

## The angora locus (*go*) in the mouse: hair morphology, duration of growth cycle and site of action

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### SUMMARY

All four coat hair types were longer in mice homozygous for angora (*go/go*) than in wild-type mice (+/+). This increase in hair length in mutant animals was due to an increase in the duration of the phase of hair growth (anagen VI). When skin recombinants incorporating mutant or wild-type epidermis and dermis were grafted to a nude host, the activity of the mutant was found to be confined to the epidermis.

### 1. INTRODUCTION

In the mouse, more than seventy of the known mutants have an effect on coat morphology (Green, 1981). Many of these mutants have no effect on hair length; others reduce it in length. Only one mutant, angora (*go*), causes an increase in the length of the coat hairs (Dickie, 1963).

Apart from its effects on hair length, nothing is known about the angora mutant. In this paper we describe the morphology of two generations of coat hairs in wild-type and angora mice, the duration of the cycle of hair follicle activity and the duration of the hair growth phase in the two genotypes, the dimensions of the hair follicle bulbs during the hair growth phase and the results of recombination experiments designed to determine the site of action of the mutant.

### 2. METHODS

#### (i) *Mouse stocks*

Wild-type (+/+) and angora (*go/go*) mice and embryos were obtained from a random-bred wild-type stock and a random-bred angora stock respectively. The angora stock was descended from two *go/go* males from the Jackson Laboratory, Bar Harbor, Maine, USA, and females from the wild-type stock. Both stocks were coloured and both were homozygous for black and tan (*a<sup>t</sup>*) at the agouti locus. The yellow stripe separating the black back from the light belly of *a<sup>t</sup>/a<sup>t</sup>* mice was used as a marker to obtain the skin and hair samples.

Heterozygous naked (*N/+*) mice, used to introduce the *N* mutation into the angora stock in order to study the hair cycle in *go/go* and +/– mice, were obtained from a random-bred coloured stock segregating for naked (*N*). This stock was closely related to the wild-type stock.

Homozygous nude (*nu/nu*) mice, used as hosts for skin recombinants, were obtained from a random-bred albino stock segregating for nude (*nu*). All stocks were maintained under conventional conditions.

(ii) *Hair morphology*

Body hair samples were obtained from *+/+* and *go/go* mice by plucking a tuft of hair from the mid-side of *a<sup>t</sup>/a<sup>t</sup>* mice. Samples of generation one coat hairs (G1) were obtained from three week old mice; G3 samples were obtained from a second group of mice at three months. Pure G3 samples were obtained by plucking G1 and G2 hairs from the area to be sampled at about the time when G2 hairs were due to pass into telogen (six weeks). The area surrounding the sample site was shaved immediately after the hairs had been plucked. Tail hair samples were obtained by plucking hairs from the middle third of the tails of two adult *+/+* and two adult *go/go* mice.

A few hairs of each morphological type (zigzags, auchenes, awls and guard hairs) were taken from each body hair sample and mounted on sticky tape on a glass slide. Paraffin oil was used as a mountant. Tail hair samples were handled in the same way.

Total lengths of all hair types and segment lengths of zigzags and auchenes were measured with the aid of a projection microscope and an image analyser (Leitz ASM). These measurements were made on 5 to 10 hairs of each type in each sample.

(iii) *Phases of the hair cycle*

The phases of the hair cycle were studied in *+/+* and *go/go* females heterozygous for the naked gene, *N* (Slee, 1951; Kindred, 1967). In *N/+* mice, observation of the changes in follicle activity during the hair cycle is facilitated because hair growth is normal during anagen but the hairs break off at the end of the growth phase and the resting phase (telogen) is extremely short. In *+/+* *N/+* and *go/go* *N/+* mice the duration of anagen VI was measured from time of hair eruption to time of hair breakage and the duration of the whole period of follicle activity (anagen I–VI plus catagen) (Chase, Rauch and Smith, 1951) was measured by determining the duration of the interval between eruption of one hair coat and eruption of its successor. Ages at eruption and breakage of successive waves of undercoat hairs (zigzags, awls and auchenes) were determined by examining the mid-sides of the mice at intervals of two or three days. Observations were commenced when the mice were about 25 days old (just before eruption of the second hair cycle) and continued until the fifth hair cycle had erupted. The duration of anagen VI was therefore determined on hair cycles 2, 3 and 4 and the duration of the period of follicle activity on intervals between cycles 2 and 3, 3 and 4 and 4 and 5.

(iv) *Follicle diameters*

Skin samples for measuring follicle diameters were obtained by fixing whole 8- and 12-day-old mice in formol saline and removing 3 × 4 mm skin samples from the mid-side after fixation was complete.

Samples were cut into horizontal 70 μ sections with a freezing microtome. These sections were attached to gelatinized slides, stained with alizarin, dehydrated and mounted. A single 70 μ section containing follicle bulbs extended as far as the mid-follicle region. Hence, with the aid of a projection microscope, the follicles could be classified into those producing zigzags, awls and guard hairs on the basis of the number of medullary cells per row in the hair. An image analyser was used to measure the maximum diameter of about 10 follicle bulbs of each type in each specimen. Diameters of all follicle types in 8 day skin were not significantly different from those of follicles in 12 day skin so the data for the two age groups were pooled.

(v) *Identification of the site of action of the mutant*

The site of action of the *go* mutant was determined from the morphology of hairs produced by dermal-epidermal recombinants of mutant and wild-type embryonic skin grafted to the nude mouse. Recombinants were prepared by splitting +/+ and *go/go* skin from 14-day embryos into epidermis and dermis with 1% trypsin and recombining mutant and wild-type epidermis and dermis in all four combinations (see Raphael and Pennycuik, 1980 and Pennycuik and Raphael, 1984 for details). The recombinants were grafted to a nude host where they remained until the follicles passed into telogen. Unseparated explants of *go/go* and +/+ skin from 14-day embryos were also grafted to nude hosts to determine the effects of grafting on hair morphology. After the follicles on the grafts had passed into telogen, hairs plucked from these grafts were examined with the aid of a projection microscope and an image analyser (see above).

(vi) *Statistics*

Because hair length measurements (and measurements of follicle diameter and intervals between hair eruption and hair breakage, etc.) were made on samples from several mice and on several hairs from each mouse, both between mouse and between hair variance contributed to the total variation in hair length. Between mouse variance proved to be an order of magnitude greater than between hair variance. In determining the significance of differences between groups, therefore, only the numbers of animals in the groups were considered.

3. RESULTS

(i) *Hair length and morphology*

Dickie (1963) reported that the coat hairs of *go/go* mice were longer at weaning than those of +/+ mice, and that adult *go/go* mice had very long, thick pelts, but he gave no details about hair lengths in the juvenile and adult coats or about hair morphology. We therefore decided to measure the lengths of the four coat hair

types (zigzags, auchenes, awls and guard hairs) in samples of juvenile (G1) and adult (G3) hair, to count the number of segments in the zigzags and auchenes, and to measure the lengths of these segments. In addition, we measured the lengths of a few tail hairs from adult  $+/+$  and  $go/go$  mice in order to determine whether angora also affected the lengths of hairs in regions other than the body.

Table 1. *Lengths (mm)  $\pm$  standard errors of body hairs of wild-type ( $+/+$ ) and angora ( $go/go$ ) mice*

Genotype	Number of mice	Hair type			
		Zigzag	Auchene	Awl	Guard
$+/+$	12	$5.7 \pm 0.21$	$6.2 \pm 0.22$	$6.3 \pm 0.53$	$9.5 \pm 0.36$
$go/go$	12	$8.1 \pm 0.63$	$8.6 \pm 0.53$	$8.7 \pm 0.76$	$14.7 \pm 1.35$
<i>t</i>		3.62**	4.11***	2.54*	3.72**

\*0.05 > P > 0.01      \*\*0.01 > P > 0.001      \*\*\*0.001 > P

Table 2. *Lengths (mm)  $\pm$  standard errors of the segments and basal region of zigzag hairs from the coats of wild-type ( $+/+$ ) and angora ( $go/go$ ) mice*

Genotype	Number of mice	Segment				
		Seg 1	Seg 2	Seg 3	Seg 4	Base
$+/+$	12	$1.4 \pm 0.06$	$1.9 \pm 0.04$	$1.5 \pm 0.08$	—	$1.0 \pm 0.11$
$go/go$	12	$1.6 \pm 0.07$	$2.2 \pm 0.12$	$1.6 \pm 0.09$	$1.2 \pm 0.08$	$1.4 \pm 0.16$
<i>t</i>		1.79	2.81*	1.33	—	2.14*

\*0.05 > P > 0.01

There was no significant difference between the length of G1 and G3 hairs within genotypes and we therefore pooled the G1 and G3 data for each hair type within each genotype. Differences between the lengths of  $go/go$  and  $+/+$  hairs of all types were significant (Table 1). The percentage increase in hair lengths was greater for guard hairs (55%) than for the other three hair types (about 40%).

The zigzags from  $go/go$  mice differed from those of  $+/+$  mice in having four segments, rather than three, above the wavy section adjoining the hair club. Most of the increase in the total length of the  $go/go$  zigzags appeared to be due to the presence of this additional segment, for the three distal segments of the  $go/go$  zigzags were only slightly longer than those of  $+/+$  zigzags. However, a slight increase in the length of segment 2, and an increase in the length of the wavy section above the hair base also contributed to the increase in the total length of the zigzags from the angora mice (Table 2).

The auchenes from  $go/go$  mice, like those from  $+/+$  mice, were composed of two segments. The lengths of the distal segments of the two genotypes were very similar ( $+/+$ ,  $2.8 \pm 0.14$  mm;  $go/go$ ,  $2.9 \pm 0.18$  mm). The length of the proximal segment of the  $go/go$  auchenes was significantly greater than that of the  $+/+$  auchenes ( $+/+$   $3.5 \pm 0.20$  mm;  $go/go$   $5.7 \pm 0.49$  mm;  $t = 4.16$ ,  $P < 0.001$ ). The increase in the total length of the  $go/go$  auchenes, like that of  $go/go$  zigzags, appeared to be due to an increase in the length of the basal region of the hair.

Tail hairs plucked from angora mice, like body hairs, were longer than those from

the tail of  $+/+$  mice ( $1.7 \pm 0.18$  mm compared with  $1.4 \pm 0.06$  mm). However, the difference between the two groups was not significant, and the percentage change in length (21%) was smaller than that observed in body hairs.

These results confirmed Dickie's (1963) findings that both the juvenile and the adult coats of *go/go* mice are longer than those of  $+/+$  mice and they established that this increase in length was due to an increase in the length of the hair base. In the case of zigzag hairs, this increase in the length of the hair base was associated with the appearance of an additional segment. Tail hairs as well as body hairs were affected by the mutant, but the increase in hair length was less marked in these short hairs than in the longer coat hairs.

Table 3. *The duration of anagen VI (days)  $\pm$  standard errors and of the interval between hair cycles on the mid-sides of wild-type ( $+/-$ ) and angora (*go/go*) females heterozygous for the naked (N) gene*

Genotype	Number of mice	Anagen VI	Between cycle interval
$+/-$ N/+	15	$7.1 \pm 0.18$	$19.5 \pm 0.35$
<i>go/go</i> N/+	13	$9.9 \pm 0.42$	$21.6 \pm 0.51$
<i>t</i>		$6.12^{***}$	$3.52^{**}$
		$**0.01 > P > 0.001$	$***0.001 > P$

#### (ii) Duration of the phase of hair growth

The increase in lengths of the hairs in the coats of *go/go* mice could have been due to an increase in the duration of the phase of hair growth (cf. angora rabbits; Fraser, 1953), or to an increase in rate of hair growth. In order to distinguish between these two possibilities, we measured the duration of anagen VI in follicles producing the undercoat (i.e. zigzags, awls and auchenes), in  $+/+$  and *go/go* mice and used these values to calculate rates of growth of these three hair types. In addition, we measured the duration of the whole hair cycle in the two genotypes.

Presence of the angora mutant increased the interval from eruption of undercoat to hair breakage from about 7 days to about 10 days, i.e. by about 40% (Table 3). When these values for anagen VI were used to calculate hair growth rates, zigzags, awls and auchenes proved to have very similar growth rates in the two genotypes (zigzags 0.80 mm/day in  $+/+$  v. 0.82 in *go/go*; awls 0.87 v. 0.87; auchenes 0.89 v. 0.88).

Increase in the duration of anagen VI in *go/go* mice was reflected in an increase in the duration of the whole hair cycle in the mutant mice (Table 3). In addition, the age at eruption of the second hair coat was delayed slightly in angora females ( $36.2 \pm 0.55$  days in *go/go* v.  $33.7 \pm 0.55$  days in  $+/+$ ).

The 40% increase in the lengths of the zigzags, awls and auchenes in angora mice, therefore, could be accounted for by the 40% increase in the duration of anagen VI. The 55% increase in the length of the guard hairs and the 20% increase in the length of the tail hairs may have been related to differences between these two hair types and the major coat hair types in the duration of anagen VI. Anagen VI is

known to be of longer duration in guard hair follicles than in follicles producing zigzags and awls (Fraser and Nay, 1955; Hale and Ebling, 1975) and it may well be of shorter duration in tail hair follicles.

(iii) *Hair follicle bulb diameters*

Variations in the duration of anagen VI could be caused by an increase in the number of stem cells in the hair follicle bulbs or by an increase in the number of divisions these stem cells underwent before the follicle passed into the resting phase. If the increase were due to an increase in the number of stem cells, this increase could be reflected in the size of the follicle bulb during anagen VI. We therefore measured the diameters of follicles producing zigzags, awls and guard hairs (auchenes, which constitute only a small proportion of the coat hairs, could not be identified with certainty in the skin sections) in skin samples taken from  $+/+$  and  $go/go$  mice during anagen VI of the first hair cycle.

Table 4. *Diameters of follicle bulbs ( $\mu$ )  $\pm$  standard errors during anagen VI in mid-side skin from wild-type ( $+/+$ ) and angora ( $go/go$ ) mice*

Genotype	Number of mice	Follicle type		
		Zigzag	Awl	Guard
$+/+$	5	444 $\pm$ 55	586 $\pm$ 57	966 $\pm$ 74
$go/go$	6	424 $\pm$ 37	546 $\pm$ 65	941 $\pm$ 63
<i>t</i>		0.30	0.46	0.26

Presence of the angora mutant appeared to have no effect on the sizes of the bulbs of follicles producing zigzags, awls and guard hairs (Table 4). There was, therefore, no evidence that angora caused an increase in the duration of anagen VI by increasing the number of stem cells in the follicle bulbs.

(iv) *Site of action of angora*

The site of action is known for several mutants affecting the structure of coat hairs (e.g. Raphael and Pennycuik, 1980) but the site of action of mutants affecting the duration of the hair growth cycle has not been identified. Recombinants of wild-type and angora epidermis and dermis were grafted to nude hosts in an attempt to identify which tissue determines the time for which the hair continues to grow.

When pieces of unseparated wild-type and angora skin (wild-type and angora explants) were grafted to a foreign host, zigzags and awls were shorter than hairs of the corresponding type plucked from the mid-sides of  $+/+$  and  $go/go$  mice (compare Tables 1 and 5), but the hairs from the  $go/go$  grafts were longer than those from the  $+/+$  grafts and the zigzags from the  $go/go$  grafts had the four segments characteristic of  $go/go$  zigzags. The difference between lengths of the awls from  $+/+$  and  $go/go$  grafts was significant ( $t = 2.72$ ,  $0.05 > P > 0.01$ ), but the difference between lengths of the zigzags from  $+/+$  and  $go/go$  grafts just failed

to reach significance because of the increased variability of lengths of the zigzags from the *go/go* grafts.

Zigzags and awls from recombinants in which the epidermis was from a *+/+* embryo were indistinguishable in length and morphology from those from *+/+* explants, irrespective of the source of the dermis. Zigzags and awls from recombinants in which the epidermis was from a *go/go* embryo were indistinguishable from those from *go/go* explants (Table 5). Two-way analysis of variance confirmed that the source of the epidermis was the only factor determining the length of the hairs on the recombinant grafts (Table 6).

Table 5. Lengths (mm)  $\pm$  standard errors of zigzags and awls plucked from wild-type (*+/+*) and angora (*go/go*) explants and from recombinants of *+/+* and *go/go* epidermis and dermis grafted to nude mice

Genotype epidermis/dermis	Number of grafts	Zigzags		Awls	
		No. of hairs	Length	No. of hairs	Length
Explants					
<i>+/+</i>	6	65	4.6 $\pm$ 0.39	60	5.4 $\pm$ 0.95
<i>go/go</i>	4	40	7.2 $\pm$ 1.11	34	8.7 $\pm$ 0.79
Recombinants					
<i>+/+/+/+</i>	5	54	4.6 $\pm$ 0.27	34	5.2 $\pm$ 0.27
<i>+/+/go/go</i>	7	70	4.8 $\pm$ 0.52	52	6.1 $\pm$ 0.40
<i>go/go/+/+</i>	7	70	6.7 $\pm$ 0.56	62	8.3 $\pm$ 0.84
<i>go/go/go/go</i>	3	30	7.4 $\pm$ 1.61	30	9.1 $\pm$ 1.25

Table 6. Analysis of variance of hair lengths (mm) in samples from skin recombinants incorporating wild-type (*+/+*) or angora (*go/go*) epidermis and dermis

Hair type	Source	d.f.	m.s.
Zigzag	Epidermis	1	27.01***
	Dermis	1	0.35
	Epidermis $\times$ Dermis	1	0.76
	Residual	21	0.19
Awls	Epidermis	1	42.27***
	Dermis	1	0.001
	Epidermis $\times$ Dermis	1	3.60**
	Residual	21	0.27

\*\* 0.01 > P      \*\*\* 0.001 > P

In spite of the reduction in hair length caused by grafting unseparated embryonic skin to a foreign host, zigzags from the *go/go* grafts retained their characteristic morphology, and both awls and zigzags from *go/go* grafts were longer than those from *+/+* grafts. The differences between the two genotypes were due to activity of the angora mutant in the epidermis.

## 4. DISCUSSION

Dickie (1963) observed that all coat hair types are longer in angora mice than in wild-type mice and that both juvenile and adult coats are affected by the mutant. Our results, which confirm Dickie's observations, show that the increase in the length of the angora hairs is due to an increase in the length of the basal region of the hair and that in zigzags, this increase results in the appearance of an additional hair segment. Our results also show that the percentage increase in hair length is greatest in the longest hairs (guard) and least in the shortest hairs (tail).

In addition to having longer coat hairs than wild-type mice, the hair cycles in *go/go* mice were two or three days longer than in *+/+* animals due to an increase in the duration of anagen VI from 7 to 10 days. This 40% increase in duration of anagen VI measured for follicles producing zigzags, awls and auchenes, was sufficient to account for the increase in the lengths of these three hair types. In guard hair follicles, anagen VI is longer than in follicles producing zigzags, awls and auchenes (Fraser and Nay, 1955; Hale and Ebling, 1975) and in tail hair follicles, anagen VI is probably shorter than in follicles producing the other hair types. These differences in the duration of anagen VI in the different follicle types may be related in some way to the differences in the percentage increase in length in guard hairs, underfur and tail hairs in *go/go* mice.

The increase in the duration of anagen VI in angora mice was not accompanied by a change in the size of the follicles producing the coat hairs. There was therefore no evidence that increase in the duration of anagen VI was due to an increase in the number of stem cells giving rise to the hair and the follicle sheaths. The increase in cell number in the mature hair in *go/go* mice, therefore, must have been due to an increase in the number of stem cell divisions before the follicle passed into the resting phase.

The recombination experiments established that the effects of the mutant are due to activity of the *go* mutant in the epidermis. That is, they suggest that the number of stem cell divisions in the hair follicle bulb is determined by the stem cells themselves rather than by the surrounding tissues. The mutant is apparently without effect on other stem cells for neither the size of the mouse nor the size of other appendages appeared to be affected by the mutant.

Several mammals besides the mouse are known to carry mutants affecting hair length e.g. rabbits, cats, dogs, goats and sheep. In rabbits, cats and dogs, like mice, long-haired animals are homozygous for a recessive allele at a locus determining hair length (rabbit, Fraser, 1953; Cray and Sawin, 1953; cat, Robinson, 1977; dog; Burns and Fraser, 1966). In the rabbit at least, the mutant produces its effect by increasing the duration of anagen VI (Fraser, 1953), this increase being more marked in the rabbit than in the mouse. In the cat and the dog the reason for the increase in hair length has not been determined. The characteristic coats of mohair goats and wool-bearing sheep are due to the action of many genes (goats, Maddocks, personal communication; sheep, Turner, personal communication). Although Lauvergne and Howell (1978) suggest that the long hairs on the spines and thighs

of Corsican goats may be due to a recessive mutant with incomplete penetrance, it is still not known whether other mammals carry a mutant analogous to the angora gene in mice.

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