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Site of beige (*bg*) and leaden (*ln*) pigment gene expression determined by recombinant embryonic skin grafts and aggregation mouse chimaeras employing sash (W^{sh}) homozygotes

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SUMMARY

Aggregation chimaeras were constructed by fusing embryos homozygous for sash (W^{sh}) with fuzzy, leaden beige (*fz*, *ln*, *bg*) homozygotes to investigate the site of action of the beige and leaden loci. The genotype of the hair follicle was identified by fuzzy alleles ($++^{fz}$ or *fzfz*). All melanocytes were derived from the fuzzy leaden beige population, as sash homozygotes do not produce functional melanocytes. Reciprocal recombinant epidermal//dermal skin grafts were constructed from 14-day embryonic skin of homozygous *fz ln bg* and either albino (*aa cc*) or pink-eyed dilution (*pp*) embryos to test for any dermal expression of leaden or beige, since the epidermal and dermal genotype of the chimaeric hair follicles could differ.

Patches of fuzzy and non-fuzzy hairs were distributed throughout the coats of two of the three chimaeras obtained. The pigmented regions were blue grey, typical of the leaden beige interactive phenotype. Large abnormal beige granules were found in fuzzy and non-fuzzy hairs. Melanocytes in both classes of growing follicles were nucleopetal, typical of leaden. Similarly, the results of the 14-day skin grafts showed that the beige and leaden loci are melanocyte-autonomous.

The chimaeras showed a pigment distribution resembling the heterozygous sash phenotype. Thus the 1:1 gene dosage of sash:wild type in heterozygotes and chimaeras has an overall effect on pigment pattern that overrides the predicted random distribution of the melanocyte precursors.

1. INTRODUCTION

Both leaden (*ln*) and beige (*bg*) are autosomal recessive mutations in the mouse that reduce the pigment intensity of the coat (Silvers, 1979). Their phenotypic effects are, however, the product of different mechanisms. Beige, a potential model for the human disorder Chediak–Higashi syndrome (Lutzner, Lowrie & Jordan,

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1967), increases pigment granule size (Pierro & Chase, 1963) at the expense of number (Pierro, 1963). Leaden, on the other hand, clumps the distribution of normal-sized pigment granules because of a change in melanocyte morphology (Markert & Silvers, 1956). Since both loci are of potential use in the development of *in vivo* somatic cell mutational testing systems (Searle, 1977, 1978; Searle & Stephenson, 1982; Stephenson, 1983) it is important to establish whether gene expression is cell-autonomous or mediated through the local tissue environment.

Site of pigment gene expression has been the subject of many investigations (see Silvers, 1979). Several methods have been used that involve the transplantation of embryonic or neonatal skin, which may have been manipulated in some way (i.e. separated into components and reconstructed) or combined with neural crest material, to sites capable of promoting normal growth and development (see Poole & Silvers, 1980). However, this approach has the limitation that it requires sampling after a specific time during the development of the skin rather than evaluating gene expression under the restraints of normal development *in situ*.

Mintz (1969*a*) appreciated the potential of using chimaeras to investigate the site of pigment gene expression, and the technique has been exploited to some extent by others (Petters & Markert, 1979). However, interpretation of the results can be difficult, if not impossible, unless the genotypes of the tissue environment and the melanocytes can be distinguished by markers independent of the loci under test.

The role of the dermis in stimulating the development of hair follicles has been known for some time (Kollar, 1966, 1970). However, certain mutants affecting the texture of the coat (e.g. tabby, *Ta*; fuzzy, *fz*; downless, *dl*) have shown that hair morphology is a product of the epidermal genotype (Sofaer, 1973; Green *et al.* 1974, 1977; Mayer, Mittelberger & Green, 1974; Mayer, Kleiman & Green, 1976; Mayer, Miller & Green, 1977; Mayer & Green, 1978; Pennycuik & Raphael, 1984). Thus alleles at these loci can be used as markers to identify the genetic origins of the follicular environment.

Similarly, the melanocyte population not carrying the gene under investigation should be: (*a*) tagged with a melanocyte-autonomous marker affecting the quality of pigmentation (e.g. pink-eyed dilution, *p*, Stephenson & Hornby, 1985); or (*b*) eliminated entirely by the use of homozygous dominant spotting mutants. Follicles in the non-pigmented regions of the coats of spotting mutants (e.g. piebald, *s*; dominant spotting, *W* and steel, *Sl*) lack cells displaying the morphological characteristics of melanocytes (Silvers, 1953, 1958). Gene expression at the *W* locus is thought to be cell-autonomous as the follicles from the non-pigmented regions of the coat are capable of supporting the development and differentiation of melanocytes derived from an external source (Silvers & Russell, 1955; Mayer, 1970, 1973, 1979; Mayer & Green, 1968; Stephenson & Hornby, 1985). Lyon & Glenister (1982) described a dominant spotting allele (sash, W^{sh}) which displays the typical homozygous black-eyed white phenotype of other *W* alleles due to an absence of neural crest-derived melanocytes (Mayer, 1970, 1973; Mayer & Green, 1968). Unlike the other *W* alleles, $W^{sh}W^{sh}$ is both viable and fully fertile. This unusual property provides an opportunity, not afforded by other mating regimes involving *W* alleles (Mayer, 1973), to produce embryos that are all genetically devoid of melanocytes which may be used for constructing chimaeras.

Homozygous fuzzy leaden beige ↔ sash (*fz ln/fz ln bgbg* ↔ $W^{sh}W^{sh}$) aggregation chimaeras were constructed to establish the site of expression of the genes at the beige and leaden loci. Both constituent embryos were homozygous for the wild-type agouti allele (*A*), since the phenotypic effects of leaden in combination with beige are best observed on an agouti background. The presence of fuzzy, which greatly modifies the morphology of the hairs (Dickie & Woolley, 1950) was used to identify the genetic origins of the follicular environment. The sash component ensured a single population of melanocytes (i.e. beige leaden). Fourteen-day recombinant embryonic skin grafts were also constructed to look for any expression of either leaden or beige mediated through the dermis, since hair follicles in the chimaera could have different epidermal and dermal genotypes.

2. MATERIAL AND METHODS

(i) *Mice*

Homozygotes for fuzzy leaden beige (*fz ln/fz ln bgbg*) were obtained from Dr J. F. Loutit at Harwell. Sash ($W^{sh}W^{sh}$) homozygotes were derived from the Harwell PM stock maintained by random breeding on a 3H1 (C3H/HeH ♀ × 101/H ♂) background. Other stocks used in this study were also obtained from the Harwell breeding stocks, namely: CBA/CaH − +*p* (homozygous for pink-eyed dilution, *p*) and JU/FaCt (homozygous for non-agouti, *a* and albino, *c*) 3H1 (F_1 C3H/HeH ♀ × 101/H ♂) and T(7; 19)145H (a male sterile stock).

(ii) *Recombinant skin technique*

Fourteen-day reciprocal recombinant embryonic grafts were constructed between fuzzy leaden beige and either albino or pink-eyed dilution skin using the method described previously (Stephenson & Hornby, 1985) following the protocol of Mayer & Fishbane (1972).

Timed matings were set up to produce 14-day-old foetuses (counting the day of the vaginal plug as day zero of gestation). Pregnant females were killed by cervical dislocation and the intact uterus was dissected out and placed in Tyrode's salt solution (Difco Laboratories). Embryos were removed and placed in fresh Tyrode's salt solution. Small pieces of skin were removed from the flanks between the developing limb-buds. The skin was placed in a 1 % trypsin solution (Sigma) dissolved in Tyrode's salt solution without Ca^{2+} and Mg^{2+} (Difco Laboratories) at 4 °C to separate the dermis from the epidermis. When complete separation was possible further digestion of the tissue was stopped by the addition of 40 % horse serum (Gibco-Bio-Cult) in Tyrode's salt solution. Epidermal tissue was transferred to an organ culture dish (Falcon) containing a semi-solid culture medium (Eagle's medium (Gibco-Bio-Cult), penicillin/streptomycin (Gibco-Bio-Cult), horse serum and 0·5 % agar). The dermis was placed on top of the outstretched epidermis and excess fluid removed. The recombinants were cultured for 12–24 h at 37 °C in a CO_2-enriched environment.

The recombinant grafts were cultured for a further three weeks by placing them beneath the testicular tunica of anaesthetized adult male mice. After this period the mice were killed and their testes dissected out and fixed in formol saline,

dehydrated and cleared in benzyl alcohol. The pigmentation of the hair produced in successful grafts was examined at the macrosopic and microscopic levels. The genotypes of the tissues in the various graft combinations are described in the text as epidermal genotype//dermal genotype.

(iii) *Construction of chimaeras*

Aggregation chimaeras were constructed according to the method of Mintz (1971*a*) with modifications to: (i) the culture media as described by Whittingham (1971) and Quinn, Barros & Whittingham (1982); and (ii) the culture conditions as defined by Cattanach, Wolfe & Lyon (1972).

Donor females were super-ovulated, by injecting 5 i.u. of pregnant mare's serum i.p. at 4.00 p.m. followed 44 h later by 5 i.u. of human chorionic gonadotropin, and mated. Three days later (two days after the observation of a vaginal plug) the females were killed by cervical dislocation and the oviducts were dissected out. Embryos were flushed from the ducts with M2 Hepes-buffered embryo culture medium (Quinn *et al.* (1982). The zonae pellucidae were removed from eight-cell embryos by pronase digestion. After several washes in fresh M2 medium, pairs of denuded embryos of different genotypes were transferred to droplets of No. 16 culture medium (Whittingham, 1971) under paraffin oil. Aggregation of the paired embryos and further development were encouraged by culturing for 24 h at 37 °C in a CO_2-enriched environment (Cattanach *et al.* 1972). Blastocysts produced from the aggregated embryos were surgically injected into the uteri of pseudo-pregnant 3H1 females mated to genetically sterile T(7;19)145H males (Cattanach *et al.* 1972).

(iv) *Analysis of chimaeras*

The distribution of pigment was noted shortly after birth. The proportion of pigmented and non-pigmented coat was measured with a Cambridge Quantimet 720 from photographs of the adult dorsum. The pigment phenotype of the coat was examined at both the macroscopic and microscopic (dry-mounted plucked hairs) levels. Patches of fuzzy and normal hair are most easily distinguished in a growing coat, since fuzzy hairs erupt slightly later than normal hairs. The distribution of the patches of fuzzy and normal hairs was therefore determined shortly after the eruption of the first pelage. Progeny testing was carried out by crossing the chimaeric offspring to mice from the recessive fuzzy leaden beige stock. Samples of venous blood were taken from the tail, smeared on glass slides, fixed in formalin vapour and stained according to the method of Sheenham & Storey (1947) with Sudan black to identify lysosomes.

3. RESULTS

(i) *Recombinant skin grafts*

Of the 18 recombinant grafts implanted 13 were recovered (Table 1). Results of control grafts between albino and pink-eyed dilution 14-day embryonic skin (i.e. *aa cc//aa cc* and *pp//pp*) were presented in an earlier publication (Stephenson &

Hornby, 1985), and demonstrate that gene expression is not influenced by the manipulative techniques. Similarly, the production of fuzzy hairs containing large clumped pigment granules by the reconstructed fuzzy leaden beige skin (Table 1) also demonstrates that the manipulation of the tissue does not affect the expression of either the fuzzy or the beige genes. There were too few hairs to score the leaden phenotype, since the identification of leaden depended on the overall macroscopic appearance.

Table 1. *Fourteen-day recombinant embryonic skin grafts constructed to determine the role of the dermis on gene expression at the* bg *and* ln *pigment loci*

Genotype		Grafts		
Epidermis (i.e. melanocyte and hair)	Dermis (environment)	Implanted	Recovered	Hair morphology and pigmentation
fz ln/fz ln bgbg	*fz ln/fz ln bgbg*	1	1	Fuzzy hairs containing large clumped pigment granules
fz ln/fz ln bgbg	*aa cc*	3	2	Fuzzy hairs containing large clumped pigment granules
aa cc	*fz ln/fz ln bgbg*	2	2	Normal non-pigmented hairs
fz ln/fz ln bgbg	*pp*	6	3	Fuzzy hairs containing large clumped pigment granules
pp	*fz ln/fz ln bgbg*	6	5	Normal yellow agouti* hairs

* A yellow-looking hair due to a reduction in black (eumalanin) pigmentation typical of the agouti pink-eyed dilution phenotype.

In the recombinant grafts between either albino or pink-eyed dilution epidermis and fuzzy leaden beige dermis (i.e. *aa cc//fz ln/fz ln bgbg* and *pp//fz ln/fz ln bgbg*) hairs displayed the normal wild-type morphology. Hairs of the *aa cc//fz ln/fz ln bgbg* recombinant grafts lacked any evidence of pigmentation (Table 1). The *pp//fz ln/fz ln bgbg* recombinant grafts produced pigmented hairs with the yellow agouti phenotype (i.e. a predominantly yellow hair with reduced black eumelanin content) typical of the epidermal melanocyte genotype (i.e. *pp*).

The reciprocal grafts between fuzzy leaden beige epidermis and either albino or pink-eyed dilution dermis (i.e. *fz ln/fz ln bgbg//aa cc* and *fz ln/fz ln bgbg//pp*) produced hairs with the aberrant fuzzy morphology, thus confirming the observations made by Mayer *et al.* (1974) that fuzzy is expressed within the epidermis. Poor hair density made it impossible to assess the pigmentation phenotype at the macroscopic level. However, large clumped pigment granules could be seen when the hairs were examined microscopically, demonstrating that beige gene expression within the follicular melanocytes does not require an interaction between the melanocyte (resident in the epidermis) and the dermal tissue environment. As noted previously, it was impossible to evaluate the behaviour of the leaden gene

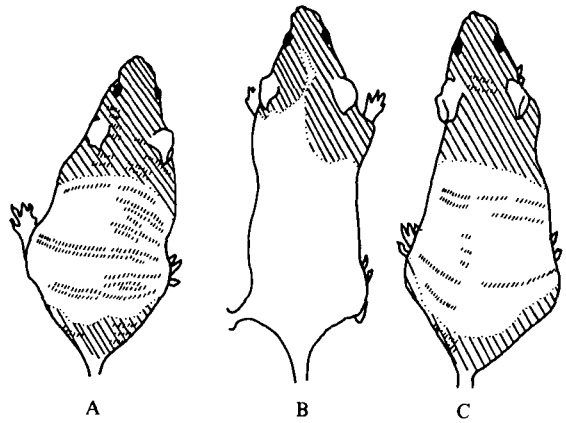


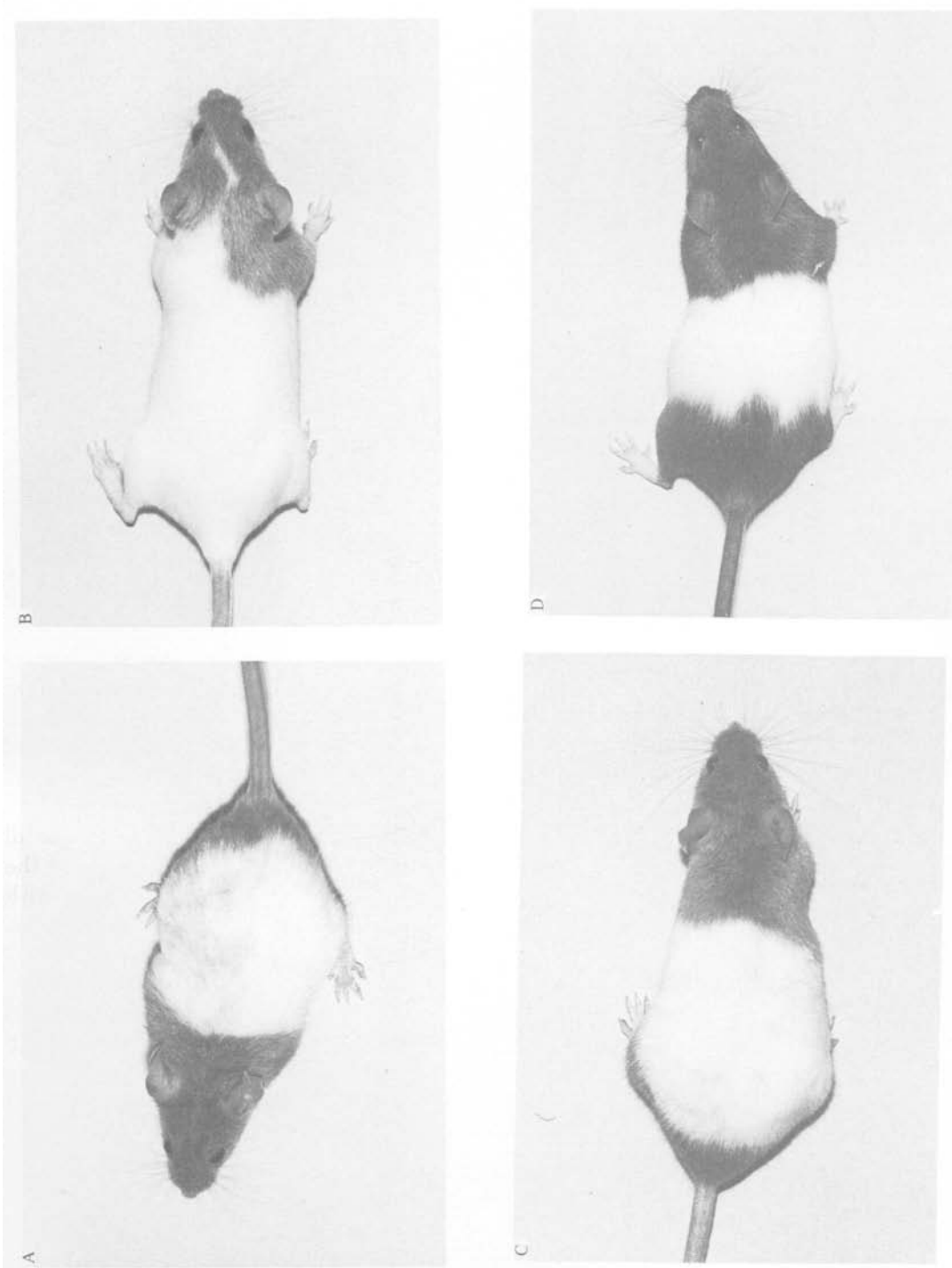
Fig. 1. Approximate distribution of the thin transverse bands of morphologically fuzzy hair ▤ recorded shortly after the eruption of the first pelage superimposed on drawings taken from the photographs of the adults shown in Plate 1.

because too few hairs were produced by the grafts to carry out unequivocal macroscopic observations.

(ii) *Chimaeras*

Three chimaeras were produced from the aggregation of fuzzy leaden beige to homozygous sash (*fz ln/fz ln bgbg*↔W^{sh} W^{sh}) eight-cell stage embryos, one male and two females (Plate 1). Pigmentation in the skin shortly after birth, and later in the coat, was confined to the head and shoulders of all the mice and the rump of chimaeras 1 and 3. Examination of the coat after the eruption of the first pelage revealed relatively thin transverse bands of poor hair growth characteristic of the fuzzy phenotype. These bands were dispersed throughout the dorsal coat of chimaeras 1 and 3 in contrast to the distribution of pigmentation (Fig. 1). At the macroscopic level, the pigmented regions of the coat had a blue-grey colour with a slight yellow haze indicative of the interactive phenotype produced by the leaden beige melanocytes. At the microscopic level, large pigment granules with a clumped distribution were clearly visible in the hair shaft of both normal and abnormal (fuzzy type) hairs plucked from the pigmented region on the head and shoulders of chimaeras 1 and 3. Phaeomelanin was absent in guard hairs of both genotypes and reduced in the other classes of hair. The melanocytes found in actively growing follicles displayed the rounded nucleopetal morphology typical of the leaden mutant (Markert & Silvers, 1956). In the non-pigmented regions both fuzzy and morphologically normal hairs lacked any detectable levels of pigmentation. The proportion of coat pigmentation (Table 2) and distribution (Plate 1) in the chimaeras was similar in phenotype to the sash heterozygote (Plate 1 D) in which the non-pigmented region occupied approximately 50% of the dorsum (Stephenson, unpublished information). Analyses of the other tissues (i.e. germ cells and white blood cells) are also presented in Table 2.

Germ-cell analysis confirmed the genetic status of the chimaeras. Both the



Distribution of pigmentation in the three *fz ln/fz ln bgbg* ↔ $W^{sh}W^{sh}$ aggregation chimaeras (Chimaera 1♀, 2♀ and 3♂ plates. A, B and C respectively) and a W^{sh} heterozygote (plate D). Note that the pigment intensity in the three chimaeras is reduced when compared with the wild-type pigmentation of W^{sh} heterozygote.

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proportion of pigmentation and the number of fuzzy leaden beige offspring displayed the same rank order for the three chimaeras (3 > 1 > 2). However, the proportion of the fuzzy leaden beige offspring from chimaeras 1 and 2 was lower than predicted by the amount of pigmentation in the coat (Table 2). The

Table 2. *Proportion of the fuzzy leaden beige component in different tissues of each chimaera*

Chimaera	Germ line*	Frequency (%)		
		Coat-pigmentation†	White blood cells‡ (1)	(2)
1♀	18·5 (10/54)	42	59·8	57·9
2♀	2·4 (1/41)	27	5·5	69·3
3♂	58·9 (66/112)	49	29·6	53·3

* Value in parentheses refers to the actual number of individuals of the total number examined.

† Pigmented (*lnln bgbg*) regions.

‡ Cells containing large (*bgbg*) lysosomes. A sample of approximately 200 cells were scored for each observation. The two samples were taken on different occasions.

proportion of the two genotypes in the white blood cell population varied with the time of sampling but this was to be expected from previous reports (Warner, McIvor & Stevenheus, 1977; Burton *et al.* 1982). Classification of white blood cell genotype, on the bases of lysosomal size (i.e. large lyosomes = beige cells (Lutzner, Lowrie & Jordan, 1967), small lysosomes = sash cells) is subject to error, since control samples yielded a 2–3 % frequency of cells with small lysosomal size in the beige homozygote and 10–11 % frequency of cells with large lysosomal size in the sash homozygote.

4. DISCUSSION

(i) *Autonomous expression at the* bg *locus*

Abnormal pigment granule morphology and size, indicative of the *bg* phenotype (Pierro, 1963; Pierro & Chase, 1963), in the fuzzy hairs of the recombinant grafts between *fz ln/fz ln bgbg* epidermis and either *aa cc* or *pp* dermis (Table 1) demonstrated that gene expression at the *bg* locus was not mediated through the dermal tissue environment. Since *bg* is hypostatic to *p* (Pierro & Chase, 1963) no conclusive information concerning the influence of *bg* dermis on pigment gene expression can be gleaned from the yellow agouti pigmentation of the hairs produced by the *pp//fz ln/fz ln bgbg* (epidermal//dermal genotype) recombinant grafts. In the chimaera study, hair structure was used to indicate the cellular genotype of the epidermally derived follicular environment (i.e. fuzzy hairs = a beige/leaden follicular environment and non-fuzzy hairs = a wild-type or non-beige/leaden follicular environment). Both the non-fuzzy hairs and the fuzzy hairs contained large abnormal granules typical of beige. This demonstrates that the *bg* locus is expressed within the melanocyte and not through the epidermal environment, since the only melanocytes in the chimaera were genetically fuzzy, leaden beige.

The presence of two populations of white blood cells, identified by large or small lysosomes, in the chimaeric mice lends further support in favour of autonomous expression of genes at the *bg* locus. Beige cells from a variety of tissues (e.g. white blood cells, liver, kidney) contain enlarged lysosomes (Lutzner *et al.* 1967; Oliver & Essner, 1973). In the kidney this is associated with abnormal β-glucuronidase activity (Brandt, Elliott & Swank, 1975; Brandt & Swank, 1976). Microtubule function is also impaired (Oliver, Zurier & Berlin, 1975) and this might explain the abnormality in lysosome size (Oliver, Kraweic & Berlin, 1976). Like lysosomes, melanosomes arise from membranous vesicles derived from the smooth endoplasmic reticulum in the region of the Golgi body (Maul, 1969), suggesting a common origin for the two subcellular structures. If melanosomes and lysosomes have a common origin then the diversity of tissues reported to contain enlarged lysosomes, the presence of two distinct populations of mast cells (Kitamura, Shimada & Hatanaka, 1977; Kitamura, Matsuda & Hatanaka, 1979) and of osteoclasts (Ash, Loutit & Townsend, 1981) in other types of chimaeric mice incorporating beige would tend to favour autonomous gene expression at the *bg* locus, rather than an interaction between cells of one type and those of the surrounding tissue environment.

In the present study, where melanocytes were exposed to dermis (as in the recombinant embryonic skin grafting experiment) or epidermis (as in the chimaera study) carrying the wild-type allele, they continue to produce large pigment granules typical of the *bg* phenotype, thus providing positive evidence in favour of autonomous gene expression at the *bg* locus.

(ii) *Autonomous expression at the* ln *locus*

The distinctive yellow agouti phenotype of the hairs produced by the *pp//fz ln/fz ln bgbg* recombinant grafts, indicative of the agouti pink-eyed dilution genotype of the melanocytes and epidermis, suggested that genes at the *ln* locus do not act through the dermis. It was not possible to confirm this from the reciprocal grafts (*fz ln/fz ln bgbg/pp* or *aa cc*) since poor hair growth prevented the macroscopic scoring of the phenotype and there were no growing hair follicles, so melanocyte morphology could not be observed. The blue-grey colour, indicative of the leaden beige phenotype, in the pigmented regions of the coat of all three chimaeras (Plate 1) regardless of the distribution of normal and fuzzy hairs suggested that *ln* gene expression is melanocyte-autonomous and not mediated through the epidermal tissue environment. This was confirmed by the observations of morphologically distinct nucleopetal leaden beige melanocytes in the follicles of non-fuzzy hairs plucked from the coat. If leaden gene expression was mediated through the tissue environment then the pigment phenotype in the chimaeras would presumably display a banding pattern. Non-fuzzy hair pigmentation would be characteristic of agouti beige phenotype (i.e. a yellow-brown colour). Fuzzy hairs would conform to the leaden beige phenotype (i.e. the observed blue-grey colour).

Markert & Silvers (1959) observed that leaden melanocytes, derived from embryonic skin implanted in the anterior chamber of the eye, displayed a range of morphological phenotypes: from nucleopetal (leaden) to nucleofugal (wild-type)

in both leaden and wild-type eyes. They concluded that nucleopetal morphology was a function of both the deficient leaden melanocyte and the ability of the surrounding tissue to resist dendrite development. This ambivalent conclusion arises out of the use of a tissue environment in which melanocytes are not normally found. Satisfactory resolution of the situation can only be obtained by considering the behaviour of the melanocytes in their normal tissue environment as investigated in the present study.

Additional evidence in favour of autonomous gene expression in the melanocyte may be derived from the chimaera studies of Mintz (1974). She demonstrated that for different loci the number of clonal bands varied according to the site of gene expression (Mintz, 1969*a*). Thus chimaeras involving loci that affect hair morphology (e.g. $++^{fz} \leftrightarrow fzfz$, $++^{a} \leftrightarrow aa$) have 150–200 clonally derived bands of hair, whilst chimaeras for loci expressed in the melanocyte (e.g. $++^{b} \leftrightarrow bb$) show an archetypal pattern based upon ~34 bands. She constructed $++^{ln} \leftrightarrow lnln$ chimaeras and described their phenotype as typical of melanocyte clones (Mintz, 1974), suggesting autonomous gene expression at the *ln* locus.

(iii) *Melanocyte distribution in the chimaeras*

The distribution of pigmentation observed in the chimaeras constructed in the present study (Plate 1 A–C) was reminiscent of that produced by the W^{sh} heterozygote (Plate 1 D). The observation that chimaeras constructed, in part, from homozygous spotting mutants (i.e. white, Mi^{wh}; dominant spotting, W; viable dominant spotting W^{v}) mimic the heterozygous phenotype of the spotting mutant is not unique (Mintz, 1969*b*, 1970, 1971*b*; Bradbury, 1983). Mintz (1971*b*) described a $WW \leftrightarrow bb\ dd$ chimaera in which the pigment cell distribution was similar to the W heterozygote whilst the pigmentation was exclusively brown dilute (*bb dd*). An intriguing question which arises from the observations of the present study and those reported by Mintz (1971*b*) is the following. Why should melanocytes (leaden beige or brown dilute) that do not carry the spotting gene produce a phenotype similar to the spotting heterozygote?

Two hypotheses have been proposed in an attempt to explain the melanocyte distribution produced by the spotting mutants. Schaible (1969), by selectively breeding mice for increased amounts of white spotting, presented a model based upon the proliferation and migration of melanocytes from 14 discrete pigment centres. Although in accord with the clonal origin of melanocytes (Mintz, 1967), the number of pigment centres is much smaller than the number of clonal bands described in chimaeras (Mintz, 1967) and X-inactivation mosaics (Cattanach, 1974) and may be due to a shortage of melanocyte precursors in the spotting mutants.

Mintz (1971*b*, 1974) proposed a pre-programmed clonal cell death model which assumes that particular clones of melanocytes are destined to die after populating a particular region of the skin, thus creating an area devoid of pigment cells. However, Deol (1971, 1973) suggested from histological studies of the eye, Harderian gland and inner ear of several spotting mutants that the melanocytes do not die but fail to differentiate.

Mintz (1971*b*) used the pre-programmed clonal cell death model to explain the

heterozygous phenotype of the $WW \leftrightarrow bb\ dd$ chimaeras and assumed that the melanocytes destined to die in the chimaeras were those carrying the spotting gene. This implies non-random assortment of the melanocyte precursors prior to their migration from the neural crest, since such chimaeras show a non-random distribution of pigmented and non-pigmented regions. In chimaeras in which both melanocyte populations are viable (e.g. $++^{c} \leftrightarrow cc$), the precursors appear to have been distributed at random (Mintz, 1967, 1970, 1971*b*, 1974). Similarly, in the present study the distribution of epidermal clones ($++^{fz} \leftrightarrow fzfz$) appears to be random. Non-random assortment does however occur with particular strain combinations (West & McLaren, 1976). They observed that melanocytes from one strain displayed a tendency to populate either the anterior or posterior end of the dorsal coat, and attributed it to differences in selection pressures acting on the two melanocyte populations during their migration from the neural crest.

The precise location of the banded region in the $W^{sh}W^{sh} \leftrightarrow fz\ ln/fz\ ln\ bgbg$ chimaeras cannot be explained by pre-programmed clonal cell death of $W^{sh}W^{sh}$ melanocytes if random assortment of melanocyte precursors were also occurring. Mayer & Green (1968) demonstrated that the absence of hair pigmentation in W^{v} homozygotes was probably due to a failure in melanoblast migration from the neural crest, since 12-day embryonic $W^{v}W^{v}$ skin was capable of supporting melanocytes derived from the wild-type neural crest, but 12-day wild-type embryonic skin cultured with $W^{v}W^{v}$ neural crest failed to produce pigmented hairs. Failure in melanoblast migration is not the cause of all spotting phenotypes (Mayer, 1970, 1973; Mayer & Green, 1968), but since in the present study we were examining the behaviour of the W locus it is assumed that the absence of pigmentation attributed to W^{v} holds for other alleles in the series. So in a chimaera, if the spotting melanocytes fail to migrate, any melanocytes that are produced by the other genotype would surely be free to populate the entire coat within the limits of the size of the melanocyte (or precursor) population and the stage of development.

Thus, if pre-programmed clonal cell death is to be accepted as an explanation for the heterozygous phenotype of $W^{sh}W^{sh} \leftrightarrow ++^{W}$ chimaeras, it would be necessary to propose that among the melanocytes (or precursors) that die are some that do not carry the spotting gene. As (i) neonatal skin grafts (Silvers & Russell, 1955); (ii) neural crest grafting techniques (Mayer & Green, 1968); and, (iii) recombinant skin studies (Mayer, 1970, 1973, 1979; Stephenson & Hornby, 1985); have demonstrated that the skin of dominant spotting mutants (including W^{sh}) is capable of supporting melanocyte development and differentiation it suggests that the apparent absence of melanocytes is not an inherent property of the local tissue environment. Furthermore the distribution of immunological markers (Mintz, 1970; Mintz & Silvers, 1970) and hair morphology mutants (as in the present study) in chimaeras shows that the non-pigmented area contains regions of a follicular environment that would normally favour melanocyte development and differentiation. The 1:1 gene dosage of spotting:wild-type alleles as in heterozygotes (e.g. $W^{sh}+$) or aggregation chimaeras (e.g. $W^{sh} \leftrightarrow fz\ ln/fz\ ln\ bgbg$) appears to have an overall effect on the pattern of pigmentation that overrides the predicted random distribution of the melanocyte precursors in the chimaeras.

In conclusion our experiments show that gene expression at the beige and leaden loci is melanocyte-autonomous and is not mediated through the local tissue environment of the hair follicle. The distribution of melanocytes in the *fz ln/fz ln bgbg* ↔ $W^{sh}W^{sh}$ chimaeras is not random but mimics that of the sash heterozygote (see Plate 1) and raises some interesting questions about the mode of gene expression at the *W* locus.

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