Role of Tyrosinase as the Determinant of Pigmentation in Cultured Human Melanocytes

Ken Iozumi,*† George E. Hoganson,‡ Raffaele Pennella,*† Mark A. Everett,† and Bryan B. Fuller*†

*Department of Biochemistry and Molecular Biology and †Department of Dermatology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma; and ‡Department of Pediatrics, University of Illinois College of Medicine, Chicago, Illinois, U.S.A.

Variations in human pigmentation among different racial groups are due to differences in the production and deposition of melanin in the skin. Although melanin synthesis is known to be controlled by the rate-limiting enzyme tyrosinase, the role of this enzyme as the principal determinant of skin pigmentation is unclear. Results from studies with human melanocyte cultures derived from different racial skin types reveal an excellent correlation between the melanin content of melanocyte cultures and the *in situ* activity of tyrosinase. Melanocytes derived from black skin have up to 10 times more tyrosinase activity and produce up to 10 times more melanin than melanocytes derived from white skin. However, the higher level of tyrosinase activity in melanocytes derived from black skin is not due to a greater abundance of tyrosinase. Results from immunotitration experiments and Western immunoblots reveal that the number of tyrosinase molecules present in white-skin melanocytes may equal the number found in highly pigmented black skin types. Moreover, approximately equivalent levels of tyrosinase mRNA are present in white and black skin cell strains. In contrast, melanocytes derived from red-haired neonates with low tyrosinase activity contain low numbers of tyrosinase molecules and low levels of tyrosinase mRNA. These results show that tyrosinase activity and melanin production in most light-skinned people is controlled primarily by a post-translational regulation of pre-existing enzyme and not by regulating tyrosinase gene activity. In contrast, melanocytes from red-haired (type I) people have low levels of tyrosinase protein and mRNA, suggesting that transcriptional activity of the tyrosinase gene is suppressed. *J Invest Dermatol 100: 806–811, 1993*

Differences in racial skin pigmentation are due to variations in the amount of melanin produced by melanocytes and deposited throughout the epidermis [1]. Differences in skin melanin content are likely to be due to several factors, including 1) the rate of synthesis and decay of tyrosinase; 2) the activity of tyrosinase in melanosomes; 3) the rate of synthesis and melanization of melanosomes; 4) melanosome size; 5) the efficiency of melanosome transfer to keratinocytes; and 6) the rate of degradation of melanosomes in keratinocytes [2,3]. At present, little is known with regard to the relative importance of any of these steps in regulating racial pigmentation. Because tyrosinase is the rate-limiting enzyme for melanin synthesis, and because defects in tyrosinase activity lead to albinism in humans [4], it seems likely that racial differences in human skin color may be due primarily to differences in tyrosinase activity in melanocytes from varying skin types. Previous studies examining tyrosinase activity in homogenates from black and white neonatal foreskins have shown that, on average, black skins have higher tyrosinase activity than white skins [5,6]. To more clearly define the role of tyrosinase as a determinant of human skin color, we examined tyrosinase activity, enzyme abundance, and tyrosinase mRNA levels in melanocyte cell cultures derived from various human skin types.

Manuscript received September 18, 1991; accepted for publication January 12, 1993.

This work was presented in part at the Annual Meeting of the Society of Investigative Dermatology, 1991, and published in abstract form in J Invest Dermatol 96:632, 1990.

Reprint requests to: Dr. Bryan B. Fuller, Department of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center, P.O. Box 26901, Oklahoma City, OK 73190.

Abbreviations:
L-DOPA: L-dihydroxyphenylalanine
PMSF: phenylmethylsulfonyl fluoride
SDS: sodium dodecyl sulfate
Tris: Tris(hydroxymethyl)aminomethane

MATERIALS AND METHODS

Reagents and Supplies L-[ring-3,5-^{3}H]tyrosine (46.7 Ci/mmol) was purchased from DuPont New England Nuclear (Boston, MA). Minimum essential Eagle's medium for suspension cultures was obtained from Flow Laboratories (McLean, VA). Ham's F-10 nutrient mixture powder and trypsin (1:250) were purchased from Gibco Laboratories (Grand Island, NY). Fetal bovine serum and horse serum were purchased from Hy Clone Laboratories Inc. (Logan, UT). [^{14}C]tyrosine (350 mCi/mmol) was obtained from ICN (Irvine, CA). Charcoal (Norit SG activated charcoal) and 2,5-diphenyloxazole were purchased from MCB Manufacturing Chemists Inc. (Cincinnati, OH). Triton X-100 came from Research Products International Corp. (Mount Prospect, IL). Bovine serum albumin, cycloheximide, geneticin, penicillin-G, dihydroxyphenylalanine (L-DOPA), Tyrode's salts, L-tyrosine, 3-isobutyl-1-methyl-xanthine, phenylmethylsulfonyl fluoride (PMSF), streptomycin, and 12-O-tetradecanoylphorbol 13-acetate were purchased from Sigma Chemical Co. (St. Louis, MO).

Cell Cultures Human melanocyte cultures were prepared from foreskins of black and white neonates and from neonates with red hair by a modified method of Eisinger and Marko [7]. The melanocyte growth medium was composed of Ham's F-10 medium, 32 nM 12-O-tetradecanoylphorbol 13-acetate, 10^{-4}M 3-isobutyl-

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1-methyl xanthine, 5% fetal bovine serum, 10% horse serum, 200 U/ml penicillin, and 200 μg/ml streptomycin. To eliminate contaminating fibroblasts, growth medium was supplemented with 25 μg/ml geneticin for as long as needed. Cells were passaged when cell densities reached 4×10^4 cells/cm². Cells were removed from flasks with Tyrode's solution containing 5 mM (disodium) ethylenediaminetetraacetic acid (pH 7.2) and were counted in a Coulter counter (Coulter Electronics Inc., Hialeah, FL). For the experiments, melanocytes were seeded into either 25-cm² flasks at 2×10^5 cells/flask (for tyrosinase measurements) or 75-cm² flasks at 6×10^5 cells/flask for cell-fractionation studies or for RNA extractions. Cell densities were never allowed to increase beyond 4×10^4 cells/cm² for any experiment. All melanocyte cell strains used for experiments had been maintained in culture for less than 4 months.

Tyrosinase Assays

Tyrosine Hydroxylase Assays: To determine *in situ* tyrosinase activity in cell cultures, growth medium was supplemented with 1 μCi/ml of [^{3}H]tyrosine and was added to cells (2.0×10^5 cells) for 24 h. The medium was then removed from flasks and was assayed for the presence of ^3H_2O using the charcoal-absorption method of Pomerantz [8]. To determine tyrosinase activity in cell homogenates, cell pellets (2.0×10^5 cells) were sonicated in 0.5 ml of 0.1 M sodium phosphate buffer (pH 6.8) containing 0.1 mM PMSF and, where indicated, 0.5% Triton X-100, and 50-μl aliquots were incubated in 0.5 ml of a reaction mixture consisting of 0.1 mM tyrosine, 2 μCi/ml of [^{3}H]tyrosine, 0.1 mM L-DOPA, and 0.1 mM PMSF in 0.1 M sodium phosphate buffer (pH 6.8). Incubations were carried out at 37°C for 2 h. To terminate the reaction, 1 ml of charcoal (10% w/v in 0.1 N HCl) was added to each assay tube, and the samples were centrifuged at $2000 \times g$ for 10 min. The supernatants were mixed with scintillation fluid (4 g/l 2,5-diphenyloxazole in toluene/Triton X-100, 2:1) and counted in a TM Analytic scintillation spectrometer.

Tyrosinase Assays in Membrane/Melanosomal and Cytosol Fractions: Melanocytes ($0.7-2.6 \times 10^6$ cells) were sonicated (Heat Systems-Ultrasonics, Plainview, NY) in 0.6 ml of 0.1 M sodium phosphate buffer (pH 6.8) containing 0.1 mM PMSF six times for 5 seconds each and were divided into two aliquots. One aliquot of homogenate was kept at 4°C; the other aliquot was centrifuged at $50,000 \times g$ for 20 min at 4°C. The supernatant fraction (cytosol) was kept at 4°C, and the pellet (membrane/melanosomal fraction) was suspended in 0.3 ml of 0.1 M sodium phosphate buffer containing 0.1 mM PMSF and was then sonicated. The tyrosine hydroxylase activity in each fraction and in the unfractionated homogenate was measured as described above.

Melanin Assays For melanin measurements, cells were removed from flasks and were centrifuged at $100 \times g$ for 5 min. The cell pellets were washed once in phosphate-buffered saline and incubated in 2 ml of 1 N NaOH for 48 h at 37°C, and the absorbance was measured at 400 nm [9].

Immunotitration Analysis To determine the relative abundance of tyrosinase in different melanocyte cell strains, increasing amounts of cell homogenates were incubated with 0.2 μl of anti-tyrosinase serum [10] in immunoprecipitation buffer [0.05 M Tris(hydroxymethyl)aminomethane (Tris)-HCl (pH 7.4), 0.5% Triton X-100, and 150 mM NaCl] for 1 h at 37°C (the total volume in each tube was 0.2 ml). Pansorbin (1.5 mg) was then added, and the tubes were vortexed every 10 min for 30 min and then centrifuged in an Eppendorf microcentrifuge for 5 min. Tyrosine hydroxylase activity remaining in the supernatant was assayed as described above.

Northern Blot Analysis Tyrosinase mRNA levels were determined by Northern blot analysis using a human tyrosinase cDNA probe [11], as described elsewhere [12,13]. Quantitation of Northern blot films was performed by densitometric scanning on a Molecular Dynamics model 300A Computing Densitometer. All tyrosinase mRNA levels were normalized to the amount of glyceraldehyde phosphate dehydrogenase present in the same sample to control for any variabilities in RNA quality or for the amount of RNA loaded onto the gels. The normalized tyrosinase mRNA values were divided by the mRNA value for one of the cell strains, 73R. Therefore, all of the data shown are the tyrosinase mRNA levels relative to that in the cell strain 73R.

Immunoblots To visualize and quantitate tyrosinase from black and white melanocytes, cells (2×10^6) were removed from culture flasks and were resuspended in 0.5 ml of 0.05 M sodium phosphate buffer (pH 6.8) containing 0.1 mM PMSF and 0.5% Triton X-100. The cell suspension was briefly sonicated and left at 4°C for 1 h to solubilize tyrosinase. The suspension was then centrifuged at $50,000 \times g$ for 20 min at 4°C. The supernatants were mixed with sodium dodecyl sulfate (SDS) sample-treatment buffer [10% SDS and 10% glycerol in 0.5 M Tris-HCl (pH 6.8) without β-mercaptoethanol], and equal amounts of cell extract (25 μg protein; approximately 8×10^4 cells) were electrophoresed in duplicate on 10% SDS polyacrylamide gels. Following electrophoresis, the position of tyrosinase was determined by incubating the gel first in 0.5 M sodium phosphate buffer (pH 6.5) for 10 min and then in 0.2% L-DOPA in 0.1 M sodium phosphate buffer (pH 6.8) at 37°C. Within 10 min, a melanin band was clearly visible on the gel. The duplicate gel was electroblotted (60 V for 2 h in 25 mM Tris, 192 mM glycine, and 15% methanol) onto an Immobilon P membrane (Millipore Corp., Bedford, MA), and tyrosinase was detected by immunoblotting using a rabbit anti-mouse tyrosinase antiserum and an Immun-Lite chemiluminescent/alkaline phosphatase detection kit manufactured by Bio-Rad Laboratories (Richmond, CA). The chemiluminescent signal was detected by exposing the blot to Kodak XAR X-ray film for 15 min, followed by development. Tyrosinase abundance was quantitated by determining the volume of the autoradiographic signal on a Molecular Dynamics model 300A computing densitometer.

Statistics To determine the correlation between two different variables, linear regression analysis was performed, and the correlation coefficient was calculated (*Fig P* scientific figure software program; Biosoft, Cambridge, UK). Tests for significance of the difference between the means of two independent samples were carried out without assuming that the two population variances were the same [14].

RESULTS

The relationship between tyrosinase activity and human pigment production was investigated in human melanocyte cell strains established from different skin types. To date, over 20 different melanocyte-cell strain cultures have been established, and all have maintained their phenotypic characteristics in culture for over a year. Melanocytes derived from black skin (black melanocytes) display high levels of melanin synthesis in culture, whereas melanocyte cultures from white skin (white melanocytes) remain lightly pigmented. Melanocytes from red-haired neonates are phenotypically similar to melanocytes from white neonates. Melanocytes from either white or red-haired neonates may be lightly pigmented, but these cells never produce as much pigment as melanocytes from black neonates.

To study the relationship between tyrosinase and melanin production in human melanocytes, we measured tyrosinase activity in 15 different melanocyte cell strains using an *in situ* enzyme assay. We also measured the amount of melanin produced by these cell strains. As shown in Fig 1, we found an almost perfect correlation ($r = 0.923$) between the level of tyrosinase activity in a melanocyte and the amount of melanin produced in that cell. As might be expected, tyrosinase activity and melanin content in all of the white-melanocyte studies were markedly lower than in black melanocytes; however, when we measured tyrosinase activity in the same cell strains using a homogenate assay instead of the *in situ* assay, the correlation between tyrosinase activity and melanin content was not as good (Fig 2; $r = 0.808$). Tyrosinase activity was higher in both black and white cell strains, as might be expected, because of the solubilization

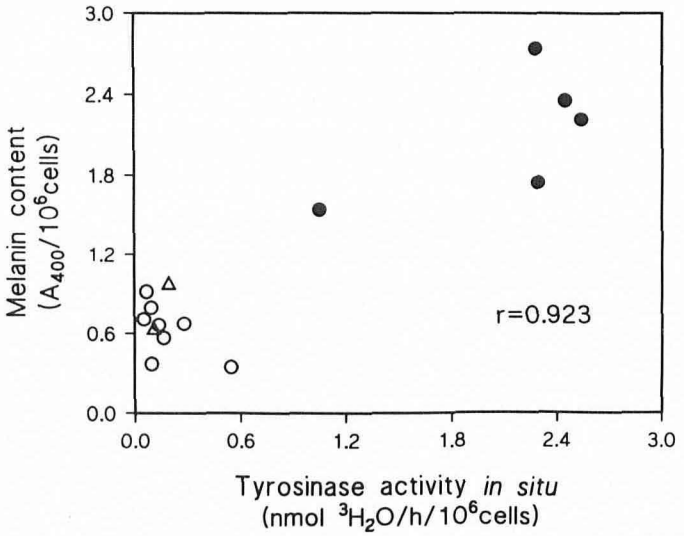

Figure 1. Correlation between melanin content and tyrosine hydroxylase activity *in situ*. Melanocytes (2.0×10^5 cells) were seeded into a 25-cm^2 flask, and melanin content and tyrosinase activity *in situ* were determined as described in *Materials and Methods*. Data points are the averages of three assays. Cell strains represented are from black *(solid circles)*, white *(open circles)*, and red-haired *(triangle)* neonates.

of membrane-bound tyrosinase by the detergent, Triton X-100. More important, however, the homogenate assays revealed that some of the white cell strains had tyrosinase activity levels that were now 60–100% that of black cell strains. In contrast, the *in situ* assays showed that white strains typically had 10% of the activity of black melanocytes. Figure 3 shows a comparison of tyrosinase activity measured *in situ* and in homogenates. This discrepancy between *in situ* and homogenate assays suggested to us that white-melanocyte cell strains (and also black strains) may contain a significant amount

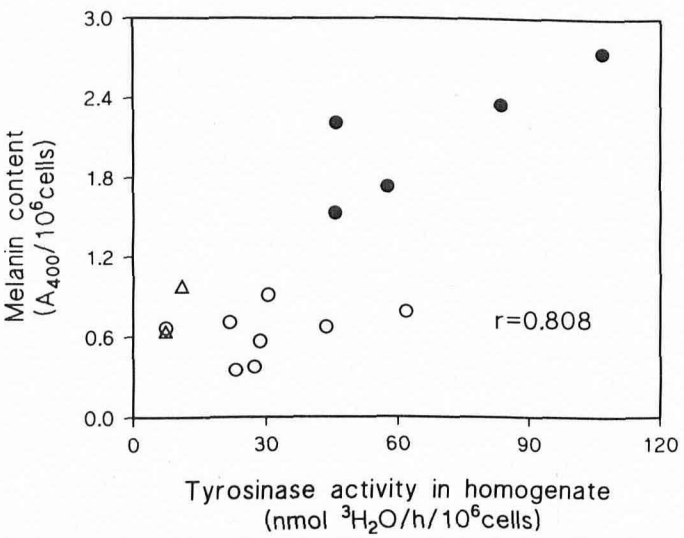

Figure 2. Correlation between melanin content (absorbance at 400 nm) and tyrosine hydroxylase activity in melanocyte homogenates. Melanocytes (2.0×10^5 cells) were removed from culture flasks with Tyrode's solution containing 5 mM ethylenediaminetetraacetic acid and centrifuged at $100 \times g$. The cell pellets were washed once in phosphate-buffered saline and then frozen at −70°C until assayed. Homogenates were prepared by sonication in 0.1 M sodium phosphate buffer (pH 6.8) containing 0.1 mM PMSF, and melanin content and tyrosinase activity were determined as described in *Materials and Methods*. Data points are the averages of three assays. Cell strains represented are the same as those shown in Fig 1 and are from black *(solid circles)*, white *(open circles)*, and red-haired *(triangles)* neonates.

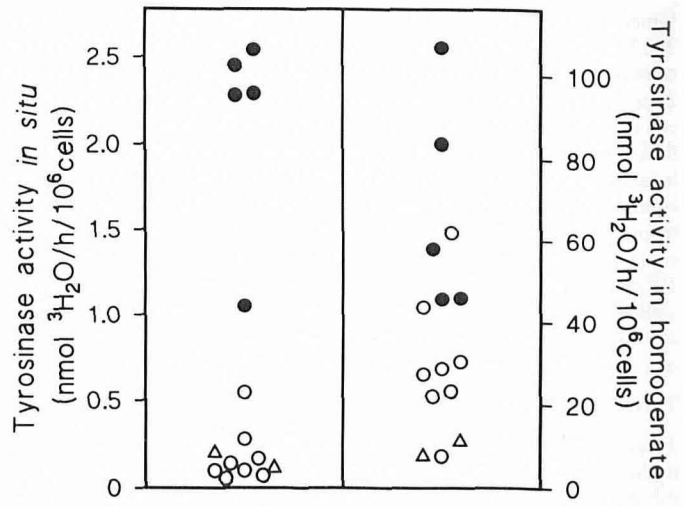

Figure 3. Comparison between tyrosinase activity *in situ (left)* and tyrosinase activity in cell homogenates *(right)*. Data from Figs 2 and 3 were compared in scattergrams. Cell strains represented are from black *(solid circles)*, white *(open circles)*, and red-haired *(triangles)* neonates.

of tyrosinase, which, in the living cell, does not exist in a catalytically optimum state. When released from the melanosome environment by detergent, this enzyme becomes active.

Although it is widely known that tyrosinase exists predominantly in the melanosome and is not found in soluble form, we carried out studies to confirm that tyrosinase in melanocytes derived from black, white, or red-haired skin types is localized primarily to the melanosome. We measured tyrosinase activity in cytosol and membrane/melanosomal fractions prepared from homogenates of these cell strains, and, as shown in Fig 4, tyrosinase was predominantly localized to the membrane fraction of the melanocyte regardless of the cell type.

Because these results indicate that white melanocytes with low *in situ* tyrosinase activity must still make significant amounts of the enzyme protein, we carried out experiments to determine the rela-

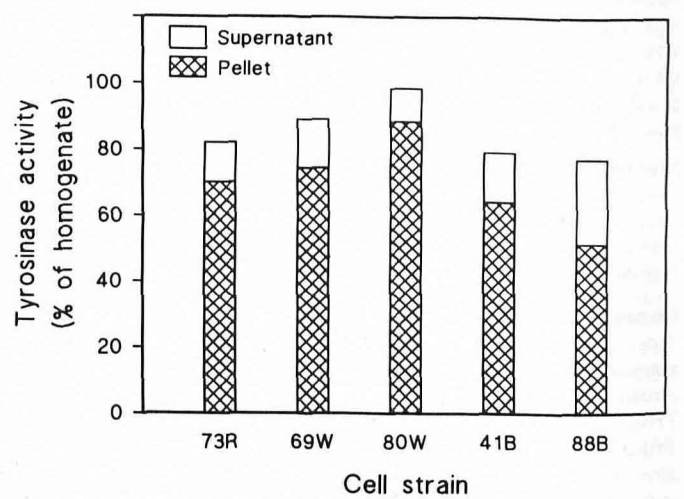

Figure 4. Determination of the intracellular distribution of tyrosine hydroxylase activity in black- and white-melanocyte cell strains and in melanocytes derived from red-haired neonates. Homogenates were prepared from five cell strains and were centrifuged at $50,000 \times g$ at 4°C for 20 min to prepare a membrane/melanosomal fraction (pellet) and a cytosol fraction (supernatant). Tyrosine hydroxylase activity in these fractions was then determined in triplicate, as described in *Materials and Methods*. Values were expressed as the percentage of the activity in the total homogenate. The cell strains used were 69W and 80W (white), 41B and 88B (black), and 73R (red-haired).

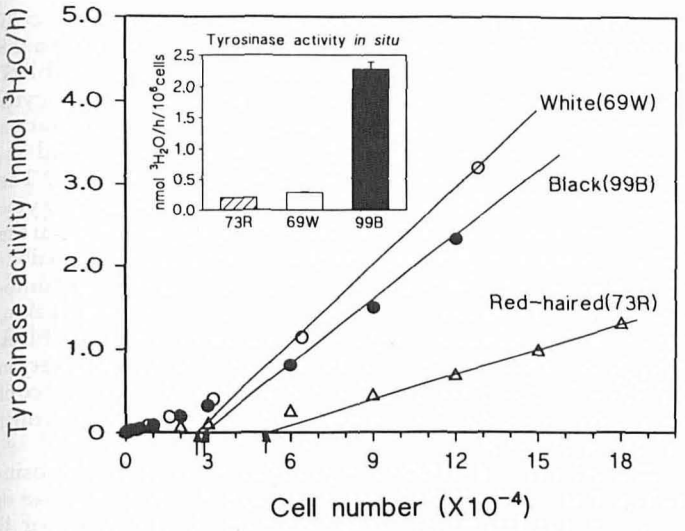

Figure 5. Immunotitration of tyrosinase in melanocyte homogenates. Increasing amounts of homogenate from a white (69W)-, black (99B)-, and red (73R)-melanocyte cell strain were incubated with an anti-mouse tyrosinase antibody, and tyrosinase activity remaining in the supernatant was determined after precipitation of the complex with Pansorbin, as described in *Materials and Methods.* The x-axis shows the number of cells in each cell homogenate sample incubated with antibody; *arrows* indicate the equivalence points. *Inset,* tyrosine hydroxylase activity *in situ* in the same cell strains used for immunoprecipitation analysis. Tyrosinase activity values are the averages of four assays ± SD.

tive abundance of tyrosinase in black and white melanocytes. For these studies, we utilized two immunologic methods: immunotitration analysis and immunoblotting protocols. The immunotitration procedure was originally described by Feigelson and Greengard [15] and was previously used by us to determine tyrosinase abundance in melanoma cell cultures [10,16]. The analysis is based on equivalence point titration. In principle, if one adds increasing amounts of antigen-containing cell extract to a fixed amount of antiserum to form an immunoprecipitate, the antibody will become saturated at the equivalence point. Thus, any further antigen added after this point is reached will appear in the supernatant following centrifugation of the antigen–antibody complex. If two different cell types contain the same number of antigen molecules per cell, the equivalence points will be the same; however, if one cell type has less antigen per cell than another, it will take more cells from the low-antigen cell type to provide enough antigen to saturate the antibody and reach equivalence. When we determined the relative abundance of tyrosinase molecules in a black- and white-melanocyte strain by this analysis, we found that the number of tyrosinase molecules in a white cell strain (69W) was approximately the same as (or even more than) that of the black cell strain (99B) (Fig 5). However, as shown in Fig 5 *(inset),* tyrosinase activity *in situ* in 99B (black strain) was eight times that of 69W (white strain). Thus, most of the tyrosinase molecules in the white cell strain (69W) are either catalytically inactive in living cells in culture or are at least functioning at a low catalytic efficiency compared with the enzyme found in black melanocytes. Because white-skinned people with red hair represent a unique skin type (type I), we also determined the relative abundance of tyrosinase in a melanocyte cell strain derived from a subject with red hair (red melanocyte). Of interest, on the basis of immunotitration analysis, this cell strain (73R) had approximately one-half as many tyrosinase molecules as the black strain (99B) and one-eleventh as much tyrosinase activity measured *in situ* (Fig 5).

We next carried out studies to visualize and quantitate tyrosinase from black and white melanocytes by SDS gel electrophoresis and immunoblotting. For these studies, we examined tyrosinase from three white cell strains with low tyrosinase activity and melanin synthesis and compared the amount of tyrosinase present in these cells to the abundance of the enzyme from a black-melanocyte cell strain. As shown in Fig 6, little difference in tyrosinase abundance was found among the four cell strains studied. The black-melanocyte strain, which had 10 times the *in situ* activity and produced over 10 times the amount of melanin as any of the white strains, had no more enzyme molecules than the 93A2 strain and at most had only 30% more enzyme than the white-melanocyte strain, with almost non-detectable *in situ* tyrosinase activity (84W). These results are in agreement with the immunotitration analysis data. Further, the immunoblots show that tyrosinase from white and black melanocytes is similar in size.

Because the immunotitration analysis and immunoblot data show that white-melanocyte cell strains may synthesize as much tyrosinase as black melanocytes, we next determined the abundance of tyrosinase mRNA in black, white, and red melanocytes by carrying out Northern immunoblot analysis [12,13]. Tyrosinase mRNA levels were determined in six melanocyte cell strains and compared with tyrosine hydroxylase activity both *in situ* and in homogenates (Fig 7). Although tyrosinase activity *in situ* was low in both white cell strains (69W and 84W; Fig 7*A*), tyrosinase mRNA levels in these cells were as high as those found in black cell strains (Fig 7*C*). As expected, tyrosinase activity was elevated in homogenates prepared from the two white-melanocyte cell strains, 69W and 84W (Fig 7*B*).

The large discrepancy between *in situ* activity and tyrosinase

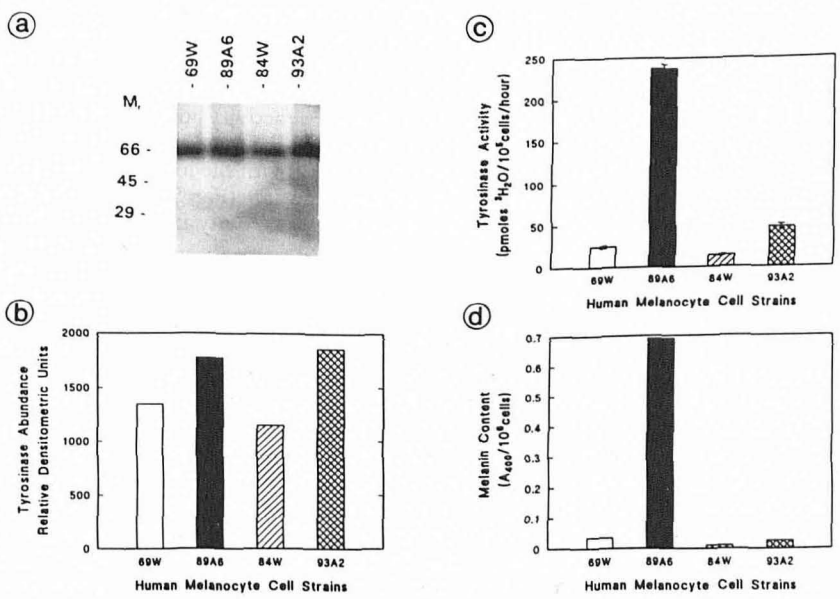

Figure 6. Immunoblotting of tyrosinase from black and white melanocytes. Cells (2×10^6) from three white-melanocyte strains (69W, 84W, and 93A2) and one black-melanocyte strain (89A6) were sonicated in 0.05 M sodium phosphate buffer (pH 6.8) containing 0.1 mM PMSF and 0.5% Triton X-100 and were centrifuged at 50,000 × *g* for 20 min, and the supernatants were electrophoresed on a 10%T, 5%C SDS polyacrylamide gel. Following electrophoresis, the cell extract was blotted to Immobilon P and reacted with an anti-tyrosinase antiserum [10]. The antibody–antigen complex was then reacted with goat anti-rabbit immunoglobulin G conjugated to alkaline phosphatase, and the resulting complex was visualized by chemiluminescence and autoradiography. *(a)* Immunoblot of human tyrosinase; *(b)* quantitation of the autoradiogram in *a* by densitometric analysis on a Molecular Dynamics Model 300A computing densitometer; *(c)* tyrosinase activity *in situ* of the cell strains used in *a*; *(d)* melanin contents of the cell strains shown in *a*. For assay details, see *Materials and Methods.* Tyrosinase activity values shown in *c* are the averages of six assays ± SD.

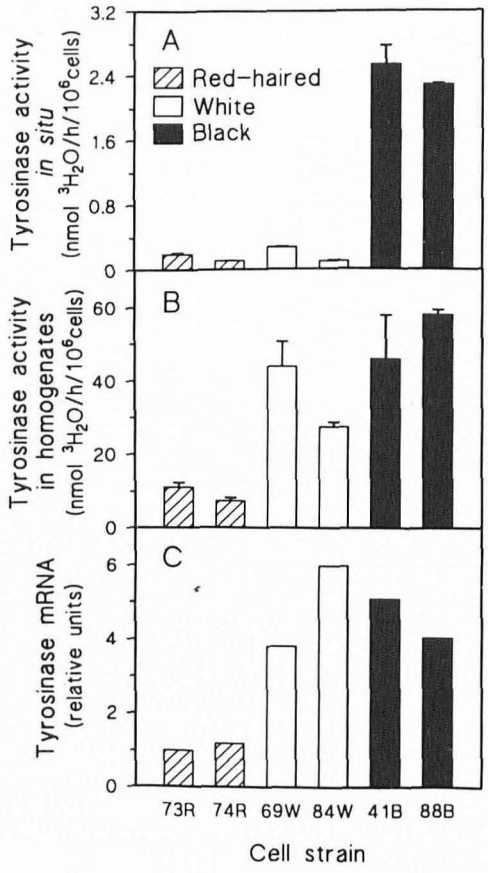

Figure 7. Comparison of tyrosinase mRNA and tyrosinase activity levels in melanocytes. Tyrosinase mRNA levels *(C)* were compared with tyrosine hydroxylase activity *in situ (A)* and in homogenates *(B)*. Total RNA was prepared from $1.0-4.0 \times 10^6$ melanocytes immediately after removal of cells from culture flasks, and tyrosinase mRNA levels were determined by Northern blot analysis, as described in *Materials and Methods.* Tyrosinase activity *in situ* and in homogenates in the small cell strains used for RNA analysis was determined as described in Figs 1 and 2. The melanocyte strains used were 73R and 74R (from red-haired neonates), 69W and 84W (white), and 41B and 88B (black). Tyrosinase activity values in *A* and *B* are averages of six assays ± SD.

mRNA levels in white melanocytes suggests that if this mRNA is being used for translation (as the data in Figs 5 and 6 suggest), much of the tyrosinase synthesized in white melanocytes from this message is being held in a catalytically repressed state in the living cells. Unlike the white-melanocyte strains, melanocyte cell strains from red-haired neonates (73R, 74R) showed low tyrosinase activity both *in situ* and in homogenates and had low tyrosinase mRNA levels. The low mRNA level in red melanocytes agrees well with the low abundance of the protein detected by immunotitration (Fig 5). Black cell strains, as expected, had high *in situ* tyrosinase activity and high tyrosinase mRNA levels.

DISCUSSION

These studies have shown that although the amount of melanin produced by human melanocytes is strictly dependent on the level of tyrosinase activity, the level of tyrosinase activity in the cell is not strictly determined by the amount of enzyme produced. Melanocytes with high tyrosinase activity do not necessarily transcribe more tyrosinase mRNA or make more tyrosinase protein than do melanocytes with low activity. Our finding of inactive (or at least catalytically less active) tyrosinase molecules in melanocytes is consistent with previous findings from our laboratory [6] and with results of other investigators who have shown that inactive tyrosinase is present in human skin and in cultured melanoma cells [10,17,18]. There are at least two possibilities that could explain the observed difference in the catalytic activity of tyrosinase in black and white melanocytes. One possibility is that white melanocytes may have at least two subpopulations of tyrosinase, one population having catalytic activity comparable to black melanocytes and another population comprising completely repressed molecules. The other possibility is that there may be one population of the enzyme in white melanocytes that functions at a lower catalytic rate than the enzyme present in melanocytes of black skin types. Either possibility would give essentially the same results as we found by immunologic analysis. In our previous studies on tyrosinase in human skin, we measured tyrosinase activity levels in skin samples from 20 black and 20 white skin types. We found that although enzyme activity in black skin averaged three times that of white skin, the abundance of the enzyme, as measured by immunotitration analysis, was similar in both skin types [6].

Although the molecular basis for catalytically repressed tyrosinase is unknown, the low *in situ* catalytic activity of tyrosinase in white compared with black melanocytes may be the result of 1) structural differences in the enzyme in black and white skin; 2) the presence of tyrosinase inhibitors in white melanocytes; or 3) differences in the structure of melanosomes in different skin types, which cause changes in tyrosinase function. With regard to the first possibility, there is no evidence at present to suggest that different tyrosinases, with different amino acid sequences, are synthesized in black and white melanocytes. Unfortunately, sequences for human tyrosinase have been determined only from tyrosinase cDNAs prepared from pigmented melanocytes or melanoma cells, not from melanocytes of defined skin types. The tyrosinase cDNA sequences that have been obtained to date are virtually identical [11,19]. We are now in the process of sequencing tyrosinase cDNAs from melanocytes derived from different skin types to determine whether amino acid differences occur among racial skin types. The possibility that tyrosinase inhibitors may regulate the enzyme in white melanocytes is indirectly supported by a considerable number of reports in the literature that have suggested the presence of such inhibitors in melanoma cells and in melanocytes [20–23]; however, in simple mixing experiments, we have not been able to demonstrate any inhibitory effect of white-melanocyte extracts on tyrosinase activity in black-melanocyte homogenates (data not shown). Finally, no evidence has yet been presented that structural differences exist in melanosomes from black and white melanocytes, although some reports have shown that there are differences between white and black skin types in the packaging of melanosomes in keratinocytes [24].

The finding that tyrosinase mRNA levels are high in melanocytes from white skin types is in agreement with recent studies from Gilchrest and co-workers [25], in which the melanin content of cultured melanocytes was found to have no correlation with the amount of tyrosinase mRNA. In their study, however, tyrosinase activity was not measured, and tyrosinase protein was not quantitated. Further, the melanocytes were not identified according to skin type.

The skin types represented by the melanocyte strains used in this study include 1) red-melanocyte cell strains representing skin type I; 2) white-cell strains representing skin type II or III; and 3) black-cell strains representing skin type VI [26]. The low levels of tyrosinase protein and mRNA in type I skin may explain the inability of people with this skin type to increase pigmentation in response to sunlight. If the tanning response involves activation of pre-existing tyrosinase, then people with type I skin may not tan because they have little tyrosinase to activate. Similarly, the ability of people with type III skin to tan may be explained by the fact that melanocytes from these skin types contain an abundance of tyrosinase, which, although largely inactive in the cell, can perhaps be activated by sunlight.

These results suggest that differences in racial human pigmentation are due to differences in tyrosinase activity levels in melanocytes but that this tyrosinase activity is regulated at a post-translational level. Studies are now in progress to identify the molecular processes involved in this regulation.

This work was supported in part by a research grant from the Presbyterian Health Foundation.

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