Regulation of Tyrosinase in Human Melanocytes Grown in Culture

R. HALABAN, S. H. POMERANTZ,* S. MARSHALL, D. T. LAMBERT, and A. B. LERNER
Department of Dermatology, Yale University School of Medicine, New Haven, Connecticut 06510; and
*Department of Biological Chemistry, University of Maryland School of Medicine,
Baltimore, Maryland 21201

ABSTRACT Tyrosinase, the enzyme that controls the synthesis of melanin, is a unique product
of melanocytes. Normal and malignant human melanocytes grown in culture were used to
study the factors that regulate the expression of tyrosinase. Immunoprecipitation experiments
showed that newly synthesized tyrosinase appeared as a protein with an apparent molecular
weight of 70,000 that was processed to a protein with an apparent molecular weight of 80,000.
Neither tunicamycin nor 2-deoxy-D-glucose inhibited this conversion, suggesting that O-
glycosylation is the major biochemical event in the posttranslational modification of tyrosinase.
Agents that stimulated the proliferation of normal melanocytes also stimulated tyrosinase
activity. Melanocytes with low levels of tyrosinase activity synthesized less tyrosinase, proc-
escended the enzyme more slowly, and degraded it more rapidly than melanocytes with high
levels of tyrosinase activity. We conclude that tyrosinase activity in cultures of human
melanocytes derived from different donors is determined predominantly by its abundance.

Epidermal melanin is synthesized by melanocytes via oxida-
tion of tyrosine. The amount of melanin in the epidermis is
determined, at least in part, by the activity of tyrosinase. For
example, the activity of tyrosinase is higher in foreskins taken
from black babies than in foreskins taken from caucasian babies (31); and melanocytes in patients with some forms of
albinism have extremely low or no detectable tyrosinase ac-
tivity (17). Knowledge of factors that regulate tyrosinase is
important because melanin protects the skin from harmful
solar radiation. In murine melanoma cells grown in vitro the
activity of tyrosinase is at least partially regulated by the
intracellular levels of cyclic adenosine monophosphate
(cAMP). Exposure of these cells to substances that increase
the intracellular levels of cAMP also causes an increase in
tyrosinase activity and melanin content (1, 6-9, 22, 33, 38).
Recently, a method for propagating normal human melanocytes
in vitro (5) was developed. This achievement made it
possible to study the control of tyrosinase activity in nonma-
lignant human melanocytes and to compare their responses
with those of murine and human melanoma cells. Our exper-
iments show that the activity of tyrosinase in cultured mela-
nocytes is stimulated by agents that stimulate the growth of
the cells and is determined by the rate of synthesis, processing,
and degradation of the enzyme.

MATERIALS AND METHODS

Materials: Eagle's minimal essential medium (MEM) and Ham's F-10
medium were purchased from Flow Laboratories, Inc. (McLean, VA). Sera and
other medium supplements were obtained from Gibco Laboratories, Grand
Island Biological Co. (Grand Island, NY). Culture dishes were purchased from
Costar (Cambridge, MA). The other chemicals were obtained from the following
sources: 4-O-methyl-12-O-tetradecanoyl-phorbol-13-acetate (TPA)1 from
Chemicals for Cancer Research, Inc. (Eden Prairie, MN), cholera toxin, dibu-
yrlyadenosine monophosphate, and other reagents used in enzyme assays and
buffers from Sigma Chemical Co. (St. Louis, MO), L-[35S]methionine from
Amersham Corp. (Arlington Heights, IL), d-[2,3H]mannose, d-[1,6-3H(N)]-
glucomannose, [9,10-3H(N)]palmitic acid, [32P]orthophosphate, sodium [35S]sul-
fate, t-3,5-[3H]tyrosine, and Econofluor from New England Nuclear (Boston,
MA), DE52 resin from Pharmacia Fine Chemicals (Piscataway, NJ), IgGorb
from New England Enzyme Center (Boston, MA), and polyacrylamide and
protein assay kit from Bio-Rad Laboratories (Richmond, CA).

Culture of Cells: Human melanocytes were grown by a method
modified from that of Eisinger and Marko (5). Foreskins from newborn babies
were cleaned of fat and incubated at 4°C overnight (~18 h) in MEM without
calcium and supplemented with 0.25% trypsin, 1 mM EDTA, 200 U/ml
penicillin, and 100 μg/ml streptomycin. The epidermis was separated from the
dermis with forceps. Melanocytes were distributed at the borders of both
segments and became detached after vigorous shaking for 1 min. The dissociated

1 Abbreviations used in this paper: dbcAMP, dibutyryl cAMP; DOPA, DOPA;
1-dihydroxyphenylalanine; IBMX, isobutylmethyl xanthine; MSH,
melanotropin; TCA, trichloroacetic acid; TPA, 4-O-methyl-12-O-
tetradecanoyl-phorbol-13-acetate; IBMX, isobutylmethyl xanthine.
cells were grown in Ham's F-10 medium containing 5% newborn calf or fetal calf serum, penicillin, streptomycin, and 10 ng/ml (16 nM) TPA and 10 nM cholera toxin.

Contaminating fibroblasts and keratinocytes were removed by differential attachment to the culture dish. Cells were detached from the culture flasks with 0.1% trypsin in MEM without calcium and subcultured in growth medium devoid of TPA for 30 min at 37°C. Most fibroblasts and keratinocytes reattached to the culture flask during this period, and the floating melanocytes were replated in medium containing TPA and cholera toxin. Confluent cultures of normal melanocytes were resuspended and seeded at a ratio of 1:3 about once every month.

Human fibroblasts obtained from foreskins were grown in Ham's F-10 medium supplemented with streptomycin, penicillin, and 10% calf serum. Human melanoma cell lines (described in reference 14), obtained from Dr. A. Houghton, Sloan-Kettering Cancer Center, NY, were grown in the same medium. Murine melanoma cells were grown as described (6, 7).

Preparation of Tyrosinase Antisera: Tyrosinase purified from a human melanoma cell line (SK MEL 23) and immune complexes precipitated from normal melanocytes was carried out under similar conditions but without SDS. The cells were washed three times with PBS (pH 6.6) over a 20-min period and incubated with 0.2% L- dihydroxyphenylalanine (DOPA) in PBS (pH 6.8) at 37°C. After 20 min, bands corresponding to active tyrosinase became visible as the DOPA was converted to melanin.

Tyrosinase Activity and Melanization: Tyrosinase activity was measured (8, 9, 29) in cell extracts containing 2-60 µg of protein in a final volume of 200 µl in PBS containing 0.5% NP-40, 50 µM tyrosine, 50 µM DOPA, and 5 µCi/ml [3H]tyrosine with a specific activity of 0.5 Ci/mmol. Measurements were made in duplicate. A unit of tyrosinase was defined as the activity of enzyme that catalyzed the oxidation of 1 µmol of tyrosine in min.

To measure the extent of melanization, cell extracts containing 20-150 µg of protein were heated at 90°C for 2 h in 1 ml of 0.1 N NaOH and centrifuged at 16,000 g for 20 min. The optical density of the supernatants was then measured at 475 nm.

RESULTS

Tyrosinase Activity and the Extent of Pigmentation in Various Cultures of Melanocytes

The extent of pigmentation in human beings differs, depending on the individual genetic background. We investigated whether such differences were also expressed in melanocytes grown in culture and whether the extent of pigmentation correlated with the level of tyrosinase activity. Tyrosinase activity and melanin content were determined in normal melanocytes cultured from the foreskins of 18 Caucasian (C) and two black (B) babies and in five melanoma cell lines. The normal melanocyte cultures were maintained for at least 3
activity of tyrosinase. Human melanoma cells grown in the absence of TPA and cholera toxin also did not respond to MSH. The lack of stimulation of tyrosinase by MSH was observed in normal and malignant human melanocytes expressing high or low tyrosinase activity. In contrast, tyrosinase activity in the murine Cloudman S91 melanoma can be increased by exposure of the cells to agents that raise intracellular levels of cAMP, such as dibutylryl cAMP (dbcAMP), isobutylmethyl xanthine (IBMX) or MSH (1, 6–9, 22, 38). The effects of these agents on the tyrosinase activity of normal human melanocytes are shown in Table I. The agents that caused a significant increase in tyrosinase activity were TPA, IBMX, and dbcAMP. Cholera toxin had only a marginal effect and aMSH or βMSH had none. The lack of response to MSH was not due to the prolonged growth of cells in the presence of cholera toxin. Normal melanocytes grown in the absence of cholera toxin for 3 mo likewise did not respond to MSH (data not presented). The substances that stimulated tyrosinase activity also stimulated cell growth (Table I, numbers in parentheses). When TPA was not present, IBMX and dbcAMP were the only substances that maintained the short-term growth of normal melanocytes. These two substances were active only when added at the high concentration indicated in Table I; lower concentrations were ineffective (data not shown). TPA was the most effective growth stimulator, and in its presence none of the other agents caused any further increase in either the rate of growth or the amount of pigment extracted from the melanocytes (Fig. 1).

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**Binding of MSH to Human Melanocytes**

Although the human melanocytes did not respond to MSH, they were able to bind [125I]βMSH. As shown in Table II, normal and malignant human melanocytes bound up to 30% of the radiolabeled βMSH in a specific manner. In contrast, human fibroblasts showed no specific binding. The highly responsive murine Cloudman melanoma cells specifically bound 85% of the [125I]βMSH.

**Immunoprecipitation of Tyrosinase with Antityrosinase Antibodies**

We used antityrosinase antibodies to correlate the level of tyrosinase activity with the amount of tyrosinase protein. The binding of the antibodies raised in a rabbit against tyrosinase purified from hamster melanomas was tested with human tyrosinase. Cell extracts, prepared from normal melanocytes (C80) metabolically labeled with [35S]methionine were reacted with immunoglobulins purified from either the anti-serum or control serum. The antityrosinase immunoglobulins precipitated material which on analysis by PAGE appeared as a

![Figure 1](image-url)
double band with an apparent molecular weight of 70,000-80,000. This double band was absent in control immunoprecipitates (Fig. 2 A). Some proteins precipitated with both the antityrosinase and the control immunoglobulins. Most of this nonspecific binding was abolished by repeated reaction of the cell extracts with control immunoglobulins (see Fig. 5). The major radioactive band of material precipitated specifically with 8 μl of a solution of antityrosinase immunoglobulins gave the highest absorbance in the scanning densitometer (Fig. 2 B). No tyrosinase activity was detected in any of the supernatants of cell extracts treated with antityrosinase antibodies and precipitated with IgGsorb. In contrast, all the activity remained in the supernatants after treatment with control serum (Fig. 2 C). Reaction of the supernatants already treated with antityrosinase antibodies with a second aliquot of antityrosinase antibodies did not result in additional precipitation of tyrosinase. In control experiments, treatment of lysates of human fibroblasts with antityrosinase antibodies did not precipitate any material corresponding to a molecular weight of 70,000-80,000 (data not shown).

The radioactive protein precipitated from normal melanocytes (C80) by the antityrosinase antibodies and a sample of tyrosinase partially purified from a human melanoma cell line (SK MEL 23) by concanavalin A-Agarose (Bethesda Research Laboratories, Inc., Gaithersburg, MD) affinity chromatography (26) were subjected to polyacrylamide gel electrophoresis under nondenaturing conditions. Fig. 3 shows that the melanoma tyrosinase visualized by reacting with DOPA and the radioactive band from the normal melanocytes have the same migration pattern.

**Cellular Processing of Newly Synthesized Tyrosinase**

The material precipitated by the antibodies against tyrosinase appeared to consist of two proteins (Fig. 2A). To determine whether the doublet band on the gel represented proteins having a precursor-product relationship, normal melanocytes (C80) were labeled for 30 min with [35S]methionine. As shown in Fig. 4, one culture was harvested immediately and the other was “chased” for 4 h with nonradioactive methionine. The results indicate that the protein with the lower molecular weight is synthesized first and is converted to a protein of higher molecular weight.

Tyrosinase is present in mammalian melanocytes in at least two, and possibly three, isozymic forms (2, 3, 11, 12, 27, 30, 35). Tyrosinase is a glycoprotein and it has been suggested that the differences in the molecular weights of the isozymes are due to differences in the content of carbohydrates (11, 12, 26). We therefore performed several experiments to determine whether the conversion of tyrosinase from the light to the heavy form includes glycosylation.

Cells were treated with two potent inhibitors of glycosylation: tunicamycin (23, 34) and 2-deoxy-D-glucose (25). The cells were exposed to tunicamycin (0.2 or 1.0 μg/ml) in the culture medium 1 h before the labeling period, during the labeling with [35S]methionine, and during the chase period. Fig. 5 shows that tunicamycin did not inhibit the synthesis or processing of tyrosinase. Tunicamycin may partially inhibit the degradation of proteins, because the specific radioactivity of [35S]methionine in protein was 50% higher at the end of the 3-h chase period in cells treated with the drug (0.2 μg/ml) than in controls.

2-Deoxy-D-glucose (3 mg/ml) added to the medium 3 h before and during the labeling did inhibit the synthesis of tyrosinase (Fig. 5). The specific radioactivity of [35S]methionine in protein was similar in cells treated with 2-deoxy-D-
FIGURE 3 Comparison of the electrophoretic mobility of tyrosinase activity and radioactive immunoprecipitated tyrosinase. Partially purified tyrosinase from human melanomas SK MEL 23 (a) and tyrosinase immunoprecipitated from a lysate of metabolically labeled C80 cells (b) were subjected to PAGE (7.5%) without SDS. The slab gel was incubated with 0.2% DOPA, dried, and exposed to film. (a) DOPA reaction on gel. (b) Radioactive band on film.

itable radioactive tyrosinase after incubation of cells with 2-deoxy-D-glucose was the result of reduction in the amount of enzyme rather than reduction in the binding activity of the antibodies to a carbohydrate-free tyrosinase. When 2-deoxy-D-glucose was present only during the chase period, the maturation of tyrosinase into a heavier isozyme was not inhibited, but there was a small reduction in the molecular weight of the processed enzyme (Fig. 5).

To better understand the effects of tunicamycin and 2-deoxy-D-glucose on the processing of tyrosinase, we metabol-
ically labeled cells with [\(^{1}H\)]mannose or [\(^{35}S\)]glucosamine in the presence or absence of either of these two inhibitors. Tunicamycin at 0.2 and 1.0 \(\mu\)g/ml concentrations inhibited the incorporation of [\(^{1}H\)]mannose into TCA-precipitable material by 80 and 98\%, respectively. However, it did not inhibit the incorporation of [\(^{35}S\)]glucosamine into TCA-precipitable material. In contrast, 2-deoxy-D-glucose inhibited the incorporation of both [\(^{1}H\)]mannose and [\(^{35}S\)]glucosamine by 70 and 94\%, respectively. In the absence of any inhibitors, tyrosinase bound \(~0.1\) and 0.8\% of the total radioactive glucosamine and mannose incorporated into glycoproteins, respectively. Tunicamycin abolished the incorporation of [\(^{1}H\)]mannose into tyrosinase, whereas incorporation of [\(^{35}S\)]glucosamine was reduced by only 40\% (Fig. 5, bottom). These results indicate that the glycosylation of tyrosinase proceeds by two different mechanisms, one sensitive and the other insensitive to tunicamycin.

There was no incorporation of [\(^{1}H\)]palmitate, [\(^{32}P\)]phosphate, or [\(^{35}S\)]sulfate into tyrosinase (data not shown); the dried gels were exposed to the film for 95, 35, and 14 d, respectively.

**Abundance, Processing, and Degradation of Tyrosinase in Melanocytes Expressing Low and High Tyrosinase Activity**

To determine whether tyrosinase activity correlates with the level of tyrosinase protein, we investigated the abundance and the rate of degradation of tyrosinase in cultures of melanocytes derived from different foreskins. We chose cultures of melanocytes having low (C9 and C33), medium (C22), and high (C8, B1, and C80) activities (Table III). Cells were labeled metabolically by incubation with [\(^{35}S\)]methionine for 4 or 6 h and then harvested immediately or at various later times. The rates of incorporation of [\(^{35}S\)]methionine into protein of each culture were similar, as were the rates of degradation (data not shown).

The labeled cells were lysed and immunoprecipitations were carried out. The tyrosinase precipitated from each lysate was analyzed by SDS polyacrylamide slab gel electrophoresis. These analyses are shown in Figs. 6–9. Several differences are seen between the results obtained from melanocytes having low tyrosinase activity and those obtained from melanocytes with high activity.

The cells in cultures C9 and C33, in which tyrosinase activity is low, appear to have synthesized 24–33\% of the amount of enzyme produced by the cultures C8, B1, and C80, in which the level of tyrosinase activity is high (Figs. 6–9 and Table III). C33 and C9 cells also processed tyrosinase more slowly and degraded it more quickly (Figs. 6–9 and Table III).

After the initial 4-h period of labeling with [\(^{35}S\)]methionine, most of the tyrosinase from C9 and C33 cells was detected only in the faster moving band of the doublet, whereas the tyrosinase from C80 cells was detected predominantly in the more slowly moving band (Figs. 6 and 9). In the experiments described above we showed that C80 cells complete the conversion of tyrosinase to the heavier isozyme within 3 h (Fig. 5), whereas C33 cells require 10 h (Figs. 6 and 7). Even at the end of a 4-h chase following a 4-h pulse, only a fraction of the label in C33 cells could be found in the slow band (data not shown). In addition, in C33 cells, 70\% of the enzyme had been degraded at the end of a 10-h chase period, whereas none was degraded during this period in C80 cells (Fig. 8).

**Table III**

<table>
<thead>
<tr>
<th>Activity</th>
<th>Labeling period</th>
<th>Chase</th>
<th>Percent of total protein</th>
</tr>
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<tbody>
<tr>
<td><strong>Activity</strong></td>
<td><strong>(h)</strong></td>
<td><strong>(h)</strong></td>
<td><strong>(%)</strong></td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<tr>
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<tr>
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<td>C33</td>
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<td>C80</td>
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<td>C80</td>
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</tr>
<tr>
<td>C8</td>
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**Melanoma cells**

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<th>Activity</th>
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<th>Chase</th>
<th>Percent of total protein</th>
</tr>
</thead>
<tbody>
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<tr>
<td>SK MEL 23</td>
<td>220</td>
<td>16</td>
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<tr>
<td>SK MEL 13</td>
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</tr>
<tr>
<td>SK MEL 28</td>
<td>16</td>
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<td>ND</td>
</tr>
<tr>
<td>SK MEL 37</td>
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<tr>
<td>SK MEL 127</td>
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<td>6</td>
<td>0.013</td>
</tr>
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</table>

Cells were metabolically labeled with [\(^{35}S\)]methionine for the time indicated and were either harvested immediately (0 chase) or incubated (chased) in regular medium for various times. Abundance of tyrosinase is given as the percent of radioactive counts in the tyrosinase band in slices of polyacrylamide gel compared with the total radioactivity of the protein used in the immunoprecipitation assay. C and B represent caucasian and black origins of melanocytes, respectively. ND, not detectable.

We calculated the half-life of tyrosinase from the linear phase of the slopes in Fig. 8 to be 3 h in C33 and 14 h in C80 cells; these half-life values probably reflect the rate of degradation of the low vs. high molecular weight forms of the enzyme, respectively.

The differences in the patterns of synthesis, processing, and degradation of tyrosinase were not limited to normal melanocytes. Such differences were also observed in cultures of human melanoma cells (Fig. 9 and Table III). Newly synthesized tyrosinase could not be detected in melanoma cells that had little or no measurable tyrosinase activity (Fig. 9, lanes i, j, m, and n, and Table III).

Tyrosinase in cells with low activity contained the same amount of carbohydrates as cells with high activity. Lysates made of C33 and C8 cells that had been metabolically labeled with either [\(^{1}H\)]mannose or [\(^{35}S\)]glucosamine for 6 h had the same amount of radioactive sugar incorporated into tyrosinase (Fig. 10). Scanning densitometer analysis of the radioactive tyrosinase bands indicated that the relative intensities of the bands were 1.00, 1.05, 0.28, and 1.2 for lanes j, 2, 3, and 4 (Fig. 10), respectively. Taking into account the specific radioactivity of each carbohydrate in protein and the 3.7-fold...
difference in the abundance of tyrosinase in C8 compared with C33 cells (Table III), it is evident that tyrosinase in C33 cells contains carbohydrate similar in amount to that in C8 cells.

DISCUSSION

Our studies on human tyrosinase give, for the first time, values for the abundance of the enzyme protein in melanocytes. Previous studies on tyrosinase used the DOPA reaction for detection of the enzyme, a technique that relies exclusively on the levels of enzyme activity.

We have shown that pigmentation of normal human melanocytes derived from different donors is expressed over a wide range, probably reflecting differences among individuals. The levels of melanization correlated positively with the levels of tyrosinase activity. The activity of tyrosinase in normal and malignant human melanocytes was determined by the abundance of the enzymes, which in turn depended on the rates of synthesis and degradation. For example, in C8 cells with 32 times more enzymatic activity, the synthesis of tyrosinase was five times greater and degradation of the enzyme was four times slower than in C9 cells. This mechanism of regulation of tyrosinase activity is not limited to human melanocytes; the rate of synthesis of tyrosinase is also increased in murine melanoma cells stimulated by MSH and dbcAMP (Halaban, R., unpublished results). In our hands, the majority of the human melanoma cell lines were amelanotic and had extremely low or no detectable tyrosinase activity, and we have not yet found a normal human melanocyte culture that did not have melanin and a detectable tyrosinase activity.
The newly synthesized tyrosinase of human melanocytes has an apparent molecular weight of 70,000 and is converted posttranslationally in the Golgi complex (10). It is evident from our results that oligosaccharides are required for the full expression of enzymatic activity.

The results of our experiments also indicate that there is a substantial lag time for the appearance of the high molecular weight species of tyrosinase. Thus, the processing of tyrosinase may be similar to that suggested for human chorionic gonadotropin, in which N-glycosylation occurs cotranslationally and the addition of O-linked carbohydrates to the polypeptide occurs postranslationally in the Golgi complex (10). It is possible that the O-glycosylation of tyrosinase confers protection against degradative enzymes, because the isozyme with low molecular weight in cells with low tyrosinase activity is degraded at a faster rate than the processed enzyme in cells with high tyrosinase activity.

2-Deoxy-D-glucose inhibited the synthesis of tyrosinase without affecting the synthesis of total protein. Inasmuch as 2-deoxy-D-glucose is an analogue of glucose, it might affect the synthesis of tyrosinase by interfering with normal sugar metabolism and not through its effect on glycosylation. The synthesis of glycoproteins in human melanocytes depend on the carbon source in the medium; depriving the cells of a carbon source caused a marked decrease in the incorporation of radioactive sugars into protein (our unpublished data).

It also is clear that tyrosinase is not affected by three other posttranslational modifications, those due to the covalent

activity. This finding may indicate that, as a result of transformation, some cells synthesize less tyrosinase or that amelanotic melanocytes undergo malignant transformation more frequently than their melanotic counterparts.

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2-Deoxy-D-glucose inhibited the synthesis of tyrosinase without affecting the synthesis of total protein. Inasmuch as 2-deoxy-D-glucose is an analogue of glucose, it might affect the synthesis of tyrosinase by interfering with normal sugar metabolism and not through its effect on glycosylation. The synthesis of glycoproteins in human melanocytes depend on the carbon source in the medium; depriving the cells of a carbon source caused a marked decrease in the incorporation of radioactive sugars into protein (our unpublished data).
linkage of fatty acid, phosphate, and glycine residues. We detected no incorporation of palmitic acid, inorganic phosphate, and sulfate into tyrosinase.

Tyrosinase activity of normal human melanocytes was stimulated by substances, such as TPA, IBMX and dbcAMP, that also stimulated cellular proliferation. Similar findings have been reported for murine Claduman melanoma cells (6, 7, 32). These murine cells require high levels of cAMP for maintaining a high rate of proliferation and high levels of tyrosinase activity. It has been reported that TPA caused a sixfold increase in the content of melanin in human melanoma cells grown in culture (15). In contrast to the findings in murine cells, we found tyrosinase activity and the rate of cellular proliferation of normal and malignant human melanocytes to be insensitive to MSH, regardless of whether the cells had low or high tyrosinase activity or originated from black or caucasian individuals. These were unexpected findings because the administration of repeated doses of adrenocorticotrophic hormone or αMSH to adult human subjects is known to cause cutaneous hyperpigmentation within 3 d (21, 24).

The differences between responses of melanocytes to MSH in vivo and in vitro may be due to the fact that melanocytes from newborns and malignant melanocytes do not display the full range of melanocytic surface proteins present in adults (14). Both normal and malignant melanocytes specifically bind 17–35% of [125I]dMSH. This level of binding is significantly higher than that of nonmelanocytic cells, such as human fibroblasts, but is much lower than the 86% binding observed in murine melanoma cells that are highly responsive to MSH. It is not as yet clear whether this difference reflects the number of receptors present on cells of different lines or a difference in the optimum receptor-binding conditions for these lines. It is possible that the lack of response to MSH in normal and malignant human melanocytes is due to binding of insufficient numbers of hormone molecules. It is also possible that other processes known to be associated with the stimulation of melanization by MSH in murine melanomas, such as internalization of the hormone (36, 37), activation of adenylate cyclase (1, 22), and activation of protein kinase (9, 22, 33), are lacking in normal human melanocytes. It is also possible that MSH indirectly stimulates melanization in vivo through a second mediator rather than by a direct action on the melanocytes. Studies of adult human melanocytes, preferably grown in the absence of TPA and cholera toxin, are needed to determine similarities and differences in the responses to MSH of human and murine normal and malignant melanocytes.

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