applying a 0.03 to 0.06 percent solution of the drug to the infected skin for 7 days. In direct comparison, a 2 percent solution of econazole or tolnaftate led to a cure rate of 0 and 50 percent, respectively [(number of animals mycologically cured/number of animals tested) × 100]. Infection of guinea pigs with *C. albicans* also responded to daily topical application of SF 86-327 for 5 days at a concentration of 1 percent.

The significance of SF 86-327 and other allylamines as a new class of compounds is underlined by their novel mode of action. This involves the inhibition of ergosterol biosynthesis at the point of squalene epoxidation (*8*). The fungicidal action is thought to result from a combination of sterol deficiency and heavy intracellular accumulation of squalene (*9*). SF 86-327 is a powerful inhibitor of squalene epoxidase preparations from *C. albicans* (Fig. 2), with apparently noncompetitive kinetics and an inhibition constant of $3 \times 10^{-8} M$. Squalene epoxidase is a key enzyme in sterol biosynthesis, being the first in the pathway to require molecular oxygen, a fact of considerable evolutionary significance (*10*). To our knowledge, the allylamines are the first specific inhibitors of this enzyme to be reported. Squalene epoxidase is also involved in mammalian cholesterol biosynthesis (*11*), but preliminary studies (*12*) indicate that the mammalian epoxidase is three to four orders of magnitude less sensitive to SF 86-327 than is the fungal enzyme. Further investigation of the molecular basis for the inhibition and its high specificity may enable even more potent inhibitors to be designed and certainly should increase our insight into the biosynthesis of sterols in general.

SF 86-327 was selected for preclinical development and clinical trials. The results of preliminary clinical investigations are encouraging (*8*). However, the full clinical spectrum of SF 86-327 is not yet known. In view of the novel mode of action of the allylamines, they might prove useful against fungal infections that are resistant to currently available antimycotics and in the treatment of other diseases caused by pathogens dependent on sterol biosynthesis.

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Action of the *e* Locus of Mice in the Response of Phaeomelanic Hair Follicles to α-Melanocyte–Stimulating Hormone in Vitro

Abstract. *Dibutyryl adenosine 3',5'-monophosphate (dibutyryl cyclic AMP) induced eumelanin synthesis in hair bulb melanocytes of recessive yellow* (e/e) *mice in vitro, whereas α-melanocyte–stimulating hormone (α-MSH) did not. In contrast, the melanocytes of lethal yellow* (A^y/a) *mice produced eumelanin in response to both dibutyryl cyclic AMP and α-MSH. These results suggest that the* e *locus controls a mechanism that determines the function of an α-MSH receptor.*

Mammalian melanocytes are capable of producing two types of pigment: black or brown melanin (eumelanin) and yellow melanin (phaeomelanin). These melanins differ in their chemical and physical properties (*1, 2*). In the house mouse, the type of melanin produced in hair bulb melanocytes is determined by the agouti (*a*) and extension (*e*) loci. Coat color of the mutants at the *a* locus ranges from extreme black (*a/a*), in which only eumelanin is deposited, to yellow (A^y/a), which is composed mainly of phaeomelanin. In contrast, the recessive yellow (*e*) gene, which is an allele at the *e* locus, is epistatic to the alleles at the *a* locus. Therefore, mice of genotype *e/e* exhibit hairs pigmented mainly with phaeomelanin regardless of their *a*-locus genotype.

Earlier studies have shown that the alleles at the *a* locus exert their effect on melanocytes indirectly by modifying the local tissue environment (*3–5*), whereas the *e* locus is considered to function within melanocytes (*6, 7*). Geschwind *et al.* found that lethal yellow (A^y/a) mice that had been producing phaeomelanin were induced to produce eumelanin when treated with α-melanocyte–stimu-

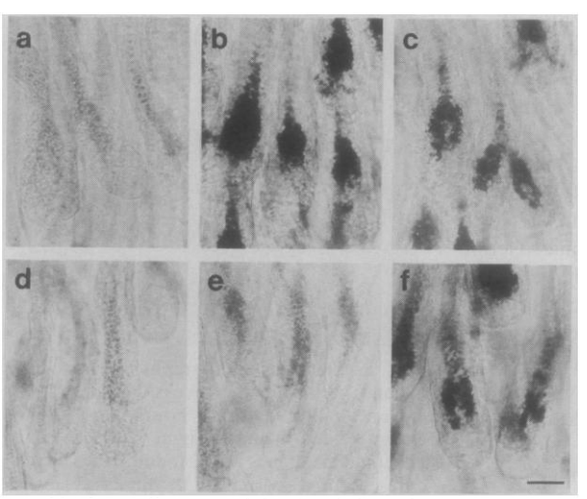

Fig. 1. Induction of eumelanin synthesis in genotypically yellow hair bulbs in vitro. Each explant was cultured in Ham's F-12 medium supplemented with 10 percent fetal bovine serum for 48 hours. Top: Hair bulbs of lethal yellow (A^y/a) explants. Only phaeomelanin was found in the explants cultured in the control medium (a). Eumelanin appeared when explants were cultured in the presence of 1 μg/ml α-MSH (b) or 1 m*M* dibutyryl cyclic AMP (c). Bottom: Hair bulbs of recessive yellow (*e/e*) explants. Only phaeomelanin was found in the explants cultured in the control medium (d) and in the explants cultured in the medium containing 1 μg/ml α-MSH (e). Treatment with 1 m*M* dibutyryl cyclic AMP gave rise to eumelanin synthesis in hair bulb melanocytes (f). Scale bar represents 100 μm.

lating hormone (α-MSH), whereas recessive yellow (*e/e*) mice were not (*8*). Although these reports have suggested that the *a* and *e* loci act on melanogenesis in different ways, the precise mechanism of the action of these genes is not well understood. We have developed an organ culture method for studying the effects of melanotropic factors on melanin synthesis in the skin of different genotypes (*9, 10*). With this procedure we have examined the effect of α-MSH and dibutyryl adenosine 3′,5′-monophosphate (dibutyryl cyclic AMP) on phaeomelanin synthesis in lethal yellow and recessive yellow mice.

Skin explants were excised from the dorsa of lethal yellow mice (C57BL/6J-A^y; genotype $A^y/a,E/E$) or recessive yellow mice (C57BL/6J-e; genotype *a/a,e/e*) at 7.5 days of age, since their hair bulbs at this stage were predominantly occupied by phaeomelanin. The explants were cultured in the presence of α-MSH or dibutyryl cyclic AMP (*9*). After 48 hours, the type of melanin in each hair bulb was identified under a light microscope. To ascertain the frequency of eumelanin synthesis, we counted the number of hair bulbs containing eumelanin as well as the total number of hair bulbs in each explant.

In the control groups not exposed to α-MSH or dibutyryl cyclic AMP, only phaeomelanin was found in A^y/a and *e/e* explants throughout the culture period (Fig. 1, a and d). When exposed to α-MSH, eumelanin was produced in A^y/a hair bulbs (Fig. 1b); this was not observed in *e/e* hair bulbs (Fig. 1e). The effect of α-MSH on the A^y/a explants was significant at doses of 0.001 to 1.0 μg/ml (Table 1). In contrast, the *e/e* explants did not produce eumelanin at any concentration examined. When exposed to dibutyryl cyclic AMP, both A^y/a and *e/e* explants were induced to form eumelanin (Fig. 1, c and f). A significant increase in the frequency of eumelanin synthesis was first observed at a concentration of 0.05 m*M* for A^y/a and 0.01 m*M* for *e/e* explants (Table 1). We reported earlier that A^y/a hair bulbs treated with α-MSH or dibutyryl cyclic AMP contained eumelanosomes (*10*). In the present study, electron microscope observation revealed that the *e/e* hair bulbs treated with dibutyryl cyclic AMP also contained typical eumelanosomes with striated longitudinal matrices (Fig. 2). These eumelanosomes were often found in genotypically black mice but not in A^y/a or *e/e* mice.

The *e* locus has been suggested to control the responsiveness of melanocytes to microenvironmental influences

Table 1. Effect of α-MSH and dibutyryl cyclic AMP concentration on the induction of eumelanin synthesis in vitro. Frequency (%) = (the number of hair bulbs with eumelanin/total number of hair bulbs in each explant) × 100. Each value represents mean ± standard error of nine experiments.

Concentrations	Frequency of hair bulbs with eumelanin (%)	
	A^y/a	*e/e*
	Control	
	5 ± 1	6 ± 1
	α-MSH	
0.001 μg/ml	65 ± 10	5 ± 1
0.01 μg/ml	93 ± 5	5 ± 1
0.1 μg/ml	90 ± 3	6 ± 1
1.0 μg/ml	96 ± 2	6 ± 1
	Dibutyryl cyclic AMP	
0.001 m*M*	5 ± 1	5 ± 1
0.01 m*M*	12 ± 8	75 ± 12
0.1 m*M*	96 ± 2	85 ± 7
1.0 m*M*	96 ± 3	88 ± 5

(*6, 11*). One of the most important pieces of evidence for this hypothesis is the discovery that *e/e* mice did not initiate eumelanin synthesis when they were injected with α-MSH (*8*). These studies, however, have left open the alternative possibility that the melanocytes of *e/e* explants have lost their ability to produce significant amounts of eumelanin under any conditions rather than their ability to respond to α-MSH.

Our study shows that the melanocytes of *e/e* hair bulbs are capable of producing eumelanin in response to dibutyryl cyclic AMP. The effect of α-MSH on mammalian melanocytes is believed to be mediated by the adenylcyclase–cyclic AMP system. Therefore, we propose that hair bulb melanocytes with genotype *e/e* exhibit defects in the function of an α-MSH receptor and that the *e* locus controls a mechanism that determines the functionality of the α-MSH receptor. This hypothesis would explain the following properties of the *e* allele: (i) it acts within melanocytes, (ii) it acts as a recessive gene, and (iii) it is epistatic to the alleles of the *a* locus.

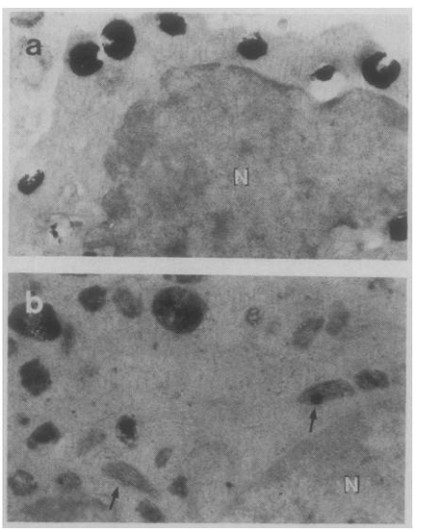

Fig. 2. Electron micrographs of hair bulb melanocytes from the recessive yellow (*e/e*) explants cultured for 48 hours. (a) Melanocytes treated with 1 μg/ml α-MSH. Only phaeomelanosomes were found. (b) Melanocytes treated with 1 m*M* dibutyryl cyclic AMP. Arrows indicate eumelanosomes; *N* indicates nucleus.

In the present study, α-MSH or dibutyryl cyclic AMP acts as an inducer of eumelanin synthesis in yellow hair bulbs in vitro. Phaeomelanin synthesis in vivo is considered to be induced by some unknown factor related to the product of the A^y or the *A* gene (*12, 13*). It seems likely that the induction of eumelanin synthesis in yellow hair bulbs occurs as a result of repression of this factor by exogenous α-MSH or dibutyryl cyclic AMP. Therefore, at present we cannot generalize that phaeomelanin synthesis is the constitutive state and eumelanin synthesis is the facultative condition. Although the nature of the interaction between α-MSH and the factor involved in phaeomelanin synthesis is still unknown, the results observed for *e/e* explants suggest that the site of the interaction is located on the cell surface, especially on the α-MSH receptor. Further investigation of the recessive yellow mutant may provide additional information on the molecular basis of α-MSH action in melanogenesis.

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