to separate the phases. Melanin content in the water layer was determined spectrophotometrically at 400 nm [7,19]. Values are expressed as micrograms of melanin per milligram wet weight of skin.

RNA Blot Hybridization Total RNA was prepared from keratome biopsies of vehicle- and retinoic acid–treated skin by cesium chloride density centrifugation [20], and size fractionated on a 1.2% formaldehyde denaturing agarose gel. The RNA was transferred to nylon membranes and hybridized to [^{32}P]labeled human tyrosinase (Pmel34) and rat cyclophilin cDNA probes [20]. The filters were subsequently washed two times for 30 min each in 2 × sodium citrate/sodium chloride buffer (SSC) plus 0.1% sodium dodecylsulfate (SDS) at 42°C, and one time with 0.2 × SSC plus 0.1% SDS at 65°C for 1 h. The transcript for cyclophilin was used as an internal control, because its level does not vary with retinoic acid treatment [21].

Semi-Quantitative PCR Reverse transcriptase/polymerase chain reaction was performed under conditions that allowed the relative levels of tyrosinase mRNA in vehicle- and retinoic acid–treated skin to be determined [22]. Rabbit globin mRNA (1.2 pg) and total RNA from vehicle- and retinoic acid–treated keratome biopsies (1 mg) were combined and reverse transcribed using random hexamer primers (25 pmol) and 200 U M-MLV H- reverse transcriptase, at 37°C for 30 min in 50 mM Tris (pH 8.3), 0.3 mM $MgCl_2$, 20 mM dithiothreitol, 500 mM dXTP, and 20 units RNAse inhibitor. Following this, three fourfold dilutions of the reverse-transcriptase reaction were made. To each dilution, specific human tyrosinase and rabbit β-globin primers (50 pmol), and TAQ DNA polymerase (2 U) were added, and polymerase chain reaction was carried out in 40 mM Tris (pH 8.3), 1.5 mM $MgCl_2$, 125 mM dXTP (final concentrations) for 20 cycles at 95°C for 30 seconds, 56°C for 30 seconds, and 72°C for 30 seconds, with a 3-second extension per cycle. Polymerase chain reaction products were separated by agarose gel electrophoresis, transferred to nylon membrane, and simultaneously hybridized with [^{32}P]labeled tyrosinase and β-globin probes (isolated polymerase chain reaction products). Radioactivity in the hybridized bands was quantified by a PhosphorImager (Molecular Dynamics). Results were analyzed as the ratio of counts in the tyrosinase band to counts in the β-globin band. Over the concentration range of human skin and rabbit globin RNA utilized, the amount of polymerase chain reaction products formed after 20 cycles was linearly proportional to the amount of RNA added.

Immunoblotting To determine tyrosinase protein content, equal amounts of supernatant protein (50 μg) prepared from vehicle-, SLS-, and retinoic acid–treated skin as described above were separated by SDS–polyacrylamide gel electrophoresis (PAGE) and transferred to nitrocellulose membranes. Following transfer, membranes were incubated for 3 h in phosphate-buffered saline (PBS) containing 5% non-fat dry milk, 0.02% Tween-20 to block nonspecific binding. The membranes were then incubated with anti-tyrosinase antibody [23] (1 : 250 dilution) overnight at 4°C and washed five times in Tween-20/PBS. The membranes were incubated with [^{125}I]goat anti-rabbit IgG in Tween-20/PBS for 2 h at room temperature and washed five times with Tween-20/PBS. The bands on the membrane were detected by autoradiography and the radioactivity in the bands measured by PhosphorImager.

Immunoprecipitation of Tyrosinase Supernatants from keratome biopsies, prepared as described above, were incubated with tyrosinase antisera (1 : 200 dilution) for 60 min at 37°C. The immunocomplex was precipitated by addition of pansorbin (20 μl) for another 30 min followed by centrifugation at 1000 × *g* for 4 min. The precipitate was dissolved in SDS-PAGE sample buffer [24], loaded on SDS-PAGE, and immunobloted using the same tyrosinase antisera used for immunoprecipitation.

DOPA Reaction Four-millimeter punch biopsies obtained from the treated areas of black and white skin were placed immediately in 2 N sodium bromide for 90–120 min at room temperature to separate epidermis from dermis. The epidermis was then incubated for 7 h at 37°C in phosphate buffer (pH 7.4) containing 0.1% DOPA [25]. After removal from the DOPA solution, the tissue was rinsed with distilled water for 5 min and mounted on a triple-welled slide with stratum corneum upwards. The epidermis was examined microscopically and the number of DOPA-positive melanocytes/unit area determined.

Statistical Analysis Differences in group means among treatment groups were analyzed by paired t test. Correlation between Northern analysis and semi-quantitative PCR was analyzed by Pearson's correlation method.

RESULTS

Tyrosinase Activity and Melanin Content in Retinoic Acid–Treated Black and White Skin To investigate the effects of treatment with topical retinoic acid (0.1%) on melanogenesis in adult human skin, ten black and eight white volunteers were treated topically with vehicle and retinoic acid (0.1%) for 4 d and keratome biopsies obtained from these individuals were analyzed for tyrosinase activity and melanin content. The results in Fig 1 indicate that tyrosinase activity and melanin content were unchanged by retinoic acid treatment in black skin. In contrast, retinoic acid treatment of white skin induced tyrosinase activity nearly threefold ($p = 0.01$).

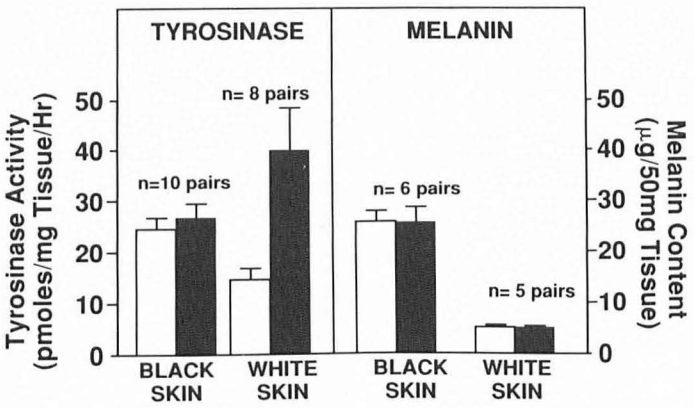

Figure 1. Tyrosinase activity and melanin content in black and white skin treated with retinoic acid. Black and white subjects were treated topically under occlusion with retinoic acid (0.1%) *(solid bars)* and retinoic acid vehicle *(open bars)* for 4 d. Tyrosinase activity and melanin content were measured in keratome biopsies as described in *Materials and Methods. Bars,* means ± SEM. Tyrosinase activity: retinoic acid versus vehicle in black skin, $p = 0.14$; in white skin, $p < 0.01$.

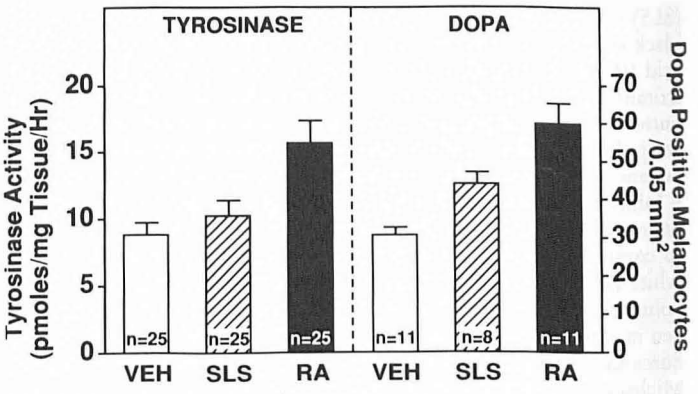

Figure 3. Tyrosinase activity and DOPA staining in white skin treated with vehicle, SLS, and retinoic acid. Subjects were treated with vehicle (VEH), SLS, and retinoic acid (RA) for 4 d under occlusion. Tyrosinase activity and DOPA staining were measured in keratome biopsies and punch biopsies, respectively, as described in *Materials and Methods. Bars,* means ± SEM. Tyrosinase activity: VEH versus SLS, $p = 0.01$; VEH versus RA, $p = 0.0002$; SLS versus RA, $p = 0.004$. DOPA staining: VEH versus SLS, $p = 0.01$; VEH versus RA, $p = 0.0005$; SLS versus RA, $p = 0.03$.

However, 4 d of retinoic acid treatment was not sufficient to produce a similar increase in melanin content (Fig 1).

Tyrosinase Activity and Melanin Content in Black and White Skin In the above study (Fig 1), tyrosinase activity and melanin content were found to be greater in vehicle-treated skin of blacks compared to whites. We therefore examined whether these differences were present in untreated black and white skin. Tyrosinase activity and melanin content were measured in supernatants prepared from keratome biopsies from ten black and ten white individuals. The results in Fig 2 demonstrate that tyrosinase activity in black epidermis is significantly greater (2.3 times, $p < 0.0001$) than in white epidermis. Melanin content was also found to be greater (3.2 times, $p < 0.0001$) in black skin compared to white skin.

Comparison of Treatment with Retinoic Acid Versus SLS on Tyrosinase Activity in White Skin Topical retinoic acid results in an erythmetous "irritant" reaction clinically and histologically similar to that observed with topical SLS treatment [26]. We therefore compared the effects of topical treatment with retinoic acid and SLS on tyrosinase activity in white skin. In this study white individuals were treated with vehicle, SLS (2%), and retinoic acid (0.1%) for 4 d under occlusion. Keratome biopsies obtained after treatment were analyzed for tyrosinase activity by enzymatic and histochemical assays. We have previously demonstrated that treatment of skin with 2% SLS causes clinical and histologic changes similar to those observed with retinoic acid [26]. The results in Fig 3 demonstrate that tyrosinase activity was increased 16% ($p = 0.01$, $n = 25$), compared to vehicle, with SLS treatment. Retinoic acid treatment resulted in a 77% increase in tyrosinase activity ($p = 0.0002$, $n = 25$), compared to vehicle, and a 52% increase ($p = 0.004$, $n = 25$), compared to SLS. These data suggest that increased tyrosinase activity following retinoic acid treatment is not solely due to non-specific irritation, because tyrosinase activity is significantly greater following retinoic acid treatment than that observed following SLS treatment. Because SLS treatment resulted in a small increase in tyrosinase activity, a small fraction of the observed increase in tyrosinase activity following retinoic acid treatment may be attributable to irritation. Whether increased tyrosinase activity following retinoic acid and SLS treatment occurs through common or distinct mechanisms remains to be determined.

Tyrosinase activity (DOPA oxidase) was also assessed histochemically (DOPA stain) in epidermis obtained from vehicle-, SLS-, and retinoic acid–treated white individuals. The results in Fig 3 demonstrate that retinoic acid treatment significantly increased the number of DOPA-staining melanocytes compared to either vehicle (100%, $p = 0.0005$) or SLS treatment (36%, $p = 0.03$). Similar to what we observed with tyrosinase activity measured biochemically, SLS treatment resulted in increased numbers of DOPA-positive melanocytes (46%, $p = 0.01$), compared to vehicle.

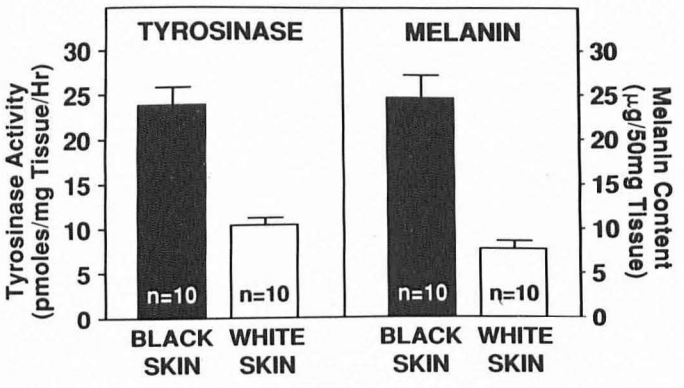

Figure 2. Comparison of tyrosinase activity and melanin content in untreated normal skin of blacks and whites. *Bars,* means ± SEM. Tyrosinase activity: black versus white, $p < 0.0001$. Melanin content: black versus white, $p < 0.0001$.

Tyrosinase mRNA Levels in White Skin To determine if the observed increase in tyrosinase activity following retinoic acid treatment of white skin was associated with increased tyrosinase gene expression, steady-state tyrosinase mRNA levels in vehicle and retinoic acid–treated white skin were analyzed by Northern blotting and semi-quantitative PCR. The results in Fig 4*A,B* demonstrate that retinoic acid treatment did not alter tyrosinase mRNA levels in six individuals. There was a significant correlation ($r = 0.93$) in the difference of relative tyrosinase mRNA levels between retinoic acid– and vehicle-treated skin as measured by Northern

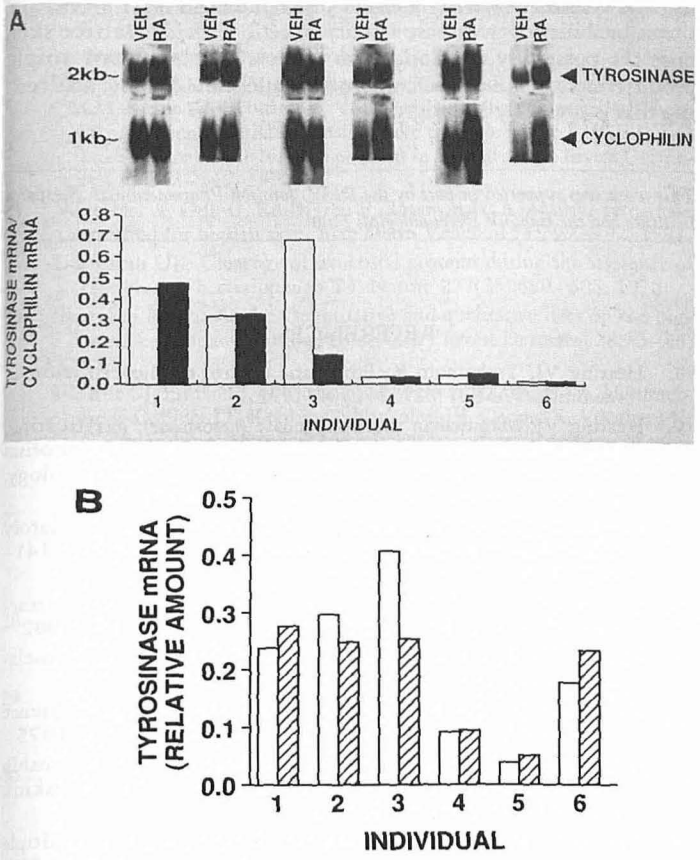

Figure 4. Tyrosinase mRNA levels in white skin treated with vehicle and retinoic acid. *(A)* Total RNA was prepared from six white subjects treated topically with 0.1% retinoic acid (RA) and vehicle (VEH) for 4 d. RNA blots *(top)* (30 μg/lane) were probed with [^{32}P]labeled cDNA for human tyrosinase and rat cyclophilin (used as an internal control). Radioactivity in the hybridized bands *(bottom)* were quantified using a PhosphorImager. *(B)* Tyrosinase mRNA levels in the same RNA samples as in *A* were determined by semi-quantitative PCR as described in *Materials and Methods.* Bands were quantified by a PhosphorImager.

analysis and semi-quantitative PCR. These results indicate that induction of tyrosinase activity in retinoic acid–treated human skin *in vivo* may not involve increased tyrosinase gene expression.

Tyrosinase Protein Levels in White Skin Treated with Retinoic Acid We next examined whether tyrosinase protein was elevated following 4-d retinoic acid and SLS treatment. Equal amounts of protein extracts from vehicle, SLS, and retinoic acid–treated white skin were immunoblotted with anti-tyrosinase antisera and detected with [^{125}I]goat anti-rabbit IgG antibody. Three prominent bands were observed with molecular weight of 66 kDa (the expected molecular weight of tyrosinase), 58 kDa, and 48 kDa. No change in intensity of the 66-kDa band was observed following retinoic acid and SLS treatment compared to vehicle (Fig 5*A*).

To further verify the above immunoblot results, solubilized supernatants from retinoic acid and vehicle–treated skin were also immunoprecipitated with anti-tyrosinase antibody and the resulting immune precipitates and supernatants immunoblotted. Western analysis of these immunoprecipitates revealed a single band of the expected molecular weight of tyrosinase (66 kDa), which was similar in intensity in samples from retinoic acid– and vehicle-treated skin (Fig 5*B*). This band was absent in the supernatants from the immunoprecipitate, which contained the 58-kDa and 48-kDa bands. These data indicate that the observed increase in tyrosinase activity in SLS- and retinoic acid–treated skin was not accompanied by a concomitant increase in tyrosinase protein.

DISCUSSION

The above data demonstrate that tyrosinase activity is induced by short-term topical retinoic acid treatment in white, but not in black, skin. The molecular basis for this difference is not known. One possible explanation for the lack of inducibility of tyrosinase activity following retinoic acid treatment in black skin may be related to our finding that the level of tyrosinase activity was 2.3-times higher in black, compared to white, skin. This difference presumably reflects differences in the genetic regulation of tyrosinase activity between black and white skin, which may confer refractoriness of tyrosinase in black skin to further induction by topical retinoic acid. The lack of inducibility of tyrosinase activity in black skin is not necessarily a general phenomen, but rather restricted to retinoic acid (and perhaps other) treatment, because black skin undergoes a delayed tanning reaction. Data indicates that delayed tanning is associated with

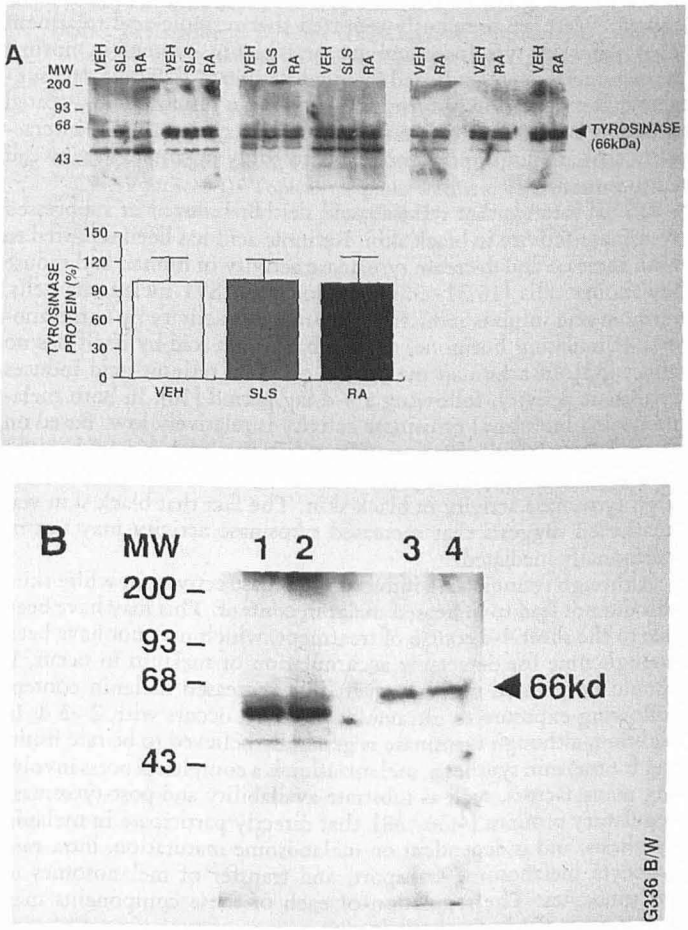

Figure 5. Immunoblot analysis of tyrosinase protein in white skin treated with vehicle, SLS, and retinoic acid. *(A)* Supernatants from detergent solubilized keratome biopsies from four subjects treated with vehicle (VEH), SLS, and retinoic acid (RA), and from three individuals treated with vehicle and retinoic acid were immunoblotted with anti-tyrosinase antibody as described in *Materials and Methods (top).* Radioactivity in the tyrosinase band (66 kDa) was quantified by a PhosphorImager *(bottom). Bars,* means ± SEM. *(B)* Immunoprecipitation of tyrosinase protein. Supernatants from detergent solubilized keratome biopsies from vehicle- and retinoic acid–treated skin were immunoprecipitated with anti-tyrosinase antibody. The supernatants (*lanes 1* and *2*) and immune precipitates (*lanes 3* and *4*) were immunoblotted with the immunoprecipitating antibody. *Lanes 1* and *3,* vehicle-treated skin; *lanes 2* and *4,* retinoic acid–treated skin. Results are representative of three experiments.

increased tyrosinase activity [27]. Our data demonstrating increased tyrosinase activity and melanin content in adult black skin is consistent with reported differences in tyrosinase activity and melanin content in black and white neonatal foreskins [7].

Induction of tyrosinase activity in white skin treated with topical retinoic acid was not accompanied by increases in either tyrosinase mRNA or protein. These data suggest that modulation of tyrosinase activity following retinoic acid treatment occurs through post-transcriptional mechanisms. Induction of tyrosinase activity by α-MSH has been reported to occur through suppression of an inhibitor [28,29]. A similar mechanism could be operating in response to retinoic acid. Alternatively, elevation of tyrosinase activity by retinoic acid may involve induction of an activating factor or direct modification, for example, through phosphorylation or glycosylation, of pre-existing tyrosinase molecules [30]. Increased tyrosinase activity in black versus white foreskins was found to be due to a higher kinetic velocity (Vmax), rather than higher levels of tyrosinase protein [7]. These data provide additional evidence for the role of post-transcriptional mechanisms in the regulation of tyrosinase activity. The molecular mechanism(s) through which retinoic acid treatment leads to increased tyrosinase activity remains to be determined. We have previously reported that retinoic acid treatment does not alter tyrosinase activity or melanin content in normal human melanocytes cultured from white skin [17]. These data suggest that modulation of tyrosinase activity in retinoic acid–treated skin may involve multiple complex secondary and tertiary interactions that are not directly amenable to study in simple *in vitro* cell culture models.

It is of interest that retinoic acid neither induced or suppressed tyrosinase activity in black skin. Retinoic acid has been reported to both increase and decrease tyrosinase activity in human and mouse melanoma cells [16,31–35]. In Cloudman S91 melanoma cells, retinoic acid inhibits induction of tyrosinase activity by α-melanocyte-stimulating hormone, although retinoic acid by itself has no effect [33]. In a human melanoma cell line retinoic acid induces tyrosinase activity, following a 4-d lag period [16]. In both melanoma cell lines basal tyrosinase activity is relatively low. Based on these *in vitro* results one would predict that retinoic acid might potentiate relatively low tyrosinase activity in white skin and reduce high tyrosinase activity in black skin. The fact that black skin was unaffected suggests that increased tyrosinase activity may not be hormonally mediated.

Although retinoic acid induced tyrosinase activity in white skin, this did not lead to increased melanin content. This may have been due to the short 4-d course of treatment, which may not have been enough time for detectable accumulation of melanin to occur. It should be pointed out, however, that increased melanin content following exposure to ultraviolet radiation occurs with 2–3 d. In addition, although tyrosinase is generally believed to be rate limiting for melanin synthesis, melanization is a complex process involving many factors, such as substrate availability and post-tyrosinase regulatory proteins [4,36–38], that directly participate in melanin synthesis, and is dependent on melanosome maturation, intra-melanocyte melanosome transport, and transfer of melanosomes to keratinocytes. The regulation of each of these components may influence melanin synthesis *in vivo*.

We have recently demonstrated that topical retinoic acid (0.1%) treatment significantly reduced hyperpigmented lesions (liver spots) associated with photodamage in white patients [39]. In normal white skin, however, we observed increased, not decreased, tyrosinase activity following retinoic acid treatment. Thus, retinoic acid treatment may be capable of modulating tyrosinase activity *in vivo* both positively and negatively depending on the degree of pigmentation and the state of the skin being treated. This suggests that mechanisms involved in cutaneous responses to retinoic acid treatment are complex.

In summary, our data demonstrate that low tyrosinase activity in white skin is induced by retinoic acid *in vivo*, whereas high tyrosinase in black skin is neither further induced or reduced. The finding of high tyrosinase activity and melanin content in black skin, and the inducibility of tyrosinase activity by retinoic acid in white skin, raise the possibility that long-term retinoic acid treatment might produce increased melanin content in white skin. A study addressing this is presently in progress.

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