DEPILATED (*dep*), A MUTANT GENE THAT AFFECTS THE COAT OF THE MOUSE AND ACTS IN THE EPIDERMIS

THOMAS C. MAYER

Department of Biology, Rider College, Lawrenceville, New Jersey 08648

NORMAN J. KLEIMAN and MARGARET C. GREEN

The Jackson Laboratory, Bar Harbor, Maine 04609

Manuscript received March 11, 1976

ABSTRACT

Depilated is a recessive mutation on Chromosome *4* in the position $b-1.93\pm 0.51-dep-3.45\pm 0.68.-$Pt. It causes severe abnormalities of hair structure. The site of action of *dep* was investigated by the method of dermal-epidermal recombination. Skins from 14-day mutant and normal mouse embryos were separated into dermal and epidermal components, recombined, and grown in histocompatible mouse testes for 20 days. The recombinations made were +/+ epidermis with +/+ dermis, +/+ epidermis with *dep/dep* dermis, *dep/dep* epidermis with +/+ dermis and *dep/dep* epidermis with *dep/dep* dermis. Grafts that contained mutant epidermis as one of the components produced hairs that were similar to those found in depilated mice. There was no observable effect of the dermis on hair types produced in this experiment.

MORE than 30 genetic loci in the mouse are known to affect the texture of the coat by altering hair structure (GREEN 1966). A number of these loci have been studied experimentally with the technique of dermal-epidermal recombination. The two components of the skin can be separated by treating pieces of 14-day embryonic skin with trypsin. The dermis and epidermis from embryos of various genotypes are then recombined and grown in a site favorable for hair growth. The type of hair produced by these recombined skins provides evidence as to whether a given locus is active within the dermis, epidermis, both components, or at some other site. Although a hair is produced by epidermal cells, the possibility that the dermis plays a role in initiating hair growth or determining and maintaining regional epidermal characteristics has been suggested (KOLLAR 1972). It, therefore, is of interest to identify sites of action of genes causing abnormal hair structure in an attempt to understand how genes affect hair morphogenesis. An understanding of this system could be expected to throw light on how genes affect developmental processes in general.

Five loci that affect hair structure in the mouse have been analyzed by the method of dermal-epidermal recombination. Dermis and epidermis from embryonic body skin were recombined between normal mice and mice mutant at the fuzzy (*fz*, MAYER, MITTELBERGER and GREEN 1974) and ichthyosis (ic, GREEN, ALPERT and MAYER 1974) loci. Both were found to act within the

epidermis. The three genes crinkled (*cr*), downless (*dl*) and tabby (*Ta*) are similar in their effects on the mouse. They produce structural abnormalities of hair and suppress hair development on the tail and behind the ears. Recombinations of tail-skin dermis and epidermis showed the epidermis to be the site of action of *dl* (SOFAER 1973) and *cr* (MILLER unpublished results). Similar studies on sex-linked *Ta* were inconclusive (SOFAER 1974).

The present study is an analysis of mice carrying the recessive gene depilated (*dep*). This mutation occurred in 1966 at the Jackson Laboratory in a stock carrying brachury (*T*) and tufted (*tf*). This report presents the effects of *dep* on hair and skin and the results of dermal-epidermal recombinations between embryonic skin of depilated and normal mice. The epidermis is shown to be the primary site of action of this gene. A summary of the data showing linkage of *dep* with loci on Chromosome *4* is included.

MATERIALS AND METHODS

The depilated stock is maintained by repeatedly crossing mice carrying *dep* to (C3HeB/FeJ-*a*/*a* X C57BL/6J)F_1 (hereafter C3B6F_1) mice by a cross-intercross system. Mutant mice and embryos were obtained by matings between C3B6F_1-*dep*/*dep* mice. Normal mice and embryos were from the C57BL/6J strain. Histocompatible C3B6F_1 males were used as hosts for the growth of recombined skins.

In order to study the course of development of depilated hair, skin from mice aged 5, 8, 11, 13, and 15 days was removed from the middorsal region, fixed in 10% formalin, dehydrated, and cleared in methyl salicylate. These preparations were hand sectioned with a razor blade and examined as whole mounts. Various other tissues from mice aged 5 and 8 days were examined in cleared whole-mount preparations for the presence and condition of melanocytes in locations other than hair follicles. This examination was considered necessary since *dep* was found to cause pigment clumps in the hair. The condition of melanocytes in areas other than hair follicles could provide evidence on whether melanocytes themselves were directly affected by the mutation.

To obtain embryos, female mice in early estrus were placed with males in the evening, and inspected for vaginal plugs the following morning. The day vaginal plugs were found was considered day 0 of pregnancy. Embryos 14 days of age were removed from pregnant females, and 1 mm square pieces of flank skin midway between the limbs were removed. The tissues were placed in Tyrode's solution containing 1% trypsin and treated at 4° for 4 hours. They were removed to a 1:1 solution of Tyrode's and fresh egg white in order to stop the action of the trypsin. Separation of the epidermis from the dermis was easily accomplished with fine watchmaker's forceps.

Recombinations were made on an agar-base culture medium consisting of Basal Medium Eagle, 20% horse serum, and 1.5% agar. The dermis was oriented with the epidermal side up, and the sheet of epidermis was gently stretched over the dermis. Excess fluid was removed from the area surrounding the skin so that the two components were firmly pressed together by the surface tension. They were then incubated overnight at 37° in a 5% CO_2 atmosphere. The next morning the tissues were removed from the agar and placed in Tyrode's solution where they were examined. Those skins with well healed epidermis on the dermis were grafted into testes of histocompatible C3B6F_1 mice. The grafts were grown initially for 14 days. Because it became apparent that this culture period was not sufficiently long for the appearance of the depilated phenotype, all subsequent grafts were grown for 20 days. At the end of the culture period, the testes were fixed in 10% formalin and examined. Successful grafts were removed from the testes, dehydrated in alcohol, and cleared in methyl salicylate. They were hand sectioned with a razor blade and examined under 100x magnification to determine hair structure.

TABLE 1

Results of cross showing linkage of dep *to* b *and* Pt *on chromosome* 4

Parents		Offspring						
Female	Male	*b dep* +	+ + *Pt*	*b* + *Pt*	+ *dep* +	*b dep Pt*	+ + +	Total
b dep + / + + *Pt*	*b dep* + / *b dep* +	227	192	7	3	2	10	441
b dep + / *b dep* +	+ + *Pt*	136	130	1	3	7	6	283

Recombinations:

b − *dep*:	Female, 2.27 ± 0.71%	Male, 1.41 ± 0.70%	both, 1.93 ± 0.51%
dep − *Pt*:	Female, 2.72 ± 0.77%	Male, 4.59 ± 1.24%	both, 3.45 ± 0.68%
b − *Pt*:	Female, 4.99 ± 1.04%	Male, 6.01 ± 1.41%	both, 5.38 ± 0.84%

RESULTS

Linkage. The results of a 3–point cross showing the linkage of *dep* with brown (*b*) and pintail (*Pt*) on Chromosome *4* are given in Table 1. The order of the loci is *b*, *dep*, *Pt*. There was no significant difference between the sexes in frequency of genetic recombination. For the sexes combined, recombination between *b* and *dep* was 1.93±0.51% and between *dep* and *Pt* was 3.45±0.68%.

Effects of dep *on hair and skin.* Homozygous depilated mice have hair that appears abnormally thin and short, although the extent of the defect is quite variable. Some mice are nearly hairless at 3 weeks, and others possess a rather substantial coat. The hair has a matted greasy appearance. When skin from mice aged 5 to 15 days was examined in cleared unstained whole-mount preparations, the most obvious abnormality was the complete disorientation of many hair follicles (Figure 1). Clumps of pigment were found that appeared to be the remains of severely affected hair follicles in an advanced state of degeneration. Other hair follicles were more recognizable but obviously misshapen. These abnormalities in depilated skin were not apparent at 5 days, but by 8 days a number of misplaced and fragmented hair follicles were found. By 11 days degenerating follicles were numerous and debris from fragmented follicles was found. The skin at 15 days was cluttered with remains of follicles and hairs oriented in abnormal directions.

A less obvious abnormality of depilated hair, which was first noticed on close examination of individual hair shafts in the grafts and is described in more detail below, was an irregular arrangement of septa in the shaft or a complete interruption of the septal pattern (Figure 2). Approximately 20% of the hairs contained such abnormalities.

A brief survey was made of melanocytes in locations other than the skin of depilated mice in an attempt to determine if the primary effect of the gene might be on the melanocyte rather than the hair follicle. Mice 5 and 8 days old were examined for melanocytes in the harderian gland of the eye, valves of the heart, membranous labyrinth of the ear, and leg muscles. Usual numbers of melano-

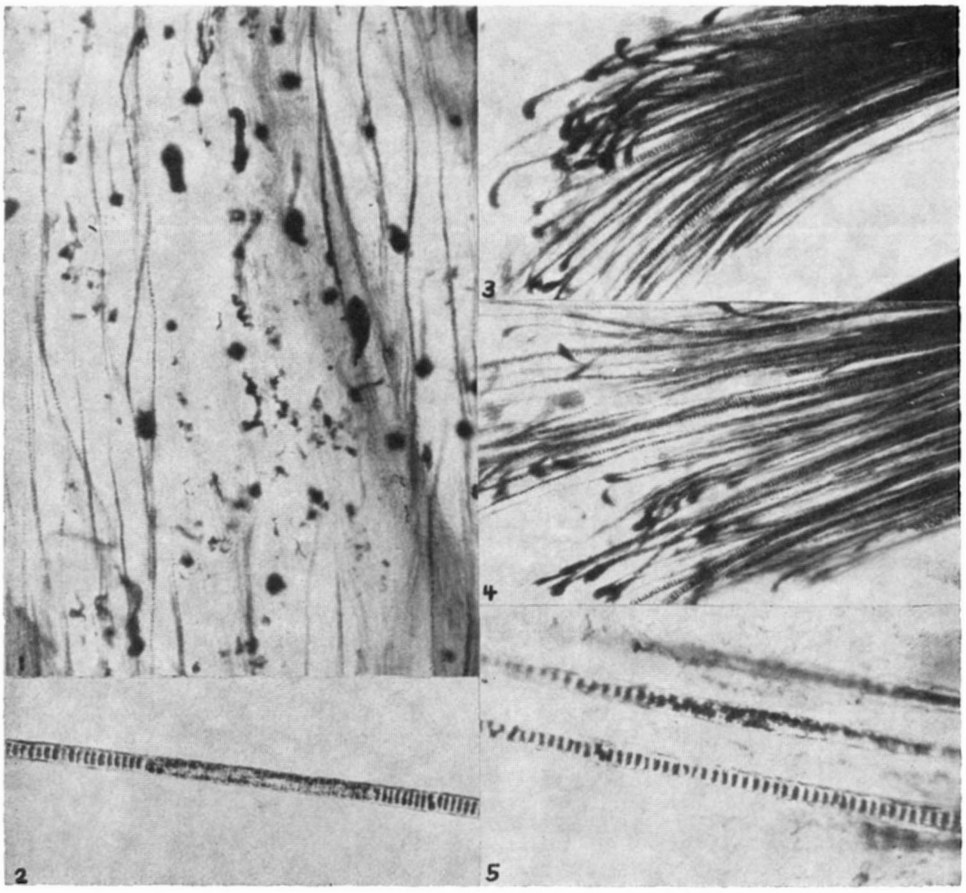

FIGURE 1.—Cleared, unstained whole-mount of sin of *dep/dep* mouse 15 days old. (X 45).
FIGURE 2.—Hair of *dep/dep* mouse showing interruption of septal pattern (X 140).
FIGURE 3.—Graft of +/+ epidermis and *dep/dep* dermis (X 80).
FIGURE 4.—Graft of *dep/dep* epidermis and +/+ dermis (X 80).
FIGURE 5.—Hairs from graft of *dep/dep* epidermis and +/+ dermis showing irregular septa and interruption of septal pattern (X 120).

cytes were found in these locations in depilated mice. The melanocytes possessed dendritic structure, and melanin granules were present with normal distribution. No clumping of melanosomes was found.

Recombination grafts. The types of recombinations made in this study were +/+ epidermis with +/+ dermis, +/+ epidermis with *dep/dep* dermis, *dep/dep* epidermis with +/+ dermis, and *dep/dep* epidermis with *dep/dep* dermis. Forty-five grafts of 20 days culture were recovered for examination from a total of 78 implantations. These older grafts were elongated in shape because the hairs were considerably longer than in the young grafts. The long hairs were arranged parallel to each other rather than oriented in many directions as were the short hairs in younger grafts. Because the older grafts contained large num-

bers of densely packed hairs, they were free-hand sectioned to make individual hairs visible.

The overall appearance of the various recombinations did not differ from each other. The orientation of the hairs was similar for all recombination types, and there were no grossly observable hair follicle malformations (Figures 3 & 4). However, on close examination of individual hair shafts, two groups of grafts were clearly distinguishable. One group possessed hair shafts that exhibited the regular arrangement of septa and septules characteristic of normal hair. Although no attempt was made to classify hair types in the grafts, some hairs were obviously of larger caliber than others, and contained two or more septules across. There were no irregularities in the positioning of these septules. This group of grafts containing hairs of normal structure consisted of 11 of the recombination type +/+ epidermis with +/+ dermis, and 14 of the type +/+ epidermis with *dep/dep* dermis (Table 2).

The second group of grafts contained hair shafts that possessed irregularities of arrangement of the septa along their length (Figure 5). These abnormalities consisted of septa that were set at an angle to each other, or a complete interruption of the septal pattern. In a given graft these structural abnormalities were found in about 20 to 30% of the hairs. All of these grafts were clearly distinguishable from those possessing normal hair structure. The grafts that contained abnormal hairs consisted of 11 of the recombination type *dep/dep* epidermis with +/+ dermis, and 9 of the type *dep/dep* epidermis with *dep/dep* dermis. With the exception of the abnormalities of hair structure, no differences were observed between the two groups.

DISCUSSION

The site of action of the depilated locus follows a pattern set by studies on

TABLE 2

Results of the dermal-epidermal recombination study

Recombination type	Number of grafts recovered	Number of grafts with abnormal hair
+/+ epidermis +/+ dermis	11	0
+/+ epidermis *dep/dep* dermis	14	0
dep/dep epidermis +/+ dermis	11	11
dep/dep epidermis *dep/dep* dermis	9	9

other mutations affecting hair structure in the mouse. The depilated locus was found to be active in the epidermis. No influence of mutant dermis on hair structure was demonstrable under the conditions of this experiment. This study, like most of the previous investigations, utilized the testis of histocompatible adult mice as a place for growing recombined skin grafts. This location appears to be a favorable one for the growth of mouse skin and hair. Hair grows in the testis at about the same rate as in the intact animal, and each graft typically produces large numbers of hairs with relatively normal structure in grafts of normal controls.

In previous studies of the fuzzy and ichthyosis loci (MAYER, MITTLEBERGER and GREEN 1974; GREEN, ALPERT and MAYER 1974), the types of hair produced by grafts containing mutant epidermis were characteristic of hair types found in the mutant mice themselves. The crinkled locus was also studied by implanting grafts into the mouse testis (MILLER unpublished results). Mice mutant at this locus not only possess hair abnormalities, but lack hair follicles on the tail and behind the ears. For this mutant, recombinations were made of tail skin in order to avoid the necessity of recognizing hair abnormalities in the grafts. Those grafts containing crinkled epidermis as one component of the tail skin failed to produce hair follicles, whereas those containing normal epidermis produced hair. Recombinations using components of body skin were not made. Two other mutations that produce effects identical to crinkled have been studied in similar experiments, but with the grafts grown on the chorioallantoic membrane of the chick embryo. In the case of downless (SOFAER 1973) the epidermis was again found to be the site of action of the locus. However, an analysis of the sex-linked gene tabby led to equivocal results (SOFAER 1974). Some recombinations possessing tabby tail epidermis formed hair on the chorioallantoic membrane, as did some grafts of whole skin taken from prospectively hairless tabby tails. A possible interpretation of these results is that under the conditions of the experiment tabby tail skin behaves like body skin, and forms hair follicles. It would be of interest to know whether the hairs produced by tabby tail skin have the characteristic structural abnormalities of hairs on tabby body skin.

The depilated-type hairs produced in recombination grafts are not identical to those produced by depilated mice. The most obvious feature of depilated skin is the abundance at certain ages of fragmented, mishapen, and disoriented hair follicles and hair shafts. This picture is never seen in the depilated hairs in recombination grafts. The similarity between the hairs of grafts and intact mice is the interruption of the septal pattern along the hair shaft. These structural abnormalities are identical in the two situations, and were not found in hairs of normal mice or in grafts possessing normal epidermis. It appears that depilated hairs in grafts escape the more serious effects of the mutation. The reason for the partial correction of depilated hair abnormalities in grafts may be the different physical environment of the testis compared to skin. The most severely affected depilated hairs are fragmented or disoriented before they reach the surface of the skin in the mouse. Hairs in grafts do not seem to grow through the epidermis, but may elongate by pushing the hair bulb itself into the interior of the

testis. They are consequently subject to stresses quite different from hair follicles in the skin of a mouse.

The possibility that depilated acts within the melanocyte in producing the hair abnormality cannot be discarded as an explanation of the depilated phenotype. A failure of melanocytes to donate melanin to hair cells in a harmonious way could result in pigment clumps in the hair, and even cause abnormalities of structure. The normal appearance of melanocytes in locations other than the hair follicle in depilated mice suggests that the melanocyte is not the primary site of gene action.

The method of dermal-epidermal recombination has now been used successfully to analyze five genetic loci that are involved in hair development, and all have proven to be epidermal in action. A role of the dermis in hair development has not been demonstrated. It is likely that certain inductive interactions are necessary between the dermis and epidermis in the initiation of hair growth. However, mutations affecting these interactions may more likely lead to complete suppression of hair growth, and not to abnormalities of hair structure. Experiments of the type presented here are important as a first step in the analysis of an interesting morphogenetic system under complex genetic control.

We are grateful to HOPE O. SWEET for producing the mutant mice for this study.

This work was supported in part by NSF grant BMS 75–03397 and ACS grant VC–17P to the Jackson Laboratory, and NSF grant BMS 74–02812 to Rider College. The Jackson Laboratory is fully accredited by the American Association for Accreditation of Laboratory Animal Care.

N.J.K. was a participant in the Jackson Laboratory Training Program supported by training grant EPP–75–06118 from the Student Science Training (SST) Program of the National Science Foundation.

LITERATURE CITED

GREEN, M. C., 1966 Mutant genes and linkages. pp. 87–150. In: *Biology of the Laboratory Mouse*. Edited by E. L. GREEN. McGraw Hill, New York.

GREEN, M. C., B. N. ALPERT and T. C. MAYER, 1974 The site of action of the ichthyosis locus (*ic*) in the mouse, as determined by dermal-epidermal recombinations. J. Embryol. exp. Morph. **32**: 715–721.

KOLLAR, E. J., 1972 The development of the integument: spatial, temporal and phylogenetic factors. Am. Zool. **12**: 125–135.

MAYER, T. C., J. A. MITTELBERGER and M. C. GREEN, 1974 The site of action of the fuzzy locus (fz) in the mouse, as determined by dermal-epidermal recombinations. J. Embryol. exp. Morph. **32**: 707–713.

SOFAER, J. A., 1973 Hair follicle initiation in reciprocal recombinations of downless homozygote and heterozygote mouse tail epidermis and dermis. Develop. Biol. **34**: 289–296. ——, 1974 Differences between tabby and downless mouse epidermis and dermis in culture. Genet. Res. **23**: 219–225.

Corresponding editor: D. BENNETT