The site of action of the fuzzy locus ($fz$) in the mouse, as determined by dermal–epidermal recombinations

By THOMAS C. MAYER¹, JAMES A. MITTELBERGER²
AND MARGARET C. GREEN²

From the Department of Biology, Rider College, and
The Jackson Laboratory, Bar Harbor

SUMMARY

The site of action of the mutation fuzzy ($fz$) 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 the mouse testis for 14 days. Grafts that contained mutant epidermis as one of the components produced many abnormal hairs characteristic of fuzzy mice. There was no observable effect of the $fz$ locus on the dermis under the conditions of this experiment.

INTRODUCTION

Mice homozygous for the mutation fuzzy ($fz$) possess hair that is generally thinner than normal, and wavy (Dickie & Woolley, 1950). In addition, the few thicker hairs present in the coat of fuzzy mice have multiple constrictions that are characteristic of zig-zag hairs of normal mice. It was originally suggested that the gene fuzzy inhibited the formation of all types of hair other than the zig-zags. A study of the developmental sequence of various hair types in the mouse revealed that four waves of hair follicle development are present in normal mice, and that these waves correspond to the sequential development of the four different types of hair (Mann, 1962). This developmental sequence is present in fuzzy mice, and Mann (1964) concluded that all types of hair are present in the mutant. The primary effect of the $fz$ mutation apparently is the alteration of hair structure, and not the suppression of certain types of follicle development.

Although developmentally a hair is the product of the epidermal ectoderm, it is likely that the dermal mesoderm plays a role in the initiation of follicle formation. Additionally, the dermis no doubt provides the condition for the orderly development and position of the hair follicle, either through the action

¹ Author's address: Department of Biology, Rider College, Trenton, N.J. 08602, U.S.A.
² Authors' address: The Jackson Laboratory, Bar Harbor, Maine 04609, U.S.A.
of the hair papilla or the surrounding dermal cells. It appeared of interest, therefore, to investigate whether the \( fz \) mutation produced its effect by acting specifically on either the dermal or epidermal tissue. The technique of separating skin from mouse embryos into dermal and epidermal components through the use of trypsin and making skin recombinations from different genotypes has been used successfully in a number of recent studies dealing with pigment (Mayer & Fishbane, 1972; Mayer, 1973) and hair (Sofaer, 1973) mutants of the mouse. The application of this technique to an analysis of the \( fz \) locus revealed that the site of action of the locus is in the epidermal ectoderm. The dermal mesoderm supported hair development normally under the conditions of this experiment regardless of whether it was derived from normal or mutant embryos.

**MATERIALS AND METHODS**

Skin from 14-day-old embryos was used in all the recombinations. Mutant embryos were produced by mating C57BL/6J-\( fz/H\) parents and normal embryos were produced by mating C57BL/6J parents. The morning on which vaginal plugs were found was considered day 0 of pregnancy.

Pieces of skin approximately 1.5 mm\(^2\) were removed from the mid-flank between the limbs of embryos in Tyrode's solution at room temperature. The skin was then put in Tyrode's solution containing 1% trypsin (Difco 1:250) and refrigerated at 4 °C for about 4 h. Following this treatment the dermal and epidermal components were separated with watchmaker's forceps, and the components were transferred to a solution of Tyrode's solution and fresh egg white (1:1) to inhibit further action of the trypsin. The tissues were kept cold from the time they were first refrigerated until the recombination was actually completed as they became soft and difficult to handle if they became warm.

The skin components were recombined on an agar-base culture medium containing Eagle's basal medium and 10% horse serum. The dermis was placed basal side down on the agar plate, and the epidermal component was positioned over the dermis. Excess fluid was removed so that the two tissues would be firmly adhered to each other by the surface tension of the fluid. They were then incubated overnight at 37 °C in an atmosphere of 10% CO\(_2\) in air. The following day the recombined skins were implanted into the testes of adult mice. These hosts were either C57BL/6J males, or \( F_1 \) hybrid males produced from C57BL/6J by CBA/Ca matings (B6CBAF\(_1\)). The grafted skins were allowed to develop in the hosts for 14 days, after which the testes were removed, fixed in 10% formalin, dehydrated, and cleared in oil of wintergreen. The skin grafts were then removed from the testes and examined for hair type and structure.
Table 1. Distribution of grafts scored on a scale of 1–4

Scores refer to percentage of abnormal hairs in a graft as follows:

1 = 0–25%; 2 = 26–50%; 3 = 51–75%; 4 = 76–100%.

<table>
<thead>
<tr>
<th>Combinations*</th>
<th>No. of grafts†</th>
<th>Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>+/+</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>+/+</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>fzl/fzl</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>fzl/fzl</td>
<td>23</td>
</tr>
</tbody>
</table>

* Epidermis
  - Dermis

† Four grafts contained normal and abnormal hairs in sharply contrasting sections. They were regarded as mosaics and are not included.

RESULTS

The four types of skin recombinations made in this study were: normal epidermis with normal dermis, normal epidermis with mutant dermis, mutant epidermis with normal dermis, and mutant epidermis with mutant dermis. A total of 119 implantations were made to the testis and 85 were recovered for study. All the grafts possessed numerous hairs arranged either parallel or in a ball, and all the hairs possessed eumelanin along their length characteristic of the non-agouti genotype of the donor mice. The implants grown in C57BL/6J or B6CBAF₁ hosts were indistinguishable from each other regarding general health of the graft and number of hairs present.

Hairs were regarded as normal if they were straight or evenly curved, not wavy, and possessed an even taper to the tip of the shaft. Abnormal hairs were thin and wavy, or large with uneven shafts and wavy toward the tip. Although the testis of the mouse generally supported normal development of skin and hair, on occasions abnormal hairs were found in normal grafts. These hairs very often were on the surface of the graft suggesting that they had been distorted by the restrictive nature of the testis environment. Hair shafts may also be distorted in the preparation of the graft for study, either in the process of handling the graft or removing it from the testis. Finally, the separation and recombination procedure itself could produce some abnormal hairs in normal skin grafts.

In order to quantify the results and eliminate as much as possible subjective judgements, each graft was rated by the percentage of abnormal hairs it con-
Figs. 1–4. Typical grafts of four combinations of +/+ and fz/fz epidermis and dermis. (1) +/+ epidermis and +/+ dermis, (2) +/+ epidermis and fz/fz dermis, (3) fz/fz epidermis and +/+ dermis, (4) fz/fz epidermis and fz/fz dermis. ×80.

The scores for the four types of recombinations are shown in Table 1. Most of the grafts were easily classified in two general categories regarding hair structure. In one category were the recombinations that contained normal epidermis and either normal dermis or fuzzy dermis (Table 1, combinations 1

...
Fuzzy locus (fz) in the mouse 711

and 2). Grafts produced by these two recombinations were indistinguishable from each other, and possessed hairs predominantly of the normal type (Figs. 1, 2). The second category of grafts were those produced by recombining fuzzy epidermis with either normal dermis or fuzzy dermis (Table 1, combinations 3 and 4). The grafts were similar to the previous category with respect to the number of hairs present in the graft and the appearance of eumelanin in the hair shaft. However, the majority of the hairs were thick and wavy (Fig. 3, 4). A smaller number of the hairs were of normal size but were also wavy, and possessed constrictions along the shaft. Only a small percentage of the hairs were of normal appearance, and some of these were curved at the tips.

χ² analysis showed that there were no significant differences between combinations 1 and 2 (χ² = 0.45, degrees of freedom = 1, P > 0.05) and between combinations 3 and 4 (χ² = 0.26, degrees of freedom = 1, P > 0.05). However, when 1 and 2 were combined and compared with 3 and 4 combined, the difference was highly significant (χ² = 55.55, degrees of freedom = 1, P < 0.01). The genotype of the epidermis therefore was the determining factor in the type of hair produced. No influence of the genotype of the dermis could be shown.

DISCUSSION

The present study was an attempt to identify the site of action of the fz locus of the mouse through the technique of dermal–epidermal recombination. Previous studies have focused on the importance of the dermis in the initiation, maintenance, and specificity of epidermal appendages. Examples of the dependence of epidermal development on the dermis are the differentiation of scales and feathers in the chick (Rawles, 1963), and hair, vibrissae and plantar surface epithelium in the mouse (Kollar, 1972). In all these cases the occurrence and type of epidermal appendage are determined by and dependent on influences from the dermal component. The results of the present study indicate that the genotype of the dermis at the fz locus plays no role in determining the type of hair formed. It appears that an inductive influence of the dermis in hair formation is not affected by the mutation. Rather, the mutation affects the ability of the epidermis to produce normal hair in response to dermal influences. This finding is consistent with the results of previous investigators (Silvers & Lane, 1958; Mann, 1964) who found that all hair types are present in fuzzy mice, but abnormal in structure. In a case of complete hair follicle suppression in the tail caused by the mutation downless (dl), Sofaei (1973) found that the epidermis of the skin was the affected component. Ichthyosis (ic), a mutation that produces hair abnormalities, has likewise been found to act in the epidermis with no observable influence on the dermis (Green, Alpert & Mayer, 1974).

It has been reported by Mintz (1970, 1971) that allophenic mice produced by fusing normal and fuzzy embryos exhibited a pattern of normal and mutant hair types arranged in 75–100 lateral bands on each side. She suggested that
these bands corresponded to the number of somites in the embryo on each side, and represented the lateral migration of dermatome cells that were derived from clones of normal or fuzzy cells. From the appearance of the allophenic mice she concluded that the dermis was the site of action of the fuzzy locus. However, in the light of the present results it is likely that clones of mutant and normal cells within the epidermis, not the dermis, account for the patterns observed by Mintz. Dermal–epidermal recombination experiments offer a much more direct means of analyzing the site of gene action of hair and skin mutants than interpretations of appearance of allophenic mice, at least until more is known about patterns of clonal expansion in these tissues. The only way in which our results could be compatible with activity of the \( fz \) locus in the dermis and not in the epidermis is under the condition that gene activity occurs transiently in the dermis at some time earlier than 14 days, and results in transmission of a message directing the epidermis to make normal or fuzzy hair. After 14 days the message must no longer be transmitted, at least under the conditions of our experiment, as the genotype of the dermis has no effect on hair type after this stage. This hypothesis cannot be completely excluded, but since the epidermis behaves in accordance with its own genotype even after recombination with dermis of a different genotype at 14 days, the most likely conclusion is that the gene at the \( fz \) locus is acting in the epidermis.

We are grateful to Mrs Hope O. Sweet for producing the mutant mice for this experiment. This work was supported in part by NSF grant GB 27487 and ACS grant VC-17-L, a Florence M. Kerrison Memorial Grant for Cancer Research in Maine to the Jackson Laboratory, and NSF grant GB 18271 to Rider College. The Jackson Laboratory is fully accredited by the American Association for Accreditation of Laboratory Animal Care. J.A.M. is a participant in the Jackson Laboratory Training Program supported by training grant GW 8212 from the Student Science Training Program of the National Science Foundation.

REFERENCES

Fuzzy locus (fz) in the mouse


(Received 12 March 1974)