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Interaction of the Murine *dilute suppressor* Gene (*dsu*) With Fourteen Coat Color Mutations

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ABSTRACT

The murine *dilute suppressor* gene, *dsu*, was previously shown to suppress the dilute coat color phenotypes of mice homozygous for the *dilute* (*d*), *leaden* (*ln*), and *ashen* (*ash*) mutations. Each of these mutations produce adendritic melanocytes, which results in an abnormal transportation of pigment granules into the hair shaft and a diluted coat color. The suppression of each mutation is associated with the restoration of near normal melanocyte morphology, indicating that *dsu* can compensate for the absence of normal *d*, *ln* and *ash* gene products. In experiments described here, we have determined whether *dsu* can suppress the coat color phenotype of 14 additional mutations, at 11 loci, that affect coat color by mechanisms other than alterations in melanocyte morphology. In no case was *dsu* able to suppress the coat color phenotype of these 14 mutations. This suggests that *dsu* acts specifically on coat color mutations that result from an abnormal melanocyte morphology. Unexpectedly, *dsu* suppressed the ruby eye color of *ruby-eye* (*ru*) and *ruby-eye-2* (*ru-2*) mice, to black. The exact nature of the defect producing these two mutant phenotypes is unknown. Histological examination of the pigmented tissues of the eyes of these mice indicated that *dsu* suppresses the eye color by increasing the overall level of pigmentation in the choroid but not the retinal pigmented epithelium. Choroid melanocytes, like those in the skin, are derived from the neural crest while melanocytes in the retinal pigmented epithelium are derived from the optic cup. This suggests that *dsu* may act specifically on neural crest-derived melanocytes. These studies have thus identified a second group of genes whose phenotypes are suppressed by *dsu* and have provided new insights into the mechanism of action of *dsu*.

THE murine *dilute suppressor* (*dsu*) locus (SWEET 1983) acts in a semidominant manner (MOORE *et al.* 1988a) and darkens, to nearly wild type, the diluted coat-color phenotype of mice homozygous for *dilute* (*d*), *leaden* (*ln*) (MURRAY 1931) and *ashen* (*ash*) (LANE and WOMACK 1979) mutations (MOORE *et al.* 1988b). Suppression results from *dsu*-mediated changes in melanocyte morphology. The melanocytes of *d*, *ln* or *ash* mice are adendritic, yet, in the presence of *dsu*, the melanocytes are dendritic. This facilitates an increase in the transportation of melanosomes into the hair shaft and produces a darkened coat color. The mechanism by which *dsu* restores normal melanocyte morphology is unknown.

To determine whether *dsu* acts specifically on melanocyte morphology in mutant mice or more generally upon pigmentation, we have examined its interaction with a number of coat color mutations that affect pigmentation in ways other than through the alteration of melanocyte morphology. There are currently 66 mouse loci reported in the literature that influence the pigmentation of the coat, eyes or extrafollicular tissues (see *Mouse News Letter* **80**). This large number reflects the numerous developmental processes that are essential for normal melanogenesis. From day 8 to day 12 of gestation the clonal initiators of melanoblasts, which originate in the neural crest, proliferate and migrate dorsa-ventrally through the mesoderm. The skin melanoblasts then penetrate the basement membrane and enter the epidermis around day 12 of development (RAWLES 1947). Those melanoblasts destined to be follicular melanocytes subsequently enter the hair follicle. After the establishment of a melanoblast population within the epidermis, the melanoblasts differentiate into melanocytes, the pigment producing cells. This differentiation process requires the formation of premelanosomes, the deposition of melanin upon the premelanosome to form the mature melanosome, and the establishment of the melanosome transportation system. The pigment granules are then transported to the keratinocytes and subsequently, into the hair. Several mouse pigment mutations have been identified that affect these various stages of melanogenesis. By observing the interaction of *dsu* with mutations representative of each of these stages, we examined whether *dsu* acts in a stage specific manner.

The two genes we examined that have been impli-

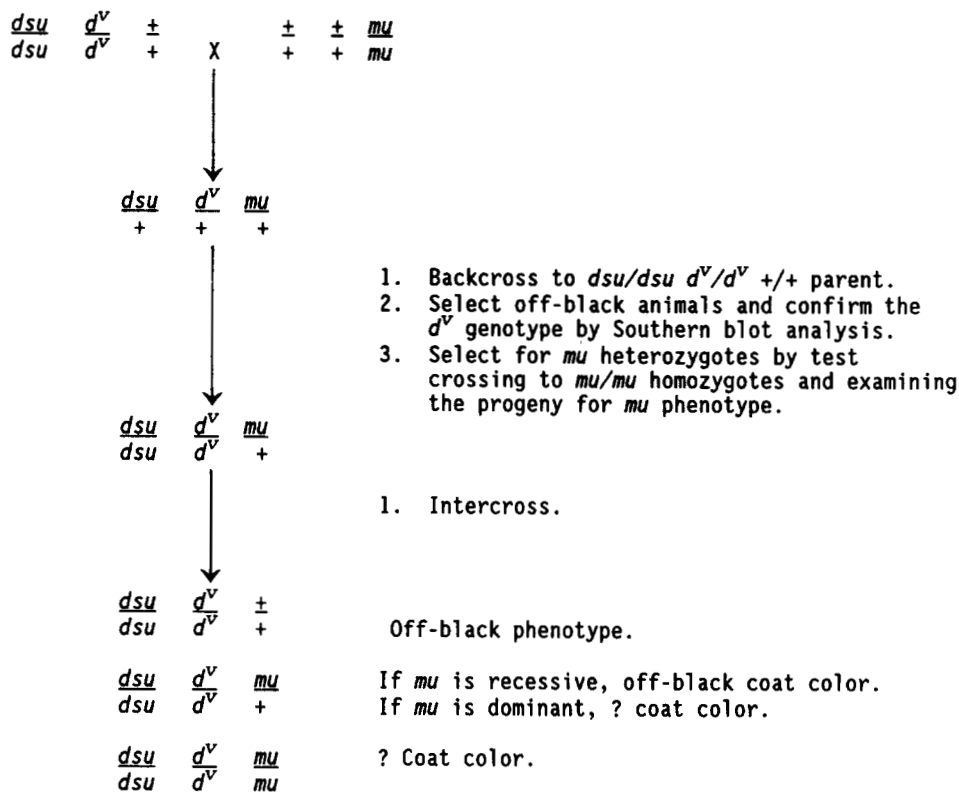

FIGURE 1.—Breeding scheme for the derivation of mutant animals homozygous for *dsu*. The symbol *mu* has been used to represent a generic mutation, it could be any of the following mutations; *p*, p^{un}, b^{J}, B^{lt}, *s*, *coa*, tp^{3J}, *m*, Mi^{wh}, *ep*, *pa*, $ru\text{-}2^{J}$, $ru\text{-}2^{hz}$, or *ru*. In addition, all mice were homozygous for *nonagouti* (*a/a*) in order to simplify the analysis of coat color. The expected coat color phenotype is shown to the right of the genotypes. The ? indicates that the coat color phenotype is the question under investigation.

TABLE 1

Affect of *dsu* on coat color and eye color phenotypes

Mutation	Gene symbol	Coat color	Eye color
Dilute[a]	*d*	Yes	—[b]
Ashen[a]	*ash*	Yes	—
Leaden[a]	*ln*	Yes	—
Pink eye	*p*	No	No
Pink eye unstable	p^{un}	No	No
Brown-J	b^{J}	No	—
Brown light	B^{lt}	No	—
Piebald	*s*	No	—
Cocoa	*coa*	No	No
Taupe-3J	tp^{3J}	No	—
Misty	*m*	No	—
Microphthalmia white	Mi^{wh}	No	No
Pale ear	*ep*	No	(?)
Pallid	*pa*	No	No
Ruby-eye-2J	$ru\text{-}2^{J}$	No	Yes
Ruby-eye-2 haze	ru^{2hz}	No	Yes
Ruby-eye	*ru*	No	Yes

[a] MOORE *et al.* (1988b).

[b] The dash indicates that the eyes of the mice carrying these mutations are black. Therefore no effect of *dsu* upon these phenotypes can be observed.

cated in early stages of melanogenesis are *piebald* (*s*) and *microphthalmia* (*mi*). The melanoblasts of both *s/s* and Mi^{wh}/Mi^{wh} mice display a cell autonomous inviability that results in the localized absence of melanocytes (MINTZ 1970, 1971). Mice homozygous for *s* are pigmented in some areas of the coat and totally unpigmented in others, giving a spotted phenotype. The *microphthalmia* locus (*mi*) also frequently gives rise to "spotted" phenotype. The allele of *mi* that was used in these studies is *microphthalmia-white* (Mi^{wh}) (GROBMAN and CHARLES 1947), which acts semidominantly. Heterozygous Mi^{wh} mice have diluted coats with white spots on the belly and black eyes. Homozygotes are white coated, red eyed mice.

The mutations we examined that affect the post migration stages of melanogenesis are *pink-eye*, (*p*), *pallid* (*pa*) (ROBERTS 1931), *ruby* (*ru*) (DUNN 1945), *brown* (*b*), and *brown-light* (B^{lt}) (MACDOWELL 1950). The B^{lt} mutation like *d*, *ln* and *ash*, affects the dendritic morphology of the follicular melanocytes. However, B^{lt} does not alter the dendritic morphology of extrafollicular melanocytes; it also affects melanosome morphology and when melanocytes from the hair bulb are displaced into the hair shaft, they are not replaced within the follicle. The other four mutations affect melanosome structure and/or the deposition of melanin upon the premelanosome. As these two processes appear to be interdependent, it is often difficult to define the exact nature of the mutation. For example, the premelanosome of *b* mice are more spheroid and less organized internally than those of wild type and upon maturation, the deposited melanin is brown in color and more flocculent than wild type (MOYER 1966). This suggests that the product of the *b* locus may provide a structural framework for the attach-

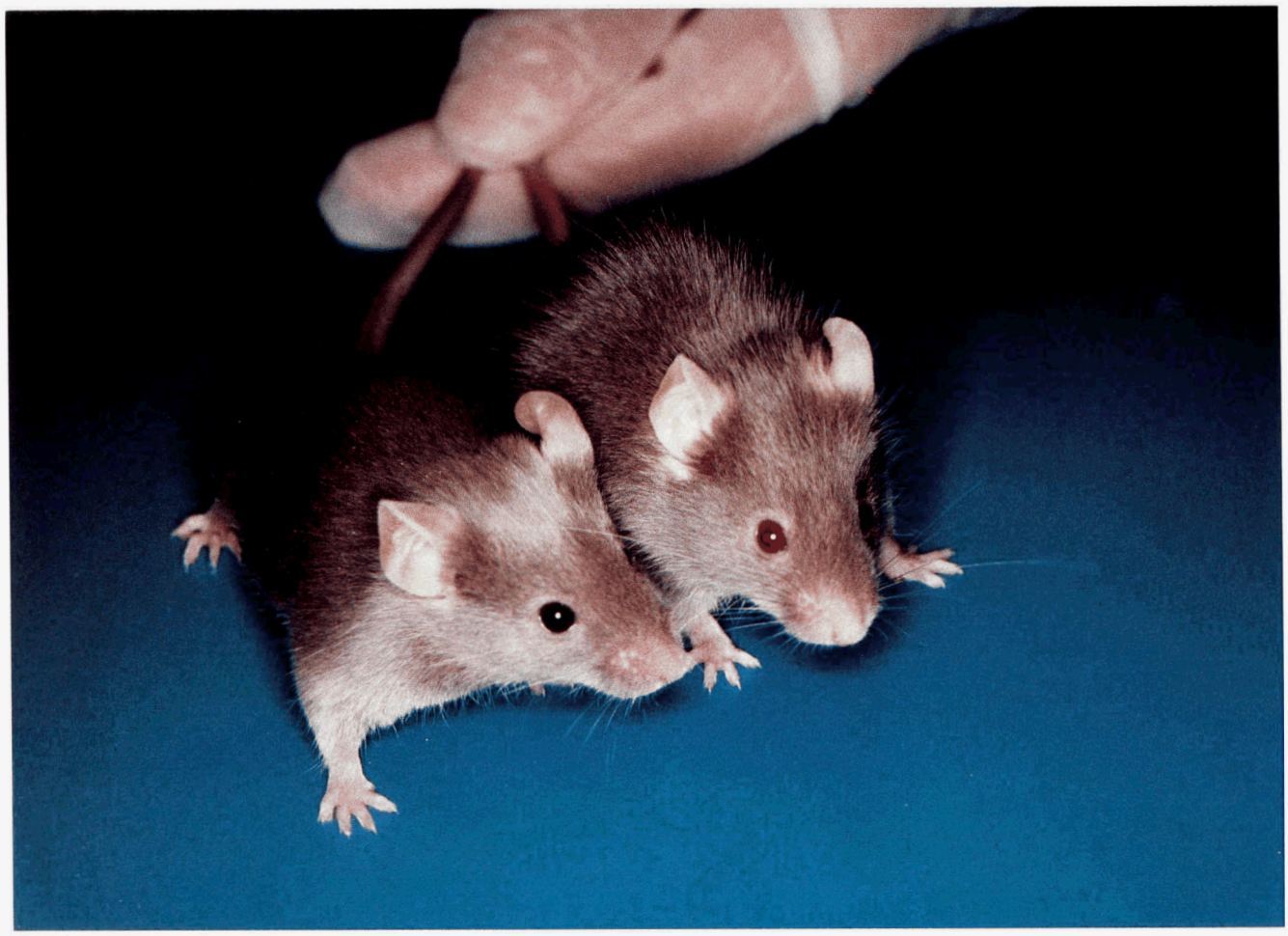

FIGURE 2.—Comparison of coat and eye color phenotypes of *ru/ru* and *dsu/dsu*, *ru/ru* d^v/d^v mice. The mouse on the right is a ruby eyed, *ru/ru* mouse. The mouse on the left is a black eyed, *dsu/dsu*, *ru-2^J/ru-2^J*, d^v/d^v mouse. The coat color of these mice is identical.

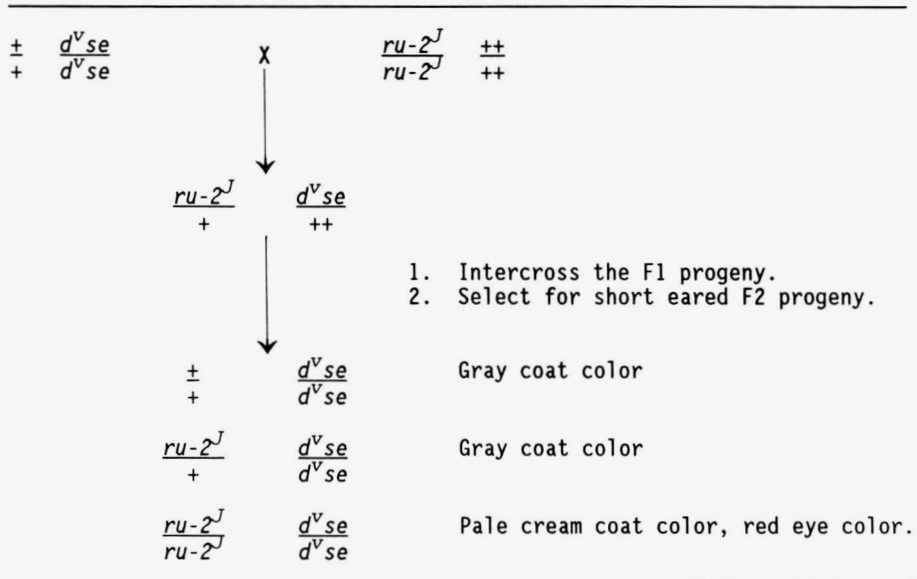

FIGURE 3.—Interaction of *ru-2^J* and d^v. The coat color and eye color phenotypes of the short-eared F_2 offspring are indicated to the right of the genotypes. All mice were also homozygous for *nonagouti* (*a/a*).

ment of the pigment synthesizing enzymes as well as affecting the activity of the pigmentation enzymes.

In addition to mutations where there is some indication of stage specificity, we also looked at coat color mutations for which no stage during melanogenesis has been implicated. These mutations are *cocoa* (*coa*) (SWEET and PROCHASKU, 1985), *taupe* (*tp*) (FIELDER 1952), *misty* (*m*) (WOOLLEY 1941), *ruby-eye-2* (*ru-2*)

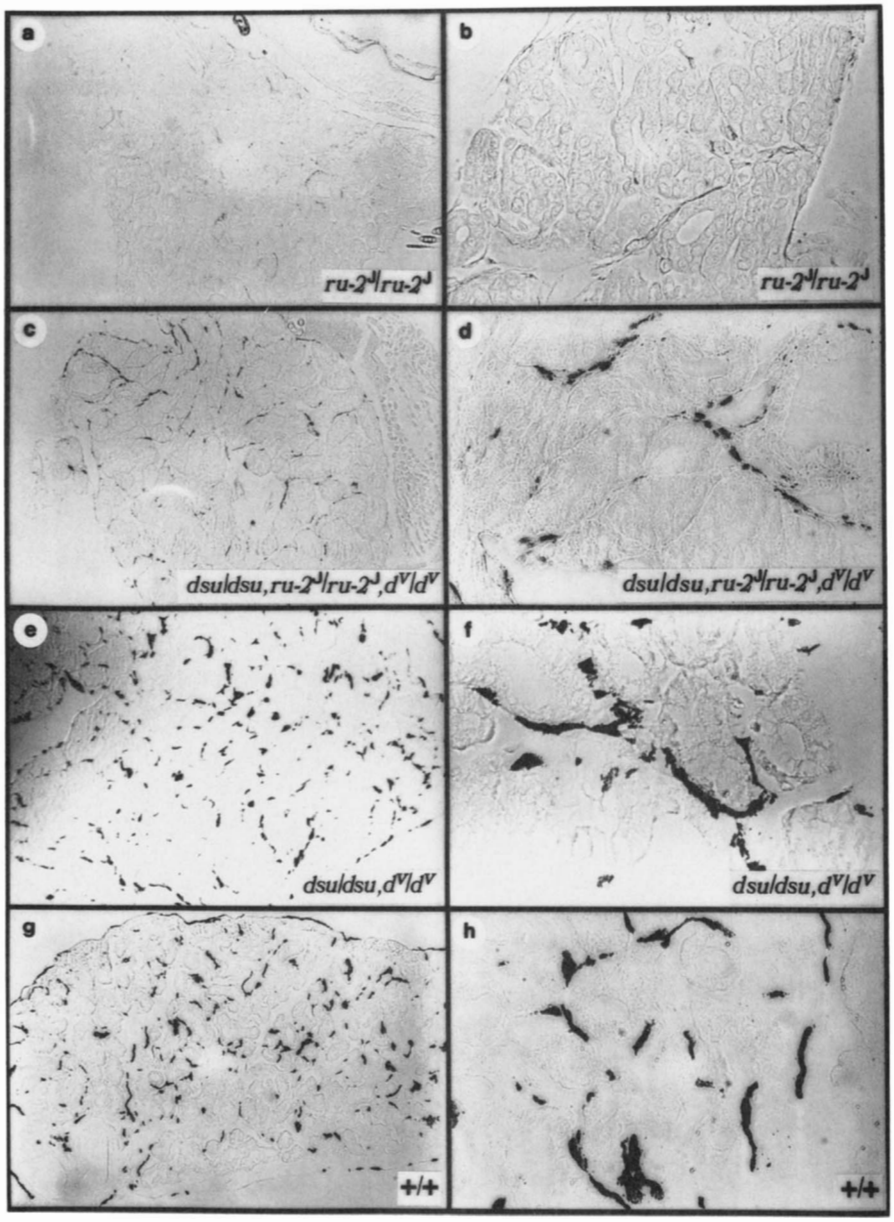

FIGURE 4.—Sections of unstained Harderian glands from 10–12-day-old mice carrying various combinations of *dilute* (d^{v}), *ruby-eye-2^{J}*-(*ru-2^{J}*) and *dilute suppressor* (*dsu*). All mice were homozygous for *nonagouti* (*a/a*). Panels a, c, e, and g are low magnification (320×) and panels b, d, f and h are high magnification (640×).

(LILLY 1966) and *pale ear* (*ep*) (LANE and GREEN 1967).

Many of the mutations we examined not only alter coat color but also affect eye color. These mutations are *p*, *pa*, *ep*, *ru*, *ru-2*, *mi* and *coa*. Eye color in the mouse is the additive effect of pigment located in a number of different tissues of the eye. The iris of the mouse is usually pigmented and can be seen as a dark ring around the periphery of the eye. A greater contribution to overall eye color comes from the pigmented layers at the back of the eye. The first layer is the retinal pigmented epithelium that lies directly behind the retina. It is a single cell layer of pigment containing hexagonal melanocytes derived from the optic cup (SEARLE 1968). This is the only pigmented tissue of the mouse that contains non-neural crest derived melanocytes. The tissue layer behind the retinal pigment epithelium, the choroid, is also a pigmented tissue that contributes to eye color. The choroid melanocytes are neural crest derived.

Homozygous *p/p* mice have vastly reduced numbers of melanocytes in both the retina and the choroid (MARKERT and SILVERS 1956) and those melanocytes that are present never obtain full melanization (HEARING, PHILLIPS and LUTZER 1973). The additive affect that *p* has upon the pigmentation of both the retina and the choroid gives rise to pink eyes. Like *p/p* mice, adult *pa/pa* mice have red eyes and vastly reduced numbers of retinal melanocytes (HEARING, PHILLIPS and LUTZNER 1973). However, there is less certainty as to the affect of the *pa* mutation upon the choroid melanocytes. HEARING, PHILLIPS and LUTZNER (1973) noted that choroidal melanocytes are affected in *pa/pa* mice although the exact comparison to wild type is not reported. The *ep* mutation also causes a dilution of eye color but only in neonates. Adult *ep/ep* mice

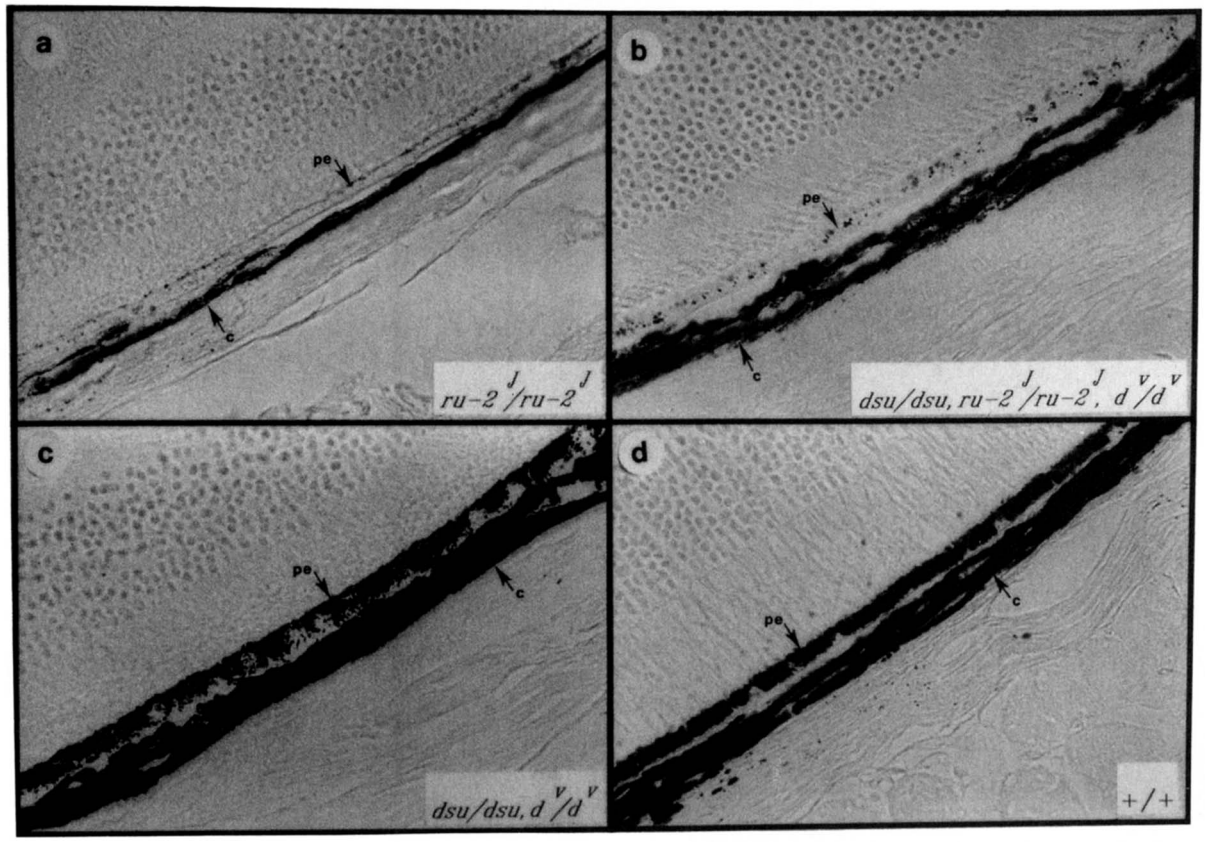

FIGURE 5.—Sections of unstained preparations of the posterior chamber of the eye from adult mice carrying various combinations of *dilute* (d^v), *ruby-eye-2*^{J} (*ru-2*^{J}) and *dilute suppressor* (*dsu*). The retina is located toward the upper left corner of each panel. The arrows marked **pe** indicate the location of the retinal pigmented epithelium and the arrows marked **c** indicate the location of the choroid. Each panel is at the same magnification (640×).

have black eyes (LANE and GREEN 1967).

Mi^{wh}/Mi^{wh} mice have red eyes due to an unpigmented choroid and a fragmented, nonpigmented retinal epithelium (DEOL 1973). In $Mi^{wh}/+$ mice the retinal epithelium is normally pigmented but the choroid is still unpigmented like that of the homozygotes. Mice homozygous for the *ru* mutation, have darker eyes than those of *p/p*, *pa/pa* or Mi^{wh}/Mi^{wh} mice, yet they are not as dark (black) as wild type. MARKERT and SILVERS (1956) reported a reduced number of melanocytes in the retina yet a normal looking contingent of melanocytes in the choroid of *ru/ru* mice. HEARING, PHILLIPS and LUTZNER (1973) in a fine structure analysis of *ru/ru* melanosomes, reported that the choroid melanosomes are mostly abnormal and are particulate in structure with only occasional patches of complete melanization. *ru-2/ru-2* mice are phenotypically identical to *ru/ru* mice having a diluted coat color and ruby eyes but the two genes, *ru* and *ru-2*, are unlinked. Until this study, the pigmented layers of the eye of *ru-2/ru-2* mice had not been examined.

In this study we have examined the interaction of the *dsu* gene with 14 coat color mutations of the mouse in an attempt to define the developmental stage or stages of melanogenesis that *dsu* influences. We were unable to show that *dsu* altered the coat color phenotypes of any of these mutations. However, *dsu* does darken the ruby colored eyes of mutations at the *ruby* (*ru*) and *ruby-2* loci (*ru-2*).

MATERIALS AND METHODS

Mice: The following strains of mice were obtained from The Jackson Laboratory (Bar Harbor, Maine); C57BL/6By-*p/p*, *Ve*/+, C57BL/6JEi-p^{un}/p^{un}, C57BL/6J-Mi^{wh}/+, C57BL/6JEi-*ru-2*^{J}/*ru-2*^{J}, C57BL/6J-*m*+/+*db*, C3HeB/FeJ-*a/a*, *s/s*, C57BL/6By-*ep/ep*, C57BL/10J-*coa/coa*, LT/SV (homozygous B^{lt}), B6D2$_p$-*ru-2*^{hz}/*ru-2*^{hz}, C57BL/6J$_p$-*ru/ru*, C57BL/6J$_p$Ei-*pa/pa*. All other strains used in these studies are maintained by the Mammalian Genetics Laboratory, NCI-Frederick Cancer Research Facility (Frederick, Maryland).

Southern blot analysis: Genomic DNA was prepared from mouse tails (SIRACUSA *et al.* 1987). The DNA was digested with 8 units *Eco*RI/μg DNA (New England Biolabs) in a high salt buffer (MANIATIS, FRITSCH and SAMBROOK 1982) containing 5 mM spermidine. The digested DNAs were electrophoresed through 0.8% agarose gels and processed as described (JENKINS *et al.* 1982), except that Zetabind (AMF Cuno) was substituted for nitrocellulose. Hybridization conditions were as described (JENKINS *et al.*, 1982). To distinguish between the d^v and + alleles of *d* we used the probe p0.3 (originally published as the Pst probe; COPELAND, HUTCHINSON and JENKINS 1983), a unique 2.6-kb cellular DNA sequence located 3′ of the *Emv-3* viral integration site in the d^v allele (RINCHIK *et al.* 1986). This probe detects an 18-kb *Eco*RI fragment in d^v chromosomes and a

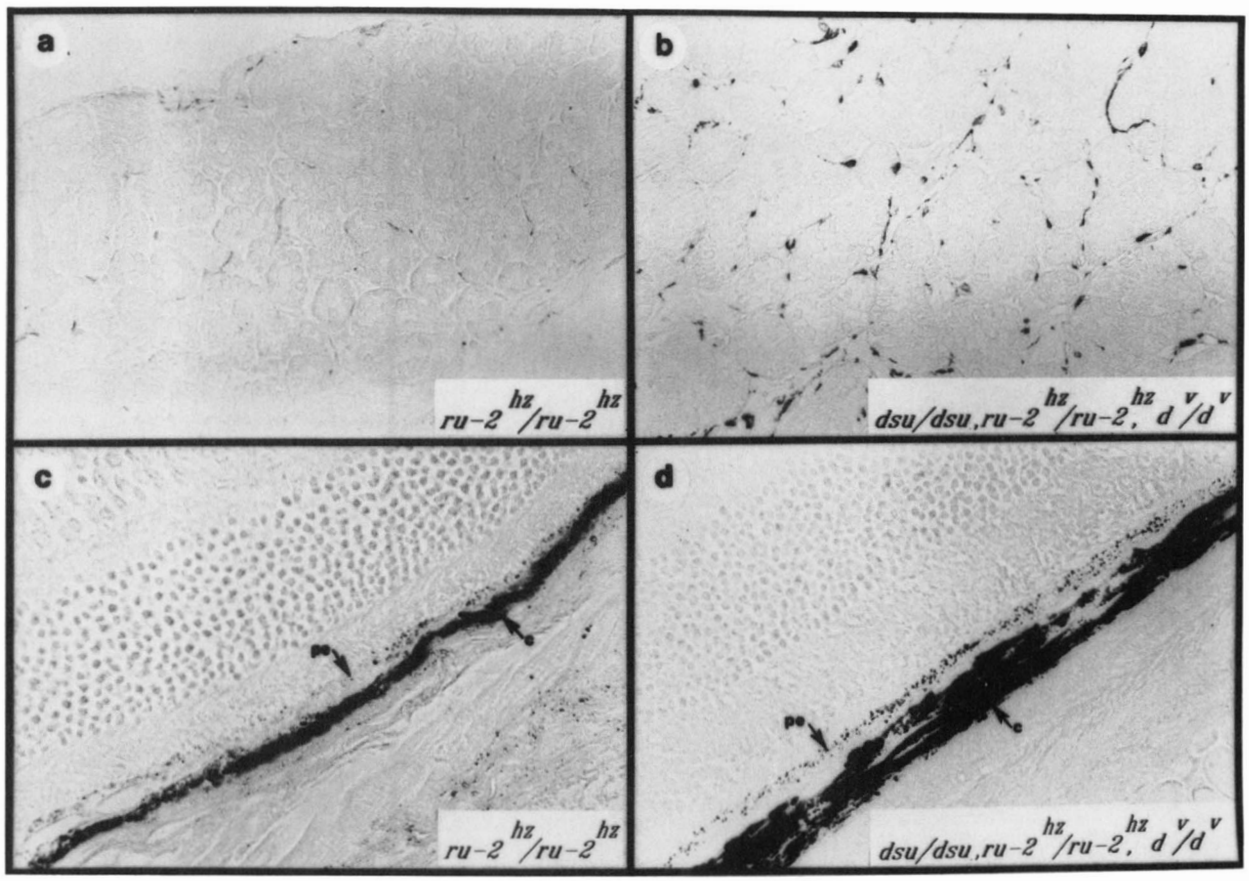

FIGURE 6.—Sections of unstained preparations of the Harderian gland and the posterior chamber of the eye. Panels a and b are low magnification (320×) of Harderian gland preparations from 10–12-day-old *ru-2^{hz}/ru-2^{hz}* and *dsu/dsu, ru-2^{hz}/ru-2^{hz}, d^{v}/d^{v}* mice, respectively. Panels c and d are high magnification (640×) of the posterior chamber of the eye of adult *ru-2^{hz}/ru-2^{hz}* and *dsu/dsu, ru-2^{hz}/ru-2^{hz}, d^{v}/d^{v}* mice, respectively. In panels c and d, the arrows marked **pe** indicate the position of the retinal pigmented epithelium and the arrows marked **c** indicate the location of the choroid.

9-kb fragment in wild-type chromosomes. Filters were washed twice at 65° in a shaking water bath with 2× SSCP, 0.1% sodium dodecyl sulfate for 20 min each, then washed 3–4 times with 0.2× SSCP, 0.1% SDS for 20 min each. Filters were autoradiographed at −70° with Kodak XAR film with 2 Dupont Lightning Plus intensifying screens for 1–5 days.

Preparation of Harderian glands and eyes: Ten- to 12-day-old mice and adult mice were euthanized and the Harderian glands, still attached to the eye, were removed for histological examination. The dissected tissues were fixed in Fekete Tellyesniczky's fluid (LILLY, 1965) and were prepared as described (SWEET 1983). The melanocytes of the Harderian gland were most readily examined in 10–12-day-old mice. The pigmented tissues of the eye were examined in both juveniles and adults.

RESULTS

For each of the mutations examined in this study, mice were made homozygous for the mutation in question, as well as *dilute* (*d^{v}*) and *dsu*. The symbol *mu* has been used to indicate a generic mutant coat color gene. It could be any of the mutations used in these studies. The *d^{v}* allele was incorporated into each stock in order to follow *dsu*, because *dsu* alone does not produce a coat color phenotype. However, mice homozygous for *dsu* and *d^{v}* have an identifiable phenotype; the coat color of *dsu/dsu, d^{v}/d^{v}* mice is almost black but slightly lighter than the black coat color of wild type mice. We have called this suppressed phenotype off-black (MOORE *et al.* 1988a). A generalized breeding scheme used to produce *mu/mu, dsu/dsu, d^{v}/d^{v}* mice is described in Figure 1. In each case, except *Mi^{wh}*, mice homozygous for the mutation (*mu/mu*) were crossed to *dsu/dsu, d^{v}/d^{v}, +/+* mice (mice heterozygous for *Mi^{wh}* were used because the homozygotes were poor breeders). The F_1 animals, which were heterozygous for *dsu, d^{v}* and *mu* were backcrossed to *dsu/dsu, d^{v}/d^{v}, +/+* mice. The backcross progeny mice that were off-black in color, and presumbly *dsu/dsu, d^{v}/d^{v}, ?/+*, were selected and analyzed by Southern blot analysis to confirm that each animal was homozygous *d^{v}*. Fifty percent of these mice should be heterozygous for the *mu* mutation and 50% should be wild type. These two genotypic classes were distinguished by crossing them to *mu* homozygotes. In such a cross only those mice that were heterozygous for *mu* will produce *mu/mu* offspring. Mice that were confirmed to be *dsu/dsu, d^{v}/d^{v}, mu/+* were intercrossed and the progeny examined for coat color. In

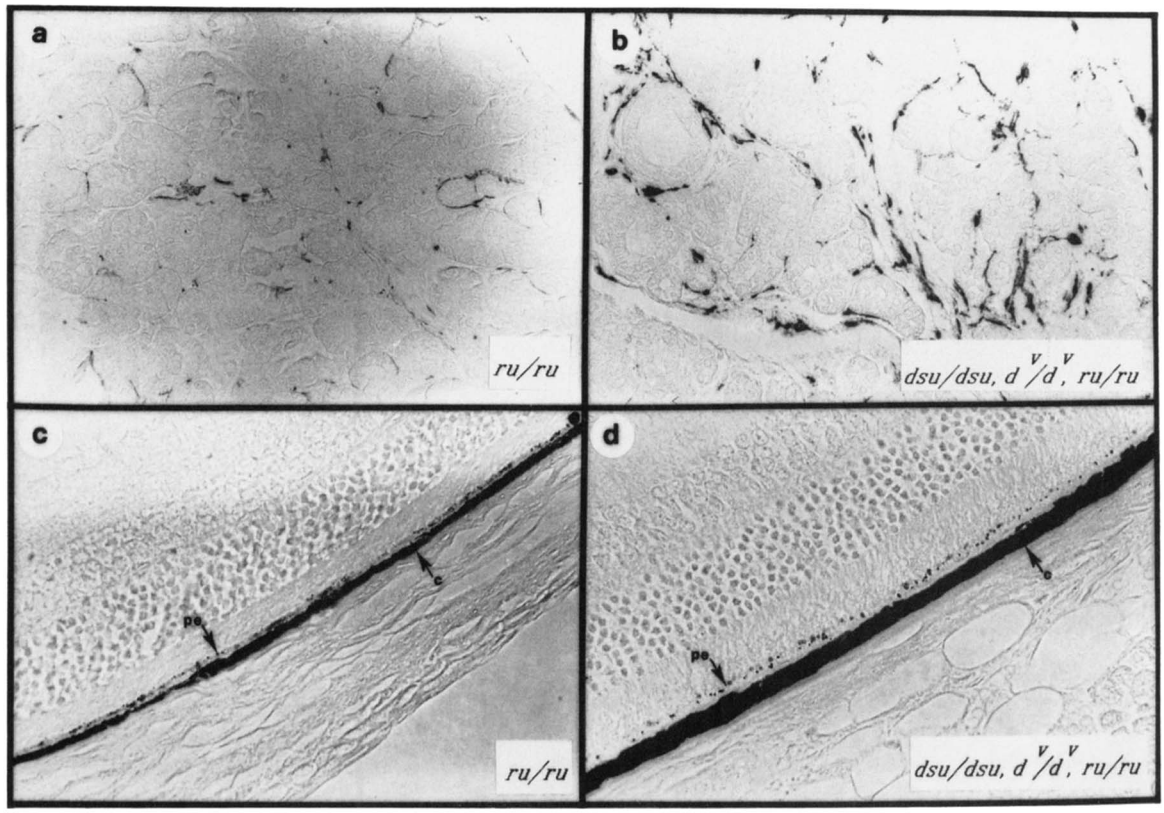

FIGURE 7.—Sections of unstained preparations of the Harderian gland and the posterior chamber of the eye. Panels a and b are low magnification (320×) of Harderian gland preparations from 10–12-day-old *ru/ru* and *dsu/dsu, ru/ru, d^v/d^v* mice, respectively. Panels c and d are high magnification (640×) of the posterior chamber of the eye of adult *ru/ru* and *dsu/dsu, ru/ru, d^v/d^v* mice, respectively. In panels c and d, the arrows marked **pe** indicate the position of the retinal pigmented epithelium and the arrows marked **c** indicate the location of the choroid.

crosses where *mu* was a recessive mutation, (*p*, *p^{un}*, *b^J*, *s*, *coa*, *tp^{3J}*, *m*, *ep*, *pa*, *ru*, *ru-2^J* and *ru-2^{hz}*), 75% of the offspring should be off-black in color (*dsu/dsu*, *d^v/d^v*, ?/+). In crosses with the semidominant mutations *Mi^{wh}* and *B^{lt}* 25% of the offspring should be off-black in color (*dsu/dsu*, *d^v/d^v*, +/+). The remaining offspring were scored phenotypically for coat color suppression by *dsu*.

Table 1 summarizes the results of these studies. *dsu* did not suppress the coat color phenotype of any of the mutations examined in this study.

As mentioned previously, many of the mutations used in this study reduce eye pigmentation as well as coat color. Therefore it was also possible to observe the affect of *dsu* upon eye color in these mice. *dsu* did not affect the eye color of *p*, *p^{un}*, *pa*, and *coa* mice (Table 1). The results on *micropthalmia white* mice were initially not clear. It has been reported that *Mi^{wh}*/+, *d^v/d^v* mice have red eyes unlike *Mi^{wh}*/+, +/+ mice that have black eyes (GROBMAN and CHARLES 1947). However, *dsu/dsu*, *Mi^{wh}*/+, *d^v/d^v* mice had black eyes. Subsequently, we generated *Mi^{wh}*/+, *d^v/d^v* mice and in contrast to the previous report their eyes were black (data not shown). Therefore the black eye color of *dsu/dsu*, *Mi^{wh}*/+, *d^v/d^v* mice is not due to suppression by *dsu*. A similar uncertainty arose over the *dsu/dsu*, *d^v/d^v*, *ep/ep* stock that had black eyes, like adult *ep/ep* mice. Again, it has been reported that *d^v/d^v*, *ep/ep* animals have red eyes while *d^v/d^v*, +/+ animals have black eyes (LANE and GREEN 1967). We are currently making mice homozygous for *ep* and *d^v* to determine whether the black eyes of *dsu/dsu*, *d^v/d^v*, *ep/ep* mice are due to the presence of *dsu*.

In contrast to the results described above (with the possible exception of *ep*), *dsu* was able to suppress the ruby eye color of *ru*, *ru-2^J* and *ru-2^{hz}* homozygous mice. Mutations of the two loci *ru*, and *ru-2*, are indistinguishable phenotypically, both having a severely diluted coat color and ruby colored eyes. However the loci are unlinked; *ruby-eye* is on chromosome *19* and *ruby-eye-2* is on chromosome 7 (EICHER 1970). We examined the interaction of *dsu* with one allele of ruby-eye (ru) and two alleles of *ruby-eye-2* (*ru-2^J* and *ru-2^{hz}*). The resulting phenotype was the same in each case (Table 1, Figure 2). There was no suppression of the diluted coat color but the presence of *dsu* suppressed the ruby-colored eyes of these mice to black.

In each case mice with black eyes were genotypically *dsu/dsu*, *d^v/d^v* and homozygous for the mutation in question. Therefore, the suppression of ruby colored

eyes could be due either to the presence of *dsu* or to an interaction between *ruby-eye* and *ruby-eye-2* alleles with d^v. To determine whether the black eyes of mice homozygous for *dsu*, *ru-2^J*, d^v was a consequence of the interaction of d^v and *ru-2^J*, we produced mice that were *ru-2^J/ru-2^J*, d^v/d^v as described in Figure 3. C57BL/6JEi-*ru-2^J/ru-2^J* mice were crossed to C57BL/6J-d^v*se*/d^v*se* mice [*short ear* (*se*) maps less than 0.2 cM from *d* (RUSSELL 1971) and was used as a visual marker to follow d^v in these crosses]. The F_1 mice were intercrossed. F_2 progeny were selected that had short ears and therefore, barring recombination between d^v and *se*, were homozygous d^v. The short eared progeny class (9/40 mice) fell into two coat color classes. The first class (6/9 mice) was gray. The genotype of these mice was presumably d^v*se*/d^v*se*, ?/+ (the ? indicates either + or *ru-2^J*). The second class (3/9 mice) had pale cream coats and ruby eyes. The genotype of these mice was presumably *ru-2^J/ru-2^J*, d^v*se*/d^v*se*. The two mutations, d^v and *ru-2^J* therefore act additively in their dilution of coat color producing a very pale cream color, but they have ruby eyes. This indicates that the black eyes of *dsu/dsu*, *ru-2^J/ru-2^J*, d^v/d^v mice are not due to an interaction of d^v and *ru-2^J*. Rather, *dsu* suppresses the ruby-eye color of *ru-2^J/ru-2^J* mice and presumably, also *ru-2^{hz}/ru-2^{hz}* and *ru/ru* mice.

To further examine the nature of the suppression of the ruby eye color of *ru*, *ru-2^J* and *ru-2^{hz}* mice by *dsu*, we examined the histology of melanocytes located in the Harderian gland and the pigmented layers of the eye. The Harderian gland is a horseshoe shaped gland that sits behind the eye and excretes a lubricating fluid into the eye cavity. The gland contains numerous neural crest derived melanocytes (RAWLES 1948) that lie around the acini. Although the pigment in the Harderian gland plays no part in the visible pigmentation of the eye, it is a readily accessible source of neural crest derived melanocytes that we could examine for any *dsu*-mediated effects upon melanocyte morphology, number, or distribution. Figure 4 shows the melanocytes in the Harderian gland of *ru-2^J/ru-2^J* (panels a and b); *dsu/dsu*, *ru-2^J/ru-2^J*, d^v/d^v (panels c and d); *dsu/dsu*, d^v/d^v (panels e and f); and wild-type mice (panels g and h). By comparing panels a and b to either panels e and f or panels g and h, one can see that the *ru-2^J/ru-2^J* mice almost completely lack pigmented melanocytes in the Harderian gland; those that can be seen are very lightly pigmented. Panels c and d show that when *dsu* is present the amount of pigment within the Harderian gland increases. At this level of analysis it is difficult to determine whether the number of melanocytes or the level of pigmentation within the melanocytes is increased.

Figure 5 shows the morphology of the pigmented layers located behind the retina of *ru-2^J/ru-2^J* (panel a); *dsu/dsu*, *ru-2^J/ru-2^J*, d^v/d^v (panel b); *dsu/dsu*, d^v/d^v (panel C); and wild type mice (panel d). Panel a shows that *ru-2^J/ru-2^J* mice partially lack pigmented melanocytes in both the choroid and the retinal pigmented epithelium. When *dsu* is present (panel b), the choroid appears considerably more pigment dense but the retinal pigmented epithelium remains deficient in pigment.

The histology of the Harderian gland and pigmented layers of the eye of *ru-2^{hz}/ru-2^{hz}* and *dsu/dsu*, *ru-2^{hz}/ru-2^{hz}*, d^v/d^v mice are shown in Figure 6 and the histology for *ru/ru* and *dsu/dsu*, d^v/d^v, *ru/ru* mice are shown in Figure 7. The results for *ru* and *ru-2^{hz}* are similar to those seen for *ru-2^J*. As the pigmentation in the choroid contributes toward the overall eye color, especially in the absence of retinal epithelium pigmentation, the increased pigmentation in the choroid of mice homozygous for *dsu* and *ru-2^J* or *ru-2^{hz}* or *ru* could easily explain the black eye color of these mice.

DISCUSSION

In previous studies we showed that *dsu* suppresses the diluted coat color produced by *d*, *ln* and *ash* mutations (MOORE *et al.* 1988b). Although the exact mechanism of suppression is not understood it has been shown that *dsu* does not affect the transcription of the *d* gene (MOORE *et al.* 1988a) or, we presume, the transcription of the *ash* and *ln* genes. Rather *dsu* acts by compensating for defective *d*, *ln* and *ash* gene products to restore a normal morphology to the adendritic melanocytes associated with each of these mutations. This allows a more efficient transportation of pigment into the developing hair shaft and thereby increases the pigmentation in the coat. These results strengthen the argument that *d*, *ln*, and *ash* are members of a multigene family (MOORE *et al.* 1988b).

To determine whether *dsu* is capable of suppressing coat color mutations that do not result soley from defects in melanocyte morphology, we extended our analysis of *dsu* by examining the interaction of *dsu* with 14 other coat color mutations. The mutations we chose affect many of the processes required for visible pigmentation. These processes include the migration of melanoblasts from the neural crest and their subsequent survival, the differentiation of melanoblasts into melanocytes, which includes the structural formation of the melanosomes and the deposition of pigment upon them, and the transportation of the pigment out of the melanocyte. The results of these studies showed that *dsu* does not suppress the coat color of any of these mutations, which included *p*, *p^{un}*, *b^J*, *B^{lt}*, *s*, *coa*, *tp^{3J}*, *m*, *Mi^{wh}*, *ep*, *pa*, *ru-2^J*, *ru-2^{hz}* and *ru*. Therefore, *dsu* appears to suppress only coat color mutations that primarily affect melanocyte morphology, like *d*, *ln* and *ash*.

In contrast to the results obtained with coat color, *dsu* was able to suppress the ruby eye color of homozygous *ru-2^J*, *ru-2^{hz}* and *ru* mice. Light microscopy revealed that the darkened eyes of suppressed mice is a consequence of an increase in the pigmentation of the choroid of the eye. The histology of Harderian gland melanocytes is also affected by *dsu*, although this tissue has no apparent affect upon eye color. It is unclear whether the *dsu*-mediated increase in pigmentation of the choroid and the Harderian gland is due to an increase in the number of melanocytes, to an increase in the number of melanosomes in otherwise nonpigmented melanocytes, or to a change in tissue organization. Electron microscopy is currently being employed to address this question. In contrast to the affects of *dsu* upon choroidal and Harderian gland pigmentation, *dsu* had no affect upon the reduced pigmentation of the retinal pigmented epithelium of *ru-2^J/ru-2^J*, *ru-2^{hz}/ru-2^{hz}* and *ru/ru* mice. It is interesting to note that unlike follicular, choroidal, and Harderian gland melanocytes, which are neural crest derived, retinal pigmented epithelial melanocytes are optic cup derived and therefore embryologically distinct. The reason that *dsu* has no affect upon the follicular melanocytes of *ru-2^J/ru-2^J*, *ru-2^{hz}/ru-2^{hz}* and *ru/ru* mice is unclear. It is possible that the developmental window of action of *dsu* is appropriate only to allow it to correct the gene defect(s) in choroid, but not follicular derived melanocytes.

It is, as yet, difficult to propose a model for *dsu* action that includes all the observed effects of *dsu*. Previously, we suggested that suppression by *dsu* results either from a direct compensation for defective *d*, *ln* and *ash* gene products or by changing the environment in which the *d*, *ln* and *ash* melanocytes reside thereby allowing the melanocytes to elaborate dendrites more easily (MOORE *et al.* 1988b). Either of these models is still equally applicable with the inclusion of the new data obtained in these studies.

The first model now has to be extended to include the *ru-2* and *ru* genes. Recent results from our laboratory have shown that the *d* gene product has strong homology with cytoskeleton proteins (unpublished results). It is not unreasonable, knowing the strong similarities of the *d*, *ln* and *ash* phenotypes, that *ash* and *ln* could encode cytoskeletal proteins and/or proteins that interact with the *d* gene product. It is also possible that *ru-2* and *ru* may also encode cytoskeletal proteins. Finally, if the product of *dsu* is also a cytoskeletal protein, models could be envisioned in which the mutant *dsu* protein can compensate for the defects in the cytoskeleton created by each of these mutations.

In the second model, we proposed that *dsu* is involved in the maintenance of the extracellular matrix. If this were the case then the mutant *dsu* product could be altering the extracellular environment of neural crest derived melanocytes thereby compensating for defects in melanocyte morphology, melanoblast migration or general tissue organization. In this regard, it is interesting to note that *dsu* maps to chromosome *1*, 6.3 cM distal to *isocitrate dehydrogenase* (*Idh-1*) (MOORE *et al.*, 1988b). This places *dsu* within 1 cM of the *fibronectin* (*Fn-1*) locus (SKOW *et al.* 1987). Fibronectins are adhesive glycoproteins present in the extracellular matrix. They are involved in many important processes including cellular adhesion, maintenance of normal cell morphology and cell migration. We are currently mapping *dsu* relative to *Fn-1* in a multipoint, interspecific hybrid mapping experiment to investigate whether *Fn-1* is a candidate for *dsu*.

Finally, it is conceivable that suppression of *d*, *ln* and *ash* by *dsu* and suppression of *ru* and *ru-2* by *dsu* occurs through different mechanisms. It will be important when distinguishing the different models described above, to know whether *dsu* acts cell autonomously. If *dsu* acts within melanocytes, we would expect its effects to be cell autonomous. If *dsu* acts in the extracellular matrix, its effects should not be cell autonomous. We hope to be able to answer this question in the future using chimeric mice. This and other studies on *dsu* should ultimately allow us to define the molecular mechanism(s) of *dsu* action.

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Note added in proof: We have recently produced mice that are homozygous for *d^v* and *ep*; they have black eyes. Therefore, the black eyes seen in *dsu/dsu*, *d^v/d^v*, *ep/ep* mice are not due to the action of *dsu*.

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