The genetics of *Sleek*: a possible regulatory mutation of the *tabby–crinkled–downless* syndrome

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SUMMARY

A new mutation, *Sleek*, similar in appearance to mutations at the *Ta*, *cr* and *dl* loci, has been investigated. It is inherited as an autosomal dominant and maps very close to *dl* on chromosome 13. Allelism with *dl* seems probable since *Sleek* interacts with *dl* but not with *cr*. The unusual occurrence of dominant and recessive alleles at the same locus which produce a similar mutant phenotype suggests that the locus might either code for a multimeric protein or a regulatory product.

1. INTRODUCTION

Crinkled (*cr*), *downless* (*dl*) and *tabby* (*Ta*) are three separate loci in the mouse having mutant forms which appear strikingly similar. Both *cr* and *dl* are autosomal recessives, *cr* being located on chromosome 13 and *dl* on chromosome 10 (Falconer, Fraser & King, 1951; Philips, 1960). *Ta* was described by Falconer (1953) and was shown to be *X*-linked. *Ta*, *dl* and *cr* homozygotes and *Ta* hemizygotes are phenotypically indistinguishable, having a thin greasy coat, bare patches behind ears, and a range of abnormalities associated with teeth and other epidermal structures.

A further mutant, *Sleek* (*Slk*), of similar phenotype has recently been discovered (Cattanach, 1975) and an examination of the teeth of affected animals by Sofaer (1977) has demonstrated a spectrum of effects similar to that found in the other mutants. This paper presents evidence to show that *Slk* is a fully viable dominant mutation, located very close to *dl* and is probably allelic with it, as it interacts with *dl* bur not with *cr*. As such it provides a unique example of a locus at which both dominant and recessive mutant alleles show a similar phenotype. Molecular models which could account for the behaviour of *dl* and *Slk* are discussed.

2. MATERIALS AND METHODS

(i) *Stocks*

The *Slk* stock is maintained by repeatedly backcrossing the mutant to the F_1 hybrid between the C3H/HeH and 101/H inbred strains. The inheritance and phenotype of *Slk* were studied on this background. Comparisons with *Ta*, specifically the Ta^F allele, were also made on this hybrid background. The *dl* mutation

is maintained in the Harwell stock CON which also carries Contrasted (Sl^{con}), whereas animals homozygous for cr alone were obtained from the Institute of Animal Genetics, Edinburgh. These mutant animals were all given supplementary feeding hoppers containing food ground up into small pieces whether or not the teeth appeared abnormal.

The following Harwell stocks were used to test for linkage with Slk: LIII, homozygous for *non-agouti* (a), *fuzzy* (fz), *leaden* (ln), *waltzer* (v) and *pale ears* (ep); VGR, homozygous for *waltzer* (v) and *grizzled* (gr) and heterozygous for Contrasted (Sl^{con}); LII heterozygous for *Varitint* (Va), *Extra toes* (Xt) and *Caracul* (Ca).

Early observations on Slk mice indicated that like Ta, dl and cr, the new mutation markedly reduced the number of secondary vibrissae. This character was therefore used as a quantitative measure of severity of effect of the mutants, either singly, or in combination.

In the present study the secondary vibrissae scored 5 days after birth were as follows: supra orbitals $(2+2)$; post orbitals $(1+1)$; post orals $(2+2)$; interamals (3); ulnacarpals $(3+3)$. Figures in brackets represent the score of wild-type animals. The normal score is therefore 19, in contrast to mutant scores which usually fell within the 8–14 range.

3. RESULTS

(i) *Phenotype*

The characteristics of the Ta–cr–dl syndrome have been observed by a variety of authors (Falconer *et al.* 1951; Falconer 1953; Sofaer, 1969 *a b*; Gruneberg, 1971). The following observatons, made on mice which were subsequently shown to be Slk heterozygotes, are consistent with the reports for the other genes in the syndrome.

The coat is thin, greasy and untidy. There is a bald area behind each ear. Analysis under the microscope of hair samples taken from a range of areas throughout the body showed that instead of the four hair types seen in a normal mouse, there is only a single type resembling a thin awl. The appearance of skin pigment and hair is delayed in young mice. The eyelids make a smaller aperture giving the appearance of squinting. Some of the adult mice develop snuffling which, on dissection of the nasal cavity, was found to be due to an accumulation of hair and other material. The incisors are noticeably affected, sometimes failing to appear, or growing at the wrong angle. Sofaer (1977) has studied the molars of Slk heterozygotes and found all the abnormalities characteristic of Ta, cr and dl, but suggested that they were present in a slightly less severe form in Slk. One characteristic of the syndrome is the reduction of hairs on the tail. This is complete in in cr, Slk and dl homozygotes and in the hemizygotes and homozygotes for the Ta^F allele. The Slk heterozygotes resemble the hemizygotes and homozygotes of two other Ta alleles, Ta^C and Ta^J, in having only partial reduction of the hairs on the tail.

(ii) *Origin and inheritance*

The mutant arose in a stock segregating for the Robertsonian translocation Rb(4.6)2Bnr. No mutagenic treatment had been given. The mating in which *Slk* was first seen consisted of a male mated to two females. One female produced 33 normal offspring, whereas the other produced 22 normal and 3 phenotypically *Slk* offspring, 2 males and 1 female. Since *Slk* was subsequently proven to be a dominant mutation these first 3 mutants seen were the product of an original mutational event in one of their parents. The probability that this result could have arisen by random segregation of the mutation in the male parent is 16% (Fisher's exact *t* test) and therefore it seems more likely that the mutation was carried as a clone by the female parent of the first three mutants.

On crossing one of the original male mutants to wild type, 76 offspring were produced, of which 32 animals of both sexes appeared *Slk* and 43 were wild type. The other 2 original mutants when mated together produced 22 mutant and 11 wild-type offspring. The inheritance of *Slk* appeared to be that of an autosomal dominant. In order to test this further, presumptive *Slk* heterozygotes from the first cross were subjected to similar investigations. The results are shown in Table 1.

When crossed to wild-type animals the mutants produced mutant and wild-type offspring in an approximately 1:1 ratio, and an approximately 3:1 ratio was obtained when the mutants were crossed to each other.

Table 1. *Inheritance of* Slk

Mating	Wild type	Mutant hairy tail	Mutant naked tail
$Slk/+ \times +/+$	52	46	0
$Slk/+ \times Slk/+$	21	43	18

It was noticed that the mutant offspring from the intercross matings could be separated into two classes, those with reduced hair on the tails like the original mutant, and those with completely naked tails. Of the 61 mutant progeny classified, 43 had hairy tails and 18 had naked tails. This suggested that those with the more extreme phenotype (naked tail) might be homozygotes. To test this hypothesis, 10 of the mutant hairy-tailed animals and 10 of the naked-tailed animals were outcrossed. In accordance with the above expectations the hairy-tailed animals produced a 1:1 ratio of wild type to mutant animals, proving them to be heterozygotes, whereas the naked tailed animals produced only mutant animals establishing them as homozygotes. The observed 2:1 ratio of hairy tailed: naked tailed mutants therefore indicates that the homozygote is fully viable. There was some reduction of fertility in the *Slk* heterozygote and homozygote females, usually associated with badly affected teeth.

(iii) *Vibrissa Scores of* Slk *and* Ta

Since the presence of tail hairs in *Slk/+* animals indicated that they might not be quite as severely affected as *Slk/Slk* mice or other mutant animals such as Ta^F/Y, *cr/cr*, *dl/dl* it was decided to use vibrissa scores to check this.

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Vibrissa number is almost invariant in normal mice (Dun & Fraser, 1959) but in cr, dl and Ta mice the number is reduced and the variability is increased (Kindred, 1967; Sofaer, 1969b). The reduction has been found to differ in the three mutant types, but this appears to be attributable to genetic background since the vibrissa scores of Ta, cr and dl homozygotes and Ta hemizygotes differ little when backcrossed to a common stock (Sofaer, 1969b). In order to make comparisons between the various mutations it is therefore necessary to eliminate these background effects. Since Slk and Ta^F are maintained on the same genetic background their vibrissa scores were considered to be directly comparable.

Table 2. *The relative effects of* Ta *and* Slk

Mating	Genotype	Tail hairs	Vibrissa scores	
$Slk/+ \times +/+$	$+/+$	Yes	19.0 ± 0.00	
$Ta^F/+ \times +/Y$	Ta^F/Y	No	8.2 ± 0.43	$P < 0.001\%$
$Slk/+ \times +/+$	$Slk/+$	Yes	11.1 ± 0.24	
$Slk/+ \times Slk/+$	Slk/Slk	No	9.0 ± 0.41	$P < 0.01\%$

The results of vibrissa scores of Ta^F hemizygotes, Slk heterozygotes and Slk homozygotes are shown in Table 2. Slk heterozygotes produced by Slk mothers showed no significant difference from Slk heterozygotes produced by Slk fathers and the results from the two types of matings have therefore been combined. Ta^F/Y and Slk/Slk scores were not significantly different from each other, suggesting that both produce the full mutant effect, but both were significantly lower than those of Slk heterozygotes. This indicates that the full mutant effect is not produced in the heterozygote, or in other words, dominance is incomplete.

(iv) *Location of* Slk

It was considered possible that Slk might be allelic with either cr or dl, but not Ta, since the latter is X-linked. In the case of cr, Slk heterozygotes were crossed to the linkage testing stock LII carrying *Extra toes*, (Xt), a dominant gene which is closely linked to cr. Those F_1 animals which were heterozygous for both Slk and Xt were crossed to wild type and the offspring scored for Slk and Xt. As can be seen from Table 3 the recombination frequency was $51.1 \pm 5.5\%$ indicating that Slk and Xt are not linked, and hence that Slk is not allelic with cr.

Linkage tests were also carried out with waltzer (v), a recessive gene which is known to be closely linked to dl (Phillips, personal communication). Slk heterozygotes were crossed to the LIII stock, homozygous for v, and the heterozygous Slk offspring were then backcrossed to the LIII stock to test the segregation of Slk and v. In this case, out of the 415 animals scored, only 4 were crossover types (Table 3). This indicated a recombination frequency of $0.5–1.7\%$ (95% confidence limits).

Since both Slk and dl were now known to be close to v it was decided to test the linkage of Slk and dl directly. To do this Slk heterozygotes were crossed to animals of the CON stock which are homozygous for dl. The Slk offspring doubly heterozygous for both Slk and dl were backcrossed to the dl homozygotes of the CON

Table 3. *Linkage of* Slk

Non-crossover types			Crossover types	
$Slk/+jXt/+$ × $++/++$	$Slk/+;+/+$	$+/+;Xt/+$	$Slk/+;Xt/+$	$+/+;+/+$
	64	50	50	71
$Slk+/+v$ × $+v/+v$	$Slk+/+v$	$+v/+v$	$Slk/+v$	$++/++$
	192	219	3	1
$Slk+/+dl$ × $+dl/+dl$	$Slk+/+dl$	$+dl/+dl$	$Slkdl/+dl$	$++/+dl$
		914 Mutant		0 Normal

Slk–Xt recombination frequency $= 51 \cdot 1 \pm 5 \cdot 5 \%$.
Slk–v recombination frequency $= 0 \cdot 5 - 1 \cdot 7 \%$ (95 % confidence limits).
Slk–dl recombination frequency $= (0 \cdot 8 \%)$ (95 % confidence limits).

stock. The two non-crossover types ($Slk+/+dl$ and $+dl/+dl$) give the mutant phenotype, and it was expected that one crossover type ($Slk\ dl/+dl$) would be indistinguishable from them whereas the other would appear normal ($++/+dl$). Thus only half the crossovers could be detected, and the recombination frequency must be adjusted accordingly. In fact, no crossovers were seen in 914 animals. Using the exact method of defining 95 % confidence limits for a binomial distribution Slk and dl can be calculated to be less than 0·8 recombination units apart with a 95 % certainty.

(v) *Tests for interaction between* Slk *and either* dl *or* cr

It was noticed from the linkage test between Slk and dl that when Slk heterozygotes were mated to dl homozygotes the resultant Slk progeny which should have been doubly heterozygous for Slk and dl had naked tails, like Slk homozygotes. This suggested that there was an interaction between dl and Slk. In order to verify this it was decided to compare the vibrissa scores of the double heterozygotes with litter-mates heterozygous for Slk alone by using the closely linked gene v as a marker. In order to do this $Slk+v/++v$ animals obtained as recombinants from the linkage testing were mated to $+dl+/++v$ animals. In the absence of crossing over the resultant mutant progeny should be of two types; $Slk+v/+dl+$ (phenotypically Slk) and $Slk+v/++v$ (phenotypically $Slk\ v$). On examination of the progeny, all of the phenotypically Slk mice had hairless tails, and all of the phenotypically $Slk\ v$ mice had hairy tails, thus confirming the original observation and indicating that no crossovers had in fact occurred. The results of vibrissa scores on these two types of animal are shown in Table 4. It can be seen that the vibrissa scores of $Slk+v/+dl+$ animals were significantly lower than those of $Slk+v/++v$ animals, indicating that dl does indeed add to the mutant effect of Slk.

The interaction of Slk and cr was investigated in a similar way using Xt which is closely linked to cr. A Slk heterozygote was mated to a cr homozygote and it was

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Table 4. The *effects of* dl *and* cr *on* Slk

Mating	Genotype	Tail hairs	Vibrassa Scores	
$Slk + v/ + + v$	$Slk + v/ + + v$	Yes	13.0 ± 0.32	$P < .05\%$
$\times$				
$+ dl + / + + v$	$Slk + v/ + dl +$	No	11.6 ± 0.37	
$Slk/ + ; + + / + +$	$Slk/ + ; Xt + / + +$	Yes	11.1 ± 0.28	NS
$\times$				
$+ / + ; Xt + / + cr$	$Slk/ + ; + + / + cr$	Yes	10.8 ± 3.1	

found that all the mutant ($Slk/ +$, $+ /cr$) progeny had hairy tails, indicating that at least for hairiness of tails there was no interaction between Slk and cr. $Slk/ +$ animals were mated to $Xt + /cr$ animals in order to score the vibrissa of the progeny which were expected to be $Slk/ + ; Xt + / + +$ or $Slk/ + ; + + / + cr$ in the absence of crossing over between Xt and cr. Vibrissa scores shown in Table 4 did not demonstrate any significant difference between the Slk heterozygotes and the double heterozygotes for Slk and cr. Thus there is no evidence of interaction between Slk and cr on the basis of either vibrissa number or tail hair.

4. DISCUSSION

The linkage data suggest that Slk and dl are allelic since no crossing over was detected. However, the data cannot exclude very close linkage of less than 0·8 centimorgans and, unfortunately, this length of chromosome could contain many genes. Allelism could only be proven by linkage data alone if tens of thousands of progeny were scored. However, the fact that Slk and dl are so closely linked and give near identical phenotypes strongly suggests that they are alleles at a single locus. The observation that they interact in the double heterozygote whereas no interaction was found between Slk and cr also favours the hypothesis of allelism. Accordingly, it is proposed that the gene symbol Slk be modified to Dl^{slk}. If Dl^{slk} and dl are indeed allelic as indicated this would be the first example in the mouse of a locus in which both dominant and recessive mutant alleles produce a similar mutant phenotype.

A variety of models could be offered to account for the observed phenotypes. For example, it has been suggested that the effects of some mutants may be caused by a reduction in concentration of an enzyme produced by a locus (Kacser & Burns, 1969). If the concentration is critical to normal metabolic function a mutant will be dominant, and if not it will be recessive. However, in the case of the dl locus which appears to have both dominant and recessive mutations of similar phenotype it is difficult to assign enzyme concentrations to each allele in a way which could account for the observed phenotypes. A more satisfactory model could be based on the production of a multimeric protein by the dl locus. If the Dl^{slk} allele was to produce a protein subunit which forms multimers either with itself or with that of the wild-type allele and both are inactive, the only active multimers would be those composed entirely of wild-type protein. The number of subunits in the multimer would not have to be very

large before the concentration of active protein in $Dl^{slk}/+$ animals is reduced severely enough to give the mutant phenotype. If the *dl* allele were to produce none of the protein the homozygote would be mutant but the heterozygote would still have half the concentration of active product and therefore would appear normal. Such a model has already been proposed to explain the behaviour of mutations at the histidase structural locus (*hut H*) of *Salmonella* (Hagen, Lipton & Magasanik, 1974).

Models based on gene regulation could also be proposed. In microorganisms mutations at regulatory loci have been reported (for review see Beckwith & Rossow, 1974) but the phenotypes produced by dominant and recessive mutations at the same locus are different. In this respect there is more similarity to mutations at the agouti (*a*) and extension (*e*) loci of the mouse than to those at the *dl* locus. The latter could, however, be understood on the basis of production of an activator molecule by the *dl* locus which is responsible for activating other genes by binding to sensor sites on them (Britten & Davidson, 1969). If the Dl^{slk} allele were to produce an activator which is slightly distorted at its sensor recognition site then this distortion could cause it to 'stick' in the sensor sites without activating the relevant genes. Therefore, in the Dl^{slk} heterozygote the Dl^{slk} activator would outcompete the normal activator and the mutant syndrome would be produced. As in the above multimer model, the *dl* allele would be recessive if it were a null allele. It should be possible to investigate the effects of dosage of wild-type product on Dl^{slk} by constructing trisomies with the genotype $Dl^{slk}/+/+$. It would be expected that the activator system would be less susceptible to extra copies of the wild-type gene than the multimer system.

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