The β Isoform of Protein Kinase C Stimulates Human Melanogenesis by Activating Tyrosinase in Pigment Cells*

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We have investigated the role of protein kinase C (PKC) in human melanogenesis. The level of PKC activity paralleled the total melanin content in cultured newborn melanocytes. Activation of PKC by treatment with 5 × 10⁻⁷ M phorbol dibutyrate acutely caused a doubling in the activity of tyrosinase, the rate-limiting enzyme in melanogenesis, known to correlate directly with melanin synthesis in these cells. When PKC was depleted to 5–10% of initial levels, there was a 40–50% parallel reduction in tyrosinase activity; and regeneration of PKC activity was associated with the recovery of tyrosinase activity. By Northern blot analysis, the α and β but not the γ isoforms were detectable in melanocytes. By Western blot analysis, the racially determined pigment level in cultured melanocytes correlated with PKC-β protein expression. In a pigmented human melanoma line (P-MM4, 20–30 ng melanin/μg protein) and its nonpigmented subclone (NP-MM4, undetectable melanin), PKC-α mRNA was expressed in both, whereas PKC-α mRNA was detectable only in P-MM4 cells. Tyrosinase protein level was comparable in both cell lines. When NP-MM4 cell lysate was incubated with melanocyte lysate known to contain PKC-β, tyrosinase activity per μg of melanocyte protein in the combined lysate increased, consistent with activation of the previously inactive tyrosinase of NP-MM4 origin. Moreover, NP-MM4 cells transiently transfected with PKC-β cDNA increased tyrosinase activity from undetectable to detectable levels. These combined data show that PKC-β regulates human melanogenesis by activating tyrosinase.

Protein kinase C (PKC)1 is a calcium- and phospholipid-dependent protein kinase activated by diacylglycerol, a minor lipid species generated when polypeptide growth factors bind to their cell surface receptors, and by tumor promoting phorbol esters (1, 2). PKC has been implicated in a wide range of biological functions including cell proliferation (3), malignant transformation (4–6), and regulation of proto-oncogenes (7–9). PKC acts in part by phosphorylating proteins such as the epidermal growth factor receptor (10), the insulin receptor (11), the protein product of the ras oncogene (12), and other intracellular substrates (13, 14).

Molecular cloning of PKC revealed that PKC is a multigene family with at least ten isoforms (reviewed in Ref. 15) that are expressed differentially among tissues. Of the better studied isoforms, PKC-α is found ubiquitously, but PKC-β is found only in some tissues, and PKC-γ appears virtually restricted to brain tissues (reviewed in Ref. 16), suggesting possible functional differences among them.

Recent results further support the postulation that different PKC isoforms have different cellular functions. The ε isoform of PKC, but not the α isoform, has been shown to be preferentially activated in response to interferon-α in Daudi cells (17). NIH 3T3 cells transfected with PKC-γ grew to a higher density and showed increased tumorigenicity when inoculated into nude mice (4). The β isoform was regulated to phospholipid hydrolysis by regulating phospholipase D activity (18). The β isoform was further implicated in cell growth stimulation in that transfected cells overexpressing PKC-β grew to a higher density, showed anchorage independence (5), and were more susceptible to transformation by activated H-ras oncogene (6). In contrast, overexpression of PKC-β in HT29 human colon cancer cells reduced growth and was tumor-suppressive in these cells (19). Thus, the role of PKC isoforms appear to depend partly on the cell type.

Recently, our laboratory reported that diacylglycerol induces human melanogenesis in cultured human melanocytes (20), suggesting that PKC may also be involved in this differentiated function of melanocytes. We now report that the β isoform of PKC specifically regulates human melanogenesis.

EXPERIMENTAL PROCEDURES

Materials—Dulbecco's modified essential medium, L15, nonessential amino acids, glutamine, medium 199, and trypsin were purchased from GIBCO. Recombinant basic fibroblast growth factor was purchased from Amgen, triiodothyronine from Collaborative Research, and hydrocortisone from Calbiochem. Phorbol dibutyrate, insulin, transferrin, and synthetic melanin were from Sigma. Choleragen was from List Biologicals. Nylon membrane was from Amersham Corp. PKC antibodies were from Seigakagu International. Bovine calf serum and fetal calf serum were from HyClone Laboratories, Inc. All radiolabeled nucleotides were purchased from Du Pont-New England Nuclear. The oligolabeling kit was purchased from Pharmacia LKB Biotechnology Inc. Lipofectin reagent was from Life Technologies, Inc.

Cells and Media—Neonatal foreskins obtained within 2 h of elective circumcision were used to culture human melanocytes as previously described (21). In brief, the epidermis was separated from the dermis after overnight incubation in 0.25% trypsin at 4 °C. Primary cultures were then established in medium 199 supplemented with 10⁻⁸ M triiodothyronine, 10 μg/ml transferrin, 10 μg/ml insulin, 1.4 × 10⁻⁴ M hydrocortisone, 10⁻⁴ M choleragen, 10 ng/ml basic fibroblast growth factor, and 5–10% fetal bovine serum; all post primary cultures were maintained in a low calcium (0.03 mM) version of this medium known to selectively support melanocyte growth (21, 22).
The MM4 human melanoma cells were obtained from Dr. U. Stierner, Göteborg, Sweden, and maintained in Dulbecco's modified essential medium (55%), L15 (27%), fetal bovine serum (15%), non-essential amino acids, 1% glutamine (2 mM), and insulin (10 μg/ml) (22).

Melanin Content of Cells—1 × 10⁶ cells were routinely used to measure melanin content. Cells were spun at 2,500 rpm for 15 min, and the resulting pellet was dissolved in 0.5 ml of 1 N NaOH. Melanin concentration was determined by OD₅₀₅, and OD₅₀₅ was measured using bovine serum albumin as a standard.

Northern Blot Analysis—Total cellular RNA was isolated by the method of Chirgwin et al. (24). Routinely, 20 μg of total cellular RNA/ lane were size-fractionated through a 1% agarose gel containing 2.2 M formaldehyde. The RNA was then transferred to a Hybond-N nylon membrane and immobilized by short ultraviolet light illumination. Blots were prehybridized for 4-24 h, hybridized with labeled specific probes, and washed according to Park and Campisi (25).

Probes: The cDNA probe for human tyrosinase (Panel 34) was a generous gift from B. Kwon (Guthrie Research Institute, Sayre, PA) (26). cDNA for glyceraldehyde-3-phosphate dehydrogenase was generated using the polymerase chain reaction (27). cDNA probes specific for PKC α, β, and γ isoforms were obtained from the American Type Cell Culture. cDNAs were radiolabeled with [³²P]dCTP, using a commercial oligolabeling kit.

Protein Kinase C Activity—Soluble cell lysates, containing both cytosolic- and membrane-associated PKC, were prepared as previously described (25). In brief, lysates were clarified by centrifugation at 100,000 × g for 60 min, and 500 μl of the supernatant, equivalent to 0.5-10 × 10⁶ cells, were mixed with 1.0 ml of DEAE-52 cellulose resin which had been equilibrated with washing buffer consisting of 20 mM Tris (pH 7.5), 2 mM EDTA, 0.5 mM EGTA, and 1 mM dithiothreitol. The resin and the lysate were allowed to react for 2-3 min, washed three times with washing buffer (5 ml each wash), and centrifuged to remove the membrane fraction. PKC-β and PKC-γ, which were extracted for 60 min at 4°C, were then used to precipitate PKC activity. The supernatant was extracted using an antibody against PKC-β. There was no detectable activity precipitated using mutated inactive PKC as a control.

Membrane fraction was separated from cytosol by centrifugation of homogenized lysate at 100,000 rpm for 60 min at 4°C. PKC activity was extracted from the resulting pellet by incubating with lysis buffer devoid of Triton X-100 containing 1% Triton X-100 for 60 min at 4°C. Cytosolic and membrane-associated PKC was subjected to partial purification using DEAE-52 resin.

Calcium- and phospholipid-stimulated PKC activity was determined using 50 μl eluates (2-5 μg protein), phosphatidylycerine, and phospholipid dibutyrate (PDBu) to activate PKC, and histone type III-S as substrate, as previously described (25). A 10-min incubation at 37°C was routinely used. For each determination, a control reaction was performed in the absence of phosphatidylycerine and PDBu. Activity was expressed as counts/min of incorporated radiactivity/μg protein/min in the presence of phosphatidylycerine and PDBu, minus the radioactivity incorporated in the absence of radiolabeled phosphatidylycerine and PDBu (background kinase activity). The background was generally less than 5-10% of the total incorporated radioactivity.

Western Blotting—Immunoblot analysis was performed for PKC, according to Park and Campisi (25) and for tyrosinase as described by Jimenez et al. (28). For PKC, cell lysate was partially purified for PKC, and 50-100 μg of partially purified protein were routinely used. Lysates enriched for tyrosinase were prepared as described under "Tyrosinase Activity," and 20-50 μg of protein were used (29). Protein samples were subjected to 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, as described by Laemmli (29), and transferred to a nitrocellulose membrane electrophoretically. The membrane was washed extensively with PBS containing 0.5% Tween 20, then with PBS containing 0.5% Tween 20, 0.05 M Tris, 0.15 M NaCl, and 0.1% (w/v) sodium dodecyl sulfate (SDS) containing 0.05% Tween 20. The membrane was washed extensively with PBS containing 0.5% Tween 20, and exposed to preflashed Kodak X-Omat film.

Tyrosinase Activity—Tyrosinase activity was measured according to Pomerantz et al. (30). In brief, 5 × 10⁶ cells were briefly sonicated in 80 mM PO₄ (pH 6.8) containing 1% Triton X-100, and tyrosinase was extracted for 60 min at 4°C. The reaction was stopped by addition of 500 μl of 10% trichloroacetic acid containing 0.2% BSA. Trichloroacetic acid-soluble material was reacted with Norit A, and released [³¹P]H₂O was measured using a scintillation counter. The activity was expressed as counts/min [³¹P]H₂O released/μg protein/h minus the nonspecific incorporation of radioactivity, determined by using lysate boiled for 30 min (background). Background was generally less than 5-10% of the sample.

Immunoprecipitation of PKC—1 × 10⁶ NP-MM4 cells were plated in 60-mm dishes 24 h prior to introducing PKC-β cDNA. PKC-β cDNA was cloned into an expression vector containing β-gal promoter system, which is composed of the Simian virus 40 early promoter and the R segment and part of the U5 sequence (R-U5') of the long terminal repeat of human T cell leukemia virus type 1 (31). 10-50 μg of PKC-β cDNA and 20 μl of lipofectin reagent, a 1:1 liposome formulation of the cationic lipid N ([(2,3-dioleoyloxy)propyl]n, n-trimethylammonium chloride and dioleoyl phosphatidylethanolamine in membrane-filtered water, were incubated at room temperature for 20-25 min and introduced onto cells. Transfection mixture was removed after 6-10 h, and cultures were maintained in standard NP-MM4 medium. After 46-72 h, cells were harvested and tyrosinase activity was measured. cDNA containing mutated inactive PKC was used as a control.

Immunoprecipitation of PKC—1 × 10⁶ NP-MM4 cells were plated in 100-mm dishes. When cells were grown to near confluency, soluble cellular lysates, containing both cytosolic and membrane-associated PKC, was prepared as described under "Protein Kinase C Activity." 300-500 μg of lysate protein were incubated in 5 μg of either antibody against β-tubulin or PKC-β for 2 h at 37°C, then further incubated with 10 μl of protein A-Sheparose for 1 h at room temperature. Samples were spun and supernatant was used for the tyrosinase experiment. Remaining pellet was subjected to 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and immunoblot was performed using an antibody against PKC-β. There was no detectable level of PKC-β.

RESULTS

Correlation of Total PKC Activity and Melanin Content—To investigate the possible role of PKC in human melanogenesis, we initially determined whether total melanin content correlates with total activatable PKC. Melanocytes were cultured under standard conditions to near confluency and total PKC activity and melanin content were measured in paired cultures. In experiments comparing four donors, PKC activity was 1,450, 1,300, 813, and 613 cpm/μg protein, and melanin content was 95, 65, 25, and 16 ng/μg protein, respectively, indicating a moderate correlation in these measurements among individual cultures (Fig. 1A). Because these cells are known to be actively melanogenic under the basal conditions (20), if PKC is involved in melanogenesis, then part of the PKC should be in an active form. Because membrane-associated PKC is considered to be active (32-34), we determined tyrosinase activity versus membrane-associated PKC activity (Fig. 1B). Approximately 30-50% of total PKC activity was associated with the membrane in all four donors, suggesting an endogenously active form of PKC, consistent with a role for PKC in human melanogenesis. The endogenous agent activating up to half the total PKC in melanocytes under basal condition is unknown, although these cells are known to respond to diacylglycerol (20) which may be continuously produced in sufficient amounts.

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Activity—if melanin synthesis is dependent on PKC activity, then melanogenic activity should return upon regeneration of PKC. Melanocytes were depleted of PKC by treating with phorbol esters for up to 2 weeks (Fig. 2C). Tyrosinase activity, known to correlate directly with melanin synthesis rates (35), was measured because it is a more sensitive indicator of changes in melanin synthesis rate than melanin content that reflects the balance between past melanin synthesis rates and cell division rates. As expected, treatment of melanocytes with PDBu caused initial activation of PKC by translocation of PKC from cytosol to membrane, maximal at 30 min to 2 h, followed by a 95% loss of PKC activity within 24 h (Fig. 2A). In parallel, tyrosinase activity was also increased from 3200 to 5700 cpm/µg/h by 2 h and then was reduced to 1500 cpm/µg/h compared to untreated cells within 24 h (Fig. 2B). Three-day exposure to PDBu caused a further reduction to 1000 cpm/µg/h in tyrosinase activity. Melanin content was not measured until day 14, when it was reduced from 80 ± 5 to 40 ± 3 ng/µg protein. Continued culturing of melanocytes in the presence of PDBu for 2–3 months caused further reduction in total melanin content, from the initial level of 80 ± 5 to 20 ± 2 ng/µg protein.

Because PKC has been implicated in growth control (3–6) and its depletion has been reported to increase proliferation in mouse melanocytes (36), depletion of PKC in human melanocytes might be expected to change the growth rate of these cells and thus indirectly reduce melanogenesis and/or melanin content per cell through dilution. To determine whether PKC depletion changes growth rate in these cells, growth curves were generated for paired untreated and PDBu-treated melanocytes for up to 2 weeks (Fig. 2C). The growth rate was unaffacted, excluding the possibility that the decreased tyrosinase activity and melanin content are secondary to altered growth rate in human melanocytes.

Recovery of Tyrosinase Activity after Regeneration of PKC Activity—If melanin synthesis is dependent on PKC activity, then melanogenic activity should return upon regeneration of PKC. Melanocytes were depleted of PKC by treating with PDBu and some cultures were then provided with fresh medium lacking PDBu for the final week to regenerate PKC. PKC activity returned to control levels (Fig. 3A), as did tyrosinase activity (Fig. 3B).

Expression of Specific PKC Isoforms in Melanocytes—Because it has been postulated that each isoform of PKC has a unique biological function, we examined which isoforms are expressed in cultured melanocytes. Total RNA was isolated and northern blot analysis was performed using cDNA probes specific for the α, β, and γ isoforms of PKC. Transcripts of both α and β isoforms, 9.5 and 4.3 kb for α and 10 and 4.2 kb for β, as previously reported (37), were expressed (Fig. 4), but the γ isoform was not detectable (data not shown). To determine if one of the isoforms is preferentially involved in melanogenesis, melanocytes derived from black and white donors were cultured, and Western blot analysis was performed. Melanocytes from the black donor showed a far higher level of PKC-β (Fig. 5, A1), and melanin contents in paired cultures were 70 and 18 ng/µg protein (Fig. 5, A2) in the black and white cultures, respectively. When melanocytes from three donors with similar pigment content (30–40 ng/µg protein) were compared by Western blot analysis in a separate experiment, their level of β isoform was similar (Fig. 5, B1). These results strongly suggest that the PKC-β protein level correlates closely with cellular ability to produce melanin.

Correlation of PKC Activity, PKC Isoform Expression, and Melanin Content in a Human Melanoma Line—To further explore the role of the PKC-β isoform in human melanogenesis, we employed a model system developed in our laboratory in which pigment level can be varied dramatically. When maintained under its standard culture conditions, the MM4 human melanoma line is moderately pigmented (P-MM4) (22). However, when passed weekly to maintain exponential growth, versus once every 3–4 weeks, pigment content decreases markedly. In at least one subculture, this loss of pigment was irreversible in that if the resulting amelanotic cells were then passed every 3–4 weeks, pigment production did not resume. Instead, the total melanin content and tyrosinase activity are undetectable, <5 ng/µg protein and <50 cpm/µg/h, respectively, in the nonpigmented MM4 (NP-MM4) cells, whereas the parent P-MM4 cells maintained under identical conditions contain 40–50 ng/µg protein of melanin and >6,000 cpm/µg/h tyrosinase activity (Fig. 6A).

![Fig. 1. Correlation of PKC activity and melanin content. A.](image-url)

**Fig. 1.** Correlation of PKC activity and melanin content. A. paired cultures of melanocytes from four donors were grown to near confluence, and PKC activity and melanin content were determined, as a fraction of near confluent cultures derived from four donors, different from those in (A), were prepared, and PKC activity of each was measured.
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FIG. 2. Effect of PDBu treatment on melanocyte PKC activity, tyrosinase activity, and growth rate. Primary cultures of melanocytes from three donors of comparable melanin content (30–40 ng/μg protein) were prepared. At the first passage, 1 × 10^6 melanocytes were plated in paired 60-mm dishes and treated with 5 × 10^−5 M PDBu or vehicle for 30 min, 2 and 4 h, and up to 3 days. At each interval, duplicate dishes in each group were harvested, cytosol and membrane fraction prepared, and PKC partially purified using DEAE resin and assayed for PKC activity (A). From the paired culture dishes, cells were harvested, and 5 μg of total cellular lysate protein were analyzed for tyrosinase activity (B). C, paired primary cultures of melanocytes from the same donors as in (A) were plated onto 60-mm dishes (1 × 10^6 cells/plate). At each interval, duplicate dishes from each donor were harvested, and cell number was determined by using the Coulter electronic particle counter. As previously noted (21), plating efficiency of melanocytes was 50–60%, and there was no significant increase in cell yield above seeding by day 3 when the first cell count was determined. Thereafter, there was good growth as previously noted in this medium (22), comparable in the two groups. Cell numbers, averaged among the three donors, were within 5% of each other at each time point.

The comparability of growth rates for P-MM4 and NP-MM4 cells was confirmed over 14 days (Fig. 6B), excluding the possibility that the difference in their pigmentation level is due to differences in growth rate.

Total activatable PKC level was measured in both the P-MM4 and NP-MM4 melanoma lines. Unlike the earlier experiment using normal melanocytes, in which total PKC activity closely correlated with melanin content, PKC activity in P-MM4 cells was only 30% higher than in NP-MM4 cells, 470 versus 360 cpm/μg protein. The specific expression of the α, β, and γ isoforms was then examined for both cell lines. As expected, the γ isoform was not expressed in either P-MM4 or NP-MM4 cells (data not shown). PKC-α mRNA was expressed in both P-MM4 and NP-MM4 cells but the level was higher in NP-MM4 cells. PKC-β mRNA was detected only in P-MM4 cells (Fig. 6C). Total level of expression, the sum of both α and β isoforms, is comparable in both lines, consistent with the similar PKC activity observed. These data...
further suggest that the level of PKC-β expression is a major determinant of melanogenesis rate.

**Activation of Tyrosinase in NP-MM4 Cells**—To determine if lack of PKC-β expression is responsible for the loss of pigmentation in NP-MM4 cells, initially the levels of tyrosinase protein were determined in NP-MM4 and P-MM4 cells using Western blot analysis and a polyclonal antibody. NP-MM4 and P-MM4 showed equal amounts of tyrosinase protein (Fig. 7). This result suggested that NP-MM4 cells either lack a factor activating tyrosinase or the tyrosinase enzyme itself has a mutation causing the enzyme to become inactive. To distinguish between these possibilities, NP-MM4 and melanocyte lysates were incubated separately and together for 1 h at 37°C, and subsequently tyrosinase activity was measured in each. As expected, the NP-MM4 cell lysate showed undetectable levels of tyrosinase activity, while the melanocyte lysate showed tyrosinase activity of 2,500 cpm/μg. When the melanocyte lysate was mixed with the NP-MM4 lysate, tyrosinase activity expressed as counts/min/μg melanocyte protein more than doubled (Fig. 8A). In a separate experiment using a different melanocyte donor line, tyrosinase activity in the NP-MM4 lysate was again undetectable and that in the melanocyte lysate was approximately 6,000 cpm/μg protein/h (Fig. 8B, lane 2). Immunoprecipitation of the melanocyte lysate with an antibody against PKC-β prior to mixing with the NP-MM4 lysate completely eliminated the previously observed increase in tyrosinase activity per μg melanocyte protein (lane 3), indicating loss of the factor in the melanocyte lysate responsible for activating tyrosinase in the NP-MM4 lysate. Immunoprecipitation of the melanocyte lysate with anti-β-tubulin antibody, as a control, did not have this effect (lane 4). These results indicate that PKC-β activates tyrosinase and that this activation of tyrosinase persists for at least 2 h, the duration of the experiment, after removal of PKC-β.

**Transfection of PKC-β cDNA into NP-MM4 Cells**—To
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**FIG. 6.** Comparison of tyrosinase activities, growth rates, and PKC isom form expression in P-MM4 and NP-MM4 cells. A, P-MM4 and NP-MM4 cells were plated into duplicate 35-mm dishes (0.5 x 10⁶/dish) and when cells were near confluency, tyrosinase activity was measured. B, P-MM4 and NP-MM4, 0.5 x 10⁶ cells per 35-mm dishes, were plated and harvested in duplicates at days 4, 6, 8, 10, 12, and 15. Cell numbers were determined using a Coulter electronic particle counter. C, three sets of Northern analyses, each set consisting of 20 μg of total RNA/lane from NP-MM4 and P-MM4 cells, were performed for the expression of PKC-α, -β, and -γ. Equal counts of each probe was used for hybridization. No hybridization was observed with the PKC-γ probe (data not shown). PKC-α mRNA transcripts (9.5 and 4.3 kb) are easily detected in both cultures (lanes 1 and 2), but PKC-β mRNA transcripts are detectable only in P-MM4, but not NP-MM4 cells (lanes 3 and 4). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe hybridization was used to confirm even loading of RNA in both blots.

**FIG. 7.** Level of tyrosinase protein in NP-MM4 and P-MM4 cells. 2.5 x 10⁶ NP-MM4 and P-MM4 cells were briefly sonicated in 80 mM phosphate buffer (pH 6.8), extracted for tyrosinase at 4 °C for 60 min, and analyzed for the level of tyrosinase protein using a polyclonal antibody as described under “Experimental Procedures.”

Further confirm that it is PKC-β activating tyrosinase, NP-MM4 cells were transiently transfected with PKC-β cDNA. After 48–72 h, cells transfected with control cDNA mutated, and inactive PKC showed no tyrosinase activity while cells transfected with PKC-β showed an activity of 270 ± 30 cpm/μg/h (two independent experiments), indicating that PKC-β activates tyrosinase in NP-MM4 cells.

**DISCUSSION**

We present data showing that the β isof orm of PKC regulates human melanogenesis through activation of tyrosinase. Total activatable PKC roughly correlates with melanin content in cultured melanocytes; and the activation of PKC increases tyrosinase activity, which directly determines melanin production (35), while depletion of PKC by PDBu treatment rapidly reduces tyrosinase activity, and recovery of PKC activity after withdrawal of PDBu conversely leads to normalization of tyrosinase activity and melanin content within 1 week. Data specifically demonstrating the role of PKC-β in human pigmentation were obtained with both melanocytes and melanoma cells. In white versus black donor cultures differing 4-fold in melanin content, PKC-α was comparably expressed...
at the mRNA and protein levels, while black donor cultures expressed far more PKC-β than did the more lightly pigmented white donor cultures. The MM4 melanoma line and its amelanotic subclone NP-MM4 differ by 30% in total activatable PKC, but only the pigmented parental line expresses detectable levels of PKC-β. A factor in melanocyte lysates causing the activation of tyrosinase in NP-MM4 lysates was identified as PKC-β by immunoprecipitation experiments, and PKC-β activation of tyrosinase was further shown by transient transfection experiments.

The near total (95%) loss of PKC activity in PDBu-treated melanocytes, however, the possibility cannot be tested directly. Moreover, since melanocyte survival depends on the presence of TPA (50). In murine melanoma cells, it is known that tyrosinase activity is largely regulated at the post-translational level in that agents inducing melanogenesis activate a preexisting pool of tyrosinase enzyme (45), consistent with phosphorylation as the mechanism for activation of human tyrosinase.

Our data suggest a direct activation of tyrosinase by PKC-β. Indeed, preliminary studies (not included in the present manuscript) strongly suggest that tyrosinase is phosphorylated when melanocytes are treated with TPA (50). In murine melanoma cells, it is known that tyrosinase activity is largely regulated at the post-translational level in that agents inducing melanogenesis activate a preexisting pool of tyrosinase enzyme (45), consistent with phosphorylation as the mechanism for activation of human tyrosinase.

Previous studies to elucidate the signal transduction pathways controlling melanogenesis, work conducted primarily but not exclusively with mouse melanoma lines, have focused on the role of cAMP. Several agents that elevate intracellular cAMP levels, such as a-melanocyte stimulating hormone, isobutylmethylxanthine and dibutyryl cAMP are known to induce pigmentation, the latter two agents in human melanocytes as well as in murine cells (reviewed in Ref. 46). However, ultraviolet light-induced pigmentation is not accompanied by an increased intracellular level of cAMP in either human melanocytes or mouse melanoma cells (39), suggesting alternative pathway(s) for pigmentation, at least in response to this physiologic stimulus. Indeed, even pigmentation induced by a-melanocyte stimulating hormone in murine melanoma cells can be blocked by depletion of PKC (47), suggesting that AMP-dependent and PKC-dependent pathways may interact (48, 49) in melanin synthesis.

Thus, the present report greatly expands our understanding of the controls for melanogenesis, as well as the role of specific PKC isoforms in mediating specific differentiated functions.

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