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Differences between *tabby* and *downless* mouse epidermis and dermis in culture

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SUMMARY

The semi-dominant X-linked gene *tabby* (*Ta*) in the mouse, and one of its recessive autosomal mimics, *downless* (*dl*) each produces a mutant syndrome that includes absence of hairs on the tail due to failure of tail hair follicle initiation. However, whereas *downless* tails failed to produce hair follicles in culture on the chick chorioallantoic membrane, which is in keeping with the adult phenotype of both *downless* and *tabby* mice, *tabby* tails produced follicles at about 40% of the control level. Furthermore, in contrast to previous findings for *downless*, the culture of mixed genotype epidermis-dermis combinations provided no evidence of a primary epidermal effect in *tabby*.

1. INTRODUCTION

The semi-dominant X-linked gene *tabby* (*Ta*) in the mouse, and two recessive autosomal mimics of *tabby*, *crinkled* (*cr*) and *downless* (*dl*), are each associated with a mutant syndrome that is characterized by abnormalities of hair, teeth and certain exocrine glands, all structures formed by the downgrowth of an epithelium into the underlying mesenchyme (Falconer, Fraser & King, 1951; Falconer, 1953; Grüneberg, 1965, 1966*a*, *b*, 1971; Sofaer, 1969*a*, *b*). In mutant mice some of these structures fail to form altogether, and those that do form are usually reduced in size and have an abnormal morphology. The normal alleles of *tabby*, *crinkled* and *downless* therefore appear to be concerned with the interaction between epithelium and mesenchyme that takes place during the development of such structures.

One of the most prominent mutant abnormalities is the absence of hairs on the tail due to failure of tail hair follicle initiation. The degree of failure to form tail hairs is influenced by genetic background, and, at least for *tabby*, by the particular mutant allele that occupies the *tabby* locus; but for certain genotypes tail hair follicle initiation appears to be completely suppressed, or at least very nearly so.

Failure to initiate a downgrowth of epithelium that would go on to form a hair follicle could be due to an abnormality in either the epidermis, the epithelial component of the system, or the dermis, the mesenchymal component; or perhaps

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both. It has been suggested that *tabby* may act through the dermis, since the fine transverse banding of the coat in *tabby* heterozygotes is reminiscent of the repetitive pattern of the somites in early development (Lyon, 1970; Mintz, 1971). However, previous work indicates that in *downless* mice the primary effect of the mutation on tail hair follicle initiation is restricted to the epidermis. Cultured combinations of $+/dl$ tail epidermis with *dl/dl* dermis produced follicles, whereas combinations of *dl/dl* epidermis and $+/dl$ dermis did not (Sofaer, 1973). The present paper describes a similar epidermis-dermis recombination experiment in which various combinations of *Ta/Ta* (or *Ta*), $+/dl$, and *dl/dl* tail epidermis and dermis were used. The experiment was undertaken to test independently the capacity of *tabby* epidermis and dermis to produce the mutant phenotype, and to compare the behaviour of *tabby* in culture with that of *downless*.

2. MATERIALS AND METHODS

The mutant stocks used were derived from *tabby* (original mutant allele *Ta*) and *downless* mice with a C3H/101 genetic background, obtained from the MRC Radiobiology Unit at Harwell. The degree of suppression of tail hair formation was determined for each stock by examining the tails of a sample of 50 adult mutant mice under a dissecting microscope for the presence of hairs in the distal, middle and proximal thirds of the tail. For comparison, the tails of 10 *downless* heterozygotes were also examined, and the mean number of hairs in the middle third of the tail was estimated. An estimate for each heterozygote was arrived at by counting the number of hairs per tail ring at the two extremes and at the centre of the middle third of the tail, and by taking the average of these three counts and multiplying by the number of middle third tail rings.

Timed matings were set up using *Ta/Ta*♀♀ with *Ta*♂♂, and $+/dl$ or *dl/dl*♀♀ with *dl/dl*♂♂. The day on which a vaginal plug was found was regarded as day zero. On day 14, two days before the first signs of hair follicle formation in normal tails, pregnant females were sacrificed and the embryonic litters were removed. Embryos from mixed litters of $+/dl$ and *dl/dl* individuals were classified on the basis of presence or absence of a postorbital tubercle, the first sign of a developing postorbital vibrissa. Heterozygotes invariably possess the tubercle whereas homozygotes do not .

The middle third of the tail of each 14-day embryo was dissected out in Tyrode's solution containing 1000 units of penicillin and 25 mg of streptomycin per litre, and the external epithelium was cut longitudinally from one end of this tail segment to the other. Segments to be used for epidermis-dermis recombination were then transferred to 2% trypsin (Difco 1:250) in Tyrode's solution and maintained at approximately 4° C for about $1\frac{1}{2}$ hours. After returning to Tyrode's solution the epidermal epithelium was peeled from the core of each tail segment, and recombinations were made between epithelium and tail cores of selected genotypes. The recombined tail segments were then carried to the chorioallantoic membrane of hen's eggs that had been incubated for

8 days. Undissociated control tail segments, not exposed to trypsin, were also explanted, with the core tissue exposed by incision of the epidermal epithelium placed in contact with the chorioallantoic membrane. Each egg received a single explant. After 8 days of incubation on the chorioallantoic membrane the explants were removed, fixed in Bouin's fluid, serially sectioned at 10 μm and stained with haematoxylin and eosin. The serial sections were then examined, and the number of early hair follicles contained in each explant was recorded.

A few additional details of the explantation technique are given in Sofaer (1973).

3. RESULTS

Table 1 shows the distribution of numbers of mutant tails of the two stocks according to the number of hairs in the distal, middle and proximal thirds of the tail. Suppression of hair formation was not complete for both *dl/dl* and *Ta/Ta* or *Ta* tails, and the incidence and position of hairs were very similar in the two mutants on this genetic background. When hairs did occur they tended to do so towards the distal rather than the proximal end of the tail.

Table 1. *The distribution of numbers of mutant tails according to the number of hairs in the distal, middle and proximal thirds of the tail*

Geno-type	Total tails	Position	Number of hairs					
			0	1–5	6–10	11–20	21–40	> 40
dl/dl	50	Distal $\frac{1}{3}$	37	8	3	0	2	0
		Middle $\frac{1}{3}$	44	5	0	1	0	0
		Proximal $\frac{1}{3}$	49	0	1	0	0	0
Ta/Ta or *Ta*	50	Distal $\frac{1}{3}$	36	11	2	1	0	0
		Middle $\frac{1}{3}$	46	4	0	0	0	0
		Proximal $\frac{1}{3}$	50	0	0	0	0	0

Table 2 compares $+dl$, *dl/dl*, and *Ta/Ta* or *Ta* adult tail phenotypes with the mean numbers of early hair follicles found per cultured undissociated embryonic tail segment. Also shown are adjusted means of hair and follicle number based on corresponding $+/dl$ values of 1. In both *dl/dl* and *Ta/Ta* or *Ta* adults the incidence of middle third tail hairs was very low compared with the $+/dl$ controls (around 1/10000 of the $+/dl$ level). However, whereas *dl/dl* explants produced no follicles at all, as was expected, *Ta/Ta* or *Ta* explants produced follicles at about 40% of the $+/dl$ control explant level. *Tabby* and *downless* therefore clearly responded in different ways to the culture system.

The results for trypsin dissociated and recombined explants of different genotype combinations are given in Table 3. Comparison with Table 2 shows that, in the change from culturing undissociated tail segments to trypsin dissociation and recombination prior to explantation, the proportion of follicle containing explants of $+/dl$ tail segments dropped from 100 % (19/19) to 40% (8/20), and that of *Ta/Ta* or *Ta* tail segments dropped from 82% (18/22) to 22% (5/23). The dis-

sociation and recombination procedure therefore affected both genotypes that produced follicles in this system in similar ways. Explants of *dl/dl* tail segments failed to produce follicles both when cultured undissociated (Table 2) and when cultured following trypsin dissociation and recombination (Table 3).

The data in Table 3 indicate that *downless* has its primary effect on the epidermis. All explants containing *dl/dl* epidermis failed to produce follicles (columns 3–5), whereas explants containing *dl/dl* dermis (except in combination with *dl/dl*

Table 2. *The mean number of hairs per middle third of adult tails, and the mean number of follicles found in cultured undissociated middle third explants*

	+/*dl*			*dl/dl*			*Ta/Ta* or *Ta*		
	N	*M*	*R*	*N*	*M*	*R*	*N*	*M*	*R*
Adult tails	10	2600 approx.	1	50	0·44	0·00017	50	0·14	0·00005
Explants	19	22·8	1	21	0	0	22	8·6	0·38

N is the number of tails or explants examined, *M* is the mean number of hairs or follicles, and *R* is the mean number of hairs or follicles relative to a *downless* heterozygote value of 1. All the +/*dl* explants, and 18 of the 22 *Ta/Ta* or *Ta* explants contained follicles. Means were based on the total number of tails or explants of each genotype examined.

Table 3. *The total numbers of explants of each epidermis/dermis genotype combination, the numbers containing hair follicles, and the average numbers of follicles found in the positive explants*

Column ...	1	2	3	4	5	6	7	8	9
Epidermis ...	+/*dl*	+/*dl*	*dl/dl*	*dl/dl*	*dl/dl*	*Ta*	*Ta*	*Ta*	+/*dl*
Dermis ...	+/*dl*	*dl/dl*	+/*dl*	*dl/dl*	*Ta*	+/*dl*	*dl/dl*	*Ta*	*Ta*
Total explants	20	24	20	21	25	23	24	23	21
Explants with follicles	8	6	0	0	0	11	9	5	4
Average follicles per positive explant	4·5	2·7	—	—	—	3·0	3·0	1·8	2·3
Positive : negative explants	14 : 30					20 : 27		9 : 35	

Ta refers to either *tabby* female homozygotes or *tabby* hemizygous males. The first four columns contain previous data (Sofaer, 1973).

epidermis – columns 2 and 7) showed some follicle yield. Explants containing *Ta/Ta* or *Ta* epidermis produced follicles (columns 6–8), and those containing *Ta/Ta* or *Ta* dermis (except in combination with *dl/dl* epidermis – columns 8 and 9) produced follicles also. This is consistent with the lack of complete mutant expression shown by cultures of undissociated *Ta/Ta* or *Ta* tails (Table 2).

Since undissociated *tabby* tail segments produced follicles at less than the control (+/*dl*) level it follows that some effect of *Ta* may be retained in the culture system and that it might be detectable in dissociated and recombined explants also. Thus, if *Ta* affects either the epidermis or the dermis, it may be possible to demonstrate a quantitative difference of follicle yield between explants containing

Ta epidermis as opposed to those containing *Ta* dermis. If *Ta* has its primary effect on the epidermis, as seems to be the case with *dl*, then explants containing *Ta* epidermis could show a lower follicle yield than those containing *Ta* dermis. Table 3 suggests that there is a tendency for the reverse to apply. There was a higher proportion of follicle containing explants among explants containing *tabby* epidermis (excluding those that also contained *tabby* dermis – columns 6 and 7) than among those containing *tabby* dermis (excluding those that also contained *dl*/*dl* or *Ta* epidermis – column 9); and the proportion for *tabby* epidermis (columns 6 and 7) was not significantly different from that for *downless* heterozygote control epidermis (columns 1 and 2). There is therefore some basis for combining columns 8 and 9 to give an overall indication of the effect of *tabby* dermis. The difference between the ratios of positive to negative explants for *tabby* epidermis (columns 6 and 7) as opposed to *tabby* dermis (columns 8 and 9) is of borderline significance. However, the difference between column 6 and column 9, and that between columns 8 and 9 as opposed to 1 and 2 are not significant. Nevertheless, while these results may be insufficient to implicate *tabby* dermis they do not provide any evidence of primary epidermal involvement in *tabby* mice.

4. DISCUSSION

The first difference disclosed between *tabby* and *downless* concerns their response to the culture system. Undissociated embryonic *downless* tail segments failed to produce hair follicles when cultured on the chick chorioallantoic membrane, whereas embryonic *tabby* tail segments produced follicles at about 40 % of the control level. There are two kinds of explanation for this. It may be that the *tabby* mutation results in a deficiency that is partly made good by the developing chick egg. The model proposed by Dun (1959) to explain the action of *tabby* is in fact based on a partial deficiency hypothesis. Alternatively, *tabby* may have its effect by inhibition of the normal developmental process. If this were the case, incomplete mutant expression could result from dilution of the inhibitory influence by the relatively massive volume of the chick egg.

Both the hypothesis of making good a deficiency and that of dilution of an inhibitor imply that a diffusible substance is involved in producing the *tabby* phenotype. Evidence for a diffusible substance comes from the observation that both 'normal' and '*tabby*' areas of the coat of *Ta* ↔ + chimaeras contain both normal and mutant hairs, only the proportion of the *tabby* effect differing in the two types of area (Cattanach, Wolfe & Lyon, 1972). Furthermore, a study of tail ring patterns in mice heterozygous or chimaeric for *tabby* has shown that only the chimaeras have phenotypically mosaic tails; the suggested interpretation being that patch size in heterozygote tails is much smaller than in chimaeras and falls within the diffusion range of a hypothetical gene product (McLaren, Gauld & Bowman, 1973).

The implications for *downless* are as follows. If *downless* results in a deficiency the chick egg must be unable to supply the explant with what is missing. On the

other hand, if *downless* acts by inhibition the inhibitor substance must be either poorly diffusible or produced in great excess.

The mode of action of the two genes might become clearer if undissociated mutant tail segments were cultured in a completely artificial system, free from the unknown factors associated with chorioallantoic grafting. It has already been reported that, in the plasma clot system, hairs developing in skin that has been taken from a prospectively hair producing area of *tabby* embryos show the characteristic mutant pattern of developmental timing and hair morphology (Hardy, 1969). The reason why the mutant characteristics of the cultured tissue are retained in this instance but not in the case of tail segments grafted onto the chorioallantoic membrane may be associated with the relative volumes of the two culture systems and the presence of a circulation in the chorioallantoic case. Both these factors would tend to allow more rapid exchange of a diffusible substance in the chorioallantoic situation.

The second difference between *tabby* and *downless* concerns the site of activity of each gene. There seems to be little doubt that the primary effect of *downless* is restricted to the epidermis. By contrast, comparison of the follicle yield between explants containing *Ta* epidermis and those containing *Ta* dermis does not provide an equally clear cut answer for *tabby*.

In all respects studied so far the phenotypes of *dl*/*dl* and *Ta*/*Ta* or *Ta* mice are qualitatively indistinguishable. Furthermore, the levels of mutant effect produced by the two genes appear to react in similar ways to the same changes of genetic background (Sofaer, 1969*b*). Nevertheless, the two genes have been shown here to respond differently to culture on the chick chorioallantoic membrane. It is possible that the difference of response may simply reflect a quantitative difference in the production of a single inhibitor. This would not necessarily be detectable under normal circumstances if there were sufficient inhibitor in *tabby* mice to cause maximum suppression of tail hair follicle initiation, but might be disclosed in culture because of the postulated dilution effect associated with chorioallantoic grafting. It is conceivable that such a difference could arise as a consequence of the difference between autosomal and X-linkage. Alternatively, since it appears possible that they may have their primary effect in different tissues, the genes may not be mimics because they produce closely related biochemical blocks, but rather because they result in abnormalities in separate but cooperating components of a single developmental system.

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