The Murine *dilute suppressor* Gene Encodes a Cell Autonomous Suppressor

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ABSTRACT

The murine *dilute suppressor* gene (*dsu*) suppresses the coat-color phenotype of three pigment mutations, *dilute* (*d*), *ashen* (*ash*) and *leaden* (*ln*), that each produce adendritic melanocytes. Suppression is due to the ability of *dsu* to partially restore (*ash* and *ln*), or almost completely restore (*d*), normal melanocyte morphology. While the *ash* and *ln* gene products have yet to be identified, the *d* gene encodes a novel myosin heavy chain (myosin 12), which is speculated to be necessary for the elaboration, maintenance, and/or function of melanocyte cell processes. To begin to discriminate between different models of *dsu* action, we have produced aggregation chimeras between mice homozygous for *dsu* and mice homozygous for *d* to determine if *dsu* acts cell autonomously or cell nonautonomously. In addition, we have further refined the map location of *dsu* in order to examine a number of possible *dsu* candidate genes mapping in the region and to provide a genetic basis for the positional cloning of *dsu*.

THE murine *dilute suppressor* (*dsu*) gene was first recognized by its ability to suppress the dilute coat color phenotype of mice homozygous for the retrovirally induced *dilute-viral* (d^v) allele (SWEET 1983). Mice homozygous for *dilute* are gray in color due to clumping and inefficient transport of pigment granules resulting from the absence of normal dendrites. Mapping studies showed that *dsu* is located on chromosome *1* (SWEET 1983), unlinked to *d* on chromosome *9*, indicating that *dsu* acts in *trans* to suppress the *d* phenotype. While *dsu* was originally reported as being recessive, additional studies showed that *dsu* acts in a semidominant fashion (MOORE *et al.* 1988a). Suppression of dilute coat color results from the ability of *dsu* to restore, to near normal, the adendritic melanocyte morphology of *d* melanocytes (MOORE *et al.* 1988b). Mice homozygous for d^v and *dsu* are off-black, as opposed to being completely black, and have nearly, but not completely, normal dendritic melanocyte morphology (MOORE *et al.* 1988b).

Two other recessive coat color mutations, *ashen* (*ash*) and *leaden* (*ln*), have also been identified that display a dilution of coat color as a consequence of adendritic melanocyte morphology (MARKET and SILVERS 1956; LANE and WOMACK 1979). Interestingly, *d* and *ash* are closely linked (~1 cM) on chromosome *9* (MOORE *et al.* 1988b) while *dsu* and *ln* are loosely linked (~9 cM) on chromosome *1* (MOORE *et al.* 1988b). The significance, if any, of this linkage remains to be determined. By breeding mice homozygous for *dsu* in addition to *ash* or *ln*, MOORE *et al.* (1988b) showed that *dsu* can suppress the coat-color phenotype of *ash* and *ln*. However, suppression was not nearly as complete as with d^v and the restoration of normal melanocyte morphology was also not as complete as with d^v.

While the *ash* and *ln* gene products have yet to be identified, the *d* gene has been shown to encode a novel myosin heavy chain, recently termed *myosin 12, Myh12* (ENGLE and KENNET 1994). This information, combined with the knowledge that *d* melanocytes are adendritic, suggests that *d* may be required for the normal elaboration, maintenance, and/or function of melanocyte cell processes. In addition to melanocytes, dilute transcripts are also expressed in many other embryonic and adult tissues and are very abundant in neurons of the central nervous system, cephalic ganglia, and spinal ganglia (MERCER *et al.* 1991). This expression pattern is interesting as most *dilute* mutations (with the rare exception of alleles such as d^v) produce a severe neurological disorder, characterized by convulsions and opisthotonus, apparent at 8–10 days after birth and continuing until the death of the animal at 2–3 weeks of age. *Dilute* may therefore play a similar role in neurons as it does in melanocytes. While *dsu* was originally identified by its ability to suppress the coat-color phenotype of d^v mice, it does not appear to suppress the neurological defect associated with most *d* alleles (MOORE *et al.* 1988a). This result raises the possibility that *dsu* functions in melanocytes but not in neurons.

In a related series of experiments, the ability of *dsu* to suppress the coat-color phenotype of 14 additional diluting mutations was assessed (MOORE *et al.* 1990). The diluted coat color of each of these mutants is not a consequence of melanocyte morphology or melanosome transport. Instead, they appear to act in other stages of melanogenesis. In no case was *dsu* able to suppress the coat color phenotype of these 14 mutations. Unexpectedly, *dsu* did suppress the ruby eye color of *ruby-eye* (*ru*)

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and *ruby-eye 2* (*ru2*) mice, to black (MOORE *et al.* 1990). The molecular basis for these two mutations has not been determined. Histological examination of the pigmented tissues of the eyes of these mice indicated that *dsu* suppresses the ruby eye color by increasing the apparent 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. *dsu* may thus act specifically on neural crest-derived melanocytes.

While the mechanism(s) by which *dsu* suppresses these phenotypes is unclear, two models have been proposed (MOORE *et al.* 1988b). In the first model, *dsu* would encode an altered protein that can partially compensate for the absence of the *d*, *ash*, and *ln* gene products or, alternatively, that suppression results from the abnormal temporal or developmental expression of an otherwise normal *dsu* product. In the second model, *dsu* would be involved in maintenance of the extracellular matrix surrounding normal melanocytes. It has been shown that *d* and *ln* melanocytes can extend dendrites when placed into a less compact tissue environment such as the anterior chamber of the eye (MARKET and SILVERS 1956). Mutant *dsu* protein could alter the normal extracellular matrix to make it less compact thereby allowing for dendrite outgrowth from mutant melanocytes.

Both models make certain testable predictions. The first model predicts that *dsu* is cell autonomous while the second model predicts that *dsu* is cell nonautonomous. In experiments described here, we have tested these predictions by making aggregation chimeras between two stocks of mice, one carrying *dsu* and one carrying *d*, and examining the coat-color phenotypes of the resultant chimeras. Concordant with the chimera studies we have also developed an interspecific backcross segregating *dsu* in order to refine the genetic map location of *dsu*, test possible *dsu* candidate genes mapping in the region, and provide a genetic basis for the positional cloning of *dsu*.

MATERIALS AND METHODS

Mice: All the mice used in this study are maintained by the Mammalian Genetics Laboratory at the NCI-Frederick Cancer Research and Development Center. The *dilute-viral* (d^v) allele was used throughout these studies (RINCHIK *et al.* 1986) in order to be able to follow the segregation of *d* by Southern blot analysis.

Aggregation chimeras: Both *dsu/dsu*, c^{2J}/c^{2J}, d^v/d^v and (DBA/2J × C57BL/6J-$d^v se/d^v se$) F_1 female mice were superovulated by intraperitoneal (i.p.) injection of 5 IU of pregnant mare's serum gonadotrophin (PMSg). Approximately 46–48 hr later, these females were injected i.p. with 5 IU of human chorionic gonadotrophin (HCG) and placed with males of the same genotype. Approximately 68–72 hr after the HCG treatment the females were sacrificed and the embryos, now 2 to 2.5 days post-coitum (5–8 cell stage) were flushed from the oviduct. The zona pellucidae were removed using 5 mg/ml proteinase K in Whitten's medium. The embryos were then twice washed in Whitten's medium. The embryos were placed, pairwise, (one of each of the two genotypes) into droplets of Whitten's medium that were overlayed with media-equilibrated paraffin oil and pushed together. The embryos were then incubated overnight at 37° in a humidified chamber containing 5% CO_2. The following morning those embryo pairs that had undergone compaction were transferred to the uteri of pseudopregnant females.

Interspecific backcross mice: Mice homozygous for *dsu* and d^v were crossed to *Mus spretus*. The resultant F_1 females (*dsu*/+, d^v/+) were backcrossed to the *dsu*, d^v parent. Fifty percent of the backcross progeny were *white-bellied agouti* (A^w/a) due to the inheritance of the dominant *white-bellied agouti* allele from *M. spretus*. These mice were discarded as *dsu* cannot easily be typed in *agouti* animals. Of the remaining backcross animals, which were *non-agouti* (*a/a*), half were heterozygous for d^v. These animals can not be typed for *dsu* in the absence of homozygous d^v. Thirty-six of these mice were saved for DNA analysis to increase the number of informative meioses for markers flanking *dsu*. The rest of these animals were not included. The remaining 25% of the backcross progeny that were *a/a*, d^v/d^v were typed for the presence of 1 or 2 copies of *dsu* by visual inspection. Those animals carrying two copies of *dsu* (*dsu/dsu*, *a/a*, d^v/d^v) were off-black while those animals carrying one copy of *dsu* (*dsu*/+, *a/a*, d^v/d^v) were gray. After typing the animals for *dsu*, they were euthanized and DNA was made for subsequent linkage analysis.

Southern blot analysis: High molecular weight genomic DNA was extracted from mouse tissues as described (JENKINS *et al.* 1982). DNAs (5 μg per lane) were digested to completion with various restriction endonucleases (8 units of enzyme/μg DNA), electrophoresed through 0.8% agarose gels and processed as described (JENKINS *et al.* 1982), except that Zetabind (Cuno) was substituted for nitrocellulose. Hybridization solutions were as described (JENKINS *et al.* 1982). Hybridization temperature and washing conditions varied with the probe in use. All blots were washed twice with 2 × SSCP, 0.1% sodium dodecyl sulfate for 20 min each, then washed 2–3 times in the final wash concentration as described below for 20 min each. For probes to the *d*, *Ctla4*, *Cryg*, *Fn1*, *Tnp1* and *Emv17* loci, the hybridization temperature and washing temperature was 65° and the final concentration of the wash solutions was 0.5 × SSCP, 0.1% sodium dodecyl sulfate, except for *d*, which was 0.2 × SSCP, 0.1% sodium dodecyl sulfate. For probes to the *Vil*, *Gpcr16* and *Mtap2* loci, the hybridization and washing temperature was 60° and the final wash concentration was 0.5 × SSCP, 0.1% sodium dodecyl sulfate. Filters were autoradiographed at −70° with Kodak XAR film and 2 DuPont Lightening Plus intensifying screens for 1–5 days.

Probes: The *dilute* probe, p0.3 (originally published as the *Pst* probe (COPELAND *et al.* 1983), is a unique 2.6-kb cellular DNA sequence located 3′ of the *Emv3* 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 9-kb fragment in wild-type chromosomes. The DNA probe, pS1.25, used for the detection of *ecotropic murine leukemia provirus 17* (*Emv17*), is a 1.2-kb *Pst*I-*Sst*I unique sequence fragment derived from cellular sequences located 5′ to the *Emv17* viral integration site of the RF/J strain of mice. The probe for *cytotoxic T lymphocyte associated protein 4* (*Ctla4*) is a 1.9-kb mouse cDNA cloned in pUC9 and was a gift from P. GOLSTEIN (Centre d'Immunologie Inserm-CNRS de Marseille-Luming, Marseille, France). The pMg4CrL probe for *gamma crystallin* (*Cryg*) is a 550-bp mouse cDNA clone in pBR322 and was a gift from J. PIATIGORSKY (National Eye Institute, Bethesda, Maryland). Two probes, pFH1 and pFH6, were used to detect the *fibronectin 1* (*Fn1*) gene; both were gifts from F. C. BARALLE (International Centre for Genetic Engineering and Biotechnology, Padriciano, Italy). pFH1 is a 2.5-kb human cDNA sequence in

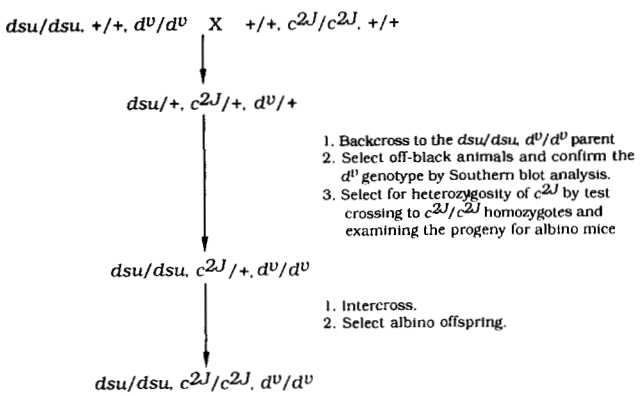

FIGURE 1.—Breeding scheme for the derivation of mice homozygous for *dsu*, c^{2J} and d^v.

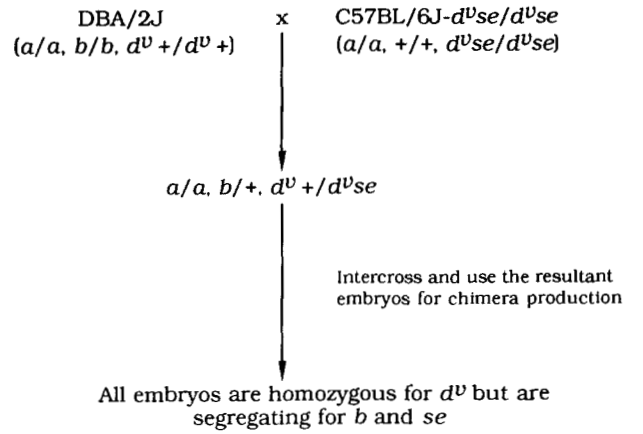

FIGURE 2.—Breeding scheme for the production of d^v/d^v embryos.

pAT153 and pFH6 is a 1.2-kb human cDNA sequence in pAT153. The probe used to detect the *villin* (*Vil*) gene is a 530-bp human cDNA sequence cloned in psP64. The *transition protein 1* (*Tnp1*) gene, defined by probe pTP1, was detected using a 450-bp mouse cDNA clone that is cloned in Bluescript. It was a gift from N. HECHT (Tufts University, Boston). The pGLC101 probe used to detect the *myosin light chain, alkali, fast skeletal muscle* (*Mylf*) gene is a 450-bp mouse cDNA sequence in the vector Bluescript. The p56 probe used to detect the *microtubule associated protein 2* (*Mtap2*) gene is a 1.2-kb mouse cDNA cloned into pUC and was a gift from N. COWAN (New York University Medical Center, New York). The *G protein coupled receptor 16* (*Gpcr16*) gene was detected using a subcloned, polymerase chain reaction-amplified cDNA fragment from mouse spermatogonia, spermatocytes and spermatids (WILKIE *et al.* 1993).

RESULTS

Chimera studies: To distinguish the clonal regions of coat color in chimeras derived from off-black *dsu/dsu*, d^v/d^v melanocytes and potentially suppressed off-black d^v/d^v melanocytes, we marked the parental *dsu/dsu*, d^v/d^v stock with a cell autonomous coat color marker, *albino 2J* (c^{2J}). This mutation prevents the synthesis of active tyrosinase needed for pigment production but does not alter melanocyte morphology. *dsu/dsu*, c^{2J}/c^{2J}, d^v/d^v mice will be phenotypically white due to the presence of the *albino* mutation. If *dsu* acts cell autonomously, the chimeras should have white hairs (from the *dsu/dsu*, c^{2J}/c^{2J}, d^v/d^v parent) and nonsuppressed gray hairs (from the d^v/d^v parent). Alternatively, if *dsu* acts cell nonautonomously, the chimeras should have white hairs and suppressed off-black hairs, or if suppression is incomplete, white, off-black, and gray hairs.

To produce a stock carrying c^{2J}, in addition to *dsu* and d^v, we crossed mice homozygous for c^{2J} to mice homozygous for *dsu* and d^v (Figure 1). Since *dsu* cannot be followed through genetic crosses in the absence of d^v (or another mutation that *dsu* suppresses), we maintained d^v in the *dsu* stock. The F_1 progeny (*dsu*/+, $c^{2J}/+$, $d^v/+$) were then backcrossed to the *dsu/dsu*, +/+, d^v/d^v parent. Backcross progeny that were off-black in color, and presumably *dsu/dsu*, +/?, d^v/d^v, were selected. Southern blot analysis was then used to confirm that these animals were homozygous for d^v. Fifty percent of these off-black mice should be heterozygous for c^{2J} and 50% should be wild type. These two genotypic classes were distinguished by crossing them to the c^{2J} homozygous parent. Mice that were confirmed to be *dsu/dsu*, $c^{2J}/+$, d^v/d^v were intercrossed. Twenty-five percent of the mice from this cross should be *dsu/dsu*, c^{2J}/c^{2J}, d^v/d^v and phenotypically albino. These mice were used to establish a *dsu/dsu*, c^{2J}/c^{2J}, d^v/d^v strain.

In our first attempts at chimera production we used either DBA/2J (which are homozygous for d^v) or C57BL/6J-d^v/d^v,*se/se* (abbreviated C57BL/6J-d^v*se*) mice for the d^v parent. These early attempts were, however, unsuccessful due to our inability to obtain a large number of healthy embryos from either strain. It has been previously reported that DBA/2J mice produce low numbers of eggs after superovulation. In these experiments we obtained, on average, only 4 embryos per superovulated DBA/2J female. C57BL/6J mice have been reported to produce large numbers of embryos after superovulation (HOGAN *et al.* 1986). However, we obtained less than 1 healthy embryo per superovulated C57BL/6J-d^v*se* female. By introducing d^v*se* into the C57BL/6J strain, the ability of C57BL/6J mice to be superovulated must have been severely reduced.

To increase the number of healthy d^v/d^v embryos we crossed DBA/2J to C57BL/6J-d^v*se* mice to produce F_1 females for superovulation (Figure 2). Although it has been reported that DBA/2J × C57BL/6J F_1 females, like the DBA/2J strain, produce low numbers of embryos, we found that our F_1 hybrid females now averaged 19 healthy embryos/superovulated female. All the embryos from the F_1 females (which were mated to DBA/2J × C57BL/6J-d^v*se* males) will be homozygous for d^v/d^v but will be heterozygous for other loci that differed between the two parental stocks including the two visible mutations *short ear* (*se*) and *brown* (*b*).

Nine phenotypically identifiable chimeras were produced from the aggregation of *dsu/dsu*, c^{2J}/c^{2J}, d^v/d^v and d^v/d^v embryos. All had coat colors that showed a contribution of both non-pigmented (white) hairs from

FIGURE 3.—Chimeric *dsu/dsu*, c^{2J}/c^{2J}, $d^v/d^v \leftrightarrow d^v/d^v$ mouse. The coat color shows white and gray hairs only. Nondilute pigmented hairs were not observed.

the *dsu/dsu*, c^{2J}/c^{2J}, d^v/d^v embryo, and pigmented hairs from the d^v/d^v embryo. In each of the nine embryos the regions of pigmented hairs were dilute in color. Six of the nine chimeras had gray and white bands of hair (Figure 3). The remaining three had dilute brown and white bands of hair. The presence of dilute brown rather than gray was due to homozygosity of the *b* allele that was segregating in some d^v/d^v embryos. The absence of off-black hairs in all chimeric mice suggests that *dsu* acts in a cell autonomous manner.

Interspecific backcross mapping panel segregating *dsu*: An interspecific backcross mapping panel segregating *dsu* was generated to test possible *dsu* candidate genes and to use as a genetic reagent for positionally cloning *dsu*. These mice were produced by mating homozygous *dsu*, d^v mice to *M. spretus* and then mating the resultant F_1 progeny back to the *dsu*, d^v parent (see MATERIALS AND METHODS). In total, 151 backcross progeny segregating *dsu* were produced. DNAs from these backcross mice were then typed for a number of *dsu* candidate genes that were known to map in the *dsu* region (SELDIN *et al.* 1992; WILKIE *et al.* 1993) including genes encoding proteins associated with the cytoskeleton such as *microtubule associated protein 2, Mtap2*; *myosin light chain, alkali, fast skeletal muscle, Mylf*; *villin, Vil*; and *gamma crystallin, Cryg*, as well as genes encoding proteins associated with the extracellular matrix such as *fibronectin 1, Fn1*. A few additional markers were also placed on the genetic map as potential landmarks for future positional cloning of *dsu* such as *cytotoxic T lymphocyte associated protein 4, Ctla4*; *transition protein 1, Tnp1*; *G protein coupled receptor 16, Gpcr16*; and *ecotropic murine leukemia provirus 17, Emv17*.

DNAs from the two parental strains were digested with several restriction enzymes and analyzed by Southern blot hybridization for informative restriction fragment length polymorphisms (RFLPs) using each of the probes listed in Table 1. Recombination distances were calculated as described by GREEN (1981) using the computer program Spretus Madness. Gene order was determined by minimizing the number of recombination events required to explain the allele distribution pattern. Thirty-six additional DNAs from backcross mice, which were not homozygous d^v and could not be typed for *dsu*, were also included in the analysis to increase the number of informative meioses. Figure 4 shows the segregation

TABLE 1

Loci symbols, loci names, probes and RFLPs used for interspecific backcross mapping

Locus	Gene name	Probe	Restriction enzyme	Fragment sizes(s) of major bands (kb)[a]		Probe reference
				dsu, d^v	*M. spretus*	
Ctla4	*Cytotoxic T lymphocyte associated protein 4*	M1797	*Msp*I	9.6	3.5	BRUNET *et al.* (1987)
Cryg	*Gamma crystallin*	pMg4CrL	*Pvu*II	10, 8.5, 6.0, 3.4	10, 6.0 3.8, 3.4	SHINOHARA *et al.* (1982)
Mtap2	*Microtubule associated protein 2*	p56	*Bgl*II	11	15	LEWIS *et al.* (1986)
Mylf	*Myosin light chain alkali, fast skeletal muscle*	pGLC101	*Eco*RI	8.2	1.8	ROBERT *et al.* (1988)
Fn1	*Fibronectin 1*	pFH1	*Hin*dIII	7.6, 4.5 2.5	4.5, 3.3, 2.5	KORNBLIHTT *et al.* (1985)
		pFH6	*Hin*dIII	8.2, 5.4 2.7, 1.0	5.4, 2.8, 2.7, 1.0	KORNBLIHTT *et al.* (1985)
Vil	*Villin*		*Bgl*II	5.0, 1.5	7.4, 1.5	PRINGAULT *et al.* (1986)
Tnp1	*Transition protein 1*	pTP1	*Hin*dIII	8.1	7.9	YELICK *et al.* (1991)
Gpcr16	*G protein coupled receptor 16*		*Pst*I	6.0	6.7	WILKIE *et al.* (1993)
Emv17	*Ecotropic murine leukemia provirus 17*	pPS1.25	*Taq*1	8.2, 1.6, 0.5	2.5	BUCHBERG *et al.* (1986)
			*Bgl*II	6.2	9.6	

[a] The RFLPs followed in the backcross animals are underlined.

analysis for the 187 backcross mice analyzed. All N_2 mice were typed for *Ctla4*, *Cryg*, *Mtap2*, *Fn1*, *Vil* and *Emv17*. *Mylf* was typed only in those animals that were recombinant in the *Cryg* to *Vil* interval, which spans the known *Mylf* map location. Similarly, *Gpcr16* was typed only in those animals that were recombinant in the *Mtap2* to *Emv17* interval and *Tnp1* was typed only in those animals that were recombinant in the *Mylf* to *Emv17* interval. Barring the highly improbable event of a double recombination within the interval examined, analyzing only those animals that already had been determined to have a recombination event within the examined interval is as effective as analyzing all N_2 animals.

The ratios of the total number of mice carrying recombinant chromosomes to the total number of mice analyzed for each pair of loci and the most likely gene order are: centromere–*Ctla4*–4/187–*Cryg*–1/187–*Mtap2*–2/187–*Mylf*–6/187–*Fn1*–1/151–*dsu*–5/151–*Vil*, *Gpcr16*, *Tnp1*–23/187–*Emv17*. The recombination frequencies expressed as genetic distance in centiMorgans ± the standard errors between each pair of loci are *Ctla4*–2.1 ± 1.1–*Cryg*–0.5 ± 0.5–*Mtap2* –1.1 ± 0.8–*Mylf*–3.2 ± 1.3–*Fn1*–0.7 ± 0.7–*dsu*–3.3 ± 1.5–*Vil*, *Gpcr16*, *Tnp1*–12.3 ± 2.4–*Emv17*. Barring intragenic recombination, these mapping results exclude any of these loci as candidate genes for *dsu*. One locus mapping proximal of *dsu*, *Fn1*, only had one recombinant with *dsu*. Two probes for *Fn1*, one representing the 5′ end and one from the 3′ end of the gene were mapped relative to *dsu* to exclude the possibility of an intragenic recombination within *Fn1* as being the *dsu* mutation. Three loci mapping distal of *dsu*, including *Vil*, *Tnp1*, and *Gpcr16* had three recombinants with *dsu* identifying them, and *Fn1*, as the closest linked flanking genes mapped in this study and potential starting points for the positional cloning of *dsu*.

DISCUSSION

The results of our chimera experiments suggest that *dsu* is a cell autonomous suppressor. This result is inconsistent with the model previously described suggesting that *dsu* acts within the melanocyte extracellular matrix but would be consistent with a model suggesting that *dsu* encodes a product that can somehow compensate for a mutant *d* (or *ash* and *ln*) gene product within melanocytes. Experiments demonstrating that *dsu* can suppress a null allele of the *dilute* locus, d^{J20J} (MOORE *et al.* 1988a), are also consistent with this compensation model.

One attractive notion is that *dsu* encodes a dilute-related protein that is only expressed in mutant *dsu* melanocytes, or alternatively, is increased in expression in *dsu* melanocytes. To date, however, we have been unable to identify any murine *dilute*-related genes by low stringency Southern blot analysis.

It is also possible that *dsu* encodes a myosin-related protein belonging to a different subclass from dilute. Myosin heavy chains are generally grouped into two distinct classes. Myosin IIs (two-headed myosins) include the conventional myosins that participate in a wide variety of cellular processes including muscle contraction and cytokinesis. Myosin IIs function as dimers and are composed of an N-terminal head region that contains ATP- and actin-binding domains and a C-terminal α-helical tail that promotes dimerization. Myosin Is (one-headed myosins) contain an N-terminal head region similar to myosin IIs, but unlike myosin IIs, they contain a non-α-helical tail and function as monomers. Myosin Is include the membrane-associated myosin from Acanthamoeba (MIYATA *et al.* 1989) and the bovine brush border myosin and have been implicated in pseudopod extension and membrane ruffling. Dilute appears to be intermediate between these two classes of

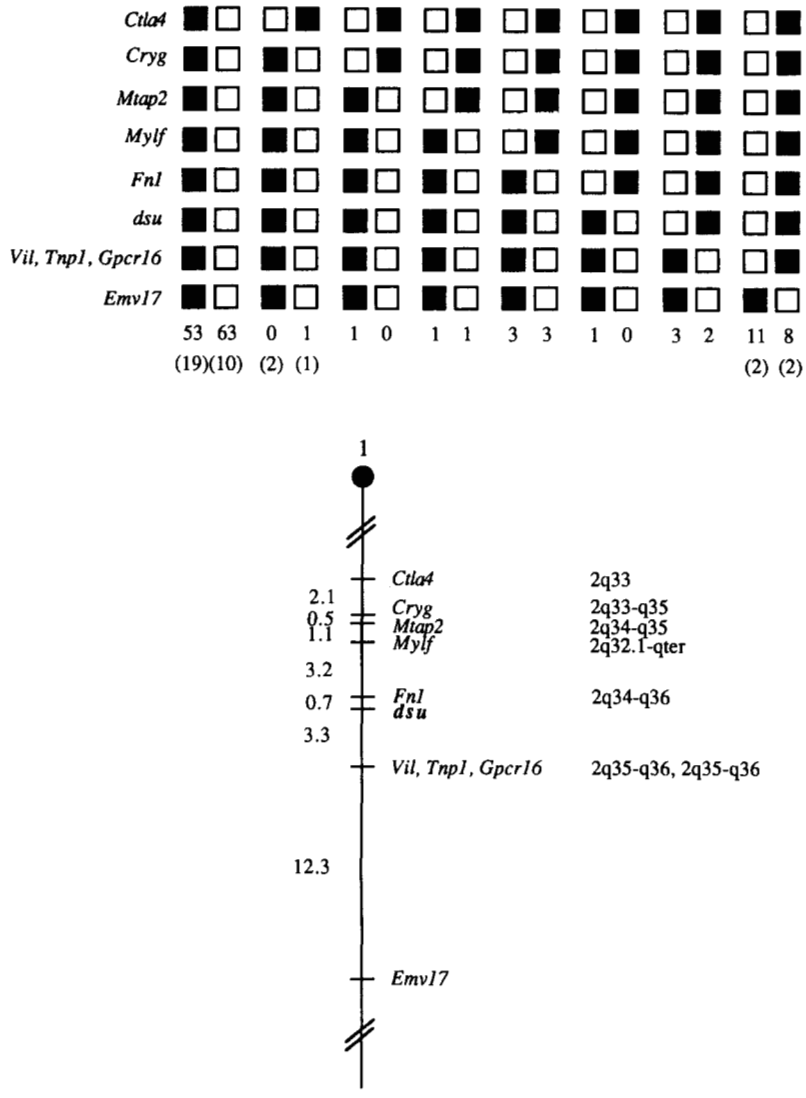

FIGURE 4.—Interspecific backcross linkage map of the *dsu* interval. A pedigree analysis of 187 N_2 progeny from the interspecific backcross segregating *dsu* is shown in the top panel. The loci followed in the backcross are listed to the left. Each column represents the chromosome identified in the N_2 progeny that was inherited from the (*dsu*/*dsu*, d^v/d^v × *M. spretus*) F_1 parent. The open squares represent the presence of a *M. spretus* allele. The black squares represents the presence of a *dsu* allele. The number of N_2 progeny (among 151 animals segregating *dsu*), carrying each type of chromosome is listed below the columns. The numbers in parentheses represent an additional 36 N_2 animals that were not typed for *dsu* but which were typed for *dsu* flanking markers. A partial chromosome *1* linkage map showing the location of *dsu* in relation to linked genes is shown at the bottom. The genetic distance, in centiMorgans, between each set of markers is shown to the left and the human map positions, where known, are shown to the right (*dsu* and *Gpcr16* are not mapped in humans). References for the human map positions of loci mapped in this study can be obtained from GDB (Genome Data Base), a computerized database of human linkage information maintained by The William H. Welch Medical Library of The Johns Hopkins University (Baltimore, Maryland).

proteins. Like myosin Is and IIs, the dilute protein contains an N-terminal head region but unlike myosin Is and IIs, the dilute C terminus contains both α-helical and non-α-helical regions (MERCER *et al.* 1991). In spite of this peculiar C-terminal structure, recent experiments utilizing the chicken homolog of dilute (ESPINDOLA *et al.* 1992; ESPREAFICO *et al.* 1992; SANDERS *et al.* 1992) have shown that dilute functions as a dimer similar to myosin IIs (CHENEY *et al.* 1993). In view of the unique nature of the dilute protein it seems unlikely that one of the previously identified myosin Is or IIs could compensate for the absence of the *dilute* gene product.

A gene that shares many of the same structural features as *dilute* is the *MYO2* gene of yeast (JOHNSTON *et al.* 1991). Yeast harboring mutations in *MYO2* fail to complete budding, show a disorganized actin cytoskeleton and an accumulation of vesicles. Surprisingly, the *MYO2* mutant phenotype can be suppressed by the overexpression of a novel member of the kinesin superfamily of microtubule-based motors (LILLIE and BROWN 1992). This raises the interesting possibility that *dsu* also encodes a non-actin-based motor such as kinesin or dynein. *In vivo* studies using anti-kinesin antibodies, have shown kinesin to be involved in the movement of pigment granules in melanophores of the teleost black tetra (RODIONOV *et al.* 1991) and with membrane bound organelles within nerve axons (HIROKAWA *et al.* 1991). Dynein has been shown to be a microtubule associated retrograde translocator (PASCHAL and VALLEE 1987). Both kinesin and dynein are encoded by a large family of genes in mammalian cells and the identification and characterization of these gene families has only just begun (VALE and GOLDSTEIN 1990). None of the current members of these gene families have been shown to map near *dsu* (N. G. COPELAND and N. A. JENKINS, unpublished observations). As new members are identified we can map them in our interspecific backcross segregating *dsu* to determine if any cosegregate with *dsu*.

That *dsu* may encode a non-actin-based motor that is either turned on or overexpressed in *dsu* melanocytes and can compensate for the absence of *d* is a very attractive notion. Alternatively, *dsu* may encode a mutant protein that can compensate for the lack of *d*. This

model could easily account for the fact that suppression of *d*, *ash* and *ln* is not complete, as it is unlikely that a non-actin-based motor could completely compensate for an actin-based motor like dilute.

The true mechanism of *dsu* suppression will likely only be known once the *dsu* gene product is cloned. As we have already excluded most preexisting *dsu* candidate genes, we have initiated a positional cloning approach to *dsu*. Many recent advances made possible by the mouse genome effort (reviewed in COPELAND *et al.* 1993) now make it feasible to contemplate positionally cloning virtually any mouse mutation. Toward this end we have begun to expand our *dsu* interspecific backcross and type additional markers that are expected to lie in the *dsu* region.

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