Mitogen-activated Protein Kinase Pathway and AP-1 Are Activated during cAMP-induced Melanogenesis in B-16 Melanoma Cells

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In mammalian melanocytes, melanin synthesis is controlled by tyrosinase, the critical enzyme in the melanogenic pathway. We and others showed that the stimulation of melanogenesis by cAMP is due to an increased tyrosinase expression at protein and mRNA levels. However, the molecular events connecting the rise of intracellular cAMP and the increase in tyrosinase activity remain to be elucidated. In this study, using B16 melanoma cells, we showed that cAMP-elevating agents stimulated mitogen-activated protein (MAP) kinase, p44mapk. This effect was mediated by the activation of MAP kinase kinase, cAMP-elevating agents induced a translocation of p44mapk to the nucleus and an activation of the transcription factor AP-1. cAMP-induced AP-1 contained FOS-related antigen-2 in association with JunD, while after phorbol ester stimulation AP-1 complexes consist mainly of JunD/C-Fos heterodimers. In an attempt to connect these molecular events to the control of tyrosinase expression that appears to be the pivotal point of melanogenesis regulation, we hypothesized that following its activation by cAMP, p44mapk activates AP-1. Then AP-1 could stimulate tyrosinase expression through the interaction with specific DNA sequences present in the mouse tyrosinase promoter.

In melanocytes and melanoma cells, melanin synthesis is controlled by a cascade of enzymatic reactions regulated at the level of tyrosinase. This enzyme synthesizes dopaquinone from tyrosine and appears to control the rate-limiting step of melanogenesis. Melanin synthesis is stimulated by a large array of effectors including 1-oleyl-2-acetyl-glycerol, ultraviolet B radiations, and cAMP-elevating agents (forskolin, IBMX,1). Tyrosinase is the rate-limiting enzyme in the melanin synthesis pathway and is controlled by tyrosinase, the critical enzyme in the melanogenesis. This enzyme synthesizes dopaquinone from tyrosine and appears to control the rate-limiting step of melanogenesis (4, 9) following post-translational modifications such as phosphorylation or glycosylation, association with an activator (11, 12), and dissociation from an inhibitor (13). Alternatively, cAMP was shown to increase tyrosinase mRNA (14, 15), resulting in an augmentation of tyrosinase amount, suggesting that cAMP stimulates tyrosinase transcription (16). However, the molecular events connecting the stimulation of tyrosinase activity or the activation of tyrosinase gene expression to the rise of cellular cAMP remain to be identified.

The proline-directed serine/threonine kinases of the MAP kinase family (p44mapk and p42mapk) are activated upon phosphorylation on both threonine 182 and tyrosine 185 by the dual specificity MAP kinase kinase (MEK) (20). MEK is itself phosphorylated and activated by Raf-1 kinase (20) or by a recently identified MAP kinase kinase (MEK) kinase (21). MAP kinases were shown to be involved in the control of cell growth (17), in the regulation of some metabolic processes such as glycogen synthesis (18, 19), and more recently in the regulation of the cell cycle and adipoocytes differentiation (22, 23). In melanocytes and melanoma cells the induction of melanogenesis is associated with cell differentiation. Thus, we hypothesized that MAP kinases could be activated during cAMP-induced melanogenesis. Using the well characterized mouse melanoma cells B-16, we demonstrated that CAMP-elevating agents such as forskolin, IBMX, and 4-norleucine-7-α-phenylalanine-α-melanocyte stimulating hormone ([Ni6,α-Phe]αMSH), a potent analog of α-MSH, stimulated p44mapk through the activation of the transcription factor AP-1 by CAMP-elevating agents. In this condition the AP-1 complex contained predominantly JunD and Fra-2. Our results provide meaningful clues concerning the molecular mechanisms triggered by cAMP in B-16 melanoma cells and suggest that the MAP kinase pathway and AP-1 could play a role in melanogenesis regulation by cAMP.

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†To whom correspondence should be addressed.

‡The abbreviations used are: IBMX, isobutylmethylxanthine; α-MSH, α-melanocyte stimulating hormone; Fra-2, Fos-related antigen-2; MEK, mitogen-activated/extracellular signal-regulated protein kinase; BSA, bovine serum albumin; CRE, CAMP-responsive element; PAGE, polyacrylamide gel electrophoresis; TPA, 12-O-tetradecanoylphorbol-13-acetate; TRE, TPA-responsive element; M + 1, [Ni6, α-Phe]αMSH plus IBMX; AEBSF, 4-(2-aminoethyl)benzenesulfonyl fluoride; CREB, CRE binding protein.
EXPERIMENTAL PROCEDURES

Materials—Dulbecco’s modified Eagle’s medium, 4-norleucine 7-
phenylalanine-o-melanoxy stimulating hormone (Nle4, D-Phe7)-MSH, IBMX, TPA, forskolin, BSA, myelin basic protein from bovine brain, protein A-Sepharose, 4-(2-aminoethyl)benzenesulfonfulylo
fluoride (AEBSF), aprotonin, and leupeptin were purchased from Sigma. Poly
cytonic rabbit antiserum to human tyrosinase (PEP-7) was provided by Dr. V. Hearing (Bethesda, MD). Antiserum to p44mapk and MEK were gener
cous gifts from Dr. E. Van Obbergen (Nice, France). Antibodies to MEK
kinase, Raf-1, c-Fos, c-jun, J unB, J unD, and the AP-1 and AP-2 syn
thetic oligonucleotides are from Santa Cruz Biotechnology. Rabbit poly
clonal anti-Fra-2 antibody was prepared using recombinant GST fusion
protein of Fra-2, [D-(L)-ring-3',5',6'-hydroxy]-tyrosine (40–60 Ci/mmol; 1 M Ci/mmol); 1,3,4-dihydroxyphenyl 3-[14C]alanine (7–12 mCi/mol, 50
µCi/ml); [α-32P]dCTP; and [γ-32P]ATP (3000 Ci/mmol) were from Amersham
(Buckinghamshire, United Kingdom).

Cell Cultures—B-16F10 murine melanoma cells (from Dr. V. Hear
ing) were cultured in Dulbecco’s modified Eagle’s medium with 10%
 fetal calf serum and penicillin/streptomycin (100 IU/50 µg/ml) in a
humidified atmosphere containing 5% CO2 in air at 37°C. 

Determination of Tyrosinase Activity and Melanin Synthesis—Tyro
sinase activity in living cells was estimated by the amount of 3H2O released into the culture medium during the hydroxylation of 3,5,6-
hydroxytyrosine to hydroxyphenylalanine, according to Oikawa et al. (24). The melanin formation assay used 1,3,4-dihydroxyphenyl 3-[14C]ala
nine (7–12 mCi/ml) as precursor (25).

Western Blot Analysis—B-16 cell lysates were separated by SDS-
PAGE (10% acrylamide gels) and transferred to Hybond-C extra mem
branes. The blots were probed with PEP-7 antibody directed to the C
terminus part of tyrosinase (26), and then proteins were visualized by the Amersham ECL system and quantified by image analysis.

Northern Blot Analysis—Total cellular RNAs were purified using the method described by Chomczynski and Sacchi (27). For Northern blot
analysis, total cellular RNA (25 µg/lane) were fractionated on 1% aga
rose, 0.66 M formaldehyde gel and transferred onto nylon membranes. Mouse tyrosinase cDNA probe (generously provided by Dr. B. Bou
chard) and human glyceraldehyde-3-phosphate dehydrogenase cDNA probe were labeled with [α-32P]dCTP using a random priming kit (Stratagene). Membranes were autoradiographed and quantified by image analysis.

Image Analysis—Morphometric measurements were performed using
a Biocom 500 (BIOCOM SA, Les Ulis, France) image analysis system
coupled to a CCD video camera and a Nikon TMS inverted light microscope. After treatment, cells were viewed using a 20 × 20µm
occasionally projected onto the video screen. Outlines of 100
 cells of each experimental condition were acquired manually, quantita
tive measurements of area (A) and perimeter (P) being performed using the Mopho software and then the dendriticity factor was evaluated as 
P/A2 according to Laour et al. (28).

Results

Effect of [Nle4, D-Phe7]-MSH plus IBMX on melanogenesis in B-16 cells

B-16 melanoma cells were cultured in the presence or in the absence of 1 µM [Nle4, D-Phe7]-MSH plus 0.1 µM IBMX (M = 1) for 24 or 48 h. 

Table 1 shows the different parameters indicated, as detailed under “Experimental Procedures.” Results are means ± S.E. of three determinations.

| Parameter | M = 1 | M = 0.1 |
|-----------|-------|--------|
| Tyrosinase activity | 3.2 ± 0.5 | 0.4 ± 0.4 |
| Tyrosinase mRNA | 3.6 ± 0.8 | 2 ± 1 |
| Dendriticity factor | 1.77 ± 0.1 | 2.3 ± 0.2 |

By the addition of [γ-32P]ATP (10 µCi/ml, 50 µmol/l) after incubation at room temperature for 20 min, assays were stopped by the addition of L-aenml sample buffer. The samples were analyzed by SDS-PAGE (10% acrylamide gel) and autoradiography.

Immunofluorescence Studies—After stimulation, B-16 cells were
washed with PBS and fixed at −20°C for 10 min with methanol/aceton (3:7, v/v). After a 10-min rehydration at 25°C in PBS containing 1% BSA (PBS/BSA), fixed cells were incubated with the primary antibody
directed to the C terminus part of p44mapk (1500) for 60 min at 25°C. 

Cells were then washed five times with PBS and incubated in PBS/BSA for 60 min at 25°C with fluorescein isothiocyanate-conjugated second
ary antibody (anti-rabbit, 1:100). Finally, cells were washed five times with PBS and examined under confocal laser scanning microscope.

Nuclear Extracts and Gel Mobility Shift Assay—Serum-starved B-16 cells were stimulated for 2 h with the different effectors, and the nuclear extracts were prepared essentially as described by Dignam (29). 

In vitro binding reaction of AP-1 in a total volume of 25 µl was per
formed by incubation of 10 µg of nuclear extract in a binding buffer containing 10 mM Hepes, pH 7.8, 50 mM KCl, 2 mM dithiothreitol, 1 mM
 EDTA, 5 mM MgCl2, 10% glycerol, 3 mM AEBSF, 2 µg of poly(dI-dC)
 mg/ml BSA. After 10 min of preincubation on ice, 50,000–100,000 cpm of
 [3H]-labeled oligonucleotide probe was added and incubated at 25 ±
 20°C for 20 min. Then DNA-protein complexes were resolved by elec
trophoresis on 4% polyacrylamide gels in TAE buffer (10 mM Tris, 9 mM
 sodium acetate/acetic acid, 275 µM EDTA) for 8 h at 120 volts, dried,
 and subjected to autoradiography. When indicated, an excess of cold
 competitor oligonucleotide was added during preincubation. For anti
body supershift assays, 1 µg of the corresponding antisera were preincu
bated with the nuclear extracts for 1 h on ice in the binding reaction
 buffer before adding the labeled probe.

RESULTS

Stimulation of Melanogenesis in B-16 Melanoma Cells—We
first studied the melanogenic activity and the morphological
changes of B-16 melanoma cells in response to cAMP-elevating
agents. In preliminary experiments, various cAMP-elevating
agents including forskolin, cholaer toxin, β-MSH, [Nle4, D-Phe7]-
MSH, and IBMX were found to increase melanin synth
esis in B-16 melanoma cells. The most important stimulation
was obtained with [Nle4, D-Phe7]-MSH plus IBMX. [Nle4, D-Phe7]-
MSH plus IBMX (M = 1) stimulated tyrosinase activity,
melanin synthesis, tyrosinase expression at the protein,
and RNA messenger levels (Table I). These results suggest that
the stimulation of tyrosinase gene expression plays a key role
in the control of melanogenesis by cAMP-elevating agents.

Simultaneously, we observed morphological differentiation
characterized by numerous and arborescent dendrite out
growths. These changes were quantified by image analysis as

described under “Experimental Procedures.” The results pre
sented in Table I show that the dendriticity factor is markedly
increased after 24 and 48 h of incubation with M + 1.

Activation of MAP Kinase by cAMP-elevating Agents in B-16
Melanoma Cells—We focused our attention on the activation of
p44mapk by cAMP-elevating agents. In this aim, B-16 mel
anoma cells were stimulated for 15 min by forskolin, [Nle4,

D-Phe7]-MSH plus IBMX (M = 1) for 24 or 48 h. Then cells were
stimulated with the different effectors, and the nuclear extracts were prepared essentially as described by Dignam (29). In vitro
binding reaction of AP-1 in a total volume of 25 µl was performed by incubation of 10 µg of nuclear extract in a binding buffer containing 10 mM Hepes, pH 7.8, 50 mM KCl, 2 mM dithiothreitol, 1 mM
 EDTA, 5 mM MgCl2, 10% glycerol, 3 mM AEBSF, 2 µg of poly(dI-dC)
 mg/ml BSA. After 10 min of preincubation on ice, 50,000–100,000 cpm of
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trophoresis on 4% polyacrylamide gels in TAE buffer (10 mM Tris, 9 mM
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 and subjected to autoradiography. When indicated, an excess of cold
 competitor oligonucleotide was added during preincubation. For anti
body supershift assays, 1 µg of the corresponding antisera were preincu
bated with the nuclear extracts for 1 h on ice in the binding reaction
 buffer before adding the labeled probe.
Activation of p44<sub>mapk</sub> and AP-1 by cAMP-elevating Agents

FIG. 1. cAMP-elevating agents stimulate p44<sub>mapk</sub> activity in B-16 cells. B-16 cells were incubated either with 10 μM forskolin (FORSK), 1 μM [Nle<sup>4</sup>, D-Phe<sup>7</sup>]-α-MSH (α-MSH), 0.1 mM IBMX, or M + I for 10 min. Then cells were solubilized, p44<sub>mapk</sub> was immunoprecipitated, and its kinase activity was measured using myelin basic protein as substrate. Results are expressed as fold stimulation of the basal p44<sub>mapk</sub> activity from unstimulated cells (CONT). Data are means ± S.E. of five experiments performed in triplicate.

D-Phe<sup>7</sup>-α-MSH, IBMX, or [Nle<sup>4</sup>, D-Phe<sup>7</sup>]-α-MSH plus IBMX. Solubilized proteins were submitted to immunoprecipitation with a specific antibody raised against the C terminus domain of p44<sub>mapk</sub>. Kinase activity was then measured using myelin basic protein as substrate and quantified by filter paper assay (Fig. 1). With forskolin, [Nle<sup>4</sup>, D-Phe<sup>7</sup>]-α-MSH, or IBMX we observed a 2.5-fold stimulation of p44<sub>mapk</sub> activity. A 4-fold stimulation was achieved with [Nle<sup>4</sup>, D-Phe<sup>7</sup>]-α-MSH, and IBMX in association (M + I). The most efficient stimulation of p44<sub>mapk</sub> activity (8-fold) was obtained with TPA (not shown). Longer stimulation time either with TPA or cAMP-elevating agents did not result in higher stimulation.

Effect of cAMP-elevating Agent on MEK and on Raf-1 Kinase—in an attempt to understand the mechanism by which cAMP stimulated p44<sub>mapk</sub>, we studied the effect of M + I on MAP kinase kinase and Raf-1 kinase activities.

The kinase activity of MEK was monitored in a cell-free system, after immunoprecipitation with specific antibody, using as substrate p44<sub>mapk</sub> extracted from unstimulated cells (Fig. 2). Lane 1 shows the basal autophosphorylation of unstimulated p44<sub>mapk</sub> at 44 kDa. A faint band at 45 kDa in lanes 3 and 4 indicates that MEK autophosphorylation was stimulated by TPA and M + I compared with the basal autophosphorylation (lane 2). When phosphorylation was performed in the presence of both MEK and p44<sub>mapk</sub>, we observed a strong phosphorylation of a protein at 44 kDa, indicating that MEK phosphorylated p44<sub>mapk</sub> (lane 5). This phosphorylation was increased when MEK was extracted from TPA or M + I-treated cells (lanes 6 and 7), demonstrating that MEK was stimulated by TPA and [Nle<sup>4</sup>, D-Phe<sup>7</sup>]-α-MSH plus IBMX in B-16 melanoma cells.

Since Raf-1 was described as operating immediately upstream of MEK, similar experiments were performed to examine the effect of M + I on Raf-1 activity. Raf-1 was isolated from B-16 melanoma cells stimulated as described above, and its kinase activity was evaluated using as substrate MEK immunoprecipitated from unstimulated cells (Fig. 3). In the first lane, we observed a band at 45 kDa corresponding to the basal autophosphorylation of MEK. The other bands appeared to be nonspecific, since they were precipitated by preimmune serum (not shown). With Raf-1 incubated alone, no autophosphorylation was observed (lanes 2–4). When MEK was added to Raf-1, no significant increase in the basal phosphorylation of MEK was observed with Raf-1 precipitated from control or M + I-treated cells (lanes 5 and 7). In contrast, MEK phosphorylation was markedly increased in the presence of Raf-1 extracted from TPA-treated cells (lane 6). These results indicate that cAMP-elevating agents did not stimulate Raf-1 activity in B-16 cells. Thus, the effect of [Nle<sup>4</sup>, D-Phe<sup>7</sup>]-α-MSH plus IBMX on p44<sub>mapk</sub> is mediated by MEK that is activated by an unknown mechanism independently on Raf-1 kinase stimulation.

Translation of p44<sub>mapk</sub> to the Nucleus—Stimulation of tyrosinase gene expression plays a key role in the regulation of melanogenesis by cAMP. Thus we hypothesized that the cAMP signal should be transmitted to transcription factors present in the nucleus. To verify this hypothesis, we studied the effect of [Nle<sup>4</sup>, D-Phe<sup>7</sup>]-α-MSH plus IBMX on p44<sub>mapk</sub> localization in B-16 melanoma cells.

The localization of p44<sub>mapk</sub> in cells treated or not treated with [Nle<sup>4</sup>, D-Phe<sup>7</sup>]-α-MSH plus IBMX was studied by immunofluorescence and confocal laser scanning microscopy (Fig. 4). Using an antipeptide to the C terminus part of p44<sub>mapk</sub>, we observed, in the absence of M + I, a strong perinuclear and a weak nuclear labeling. After a 60-min exposure to M + I, the cytoplasm and the nucleus appeared equally labeled, indicating that p44<sub>mapk</sub> translocated to the nucleus. This phenomenon was transient, since after 150 min in presence of M + I, nucleus labeling decreased, suggesting that p44<sub>mapk</sub> returned to the cytoplasm.
Activation of Transcription Factors by cAMP-activating Agents—Since p44mapk is activated and translocated to the nucleus by M + I, it is tempting to propose that these phenomena are followed by an activation of transcription factors. Among the transcription factors activated by p44mapk, AP-1 was reported to stimulate gene expression through the binding to TRE sequences. The presence of TRE-like sequences in the mouse tyrosine kinase promoter prompted us to study the activation of AP-1 by [Nle4, D-Phe7]-α-MSH plus IBMX. B-16 melanoma cells were stimulated either with TPA as positive control or with M + I. Then nuclear extracts were prepared, and the gel retardation assay was performed with a labeled oligonucleotide containing the TRE or CRE sequence. DNA binding activity was measured in the same condition using a labeled oligonucleotide presenting the AP-2 consensus binding site.

Characterization of AP-1 Complexes Induced by cAMP and TPA—Interestingly, the AP-1 complex induced by M + I migrated more slowly than that observed with TPA, suggesting that these AP-1 complexes contain different components. AP-1 consists in either homodimers of Jun family proteins or in heterodimers of Jun/Fos family proteins. Three Jun proteins (c-Jun, JunB, JunD) and at least four Fos proteins (c-Fos, FosB, Fra-1, Fra-2) were found in AP-1 complexes. The composition of AP-1 complexes in both TPA and M + I conditions was investigated by supershift experiments using specific antibodies to c-Jun, JunB, JunD, and c-Fos antibodies (Fig. 6). With a nuclear extract from TPA-treated cells, AP-1 complexes were almost totally shifted by anti-c-Jun antibodies (lane 10) and anti-c-Fos antibodies (lane 11). AP-1 complexes were also shifted, but to a lesser extent, by antibodies to JunB (lane 8), c-Jun (lane 9), and Fra-2 (lane 12).

In conclusion, we characterize the molecular mechanisms by which cAMP stimulates melanin synthesis in melanocytes and melanoma cells remain to be identified. In this aim, we characterized the molecular events triggered by cAMP in B-16 melanoma cells. Our results demonstrate that cAMP activated p44mapk through the stimulation of MEK, the enzyme immediately upstream from MAP kinases. The molecular mechanisms of MEK activation by cAMP in B-16 melanoma cells differ from those already reported in other cell types.
types (30). Indeed, neither Raf-1, which is not activated by cAMP, nor MEK kinase, which is not detected in B-16 melanoma cells, is apparently involved in MEK activation by cAMP. The involvement of another member of the Raf kinase family, i.e. A-Raf or B-Raf that is mainly expressed in neuronal cells (31) may be suggested. However, the inhibition of B-Raf kinase activity by cAMP observed in PC12 cells (32), makes this hypothesis unlikely. It remains possible that in B-16 melanoma cells cAMP activates an isoform of MEK kinase, different from that previously described by Lange-Carter (21). Alternatively, inhibition by cAMP of phosphatase 2A activity, which was reported to dephosphorylate and deactivate MEK (33), can be also suggested.

Following its activation by cAMP, we observed a transient translocation of p44 mapk to the nucleus. Similar observations were reported in serum-treated fibroblast (34) or in NGF-stimulated PC12 cells (35). In the nucleus, p44 mapk is thought to phosphorylate and activate numerous transcription factors such as p62 c-fos (36), c-Myc (37), and AP-1 (38). In B-16 melanoma cells, we showed that cAMP stimulated AP-1 binding to an oligonucleotide containing a TRE sequence. cAMP-induced AP-1 contained mainly JunD and Fra-2 components, while in TPA-induced AP-1, we found J unB, c-j un, J unD, c-F os, and Fra-2, J unD and c-F os being the major components of these AP-1 complexes. Recently Tamir et al. (39) reported the activation of AP-1 by cAMP in lymphocyte and ascribed the activation of AP-1 by cAMP to the inactivation of the AP-1 inhibitory protein, IP-1, upon phosphorylation by cAMP-dependent kinase (protein kinase A) (40). However, AP-1 can be also activated following the phosphorylation of serines 63 and 73 of the N terminus domain of J un proteins. These sites are phosphorylated by J un N-terminal kinases (41, 42) and by MAP kinases (38), suggesting that MAP kinases are involved in AP-1 activation. Additionally, a recent report indicates that MAP kinases are involved in the regulation of the expression of F os family proteins, leading thereby to the stimulation of AP-1 activity (43). Thus, it is tempting to propose that p44 mapk through J unD phosphorylation or Fra-2 up-regulation is accountable for AP-1 activation by cAMP in B-16 melanoma cells.

In this study we showed that melanin synthesis, tyrosinase activity, and amount were simultaneously increased by [Nle6, D-Phe7]-l-MSH plus IBMX. These effects appear to be the consequence of the augmentation of tyrosinase mRNA. These observations confirmed previous reports (14, 15) suggesting that the control of tyrosinase mRNA expression is a key step in the cAMP-mediated stimulation of melanogenesis in B-16 melanoma cells. Usually, regulation of gene expression by cAMP is mediated by CRE through the binding of C Reb family transcription factors that are phosphorylated and activated by protein kinase A (44). However, no canonical CRE was found in the mouse tyrosinase promoter. The presence of two TRE-like sequences (2.1- and 0.18-kilobase upstream transcription start site) in the mouse tyrosinase promoter suggests that the stimulation of AP-1 by cAMP could lead to an increased tyrosinase gene expression. AP-2, another transcription factor, was also shown to mediate the effect of cAMP on gene expression (45). The presence of a putative AP-2 binding site in the mouse tyrosinase promoter and its activation by cAMP suggests that AP-2 could participate, in coordination with AP-1, in the regulation of mouse tyrosinase gene expression. Interestingly, TPA and cAMP display a common set of cellular responses, i.e. activation of p44 mapk and of AP-1, but they promote opposite effects on melanogenesis (14, 46). This could be explained by the respective nature of TPA and cAMP-induced AP-1 complexes, suggesting that J unD/F ra-2 would transactivate tyrosinase gene expression while J unD/c-F os would inhibit, directly or indirectly, tyrosinase gene transcription.

Dendritogenesis, another feature of melanocyte differentiation is stimulated during cAMP-induced melanogenesis in B-16 melanoma cells. Interestingly, cAMP-elevating agents induce in PC12 a differentiated phenotype characterized by neurite outgrowth and an activation of p44 mapk (47-49). Further, the transfection of these cells with a constitutively active MEK leads to spontaneous neuritogenesis (22), demonstrating that the MAP kinase pathway plays a pivotal role in the regulation of PC12 differentiation. Since dendritogenesis and neuritogenesis are closely related processes, we hypothesize that MAP kinase could play a critical role in the control of differentiation in neural crest-derived cells.

In summary, the data gathered in this study demonstrate that the MAP kinase pathway and AP-1 are activated during cAMP-induced melanogenesis. The role of p44 mapk and that of AP-1 in the regulation of melanogenesis remain to be proved. Nevertheless, we would like to suggest that p44 mapk, possibly through the regulation of AP-1, plays a pivotal role in the control of tyrosinase gene expression and thereby in the regulation of melanogenesis by cAMP in B-16 melanoma cells.

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Activation of p44\textsuperscript{mapk} and AP-1 by cAMP-elevating Agents

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