マウスの表皮メラノサイト数の遺伝的支配

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Genetic control of the population size of the melanocyte in the mouse epidermis

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ABSTRACT

Hair bulb melanocytes involved in the expression of the coat colors of mice are derived from epidermal melanocytes. The biological study of epidermal melanocytes is important to understand the expression of the coat color.

Analysis of the melanocyte and melanoblast-melanocyte population in the mouse epidermis has shown marked strain differences, suggesting that the two populations are regulated by genetic factors. From the results of genetic crosses between C57BL/10J and C3H/He mice semidominant genes were shown to be involved in regulating the two populations. The two populations in C57BL/10J-*A*/*A* congenic mouse did not differ from those in C57BL/10J. The distributions of the two populations in C3H/He were gradually transferred to those in C57BL/10J by repeated backcrossing.

The semidominant genes are thought to affect the melanocyte proliferation, melanocyte differentiation, or induction of tyrosinase activity by MSH. Such effects may change the pigmentation in the hair bulb and produce a variety of the hair color intensity of mice, which may in turn be influenced by selection, mutation, random mating, and environment.

1. INTRODUCTION

A number of genetic variations have been described in the coat colors of mice. These color mutations have been shown to affect melanocyte differentiation and the nature of the pigment formed in the melanocytes (Silvers 1979). This indicates that each step of the melanocyte differentiation and melanin synthesis is controlled by numerous genes.

Several investigators (Foster 1965; Bagnara *et al.* 1979) have been interested in the evolution of animal pigmentation. However, the identification of the major trends in the evolution of mammalian pigmentary systems have not been precisely studied. Similarly, there are few reports on the evolutionary biology of mouse melanocytes in natural populations, that are concerned with aspects of coat color polymorphisms, frequencies of the coat color mutant genes, and genetic variations of melanocyte numbers in the skin. A great deal of work is required to perform these studies in natural populations, since the number of mice that can be captured by trap is limited. One possible approach is to analyze the genetic constitutions of the present inbred mice possessing several mutant genes. Indeed, the melanocyte population in the

mouse epidermis was found to differ dramatically among the several inbred strains of mice that were tested.

The aim of this review was to survey the study on genetic control of the melanocyte population.

2. STRAIN DIFFERENCES IN THE MELANOCYTE POPULATION

Hair bulb melanocytes that are involved in the expression of the coat colors of mice are derived from epidermal melanocytes. Therefore, biological research on epidermal melanocytes is important in order to understand the expression of the coat colors of mice.

The epidermal melanocytes derive from melanoblasts originating within the neural crest. The neural crest cells begin to migrate latero-ventrally from day 8 of embryonic age, and melanoblasts originating from the neural crest have reached all the major body regions by day 12 (Rawles 1947; Mayer 1973). By day 13 or 14 melanoblasts have sufficiently colonized the epidermis of the trunk skin (Mayer 1973), and begin to produce histochemically detectable substances (Hirobe 1984a). Thus, epidermal melanoblasts can be detected by the combined dopa-premelanin reaction (Mishima 1964: Hirobe 1982b) on day 14 and increased in number up to day 17 and thereafter remained constant (Hirobe 1984a). However, the activity of tyrosinase, the key enzyme of melanin synthesis (Hearing and Ekel 1976), which can be detected by the dopa reaction was induced within the cells on day 16 and gradually increased.

The dopa reactive cells (melanocyte population) increased dramatically after birth (Quevedo, *et al.* 1966; Takeuchi 1968; Weiss and Zelickson 1975; Hirobe and Takeuchi 1977a, b, 1978). The number of epidermal melanocytes of the dorsal skin in several strains of mice increased until day 3 or 4, then gradually decreased. However, the combined number of melanoblasts and melanocytes (the combined dopa-premelanin reaction reveals melanoblasts with only unmelanized stage I and II melanosomes as well as differentiated melanocytes, Hirobe 1982b: melanoblast-melanocyte population) remained constant until day 3 or 4, after which it decreased (Hirobe 1982a). The finding that melanocytes increased but the total number of cells capable of differentiating into melanocytes was constant indicates that the melanoblasts decrease rapidly after birth. Despite the similarity of their changes in different strains, there were dramatic differences in the numbers of cells between strains (Table 1). The melanocyte population/0.1mm^2 of epidermis at day 3 was greatest in C57BR/cdJ strain (Hirobe 1984b). The value was greater than those of C57BL/10J (Hirobe 1982a), DBA/1J (Hirobe 1980), C57BL/6J (Tamate *et al.* 1986), C57BL/6J-*e*/*e* (Tamate *et al.* 1986), C3H/HeJms (Hirobe 1982a), and C57BL/6J-A^v/*a* (Tamate *et al.*, 1986). Similarly, the melanoblast-melanocyte population/0.1mm^2 at day 3 was greatest in C57BR/cdJ (Hirobe 1984b)

Table 1. *Number of melanocytes and melanoblast-melanocyte population in the dorsal epidermis of several strains of mice at day 3 after birth*[a]

Strain	Number of melanocytes	Size of melanoblast-melanocyte population
C57BR/cdJ	128.0±6.2	133.3±5.0
C57BL/10J	89.8±5.4	132.5±3.4
DBA/1J	36.0±4.3	76.3±3.7
C57BL/6J	28.8±4.6	66.7±8.8
C3H/HeJms	8.9±1.8	32.5±6.4
C57BL/6J-*e*/*e*	9.4±2.4	16.8±3.2
C57BL/6J-A^v/*a*	0.9±1.8	24.1±3.6

[a]Numbers of melanocytes (cells positive to the dopa reaction) and melanoblast-melanocyte (cells positive to the combined dopa-premelanin reaction) per 0.1 mm² of the interfollicular epidermis of the dorsal skin of mice at day 3. Each value is the mean±S. E. (standard error).

or C57BL/10J (Hirobe, 1982a). The values were greater than those of DBA/1J (Hirobe 1980), C57BL/6J (Tamate *et al.* 1986), C3H/HeJms (Hirobe 1982a), C57BL/6J-A^v/*a* (Tamate *et al.* 1986), and C57BL/6J-*e*/*e* (Tamate *et al.* 1986). These results suggest the possibility that melanocyte and melanoblast numbers are regulated by genetic factors.

3. SEMIDOMINANT GENES CONTROLLING THE MELANOCYTE POPULATION

In order to understand the genetic basis for the difference in the melanocyte and melanoblast-melanocyte populations, C57BL/10J and C3H/HeJms mice were selected for the study of genetic crosses (Hirobe 1982a).

The observed number of melanocytes and melanoblasts plus melanocytes of the F_1 from the reciprocal crosses was intermediate between the numbers in the parent strains at day 3 after birth. Histogram (Fig. 1a) of the distribution of the melanocyte population of the F_1 at day 3 located between those of the two strains. The distribution in the F_2 was multimodal (Fig. 1b). The distributions of the reciprocal backcrosses were bimodal (Fig. 1c, d). When the populations in the F_2 generation were classified into three groups shown on the histogram of Fig. 1b, namely, 0–20 (C3H/He type), 21–60 (F_1 type), and 61–110 (C57BL/10J type), the ratio of segregation was 1:2:1. Similarly, in the backcrosses between F_1 and the parents, F_1 type and parental type were present in a ratio of 1:1. Similar results were obtained in the melanoblast-melanocyte population (Fig. 2). These results suggest that semidominant genes are involved in regulating the population size of the melanocyte and melanoblast-melanocyte in the epidermis (Hirobe 1982a).

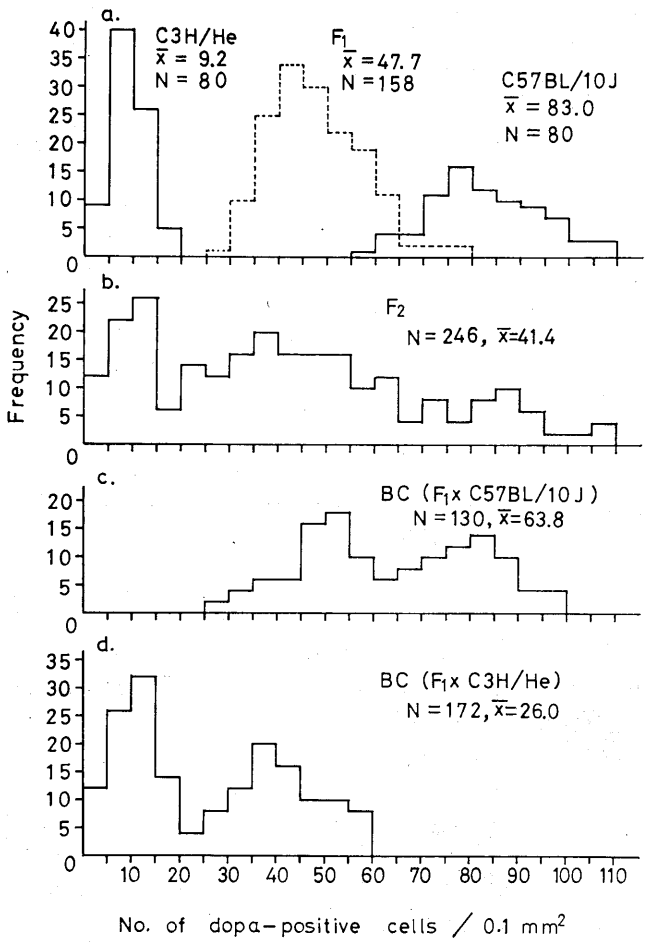

Fig. 1. Frequency histograms showing the melanocyte population (cells positive to the dopa reaction) in the dorsal epidermis of C57BL/10J, C3H/He, and F_1 (dotted line) (a) as well as F_2 (b) and backcross [$F_1 \times$C57BL/10J, (c); $F_1 \times$C3H/He, (d)] at day 3 after birth. Dorsal skins of mice at day 3 were fixed with buffered formalin and subjected to dopa reaction. Each value is the mean of the melanocyte population of three specimens per dorsum.

The only genetic difference in loci known to control melanogenesis between C57BL/10J and C3H/He is an allelic difference at the agouti locus. Agouti mice in the backcross generation between F_1 and C57BL/10J were crossed with C57BL/10J, and repeatedly backcrossed. Thus, the *A* allele at the agouti locus in C3H/He was subsequently transferred to C57BL/10J genetic background by repeated backcross matings.

The distributions of two populations were investigated for offspring from the second, third, fifth, seventh, and ninth backcross generations (Hirobe

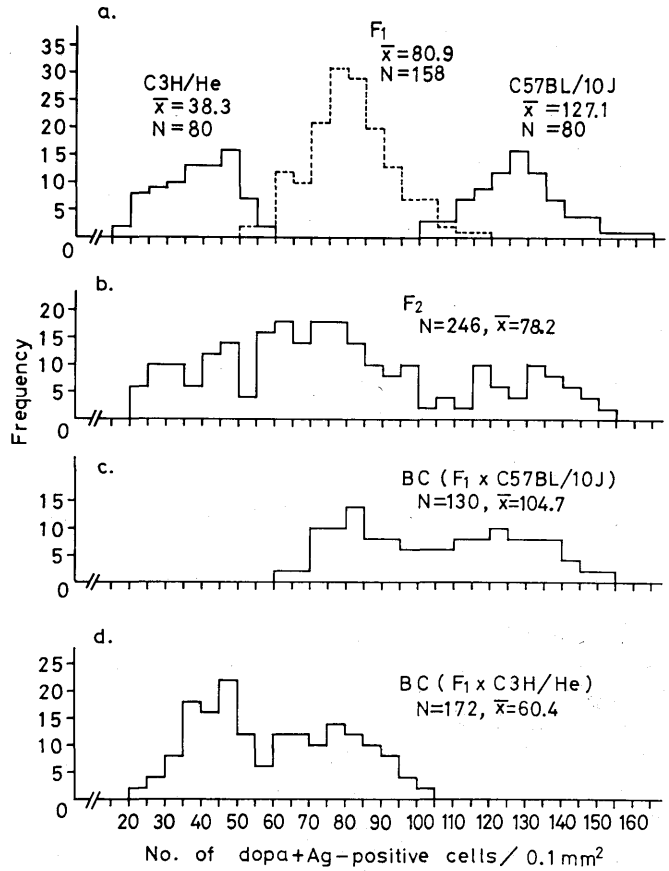

Fig. 2. Frequency histograms showing the melanoblast-melanocyte population (cells positive to the combined dopa-premelanin reaction) in the dorsal epidermis of C57BL/10J, C3H/He, and F_1 (dotted line) (a) as well as F_2 (b) and backcross [$F_1 \times$C57BL/10J, (c); $F_1 \times$C3H/He, (d)] at day 3 after birth. Dorsal skins of mice at day 3 were fixed with buffered formalin and subjected to combined dopa-premelanin reaction. Each value is the mean of the melanoblast-melanocyte population of three specimens per dorsum.

1985). The distributions gradually approached those of parental C57BL/10J. The difference in the distributions of the two populations between C57BL/10J and the second backcross was statistically significant, but not between C57BL/10J and the third, fifth, seventh, and ninth backcrosses. Thus, the distributions of the two populations in C3H/He are transferred to those of C57BL/10J by crossing four times (the third backcross). Moreover, the melanocyte (Fig. 3) and melanoblast-melanocyte (Fig. 4) populations in congenic C57BL/10J-*A*/*A* (N12F4-N12F5) did not differ from those in C57BL/10J. Thus, the possibility that the agouti locus determines the size of the two populations

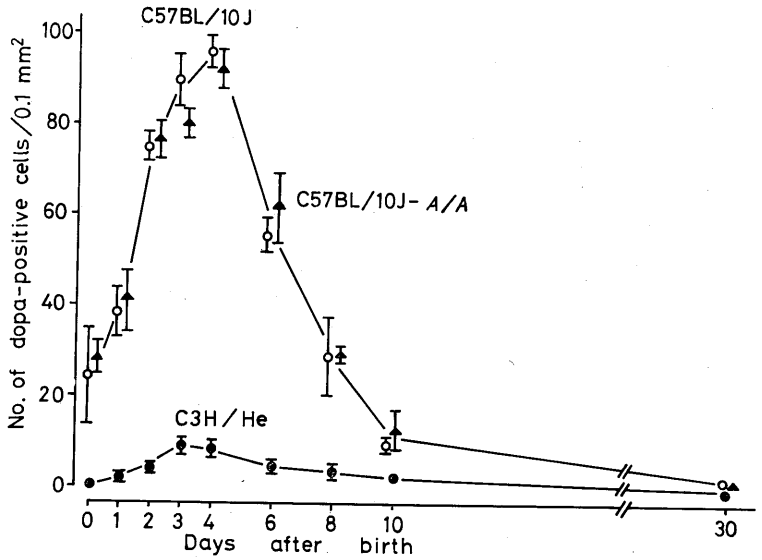

Fig. 3. Changes in the size of the melanocyte population (cells positive to the dopa reaction) of the dorsal skin of newborn C57BL/10J (○), C3H/He (●), and C57BL/10J-*A*/*A* (▲) mice per 0.1 mm² of the interfollicular epidermis. Bars indicate S. E. (standard error).

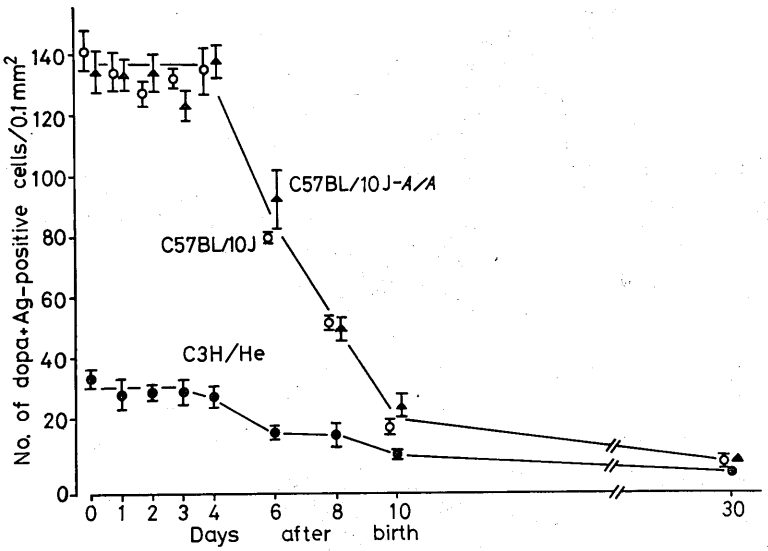

Fig. 4. Changes in the size of the melanoblast-melanocyte population (cells positive to the combined dopa-premelanin reaction) of the dorsal skin of newborn C57BL/10J (○), C3H/He (●), and C57BL/10J-*A*/*A* (▲) mice per 0.1 mm² of the interfollicular epidermis. Bars indicate S. E. (standard error).

is excluded. Therefore, it is conceivable that semidominant genes are involved in regulating the melanocyte and melanoblast-melanocyte populations in the epidermis of the mouse skin.

4. COMPARISON BETWEEN MOUSE AND HUMAN EPIDERMAL MELANOCYTE POPULATION

In man, the epidermal melanin unit (Fitzpatrick and Breathnach 1963) is a functional unit that is composed of an epidermal melanocyte and a group of keratinocytes. The numbers of epidermal melanocytes appear to be the same in almost all races of man (Szabó 1967), but the number of epidermal melanocytes per unit area of human epidermis varies significantly in different regions of the body (ca. 80–250 cells/0.1mm^2, Szabó 1967). Human skin color differences among various races exist as a result of differences in melanosome transfer, melanosome melanization, ie stage I, II, III, or IV, variation in size of melanosomes, and organization of melanosomes—singularly or clumped (Szabó *et al.* 1969).

It has been reported that mouse melanocyte proliferation can be induced or enhanced by ultraviolet irradiation (Quevedo and Smith 1963; Rosdahl and Szabó 1978) or wound formation (Hirobe 1983b). In mice of strain C57BL/10J, a 2.5-fold increase in the number of melanocytes (ca. 250 cells/0.1mm^2) was shown in the epidermis adjacent to a cut edge. The number of mouse epidermal melanocytes that are increased during the process of skin wound healing is comparable to the numbers of human epidermal melanocytes that are present in high density areas such as the foreskin or cheek (Szabó 1967). These results lead us to speculate that the property of epidermis to support a difinite density of melanocytes is unchanged in the process of mammalian evolution. However, the questions as to why the melanocyte population varies in different regions of the human body and how the human melanocytes have obtained the property during the process of evolution remain to be answered.

5. ROLE OF SEMIDOMINANT GENES CONTROLLING THE MELANOCYTE POPULATION

The melanoblast-melanocyte population in the epidermis is thought to correspond to the total number of melanoblasts that invade the epidermis from the dermis. Therefore, it is possible that the genes involved in regulating the melanoblast-melanocyte population determine the population of melanoblasts that settles in the epidermis. It might be assumed that the genes regulate the proliferation of melanoblasts or the migration of melanoblasts from the dermis into the epidermis.

The semidominant genes involved in regulating the melanocyte population

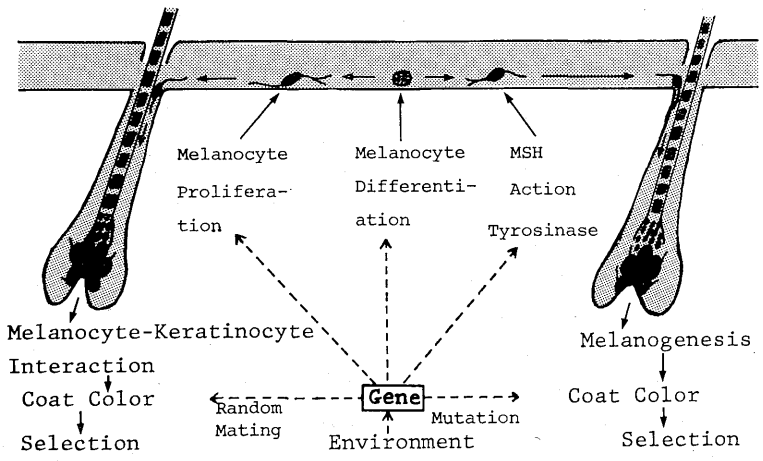

Fig. 5. Schematic drawing of the role of the semidominant genes in the pigmentary system in mice. The semidominant genes involved in regulating the melanocyte and melanoblast-melanocyte populations in the epidermis are assumed to affect the proliferation, differentiation, action of MSH, and tyrosinase activity in the epidermal melanocytes. These changes may produce a variety of the hair color intensity of mice through hair bulb melanocytes.

are thought to determine the proportion of melanocytes in the melanoblast-melanocyte population. The increase in the melanocyte population after birth seems to be related to the induction of tyrosinase activity (Coleman 1962; Pomerantz and Chuang 1970; Hirobe 1983a) in the melanoblasts that contain unmelanized stage I and II melanosomes. Therefore, it seems likely that the genes determine the extent to which tyrosinase activity can be induced in the total number of melanoblasts.

Melanocyte stimulating hormone (MSH) is known to be involved in mammalian seasonal color change (Rust 1965) and to increase tyrosinase activity or stimulate melanogenesis of mammalian melanocytes of various species including man (Lerner and McGuire 1961; Kitano 1976), mouse (Geschwind 1966; Pomerantz and Chuang 1970; Hirobe 1978a, Burchill *et al.* 1986), hamster (Weatherhead and Logan 1981), guinea pig (Snell 1964). Also, MSH induces eumelanogenesis of hair bulb melanocytes of the trunk skin in lethal yellow (Tamate and Takeuchi 1981) and viable yellow (Thody *et al.* 1984; Burchill *et al.* 1986) mice, in which normally pheomelanogenesis occurred. In addition to these effects, MSH has a inducible effect on the melanocyte differentiation (Hirobe and Takeuchi 1977a, 1978; Ito and Takeuchi 1981). The initiation of melanin synthesis in melanoblasts in neonatal epidermis was induced by injecting MSH in both C57BL/10J (Hirobe and Takeuchi 1977a) and C3H/He (Hirobe 1978b) mice. Almost all the melanoblasts of C57BL/10J and C3H/He mice were competent for the induction of melanin synthesis by MSH, though the proportion of undifferentiated melanoblasts in C3H/He was

much greater than that in C57BL/10J under normal circumstances. It might be assumed that the semidominant genes involved in regulating the melanocyte population control the action of MSH in the melanocytes.

The semidominant genes are thought to affect the melanocyte proliferation, melanocyte differentiation, or the induction of tyrosinase activity by MSH. The increase in the number of epidermal melanocytes may bring about the increase in the number of hair bulb melanocytes, the pigmentation of hairs, and the altered melanocyte-keratinocyte interaction. These changes in the pigmentary system in mice may produce a difference in the hair color intensity, which may in turn be influenced by selection, mutation, random mating, and environment (Fig. 5).

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