Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 8131–8135, November 1988
Genetics

Dilute suppressor *dsu* acts semidominantly to suppress the coat color phenotype of a deletion mutation, d^{l20J}, of the murine dilute locus

(deletion allele)

KAREN J. MOORE, PETER K. SEPERACK, MARJORIE C. STROBEL, DEBORAH A. SWING, NEAL G. COPELAND, AND NANCY A. JENKINS

Mammalian Genetics Laboratory, Bionetics Research Inc., Basic Research Program, National Cancer Institute–Frederick Cancer Research Facility, Frederick, MD 21701

Communicated by L. B. Russell, July 25, 1988

ABSTRACT **The murine dilute suppressor (*dsu*) gene is the only unlinked trans-acting suppressor identified in mammals. *dsu*, which was originally reported to be recessive, was recognized by its ability to suppress the coat color phenotype of a retroviral insertion mutation, d^v, of the murine dilute (*d*) locus. This insertion mutation resulted from the integration of an ecotropic murine leukemia virus into noncoding sequences of the dilute gene. Therefore, *dsu* may act like other allele-specific recessive suppressors identified in *Drosophila melanogaster* and yeast that suppress mutations induced by retrotransposon insertions. To investigate this possibility, we have examined whether *dsu* could suppress a spontaneously arising allele of *d*, d^{l20J}, which is shown here to result from a 3.5-kilobase deletion. These studies indicate that *dsu* does not function like other eukaryotic suppressor genes that suppress retrotransposon-induced mutations. We also show that *dsu* is not, as originally reported, a recessive gene but is semidominantly inherited. Collectively, these results allow us to propose a mechanism for the suppressor activity of *dsu*.**

Suppression of mutant phenotypes by unlinked, trans-acting, recessive suppressor genes has been recognized in many genetic systems. A common theme has arisen for a subset of these suppressor genes in that they suppress the phenotypes of mutations caused by retrotransposon insertions. In *Drosophila melanogaster*, this class of genes includes the suppressors of hairy wing *su(Hw)* (1, 2), of white apricot *su(Wa)* (3), and of forked *su(f)* (4). These loci all act on mutations induced by the copia-like family of retrotransposons. In yeast, the suppressor *spt* genes suppress mutations induced by the yeast retrotransposon *Ty* (5). In both cases the suppressed phenotype is likely to result from the interaction between the product of the suppressor and the retrotransposon genome (6). The recently discovered murine *dsu* gene was identified as a recessively inherited trans-acting gene of mouse chromosome 1, which suppresses the coat color phenotype of a retrovirally induced mutant allele of the dilute (*d*) locus d^v (7). Therefore, many parallels may be drawn between *dsu* and the class of suppressor genes described above. Our experimental objective was to determine whether *dsu* suppresses only retrovirally induced mutations of *d* and, consequently, whether these parallels were justified.

The original *d* mutation, now designated d^v, is a recessive mutation located on mouse chromosome 9. The visible pigmentation in the coat hairs of d^v/d^v mice is less than that of wild-type mice as a consequence of a change in melanocyte morphology: wild-type melanocytes have thick dendritic processes, whereas the dilute melanocytes have vastly reduced numbers of such processes (8). This alteration affects the transport of pigment granules from the melanocyte into the hair shaft, thereby changing the distribution of pigment granules and lightening the coat color. The quantity of pigment synthesized in d^v/d^v mice is normal (9). The d^v mutation is caused by the insertion of an ecotropic provirus, *Emv-3*, into noncoding sequences of the *d* gene (10, 11). On a nonagouti background d^v/d^v mice have a slate gray coat color, whereas *dsu/dsu*, d^v/d^v mice have a much darker, nearly black, coat color. However, suppression is not complete and the suppressed phenotype is distinguishable from true nonagouti black mice. We have called this suppressed phenotype off-black. In the absence of d^v, *dsu* has no effect on coat color: *dsu/dsu*, $d^v/+$ and *dsu/dsu*, $+/+$ mice are true nonagouti black. Histological examination of melanocytes from *dsu/dsu*, d^v/d^v mice showed that the suppression of dilute coat color by *dsu* was associated with the restoration of normal dendritic melanocyte morphology (7).

In addition to d^v, numerous spontaneous and mutagen-induced *d* alleles have been identified (12–14). Unlike d^v, which only affects coat color, most of these alleles, designated dilute-lethal (d^l) or dilute-opisthotonus (d^{op}), when homozygous result in a severe neurological defect in addition to the light coat color. This neurological defect is called opisthotonus (a convulsive arching upward of the head and neck). Mice displaying this phenotype usually die by 18–21 days postpartum. The postnatal lethality of d^l is itself recessive, since d^l/d^v mice exhibit only the dilute coat color. *dsu* has been shown by Sweet (7) to suppress the coat color but not the neurological defect of one of these d^l alleles. However, the structural alteration of this *d* allele is unknown, and it is conceivable that this d^l mutation is retrovirally induced.

Recently, a spontaneous d^l allele, d^{l20J}, was identified at The Jackson Laboratory. Like other d^l alleles studied, d^{l20J} homozygotes display both coat color and neurological defects. Subsequent analysis of this mutation, described below, showed that it resulted from a 3.5-kilobase (kb) deletion of DNA from the dilute locus. This mutation, therefore, offers a unique opportunity to determine whether *dsu* can suppress a dilute mutation that is not retrovirally induced.

MATERIALS AND METHODS

Mice. All the mice used in this study (C57BL/6J, the *dsu/dsu*, d^v/d^v stock, and C57BL/6J-d^{l20J} $+/d^v$ *se*) are maintained by this laboratory.

Southern Blot Analysis. Genomic DNA was made from mouse tails as described by Siracusa *et al.* (15). The DNA was digested with 10 units of *Eco*RI per μg of DNA (New England Biolabs) in a high salt buffer (16) containing spermidine. The digested DNA was run on 0.8% agarose gels, which were then processed as described by Jenkins *et al.* (16) except that

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Zetabind (AMF Cuno, Meriden, CT) was substituted for nitrocellulose. Hybridization conditions were as described by Jenkins *et al.* (16). The DNA probe used for the typing of different *d* alleles is p0.3, a unique 2.6-kb cellular DNA sequence located 3′ to the *Emv-3* viral integration site (10). This probe detects a 9-kb *Eco*RI fragment in wild-type chromosomes and, due to the presence of the 9-kb provirus, an 18-kb *Eco*RI fragment in d^v chromosomes. The blots were washed at 65°C in a shaking water bath with 2× SSCP (1× SSCP = 120 mM NaCl/15 mM sodium citrate/15 mM Na_2HPO_4/4.6 mM NaH_2PO_4)/0.1% sodium dodecyl sulfate (SDS) twice for 20 min each, then with 0.2× SSCP/0.1% SDS three or four times for 20 min each. Filters were autoradiographed at −70°C with Kodak XAR-5 film with two Dupont Lightning Plus intensifying screens for 1–5 days.

Genomic Cloning of the d^{l20J} Allele. C57BL/6J-d^{l20J}/d^{l20J} spleen DNA, digested with *Eco*RI, was resolved on a 0.8% agarose gel in 40 mM Tris acetate/1 mM EDTA, pH 8.3 (17). DNA (4–6 kb) was recovered by electrophoresis onto and elution from NA-45 membrane (Schleicher & Schuell). The size-selected mouse DNA was cloned into *Eco*RI-digested λgt10 (Protoclone; Promega) essentially as described by the distributor. Recombinant phage, containing the d^{l20J} deletion breakpoint fusion fragment, were detected by filter hybridization (17) with the p0.3 genomic probe (10). For restriction mapping of the d^{l20J} deletion breakpoint fragment, this *Eco*RI fragment was subcloned into the pUC18 vector.

RNA Preparation and Northern Blot Analysis. Total RNA was prepared from the brains of mice that had undergone euthanasia. The tissues were either frozen in liquid nitrogen for later use or immediately placed in lithium urea buffer (10 ml per g or tissue) (3 M LiCl/6 M urea/50 mM Tris·HCl, pH 7.4/5 mM EDTA/0.1 M 2-mercaptoethanol/0.1% sarkosyl) (18). The tissue was homogenized for 30–60 sec with a Polytron (Kinematic) at setting 5. RNA was precipitated from this homogenate by storing overnight at −20°C followed by an 8000× *g* spin for 90 min. The pellet was dissolved in 10 mM Tris/1 mM EDTA, pH 8.0/0.5% SDS and treated with proteinase K (100 μg/ml) very briefly, followed by two extractions with phenol/chloroform/isoamyl alcohol (50:49:1). The RNA was precipitated with ethanol. The RNA pellet was dissolved as described (19) and RNA was passed over an oligo(dT) column twice [as described by the distributor (Collaborative Research, Waltham, MA)]. The poly(A)-selected mRNAs were electrophoresed on 1% agarose/6.0% formaldehyde gels (19). After gels were run, they were soaked in 20× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate) for 30 min before transfer onto Zetabind (AMC Cuno).

Filters were prehybridized and hybridized by a modified method of Wahl *et al.* (20) in 5× SSC/45% formamide/25 mM phosphate buffer, pH 7.2/1% glycine/5× Denhardt's solution (21)/sonicated salmon sperm DNA (100 μg/ml)/1% SDS for 2–3 hr at 42°C. The probe used to detect the dilute transcripts was a 2.5-kb cDNA clone for the *d* gene. This clone was isolated from a cDNA library made from the mRNA of B16 melanoma cells (22), which are wild-type at *d*, (P.K.S., M.C.S., C. Raskin, N.G.C., and N.A.J., unpublished data). The filters were washed twice at room temperature in 2× SSC/0.1% SDS, twice at 42°C with 2× SSC/0.1% SDS, twice at 42°C with 0.1× SSC/0.1% SDS, and twice with 0.1× SSC/0.1% SDS (23). Filters were exposed to Kodak XAR-5 film with Dupont Lightning Plus screens. Subsequently, the blot was stripped and rehybridized with a chicken β-actin probe (24) to confirm equal amounts of mRNA in each lane.

RESULTS

Identification and Analysis of the d^{l20J} Allele. The spontaneously arising *d* allele, d^{l20J}, was identified because of the noncomplementation of this allele with d^v, resulting in a mouse with a diluted coat color. Southern blot analysis of genomic DNA from d^{l20J} mice using p0.3, a 2.6-kb cellular sequence probe from the *d* gene (10), suggested that this allele contained a deletion (see below).

Fig. 1 (*Inset*) shows the *Eco*RI restriction fragments detected by Southern blot analysis of genomic DNA of mice carrying three different *d* alleles, +, d^v, and d^{l20J}. The genomic *Eco*RI fragment from a mouse wild type at *d* is 9 kb. The insertion of a 9-kb ecotropic provirus, *Emv-3*, into this fragment in the d^v allele generates an 18-kb fragment. When compared to the wild-type allele, the d^{l20J} *Eco*RI fragment was smaller, only 5.5 kb. This aberrant 5.5-kb *Eco*RI fragment was cloned from d^{l20J} mice. The restriction enzyme map of this clone was compared to restriction enzyme maps of previously identified clones of (*i*) the d^v allele containing the *Emv-3* provirus in Charon 9 λ phage and (*ii*) the 9-kb wild-type allele containing the viral preintegration site in λgtwes.λB (10). The restriction maps of the d^{l20J}, +, and d^v alleles are shown in Fig. 1. This analysis confirmed that the d^{l20J} mutation resulted from a 3.5-kb deletion that begins ≈2 kb 3′ from the proviral integration site in the original d^v allele.

Northern blot analysis of mRNA from mice homozygous for the d^{l20J} allele and from mice wild-type at *d* (C57BL/6J) is shown in Fig. 2. The tissue used as a source for the mRNA is brain because it is a rich source of *d* gene transcripts, and the pattern of dilute transcripts seen (11, 9, and 7 kb) is no different from that seen for most other tissues examined (P.K.S. *et al.*, unpublished data). This analysis revealed that the d^{l20J} deletion causes aberrantly sized *d* transcripts that are present in greatly reduced levels. Also, DNA sequence analysis of the region deleted in d^{l20J} showed that the deletion removes *d* gene exon sequences (M.C.S., P.K.S., N.G.C., and N.A.J., unpublished data). These two results suggest that the d^{l20J} allele is functionally null. Our finding that the d^{l20J} allele is caused by a 3.5-kb deletion in the *d* gene and that homozygotes for this allele display both the dilute coat color and the neurological abnormalities strongly suggest that both these phenotypes are the consequence of mutation at a single locus (the *d* locus) rather than two very closely linked genes.

Suppression by *dsu*. To determine whether *dsu* is capable of suppressing the coat color and/or neurological phenotypes of the d^{l20J} allele, we mated a *dsu*/*dsu*, d^v/d^v mouse to a +/+, d^v/d^{l20J} mouse. Two classes of F_1 progeny resulted from this mating: *dsu*/+, d^v/d^v and *dsu*/+, d^v/d^{l20J}. Southern blot analysis of genomic DNA from the F_1 mice using the *d* gene probe p0.3 allowed us to distinguish between these genotypes (see Fig. 1). *dsu*/+, d^v/d^{l20J} mice were selected and intercrossed. At 10–14 days of age, the F_2 mice were visually inspected for coat color and neurological behavior. Of the 25 opisthotonic F_2 mice identified (Table 1), 14 were characterized by Southern blot analysis and were confirmed to be d^{l20J} homozygotes. Twenty-six of the 84 dilute (gray) nonopisthotonic mice identified (Table 1) were also characterized by Southern blot analysis and were shown, as expected, to be either d^v/d^v or d^v/d^{l20J}. The 25 mice that were opisthotonic and, therefore, d^{l20J} homozygotes fell into two coat color classes (Table 1). Seventeen were gray and eight were off-black. One-sixteenth (6.3%) of the F_2 mice would be expected to be the double homozygotes *dsu*/*dsu*, d^{l20J}/d^{l20J}. The off-black opisthotonic class fitted this expectation and represented 8 of a total of 134 mice analyzed (6.0%). The fact that these mice were off-black in coloration indicates that *dsu* is capable of suppressing the dilute coat color phenotype of d^{l20J}. However, these mice still displayed opisthotonus and died by 18–21 days postpartum, indicating that *dsu* cannot suppress the dilute-associated neurological and lethal phenotypes. These results are consistent with those reported previously for the d^l allele (7) and with the results of some recent studies on radiation-induced lethal alleles of *d* (25).

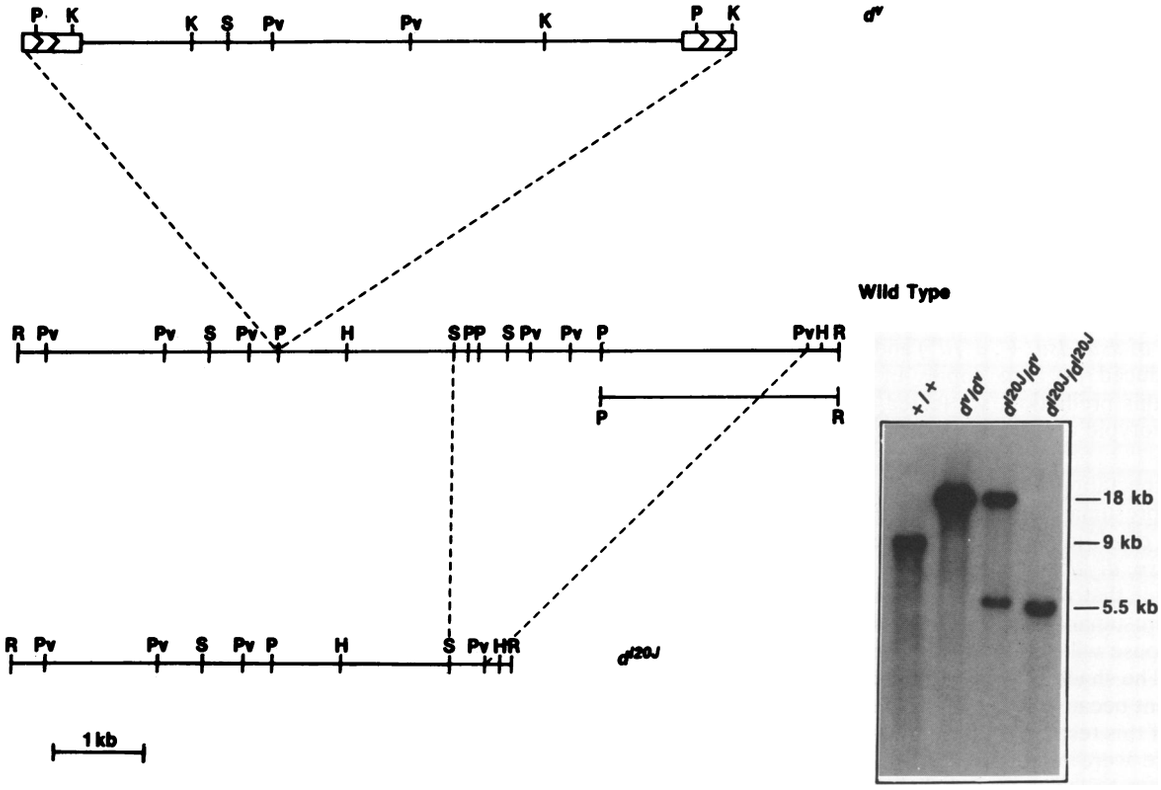

FIG. 1. Comparative restriction enzyme maps and Southern blot analyses of three *d* alleles (+, d^v, and d^{l20J}) are shown. K, *Kpn* I; P, *Pst* I; S, *Sst* I; Pv, *Pvu* II; R, *Eco*RI; and H, *Hin*dIII. The proviral sequences representing *Emv-3* are drawn 5′ to 3′ with respect to viral RNA transcription and are bounded by rectangles indicating the location of the viral long terminal repeats. The dashed line drawn between the wild-type and d^{l20J} alleles represents deleted sequences in d^{l20J}. (*Inset*) *Eco*RI genomic restriction enzyme fragments that are diagnostic for the different *d* alleles using p0.3 as the probe.

Fig. 2 shows, in addition to the dilute transcripts of wild-type and d^{l20J} homozygote mice discussed above, transcripts

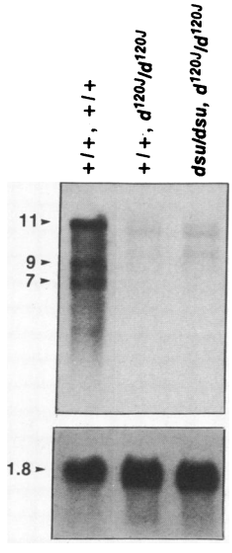

FIG. 2. Northern blot analysis of *d* gene transcripts from mice homozygous for the wild-type, d^{l20J} and *dsu*, d^{l20J} alleles. The tissue from which the mRNA was extracted was brain, as this is an abundant source of *d* gene transcripts. Five micrograms of twice poly(A)-selected mRNA was loaded into each lane. The blot was hybridized with a cDNA probe used for the *d* gene (P.K.S. *et al.*, unpublished data) and resulting transcripts are shown (*Upper*). Subsequently, the blot was stripped and rehybridized with a chicken β-actin probe (24) to confirm the loading of equivalent amounts of mRNA in each lane (*Lower*). Numbers on left are kb.

of *dsu*, d^{l20J} homozygote mice. As shown, no difference between this pattern and that of d^{l20J} homozygotes carrying a wild-type allele at *dsu* is evident. Therefore, *dsu* does not appear to affect the transcription from the d^{l20J} allele in brain. These results are, however, not conclusive because dilute transcripts were characterized in brain, while the only currently known site of action of *dsu* is in skin melanocytes. The problems inherent in the isolation of large quantities of skin melanocytes has so far precluded studies of the effects of *dsu* and *d* melanocyte transcription.

Collectively, these studies suggest that the mechanism of action of *dsu* is unlike that of the eukaryotic suppressor genes of *D. melanogaster* and yeast that exclusively suppress retrotransposon-induced mutations.

Semidominant Inheritance of *dsu*. Another characteristic of *dsu* that distinguishes it from the eukaryotic recessive suppressor genes mentioned previously, is its mode of inheritance. Although *dsu* was originally reported as recessive, our results described below show that it acts semidominantly; different gene dosages of *dsu* can be identified by their differential effects on d^v. To obtain mice that carry different gene dosages of *dsu*, mice that were *dsu*/*dsu*, d^v/d^v were crossed to C57BL/6J (+/+, +/+) mice, and the progeny were backcrossed to *dsu*/*dsu*, d^v/d^v mice. The N2 backcross progeny were analyzed by visual inspection. Gray mice (*dsu*/+, d^v/d^v) were intercrossed. The resulting progeny from this cross were homozygous d^v but segregated for zero, one, or two copies of *dsu*. Three phenotypic classes were distinguishable (Table 2). The most obvious was the *dsu*/*dsu*, d^v/d^v class, which was off-black as described above. The other mice fell into two phenotypic classes, both of which were gray. The shades of gray were only very subtly different and were referred to as dark gray and light gray. We

Table 1. Segregation of *dsu* and alleles of *d* in the progeny of an intercross of *dsu*/+, d^v/d^{l20J} mice

Phenotype	Genotype	Observed	Expected
Gray	+/(*dsu* or +), $d^v/(d^{l20J}$ or $d^v)$	84	75
Gray, opisthotonus	+/(*dsu* or +), d^{l20J}/d^{l20J}	17	25
Off-black	*dsu*/*dsu*, $d^v/(d^{l20J}$ or $d^v)$	25	25
Off-black, opisthotonus	*dsu*/*dsu*, d^{l20J}/d^{l20J}	8	8

The number of progeny expected in each class was calculated assuming that *dsu* suppresses the coat color but not the neurological defect of d^{l20J}, as observed. The observed number did not differ significantly from the expected ($\chi^2_3 = 3.65$; $0.5 > P > 0.25$).

hypothesized that the darker shade of gray was produced by one copy of *dsu* (*dsu*/+, d^v/d^v) and the lighter shade of gray was produced by zero copies of *dsu* (+/+, d^v/d^v). To confirm this prediction, we tested mice from each of these two phenotypic classes by crossing them to *dsu*/*dsu*, d^v/d^v mice.

The expectations from such a test are that the darker gray parent (presumably *dsu*/+, d^v/d^v), when crossed to a *dsu*/*dsu*, d^v/d^v mouse, will produce two classes of offspring: (*i*) *dsu*/*dsu*, d^v/d^v mice that will be off-black and (*ii*) *dsu*/+, d^v/d^v mice that will be dark gray. In contrast, the light gray parent (presumably +/+, d^v/d^v) when crossed to a *dsu*/*dsu*, d^v/d^v mouse will produce only dark gray progeny (*dsu*/+, d^v/d^v). The shade of gray would be darker than the original test parent because of the presence of one copy of *dsu*. The results of this test cross are summarized in Table 2. Twenty-nine mice were originally classified as light gray and were assumed to be +/+, d^v/d^v. Ten of these mice were tested and nine gave the predicted phenotypic classes of progeny described above. The other mouse was apparently misclassified and behaved like the darker gray class. Nine mice of the darker gray phenotypic class were tested. All proved to be, as predicted, *dsu*/+, d^v/d^v.

The results of this progeny test confirmed that *dsu* acts in a dosage-dependent manner and is, therefore, not a recessive gene but a semidominantly inherited gene. The subtle phenotypic distinction of zero and one copies of *dsu* was not noticed in previous experiments so the two gray phenotypic classes were reported as one in Table 1. After these findings, mice from the *dsu*, d^{l20J} crosses have been observed that are light gray as well as dark gray.

DISCUSSION

The data presented in this paper show that *dsu*, the murine dilute suppressor gene, is a semidominantly inherited gene whose action is not dependent solely on proviral mutations of the dilute gene. The results show that *dsu* can also suppress the coat color phenotype of a deletion mutation of *d*. This result is consistent with recently reported studies of the action of *dsu* on radiation-induced large deletions of the dilute gene and surrounding sequences (25). It is also consistent with the ability of *dsu* to suppress the coat colors of both ashen (*ash*) and leaden (*ln*) mice (26).

Table 2. Phenotypic and genotypic classes of progeny observed in an intercross of *dsu*/+, d^v/d^v mice

Coat color	No. of mice	Genotype	No. of progeny tested	No. with expected genotype
Light gray	29	+/+, d^v/d^v	10	9
Dark gray	42	*dsu*/+, d^v/d^v	9	9
Off-black	16	*dsu*/*dsu*, d^v/d^v	ND	ND

ND, not done. The observed number of progeny in each class did not differ significantly from the expected number ($\chi^2_2 = 3.99$; $0.25 > P > 0.1$).

A simple interpretation of the experimental results obtained in these studies is that *dsu* produces a mutant protein that can substitute for the lack of the *d* gene product in melanocytes but not neural tissue or, alternatively, that suppression results from the abnormal temporal or developmental expression of an otherwise normal *dsu* gene product. Either model is compatible with the observation that *dsu* acts in a dosage-dependent manner on *d* and that the product of *dsu* on a wild-type genetic background [*dsu*/*dsu*, +/+ (at *d*)] has no effect on coat color. Consistent with these hypotheses, *dsu* does not appear to affect the level or sizes of *d* transcripts identified in brain tissue of d^{l20J} homozygous mice.

dsu is not the only known extragenic suppressor that appears to act in a manner consistent with compensation of a defective gene product. In the case of holoenzymes, a mutation in one subunit can be compensated for by a mutation in a second subunit, resulting in a suppression of the mutant phenotype. An example of this can be seen in the DNA polymerase III of *Salmonella typhimurium* where a mutation in the *dnaZ* gene, which encodes the γ subunit, is suppressed by mutations in the *dnaN* gene, which encodes the β subunit (27). It is unlikely that *dsu* acts in this manner as it can suppress a presumed functionally null allele of *d*, d^{l20J}. Rather, the suppression of *d* by *dsu* is more analogous to the suppression of β-tubulin mutations in *Saccharomyces cerevisiae*. Weil *et al.* (28) have identified two extragenic suppressors of *benA33*, a heat-sensitive β-tubulin mutation, which probably encode previously unidentified nontubulin proteins involved in microtubule function. However, unlike the interaction of *dsu* with *d* alleles, the interaction of *benA33* suppressors, *mipA* and *mipB*, are allele specific and do not suppress all *benA* alleles.

The compensatory model of action of *dsu* suggests that *d* and *dsu* may be evolutionarily and functionally related. If so, probes obtained from various *d* locus transcripts may ultimately be identified that cross-hybridize to *dsu* transcripts, thus facilitating the identification and molecular characterization of *dsu* and our understanding of the nature of this suppression at the biochemical level.

We thank Linda Brubaker for typing the manuscript and Hope Sweet (The Jackson Laboratory, Bar Harbor, ME) for providing us with the d^{l20J} allele. M.C.S. is the recipient of a postdoctoral fellowship from the National Institutes of Health (HD 07014). This research was supported in part by The National Cancer Institute, Department of Health and Human Services, under Contract NO1-CO-23909 with Bionetics Research, Inc.

1. Parkhurst, S. M. & Corces, V. G. (1986) *Mol. Cell. Biol.* **6**, 47–53.
2. Modollel, J., Bender, W. & Meselson, M. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 1678–1682.
3. Zachar, Z., Davison, D., Garza, D. & Bingham, P. M. (1985) *Genetics* **111**, 495–515.
4. Parkhurst, S. M. & Corces, V. G. (1985) *Cell* **41**, 429–437.
5. Roeder, G. S. & Fink, G. R. (1983) in *Mobile Genetic Elements*, ed. Shapiro, J. A. (Academic, New York), pp. 299–328.
6. Kubli, E. (1986) *Trends Genet.* **2**, 204–209.
7. Sweet, H. O. (1983) *J. Hered.* **74**, 305–306.

8. Market, C. L. & Silvers, W. K. (1956) *Genetics* **41,** 429–450.
9. Russell, E. S. (1948) *Genetics* **33,** 228–236.
10. Copeland, N. G., Hutchinson, K. W. & Jenkins, N. A. (1983) *Cell* **33,** 379–387.
11. Jenkins, N. A., Copeland, N. G., Taylor, B. A. & Lee, B. K. (1981) *Nature (London)* **293,** 370–374.
12. Rinchik, E. M., Russell, L. B., Copeland, N. G. & Jenkins, N. A. (1986) *Genetics* **112,** 321–342.
13. Russell, L. B. (1971) *Mutat. Res.* **11,** 107–123.
14. Russell, W. L. (1951) *Cold Spring Harbor Symp. Quant. Biol.* **16,** 89–96.
15. Siracusa, L. D., Russell, L. B., Jenkins, N. A. & Copeland, N. G. (1987) *Genetics* **117,** 85–92.
16. Jenkins, N. A., Copeland, N. G., Taylor, B. A. & Lee, B. K. (1982) *J. Virol.* **43,** 26–36.
17. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
18. Kato, N., Pfeiffer-Ohlsson, S., Kato, M., Larson, E., Ryndert, J., Ohlsson, R. & Cohen, M. (1987) *J. Virol.* **61,** 2182–2191.
19. Goldberg, D. A. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5794–5798.
20. Wahl, G. M., Stern, M. & Stark, G. R. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 3683–3687.
21. Denhardt, D. T. (1966) *Biochem. Biophys. Res. Commun.* **23,** 641–646.
22. Hu, F. & Lesney, P. F. (1964) *Cancer Res.* **24,** 1634–1643.
23. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5201–5205.
24. Kost, T. A., Theodorakis, N. & Hughes, S. H. (1983) *Nucleic Acids Res.* **11,** 8287–8301.
25. Russell, L. B. & Montgomery, C. S. (1987) *Genetics* **116,** s7 (abstr.).
26. Moore, K. J., Swing, D. A., Rinchik, E. M., Mucenski, M. L., Buchberg, A. M., Copeland, N. G. & Jenkins, N. A. (1988) *Genetics* **119,** 933–941.
27. Engstrom, J., Wong, A. & Maurer, R. (1986) *Genetics* **113,** 499–515.
28. Weil, C. F., Oakley, C. E. & Oakley, B. R. (1986) *Mol. Cell. Biol.* **6,** 2963–2968.