Genet. Res., Camb. (1977), **29**, *pp.* 279–284
With 2 plates
Printed in Great Britain

Evidence from chimaeras for the pattern of proliferation of epidermis in the mouse

By MARGARET C. GREEN, DIANNE DURHAM, THOMAS C. MAYER and PETER C. HOPPE

The Jackson Laboratory, Bar Harbor, Maine 04609, U.S.A., and the Department of Biology, Rider College, Lawrenceville, N.J. 08648, U.S.A.

(*Received* 8 *March* 1977)

SUMMARY

The recessive mutant gene downless (*dl*) causes abnormal texture of the coat and absence of hair on the tail. The *dl* locus had previously been shown to act in the epidermis and not in the dermis. To obtain evidence on the pattern of proliferation of epidermis, downless ↔ normal chimaeras were produced by embryo aggregation, and the pattern of normal and mutant hair in the coat was examined. The chimaeras showed a pattern of narrow transverse stripes of normal and abnormal hair. This pattern was similar to that found in mice chimaeric for alleles at the agouti locus known to act in the dermis. This evidence supports the conclusion that the pattern of cell proliferation is similar in dermis and epidermis, and is compatible with the hypothesis that both tissues proliferate by lateral coherent clonal growth from a randomly mixed array of longitudinally arranged cells.

1. INTRODUCTION

The pattern of cell proliferation in tissues of the developing organism is an incompletely known aspect of descriptive embryology. Evidence bearing on this question for a given tissue is provided by chimaeras derived by aggregating normal embryos with embryos that are mutant for a gene known to be effective in that tissue. Given that the phenotype of the tissue reflects the genotype of the component cells, the size and shape of mosaic patches of the tissue in such chimaeras should reflect the history of cell mixing and coherent clonal growth in development of that tissue.

Cell proliferation patterns in the skin can be easily studied using genes acting in the skin and causing abnormal coat texture or colour. Previous experiments have shown that mice chimaeric for genes known to act in the dermis have a pattern of narrow transverse stripes. For example, in chimaeras composed of agouti (A/A) and nonagouti (a/a) cells, the typical pattern consists of narrow transverse stripes of agouti (yellow-banded) and non-agouti (black) hairs. A clear illustration of one such chimaera is given by Mintz & Silvers (1970). A similar pattern, described as resembling tiger stripes, was seen by Bhat (1949) in a mouse

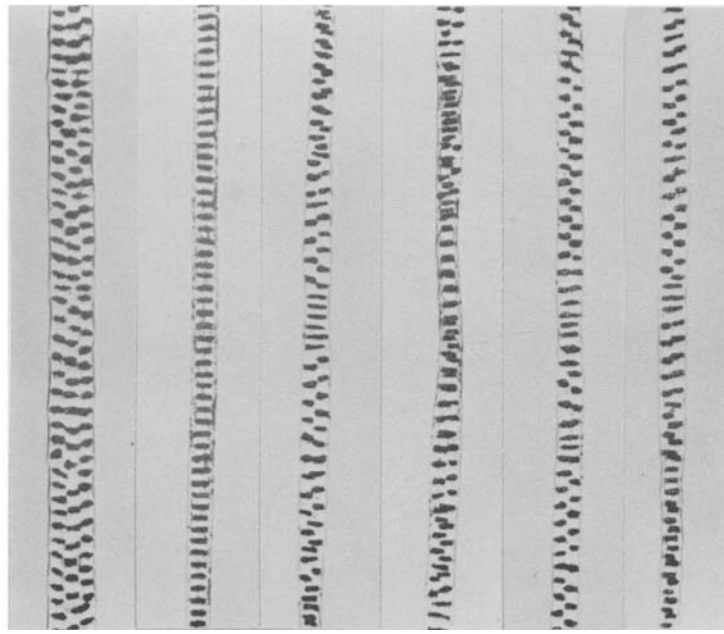
mosaic for a and A^w by virtue of a somatic mutation from a to A^w. Several mosaics of this kind have occurred in non-agouti strains at the Jackson Laboratory (M. M. Dickie & E. M. Eicher, unpublished), and have shown areas of characteristic tiger striping. Since the A^w–a difference, and presumably also the A–a difference, is expressed in dermis (Mayer & Fishbane, 1972), this evidence suggests that dermis may proliferate by lateral coherent clonal growth from a longitudinally arranged population of randomly mixed cells. From the number of stripes in maximally patterned areas of a-locus chimaeras, Mintz (1969) has concluded that there are 75–100 clones on each side. In the trunk region the number of stripes corresponds roughly to the number of somites and suggests that the dermal clones arise from somites (Mintz, 1970).

The evidence from chimaeras on the pattern of proliferation of epidermis is less conclusive. The autosomal gene fuzzy (*fz*), which causes all the hairs to be thin and wavy (Dickie & Woolley, 1950), was studied in chimaeras by Mintz (1970). In $fz/fz \leftrightarrow +/+$ chimaeras, she described, but without illustration, a pattern of fine transverse bands of mutant and normal hair. Mayer, Mittelberger & Green (1974) later showed, by dermal–epidermal recombinations, that *fz* acts in the epidermis. Mintz's results, therefore, suggest that the pattern of proliferation of epidermis is similar to that of dermis.

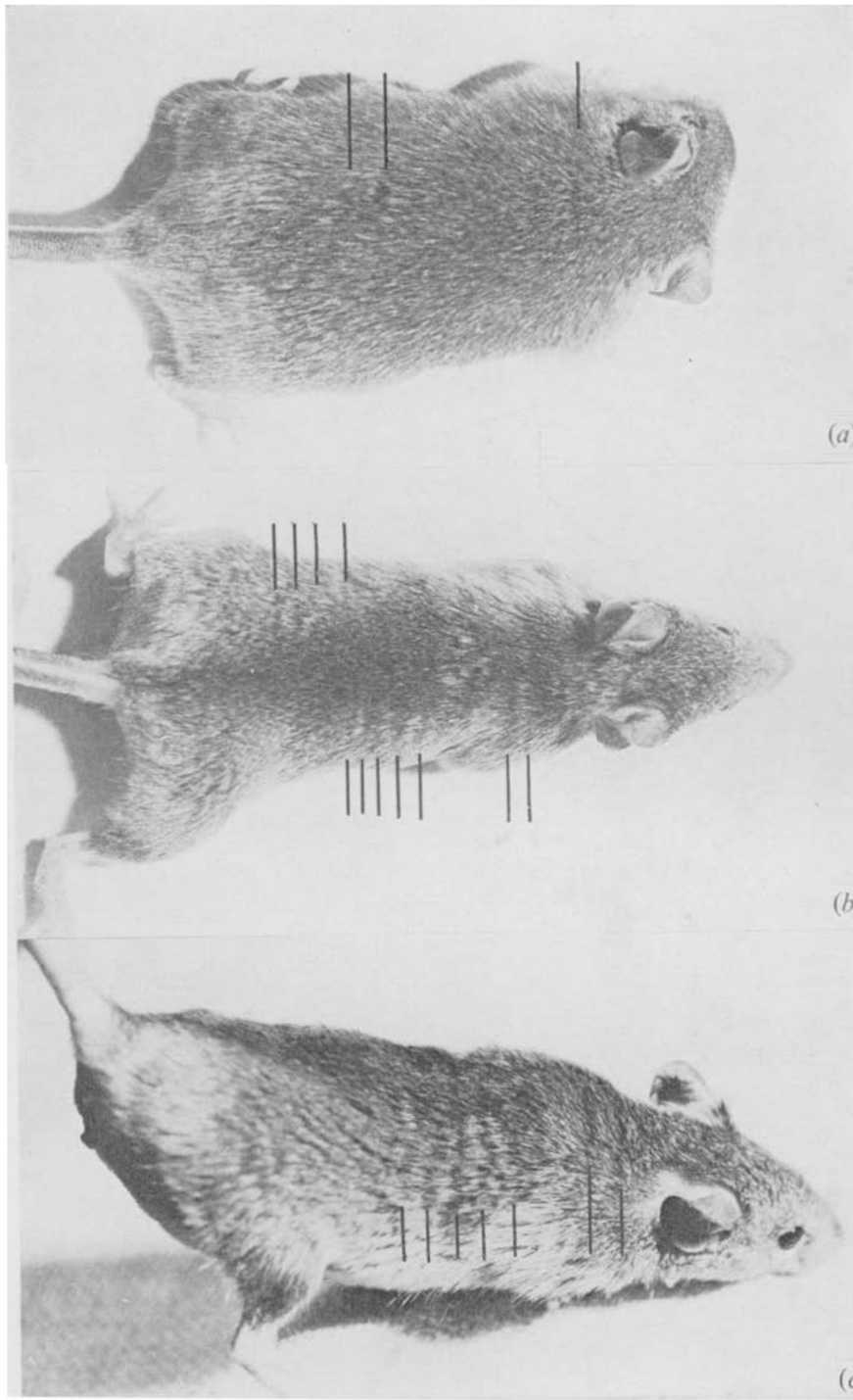
In this paper we describe an attempt to obtain further evidence on the pattern of proliferation of epidermis using the autosomal recessive mutant gene downless (*dl*). Downless causes abnormal texture of the coat and baldness of the tail (Sofaer, 1969), and has been shown by tail–skin recombinations to act in the epidermis (Sofaer, 1973, 1974), but has not previously been examined in chimaeras. The X-linked gene tabby (*Ta*), which is apparently identical to *dl* in its effect on the coat, has been studied extensively in chimaeras, as well as in heterozygous females ($Ta/+$) that are mosaic for cells with one or the other of the X chromosomes inactivated according to the X-inactivation hypothesis of Lyon (1961). $Ta/+$ females show a pattern of narrow tranvserse stripes of mutant and normal hair. Chimaeras made by aggregating Ta/Ta or Ta/Y and $+/+$ or $+$/Y embryos (Cattanach, Wolfe & Lyon, 1972) have coat patterns similar to those of $Ta/+$ females but more variable. The width of the stripes is similar to that in a-locus chimaeras. Since the effect of *Ta* is so similar to that of *dl*, *Ta* might also be presumed to act in the epidermis. Unfortunately, tail skin recombination experiments with *Ta* have given equivocal results (Sofaer, 1974), so that it is not known whether dermis or epidermis is the site of action or *Ta*.

The pattern in chimaeras involving *Ta* is easily seen. We chose to use the *dl* gene for this experiment because it so closely resembles *Ta* in its effect on the coat. We anticipated that the pattern in $dl/dl \leftrightarrow +/+$ chimaeras would be clear, and any departure from the pattern in *Ta*-locus chimaeras and in the similar a-locus chimaeras, such as wider bands or randomly shaped patches, would be easily discernible.

Although our experiments produced only two mice with obviously chimaeric coats, a pattern of narrow transverse stripes similar to that in *Ta*-locus and



Hairs of normal and *dl*/*dl* mice, and of *dl*/*dl* ↔ +/+ chimaeras. (*a*) Normal awl. (*b*) Region between constriction of a normal zigzag. (*c*, *d*) Single type of hair, resembling an abnormal awl, of *dl*/*dl* mice. (*e*) Region between constructions of an abnormal zigzag hair of Chimaera 1. (*f*) Downless-type hair cf Chimaera 2. × 100.



Chimaeras showing striped areas. Lines indicate some of the dark stripes of predominantly downless-type hairs. (*a*) Chimaera 1, 6 weeks old. (*b*) Chimaera 2, 6 weeks old. (*c*) Chimaera 2, 8 weeks old.

https://doi.org/10.1017/S0016672300017353 Published online by Cambridge University Press

a-locus chimaeras was clear in both of them. The similarity of *dl*- and *a*-locus chimaeras supports the view that the pattern of cell proliferation is similar in dermis and epidermis.

2. MATERIALS AND METHODS

The downless allele used was a recurrent mutation (dl^J) that occurred at the Jackson Laboratory in a linkage cross in 1964. Since downless affects coat colour of agouti mice by reducing the number of hairs with yellow agouti bands, we anticipated that a mosaic pattern would be more obvious in agouti mice and therefore used agouti parent stocks. Downless mice (dl^J/dl^J) from an agouti stock that had been brother–sister mated for 20 generations were crossed to mice of the agouti C3HeB/FeJ strain, and dl^J/dl^J mice recovered from the F_2 and subsequent generations were used as parents for the dl^J/dl^J embryos. C3HeB/FeJ mice from the Production Department of the Jackson Laboratory were used as parents for the wild-type (+/+) embryos.

Downless and wild-type embryos at the 8-cell stage were aggregated by a method previously described (Eicher & Hoppe, 1973). The aggregated embryos were cultured overnight and then transplanted into pseudopreganant hosts, (SJL/Wt × C57BL/10Wt)F_1, using the surgical method of Mullen & Carter (1973). The hosts were housed singly and allowed to give birth.

The offspring were examined visually for evidence of chimaerism. Those showing obvious patterns were photographed at 12 days and again at 6 and at 8 weeks of age. All offspring were examined for hair type by plucking a small patch of hair from the mid-dorsal region, spreading the hairs on a microscope slide flooded with 100 % alcohol, and counting hair types under a dissecting microscope. Mice showing obvious patches of chimaeric skin and hair were sampled for hair type from the chimaeric region as well as from a nonchimaeric mid-dorsal region. Samples from chimaeric regions included hair from both light and dark bands, as the bands were too narrow to allow sampling from each kind separately. Similar samples of hair from the downless and normal parent stocks were examined as controls.

3. RESULTS

Abnormalities of the hair of tabby mice have been described in detail (Grüneberg, 1966, 1969). The hair of downless mice is said to be similar to that of tabby mice (Sofaer, 1969), but this statement appears to be based on the general appearance of the coat and not on microscopic examination of the hair. We therefore examined samples of the hair of dl^J/dl^J mice. Only one type of hair was found. It closely resembled the single type of hair described in *Ta*/*Ta* or *Ta*/Y mice by Grüneberg (1966, 1969), and thought to be an abnormal awl. The septa were irregular in shape and varied in number along the shaft, with corresponding irregularities in calibre of the shaft (Plate 1*c*, *d*). The septal pattern was clearly distinguishable from that of normal awls (Plate 1*a*) or normal zigzags (Plate 1*b*).

From the aggregated embryos transplanted into pseudopregnant host females, eight offspring were recovered. As judged by external appearance alone, one was

totally downless, five were totally normal, and two were chimaeric (one male and one female). The chimaeric pattern was obvious when the coat was sufficiently developed by about 12 days.

The male chimaera (Plate 2*a*) was mostly normal in appearance, but possessed an obvious thin dark transverse stripe across the shoulder that included both right and left sides and extended almost to the midventral line. Three dark transverse stripes were obvious on the pelvic region, and they also included both sides. Two of these stripes extended to the midventral line. The stripes were about the same width as those in mice chimaeric at the agouti locus. Counts of hair types were made by plucking small patches of hair from the chimaeric region, and from a mid-dorsal region that was not obviously chimaeric. The results are shown in Table 1. No downless-type hairs were found in the nonchimaeric regions, and the percentages of zigzags, auchenes, awls, and guard hairs were within the expected levels for normal mice (Falconer, Fraser & King, 1951). Proportions of hair types present in the chimaeric area did not deviate significantly from the normal.

Table 1. *Hair types found in eight mice produced by aggregating downless and normal embryos: samples were plucked at 10 weeks of age*

			Hair types				
Mouse	Appearance	Region sampled	Zigzag	Auchene	Awl and guardhair	Downless type	Zigzag (%)
1	Chimaeric	Mid-dorsal*	319	24	134	0	67
		Chimaeric	167	8	79	3	65
2	Chimaeric	Mid-dorsal*	0	0	0	all	0
		Chimaeric	31	15	79	80	15
3	Normal	Mid-dorsal	334	19	151	0	66
4	Normal	Mid-dorsal	326	20	147	0	66
5	Normal	Mid-dorsal	188	29	141	0	53
6	Normal	Mid-dorsal	324	14	163	0	65
7	Normal	Mid-dorsal	278	17	152	0	62
8	Downless	Mid-dorsal	0	0	0	all	0

* Mid-dorsal areas not showing obvious chimaerism.

However, about 1% of the hairs in this region were of the downless type, and many of the zigzag hairs showed hair shaft abnormalities. The defects of zigzags consisted of interruptions of the normal septal patterns, and constrictions at random along the hair shaft (Plate 1*e*), similar to the shaft constrictions and septal interruptions typically present in the downless hair type. These abnormal zigzags are similar to abnormal hairs found in *Ta*/+ females and in tabby ↔ normal chimaeras (Grüneberg, 1969).

The coat of the second chimaeric mouse, a female, was mostly of downless type in appearance and the tail was largely hairless. There were areas of thin transverse light and dark bands from the shoulder level to the base of the tail (Plate 2*b*, *c*). Many of these stripes crossed the mid-dorsal line but were not as obvious there as on the sides, and many extended to the midventral line. There were about 30–40

alternating dark and light stripes in this area. The stripes were similar in width to those of chimaera 1. A count of hair types in the chimaeric area showed that all four normal types were present (Table 1). Zigzags were markedly reduced in number, and those present possessed irregularities of septa and hair shaft. In addition, typical downless hairs with irregularities in septa and shaft calibre were found (Plate 1*f*). Hairs plucked from an area in the mid-dorsal region of the rump not showing obvious evidence of chimaerism were all of the downless type. The striking similarity of the hair types in downless chimaeras to those in tabby chimaeras and *X*-inactivation mosaics (Grüneberg, 1969) is evidence that these two loci affect hair development through a common mechanism.

The five normal and one downless offspring showed no evidence of chimaerism of the coat. These mice were examined for hair types by plucking small patches from the mid-dorsal region. The normal mice possessed all hair types in proportions comparable to normal mice. The 'downless' mouse possessed hairs that were abnormal in structure and typical of those found in natural *dl*/*dl* animals.

4. DISCUSSION

Our experiments produced only two mice recognizably chimaeric with respect to the coat. Despite the small number, ascertainment of the nature of the pattern was unequivocal and unlikely to be changed by observing larger numbers. Although the two mice were quite different in the relative amount of normal and mutant coat, they both showed the same pattern of narrow transverse stripes in the maximally patterned areas rather than some other pattern, such as large randomly distributed patches.

In the two chimaeras, there was good correlation between the appearance of the coat and the proportion of abnormal hairs. We assume that the dark bands represent mutant hair and the light bands represent normal hair. In Chimaera 1, there were only a few thin dark mutant stripes, and samples of hair from the striped areas were found to contain mostly normal hairs. In Chimaera 2, the coat was mostly of mutant type, but with many light normal-type stripes. Samples of hair from the striped areas contained a high proportion of abnormal hairs. The abnormal zigzags found in both chimaeras are not found in either +/*dl* or *dl*/*dl* mice and presumably result from follicles composed of mixed +/*dl* and *dl*/*dl* epidermal cells.

The conclusions from this study rest on the assumption that the observed chimaeric pattern is an accurate reflexion of the distribution of cells from the downless and normal embryos. The phenotype of the hair must reflect the phenotype of the hair follicle, a multicellular organ which, in chimaeras, can comprise cells of both genotypes (Mintz & Silvers, 1970). Because we were not able to sample the dark and light stripes separately, we cannot say how the two areas differed in the proportion of normal and abnormal hair types. Kindred (1967) has examined the hair types of the dark and light stripes of *Ta*/+ mice in which the stripes were wide enough to permit separate sampling, and found a clear difference in the

proportion of zigzags and abnormal awls but otherwise considerable similarity of hair types. The presence of intermediate-type hairs in downless chimaeras and in tabby chimaeras and mosaics suggests that the stripes do not strictly represent clones of mutant and normal cells. Rather, they probably consist of mixtures of mutant and normal cells, with mutant cells predominating in the dark stripes and normal cells predominating in the light stripes.

Since the relationship of genotype of cells to phenotype of hair is thus not well established, conclusions about the pattern of cell proliferation in the skin must be tentative. Nevertheless, the pattern of narrow transverse stripes seen in mice chimaeric for genes affecting both the dermis (*a* locus) and epidermis (*fz* and *dl* loci) is compatible with development by lateral coherent clonal growth from a randomly mixed longitudinally arranged linear array. It is somewhat surprising that the pattern for epidermal genes is as regular as the pattern for dermal genes, since epidermis, in contrast to dermis, proliferates without morphological evidence of segmentation. The similar size of the stripes in mice chimaeric for genes acting in the dermis and in the epidermis is evidence that coherent clonal growth of the two cell layers begins at about the same time and involves about the same number of clones.

We thank Mrs Hope O. Sweet for producing the downless mice, Messrs Darrold L. Dorr and Stetson C. Carter for expert technical assistance, and Miss Janice L. Southard for providing photographs of the agouti-locus mosaics found by Dr M. M. Dickie.

This investigation was supported in part by NSF Grant BMS75-03397 and NIH Grant HD-04083 to the Jackson Laboratory and by NSF Grant BMS75-02812 to Rider College. The Jackson Laboratory is fully accredited by the Association for Accreditation of Laboratory Animal Care.

D.D. was a participant in the Summer Program for College, Graduate, and Medical Students of the Jackson Laboratory, supported in part by the Clark Foundation, New York, N.Y.

REFERENCES

BHAT, N. R. (1949). A dominant mutant mosaic house mouse. *Heredity* **3**, 243–248.

CATTANACH, B. M., WOLFE, H. G. & LYON, M. F. (1972). A comparative study of the coats of chimaeric mice and those of heterozygotes for *X*-linked genes. *Genetical Research* **19**, 213–228.

DICKIE, M. M. & WOOLLEY, G. W. (1950). Fuzzy mice. *Journal of Heredity* **41**, 193–196.

EICHER, E. M. & HOPPE, P. C. (1973). Use of chimeras to transmit lethal genes in the mouse and to demonstrate allelism of the two X-linked genes *jp* and *msd*. *Journal of Experimental Zoology* **183**, 181–184.

FALCONER, D. S., FRASER, A. S. & KING, J. W. B. (1951). The genetics and development of 'crinkled', a new mutant in the house mouse. *Journal of Genetics* **50**, 324–344.

GRÜNEBERG, H. (1966). More about the tabby mouse and about the Lyon hypothesis. *Journal of Embryology and Experimental Morphology* **16**, 569–590.

GRÜNEBERG, H. (1969). Threshold phenomena versus cell heredity in the manifestation of sex-linked genes in mammals. *Journal of Embryology and Experimental Morphology* **22**, 145–179.

KINDRED, B. (1967). Some observations on the skin and hair of tabby mice. *Journal of Heredity* **58**, 197–199.

LYON, M. F. (1961). Gene action in the *X*-chromosome of the mouse (*Mus musculus* L.). *Nature* **190**, 372–373.

MAYER, T. C. & FISHBANE, J. L. (1972). Mesoderm–ectoderm interaction in the production of the agouti pigmentation pattern in mice. *Genetics* **71**, 297–303.

MAYER, T. C., MITTELBERGER, J. A. & GREEN, M. C. (1974). The site of action of the fuzzy locus (*fz*) in the mouse, as determined by dermal–epidermal recombinations. *Journal of Embryology and Experimental Morphology* **32**, 707–713.

MINTZ, B. (1969). Genetic control of the mouse pigmentary system. *Genetics* **61**, s 41 (abstr.).

MINTZ, B. (1970). Gene expression in allophenic mice. In *Control Mechanisms in the Expression of Cellular Phenotypes* (ed. H. A. Padykula), pp. 15–42. New York: Academic Press.

MINTZ, B. & SILVERS, W. K. (1970). Histocompatibility antigens on melanoblasts and hair follicle cells. Cell-localized homograft rejection in allophenic skin grafts. *Transplantation* **9**, 497–505.

MULLEN, R. J. & CARTER, S. C. (1973). Efficiency of transplanting normal, zona-free, and chimeric embryos to one or both uterine horns of inbred and hybrid mice. *Biology of Reproduction* **9**, 111–115.

SOFAER, J. A. (1969). Aspects of the tabby-crinkled-downless syndrome. II. Observations on the reaction to changes of genetic background. *Journal of Embryology and Experimental Morphology* **22**, 207–227.

SOFAER, J. A. (1973). Hair follicle initiation in reciprocal recombinations of *downless* homozygote and heterozygote mouse tail epidermis and dermis. *Developmental Biology* **34**, 289–296.

SOFAER, J. A. (1974). Differences between *tabby* and *downless* mouse epidermis and dermis in culture. *Genetical Research* **23**, 219–225.