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# Mammalian tyrosinase: Biosynthesis, processing, and modulation by melanocyte-stimulating hormone

(melanogenesis/melanoma/melanotropin/pigmentation/monophenol monooxygenase)

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ABSTRACT **We have examined the rate of synthesis and degradation of tyrosinase (monophenol, 3,4-dihydroxyphenylalanine:oxygen oxidoreductase, EC 1.14.18.1), the critical enzyme involved in mammalian pigmentation, using pulse–chase metabolic labeling of murine melanoma cells and immunoprecipitation of protein extracts with antibodies directed specifically against the enzyme. We have found that tyrosinase is synthesized and glycosylated within melanocytes rapidly, since significant quantities of pulse-labeled enzyme could be detected within 30 min. The maximum amount of enzyme was processed within 4 hr, and the $t_{1/2}$ of tyrosinase *in vivo* was 10 hr (compared to 120 hr with purified enzyme), suggesting that tyrosinase activity in melanocytes is at least in part regulated by rapid synthesis and active degradation. We also have examined the melanogenic stimulation caused by melanocyte-stimulating hormone, using metabolic labeling, radiometric assays, and immunofluorescence techniques; responding cells increased their melanogenic potential more than 7-fold within 4 days without increasing their levels of tyrosinase synthesis. The results demonstrate that a pool of inactive tyrosinase exists in melanocytes and that rapid increases in enzyme activity elicited by melanocyte-stimulating hormone reflect an alteration in the activity of a preexisting pool of intracellular tyrosinase.**

Tyrosinase (monophenol, 3,4-dihydroxyphenylalanine:oxygen oxidoreductase, EC 1.14.18.1), a single-chain glycoprotein enzyme essential to pigment formation in mammals, is specifically localized in melanocytes, which occur primarily in the skin, hair bulbs, and eyes. The principal subcellular site of tyrosinase activity has been determined to be the melanosome; however, tyrosinase is demonstrable in the soluble, ribosomal, endoplasmic reticulum and Golgi apparatus fractions. Although the exact sequence of posttranslational processing is still unknown, tyrosinase appears to be synthesized on ribosomes, transferred through the endoplasmic reticulum and the Golgi apparatus, where it is glycosylated and packaged into vesicles prior to fusion with premelanosomes (1–5).

Tyrosinase occurs in four distinct microheterogeneous forms as identified by PAGE (6–8); the high molecular weight membrane-bound form of tyrosinase found in melanosomes where physiologic melanin production occurs is termed $T_4$ tyrosinase. The other three forms ($T_1$, $T_2$, and $T_3$) appear to be precursors of the $T_4$ form of the enzyme, differing only with respect to their posttranslational modifications (9). After solubilization of melanosomes with ionic detergents or trypsin, $T_4$ tyrosinase is dissociated into monomeric units and shows the same electrophoretic mobility as $T_1$ tyrosinase (10, 11), with a $M_r \approx 70{,}000$. Recently, some of us have succeeded in producing monoclonal antibodies specifically directed against the mature glycosylated $T_4$ form of murine tyrosinase (12); these have proved to be useful in the identification and study of normal and transformed human melanocytes (13, 14). In addition, specific antibodies against synthetic peptides encoded by the tyrosinase gene have been prepared (15).

In this study we have examined the rate of synthesis and degradation of tyrosinase in murine melanocytes using these specific antibodies for immunoprecipitation of pulse–chase metabolically labeled cells. Further, we have used this model system to analyze the mechanisms involved in the modulation of melanogenesis in response to melanocyte-stimulating hormone (MSH), a specific differentiating stimulus that causes a dramatic increase in tyrosinase activity and pigmentation in melanocytes (16–20).

## MATERIALS AND METHODS

**Cell Lines and Culture Techniques.** B16, S91, and JB/MS melanoma cells were obtained, cultured, harvested, and counted as detailed (21–23).

**Metabolic Labeling and Solubilization of Tyrosinase.** Cells routinely were labeled for 30 min with [$^{35}$S]methionine (500 $\mu$Ci/ml; 1100 Ci/mmol, New England Nuclear; 1 Ci = 37 GBq) in methionine-free medium and were chased for 60 min, unless otherwise noted. For solubility studies, B16 cells were labeled for 30 min and chased for 1, 6, or 30 hr; then the cells were extracted for 1 hr at 4°C with 1% Nonidet P-40. Insoluble material was treated for 16 hr at 23°C with 1% NaDodSO$_4$; melanin and insoluble material were completely solubilized with 8 M urea for 48 hr at 60°C. Each extract was dialyzed against phosphate-buffered saline and immunoprecipitated as detailed below.

**MSH Treatment.** Cells were seeded at $3 \times 10^5$ per 75-cm$^2$ flask; 24 hr later, the medium was replaced with fresh medium with or without MSH and was changed daily thereafter. We used purified synthetic $\alpha$-MSH (from Calbiochem or from J. Pawelek, Yale University School of Medicine) at 0.2 $\mu$M (unless otherwise noted) because this concentration has been effective in our studies and allows our results to be directly compared with those previously published.

**Peptide Synthesis and Antibody Preparation.** A peptide corresponding to residues 500–513 (the carboxyl terminus) of the pMT4 tyrosinase clone (15) was prepared with a Beckman 990 solid-phase peptide synthesizer (24); the peptide was conjugated to hemocyanin (25) and used to prepare specific antibodies (termed "anti-PEP1") in rabbits as detailed (26); rabbit IgG were purified over an immobilized protein A column (Pierce).

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Abbreviation: MSH, melanocyte-stimulating hormone.

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**Immunoprecipitation.** This was performed as detailed (12); briefly, $5 \times 10^6$ dpm of [$^{35}$S]methionine-labeled Nonidet P-40-solubilized proteins were incubated with 50 μl of TMH-1 (a tyrosinase-specific monoclonal antibody) culture supernatant or with 40 μg of anti-PEP1 Ig for 60 min at 37°C; when TMH-1 antibody was used, 1 μl of rabbit IgG anti-rat IgG (Dako, Santa Barbara, CA) was subsequently added and incubated another 60 min at 37°C; 100 μl of 10% protein A-Sepharose (Pharmacia) was added and mixed for 30 min at 23°C. The immunoconjugates were then washed thoroughly, eluted with $NaDodSO_4$ sample buffer, and resolved by $NaDodSO_4$/PAGE on 7.5% gels (27). After fixation the gels were incubated in Fluoro-Hance (Research Products International, Mt. Prospect, IL) for 15 min, dried, and autoradiographed on XAR-2 film with Cronex-II intensifying screens.

**Immunofluorescence Techniques.** Cells were grown on LabTek tissue culture slides (Miles), stained for antibody localization, and photographed as described (12, 13). Flow cytometry was performed as detailed (23).

**Melanogenic Activity Determinations.** Melanogenic activities were radiometrically assayed in extracts as detailed (28, 29); briefly, the tyrosinase assay uses [3,5-$^3$H]tyrosine (1 Ci/mmol, New England Nuclear) and measures the $^3H_2O$ released as tyrosine is hydroxylated to dihydroxyphenylalanine. The melanin formation assay uses L-[U-$^{14}$C]tyrosine (100 mCi/mmol, New England Nuclear) and measures the entire melanogenic pathway; it reflects the many separate enzymatic and nonenzymatic steps required to produce melanin.

## RESULTS AND DISCUSSION

MSH was shown to influence rates of melanogenesis within integumental melanocytes some time ago (30), but it was more than a decade later before Wong and Pawelek (16) demonstrated that S91 murine melanoma cells in culture were able to respond to the hormone. Since that time, a number of studies have examined the mechanisms involved in the interaction of melanocytes with MSH, and the following sequence of events has been noted: (*i*) the hormone binds to melanocyte-specific surface receptors, (*ii*) this interaction results in a stimulation of adenylate cyclase activity and increased levels of intracellular cAMP, (*iii*) the elevated levels of cAMP precede increases in tyrosinase activity and melanin deposition, and (*iv*) there are long-term changes in the morphology and proliferation rates of the cells (for a review, see ref. 31). Several studies have shown that the effects of MSH on melanogenesis are dependent on continued translation and transcription (19, 20), which in turn suggests that MSH stimulates *de novo* synthesis of tyrosinase and/or other melanogenic factors. Thus, although many laboratories have studied the regulation of proliferation and pigmentation of melanocytes responding to MSH, the events that follow the binding of the hormone to pigment cells are complex, and most details of these interactions are still unknown. A number of questions have yet to be answered, including the following ones. (*i*) Are new molecules of tyrosinase synthesized in response to MSH? (*ii*) Are preexisting molecules of the enzyme activated? (*iii*) Is a melanogenic inhibitor inactivated? (*iv*) Are other control points in the melanogenic pathway regulated? (*v*) Are various combinations of the above occurring?

The TMH-1 antibody does not recognize the primary structure of tyrosinase but rather a specific posttranslational modification of that enzyme; its preparation and specificity has been detailed (12, 13). We have now synthesized a peptide corresponding to the carboxyl terminus of tyrosinase and prepared polyclonal antibodies specific to that primary sequence, which recognize native, enzymatically active tyrosinase (32). We have used these antibodies with their unique epitopes to examine the rates of normal tyrosinase processing, and the responses of melanocytes to MSH at the translational and posttranslational levels to resolve the questions posed above.

**Solubility Characteristics of Labeled Tyrosinase.** The major proportion of tyrosinase present in melanocytes is associated with the melanin granule fraction; since melanin is extremely insoluble, we initially determined the optimal solubilization conditions required to recover *de novo* synthesized tyrosinase. Tyrosinase was virtually completely extracted (>99%) with Nonidet P-40 at all of the labeling times examined (Fig. 1*A*); <1% was extracted with $NaDodSO_4$, and none was extracted with urea. Since the latter two solvents completely solubilize even intact melanosomes (33), these results show that after transport to the melanosome, active tyrosinase remains associated with the limiting membrane and can be released with nonionic detergent; these data are consistent with enzymatic studies on tyrosinase solubility (29). Estimation of label incorporated into tyrosinase indicates that it represents about 0.04% of total protein synthesized, a value comparable to estimates from enzyme purification and recovery (29).

**Specificity of Anti-PEP1 Ig.** The reactivity and specificity of the anti-PEP1 Ig is shown in Fig. 1*B*, where a single protein was immunoprecipitated from labeled extracts of B16 cells; it comigrated with that precipitated with TMH-1. Although a complete report of our studies with anti-PEP1 Ig and other antibodies specific for other tyrosinase-encoded peptides will be published separately (M.J., W.L.M., and V.J.H., unpublished data), anti-PEP1 Ig reacts specifically with mammalian tyrosinase, can be blocked by competition by the immunizing peptide (but not irrelevant peptides), and can precipitate enzymatic activity.

**Cellular Processing of Newly Synthesized Tyrosinase.** Our pulse–chase labeling studies showed that tyrosinase was synthesized and glycosylated within melanocytes extremely rapidly, since significant quantities of enzyme could be detected by TMH-1 in as little as 30 min (Fig. 2). The

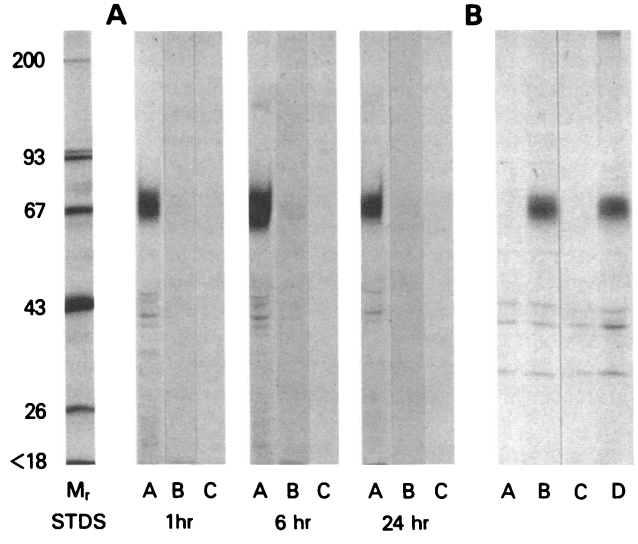


FIG. 1. Solubilization and immunoprecipitation of tyrosinase. (*A*) B16 cells were labeled with [$^{35}$S]methionine for 30 min and chased for 1, 6, or 24 hr as noted; the cells were then harvested and solubilized with Nonidet P-40 (lanes A), $NaDodSO_4$ (lanes B), and finally urea (lanes C); each extract was dialyzed, immunoprecipitated with TMH-1 antibody and visualized by fluorography after $NaDodSO_4$/PAGE. Molecular weight marker proteins are noted $\times 10^{-3}$. (*B*) B16 cells were labeled with [$^{35}$S]methionine, solubilized with Nonidet P-40, immunoprecipitated with medium (lane A), medium containing TMH-1 (lane B), normal rabbit Ig (lane C), or anti-PEP1 Ig (lane D) and visualized as above.

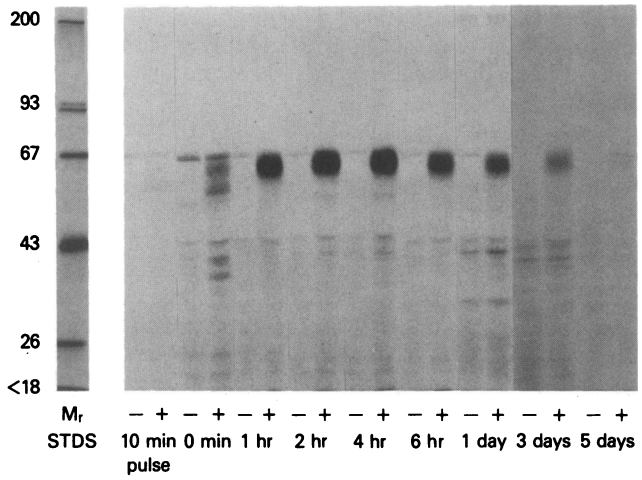


FIG. 2. Synthesis and degradation of tyrosinase *in vivo*. B16 cells were pulse-labeled for 30 min with [$^{35}$S]methionine and chased for the times indicated; labeled proteins were solubilized, immunoprecipitated with (+) or without (−) TMH-1 antibody, and visualized as in Fig. 1. Molecular weight marker proteins are noted × $10^{-3}$.

maximum amount of enzyme was processed within 4 hr, and demonstrable quantities of labeled enzyme declined steadily thereafter, with an estimated *in vivo* $t_{1/2}$ of 10 hr. Identical patterns of synthesis and degradation of tyrosinase were obtained with the anti-PEP1 Ig (not shown). We have reported (5) the remarkable stability of purified tyrosinase ($t_{1/2}$ of 5 days at 37°C), but the sum of these pulse–chase experiments suggests that tyrosinase activity within melanocytes is regulated, at least in part, by a relatively rapid synthesis and degradation. A recent study using polyclonal antibodies to tyrosinase estimated the $t_{1/2}$ of the enzyme in S91 cells at 18 hr (34), whereas another study (35) estimated the $t_{1/2}$ of tyrosinase catalytic activity in S91 cells to be 12 hr.

The earliest labeling time demonstrable for tyrosinase in this study (30 min) corresponds well with an earlier *in vivo* study (10) in which tritiated amino acids were used for metabolic labeling, and newly synthesized tyrosinase, detected by polyclonal antibodies, was localized in the microsomal fraction after 30 min, *en route* to the melanosomes.

**Response of Melanoma Cells to Treatment with MSH.** Dramatic increases in tyrosinase activity in melanocytes after treatment with MSH have been shown by many laboratories (16–20), but whether this activity resulted from synthesis of new enzyme, activation of quiescent tyrosinase, inactivation of melanogenic inhibitors, etc., has never been fully clarified. Since the TMH-1 antibody recognizes only the mature, processed $T_4$ form of tyrosinase (12), we have attempted to resolve this question by quantifying *de novo* enzyme synthesis and processing in response to MSH treatment. Immunofluorescence microscopy with TMH-1 was used to observe B16 and S91 melanoma cell lines before and after treatment with MSH (Fig. 3). B16 cells, which are usually slightly pigmented, reacted with TMH-1 even in untreated cells, but the level of reactivity was increased only slightly after MSH treatment for 4 days or even 8 days. S91 melanoma cells, which are essentially amelanotic and poorly reactive with TMH-1, also responded to MSH with increases in intracellular tyrosinase but took 8 days to do so. There was no increase in enzyme concentration in S91 cells after only 4 days of treatment with the hormone, even though increases in enzyme activity and visible pigmentation of these cells occurred (see Table 1 below).

The response of melanoma cells to MSH treatment has been measured with respect to cell growth, tyrosinase activity (measured enzymatically), and tyrosinase synthesis (measured by metabolic labeling and immunoprecipitation). The changes in these parameters for B16 cells responding to MSH over a 4-day period (as they reached confluence) are shown in Fig. 4; there were no significant differences in the growth rates of MSH-treated and untreated cells over the course of this (or any other) short-term experiment. The tyrosinase activity in B16 cells showed only a slight increase in MSH-treated cells compared to untreated controls after 1 day, but by day 2 the enzyme activity in MSH-treated B16 cells had begun to significantly increase; this increase was maximal at day 4 and then declined toward control levels by day 8 (not shown).

In similar experiments (not shown), JB/MS cells responded even more quickly to MSH than did B16 cells; tyrosinase activity had doubled within 24 hr of MSH treatment, but again, maximal response was seen by 4 days. In contrast, the rate of enzyme synthesis by these same cells throughout the course of the experiment was virtually constitutive, with or without the addition of MSH. The tyrosinase synthesis results shown in Fig. 4 were measured by reactivity with TMH-1, but similar results were found with the anti-PEP1 Ig (Fig. 5). We have occasionally detected increases in synthesis of tyrosinase elicited by MSH (cf. Fig. 5 at day 3), but these responses have never occurred before 3 days of treatment, nor has more than a 2- to 3-fold increase in synthesis ever been noted. These results are consistent with our immunofluorescence studies (Fig. 3) and indicate that melanoma cells require at least 3 days to begin responding to MSH by increasing synthesis of tyrosinase, although dramatic increases in enzyme activity precede this (see below).

To investigate whether the effects of MSH on melanoma cells might be related to changes in the rate of degradation of tyrosinase, we examined its metabolic processing in cells treated with MSH. B16 melanoma cells were incubated with MSH and, on day 4 (at maximal response) were pulse–chase-labeled and immunoprecipitated with TMH-1 and anti-PEP1 Ig as in Fig. 2. Those studies (not shown) showed that there was no difference in the degradation rate of tyrosinase in cells

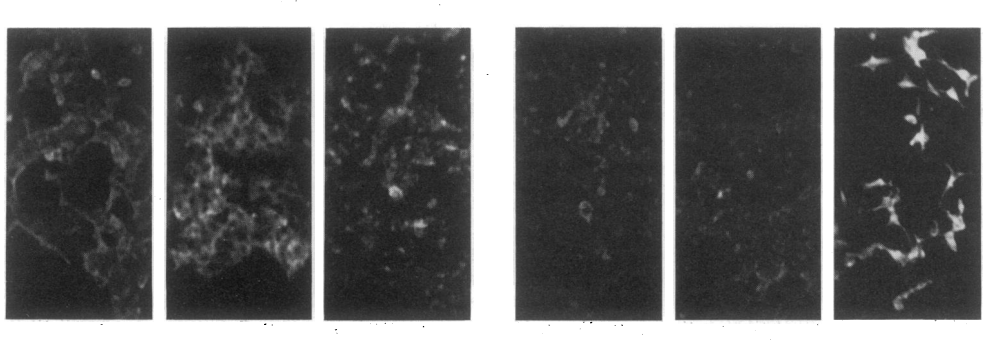


FIG. 3. Fluorescence microscopy of tyrosinase content after MSH treatment. B16 and S91 cells were grown on tissue culture slides in medium without or with MSH for 4 or 8 days; the slides were stained with TMH-1 and viewed by indirect immunofluorescence. All photographs were taken with identical exposures and magnifications (×50) to enable direct comparison.

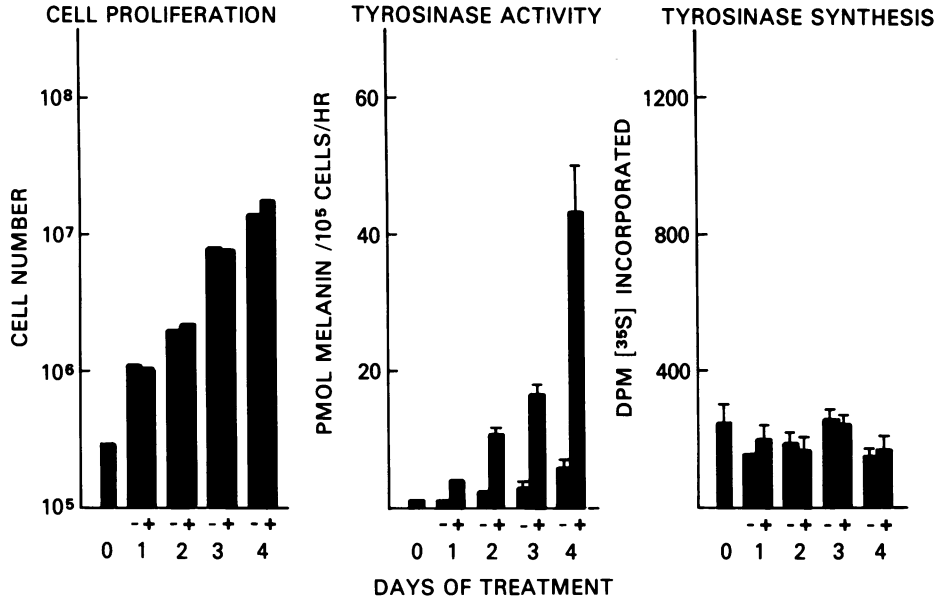


FIG. 4. Kinetic responses of melanoma cells to MSH. B16 cells were grown in identical flasks with (lanes +) or without (lanes −) MSH. Flasks were harvested each day, the cells were counted, solubilized with Nonidet P-40, and assayed for melanogenic activity; identical flasks were concurrently labeled with [$^{35}$S]methionine, harvested, solubilized with Nonidet P-40, and immunoprecipitated with TMH-1 to determine tyrosinase synthesis.

after MSH treatment compared to controls, and thus there was no change in the $t_{1/2}$ of tyrosinase as the result of hormonally induced melanogenesis. These data agree well with other studies on S91 cells that used immunologic and enzymatic methods (34, 35) to show that MSH had no effect on the $t_{1/2}$ of tyrosinase in S91 cells and, thus, that the level of action of MSH is not on enzyme stability.

In an effort to quantify the intracellular concentrations of tyrosinase in melanocytes responding to MSH, we used TMH-1 with flow cytometry. Table 1 shows a comparison of three different melanoma cell lines that respond to the hormone; B16 cells, which are slightly pigmented in culture, became highly pigmented after 4 days of MSH treatment, whereas the S91 and JB/MS amelanotic melanoma cells became only moderately pigmented under identical conditions. The melanogenic potential, determined by the melanin formation assay, reflected the visible response; B16 cells increased their rate of pigment formation about 7-fold in this experiment, while the S91 and JB/MS cell lines increased 22-fold and 40-fold, respectively. However, determination of the intracellular tyrosinase concentrations within these same cells by flow cytometry revealed only a slight increase in MSH-treated B16 cells after 4 days and no significant increase in MSH-treated S91 or JB/MS cells after 4 days. These data clearly indicate that increased synthesis of tyrosinase cannot explain the stimulation of the rates of melanogenesis observed in any of these lines. In addition, it should be noted that although the levels of enzymatic activity of untreated S91 and JB/MS cells are similarly low, their tyrosinase contents are dramatically different—the JB/MS cells have tyrosinase levels virtually identical to those found in B16 cells. This suggests the presence of a potent inhibitor of tyrosinase in JB/MS cells; in experiments in which we have purified tyrosinase from JB/MS cells (not shown), the inhibition was removed after partial purification, and quantities of tyrosinase similar to those found in B16 cells were isolated. A recent study (34), which used an unrelated approach (immunotitration) for the detection of tyrosinase, also concluded that an inactive precursor pool of the enzyme exists in S91 cells.

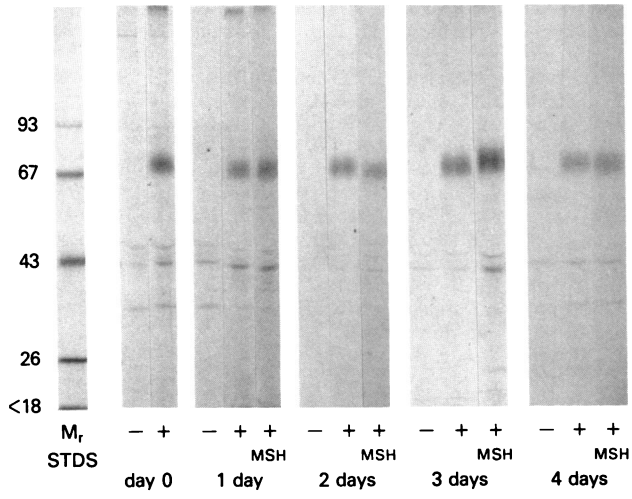


FIG. 5. Synthesis of tyrosinase in response to MSH stimulation. B16 cells were seeded into identical flasks and grown with or without MSH as noted; each day, the cells were pulse-labeled with [$^{35}$S]-methionine, immunoprecipitated with normal rabbit Ig (lanes −) or with anti-PEP1 Ig (lanes +), and visualized as detailed for Fig. 1. Molecular weight marker proteins are noted × $10^{-3}$.

**Effects of MSH on Posttyrosinase Controls of Melanogenesis.** It has been recently proposed (36–39) that several posttyrosinase regulatory steps exist in the melanin biosynthetic pathway. Although the nature and regulatory significance of posttyrosinase factors in melanogenesis have not yet been fully elucidated, the stimulatory effects of MSH on pigmentation theoretically could result from their modulation and not from direct effects on tyrosinase activity (36, 38). We have examined this possibility by measuring tyrosinase activity and melanin formation in MSH-treated and untreated cells; the tyrosinase assay specifically reflects that enzyme's

Table 1. Tyrosinase activity and concentration in MSH-treated melanoma cells

| | Visible melanin | | Melanin formation, pmol/$10^5$ cells/hr | | | Tyrosinase content, mean fluorescence intensity | | | |
|---|---|---|---|---|---|---|---|---|---|
| Cells | − | + | − | + | Fold increase | Bkg | − | + | % increase |
| B16 | + | +++ | 18 | 125 | 7 | 10 | 70 | 88 | 130 |
| S91 | − | ++ | 1 | 22 | 22 | 17 | 26 | 24 | 78 |
| JB/MS | − | ++ | 1 | 40 | 40 | 11 | 68 | 71 | 105 |

Melanoma cells were cultured in the absence (−) or presence (+) of 0.2 μM MSH for 4 days; they were examined for visible pigment (− to +++ scale) and then harvested and solubilized; the melanin formation assay was carried out in triplicate (±SEM < 10%); tyrosinase content was determined by flow cytometry.

Table 2. Effect of MSH on melanogenic activities of melanoma cells

| Day | Cells $\times 10^{-6}$ | | Tyrosinase activity, pmol/$10^5$ cells/hr | | | Melanin formation, pmol/$10^5$ cells/hr | | | M/T |
|---|---|---|---|---|---|---|---|---|---|
| | − | + | − | + | % increase | − | + | % increase | |
| B16 melanoma cells | | | | | | | | | |
| 1 | 0.4 | 0.7 | 223 | 282 | 126 | 5.0 | 7.6 | 152 | 1.2 |
| 2 | 1.6 | 1.4 | 147 | 240 | 163 | 5.1 | 8.0 | 156 | 1.0 |
| 3 | 3.0 | 4.5 | 87 | 400 | 460 | 4.7 | 12.5 | 266 | 0.6 |
| 4 | 6.0 | 6.3 | 80 | 485 | 606 | 5.5 | 17.6 | 320 | 0.5 |
| JB/MS melanoma cells | | | | | | | | | |
| 1 | 0.2 | 0.2 | 23 | 43 | 187 | 1.9 | 3.1 | 163 | 0.9 |
| 2 | 0.8 | 1.1 | 16 | 48 | 300 | 1.5 | 5.3 | 353 | 1.2 |
| 3 | 1.9 | 1.9 | 11 | 76 | 691 | 1.6 | 26.4 | 1650 | 2.4 |
| 4 | 2.3 | 2.1 | 14 | 146 | 1043 | 2.1 | 41.1 | 1957 | 1.9 |

B16 or JB/MS melanoma cells were cultured in the absence (−) or presence (+) of 0.2 $\mu$M MSH for the times noted, harvested, counted, and solubilized. Melanogenic activities were assayed in triplicate ($\pm$ SEM < 10%). M/T reflects the ratio of MSH stimulation of melanin formation to tyrosinase activity.

activity, whereas the melanin-formation assay reflects not only tyrosinase activity but also many other significant melanogenic factors (28, 29, 39). Our kinetic studies on the response of B16 and JB/MS cells to MSH over a 4-day period are shown in Table 2. Tyrosinase activity in B16 cells was stimulated approximately 6-fold after 4 days, while melanin formation was increased only 3-fold in this experiment. The ratio of the stimulations elicited by MSH of melanin formation over tyrosinase activity is shown in the far right column (M/T) of Table 2; a ratio of 1.0 would result if there were no effect of MSH on posttyrosinase melanin formation (that is, if MSH stimulated only tyrosinase activity), whereas the ratio might increase or decrease if MSH had an effect, depending on which factor were modulated. If blocking factor activity, for example, were reduced (suggested in ref. 36), an increase in melanin formation relative to tyrosinase would result. In fact, in B16 cells, the M/T ratio actually decreased with time of MSH treatment—opposite to what might be expected were posttyrosinase steps in melanin formation responsible for increased synthesis of pigment.

Similar studies on JB/MS cells are also shown in Table 2; after 4 days of MSH treatment, the tyrosinase activity in this experiment was stimulated 10-fold. With JB/MS cells, however, the melanin-formation assay showed a 20-fold stimulation after 4 days of MSH treatment, suggesting that modulation of posttyrosinase melanogenic factors may result in an additional 2-fold increase in melanin formation. However, the 10-fold stimulation of tyrosinase activity observed directly reflects tyrosinase activation and indicates that this is the more significant response to MSH.

Thus, it seems clear that tyrosinase is synthesized and glycosylated in melanocytes more quickly than previously thought; pulse-labeled enzyme could be detected within 30 min. The $t_{1/2}$ of tyrosinase *in vivo* was found to be 10 hr, which suggests that melanogenesis is at least in part regulated by a relatively rapid synthesis and degradation. Further, it is clear that MSH elicits responses in melanocytes that are at least biphasic in nature—there is an increase in the rate of synthesis of tyrosinase after 4 or more days, but a more rapid stimulation of melanogenic potential occurs, which represents activation of preexisting enzyme. This rapid response to MSH results primarily from a direct effect on tyrosinase but also may partly reflect complementary effects on distal points in the melanin biosynthetic pathway. The fact that similar responses to MSH were observed on three distinct murine melanomas of diverse origin by two unique immunological probes suggests that the scheme elaborated here may reflect a general phenomenon in the response of mammalian melanocytes to this melanogenic stimulus.

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