MUTATIONS AND CHROMOSOMAL REARRANGEMENTS AFFECTING THE EXPRESSION OF SNAIL, A GENE INVOLVED IN EMBRYONIC PATTERNING IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

Mutants at the snail locus are zygotically acting embryonic lethals that affect dorsoventral patterning. A comparison of seven mutant alleles shows considerable variation in expressivity and a graded effect along the dorsoventral axis: more extreme alleles result in the abnormal development of the dorsally derived ectoderm as well as the ventrally derived mesoderm, whereas weaker alleles affect only development of the mesoderm. Animals transheterozygous for different mutant alleles occasionally survive to adulthood; they frequently have missing halteres and more rarely are hemithorax. The mutant phenotype of snail is shown here to be enhanced zygotically by haploidy of two nearby regions on the second chromosome: the elbow to no-ocelli region and the interval defined by *l(2)br36* and *l(2)br37*. It is concluded that the products of all of these genes function together in the process of specification of pattern in the embryo.

A defined sequence of tissues develops along the dorsoventral axis of Drosophila embryos. The ectoderm is derived from cells in the dorsal part of the blastoderm, whereas the mesoderm develops from cells situated laterally and ventrally; the endoderm derives from an anteroventral and posterior position. These three tissues form the germ layers of the embryo (POULSON 1950; LOHS-SCHARDIN, CREMER and NÜSSLEIN-VOLHARD 1979). At gastrulation the presumptive mesodermal cells invaginate by means of a ventral furrow extending most of the length of the embryo. Homozygous mutant snail embryos fail to form a ventral furrow, and subsequent development leads to differentiation of a twisted larval cuticle enclosing very few or no internal tissues (ANDERSON and NÜSSLEIN-VOLHARD 1983; SIMPSON 1983). A synergistic interaction between snail and the gene dorsal leading to lethality of snail heterozygotes has been described (SIMPSON 1983). dorsal acts maternally, and, when mutant, causes complete dorsalization of embryos such that structures normally made only on the dorsal side are formed in all regions of the embryo (NÜSSLEIN-VOLHARD 1979). dorsal and snail are, therefore, thought to be involved in the same developmental pathway.

Here, we describe a number of snail alleles and explore the effects of two other second chromosome regions on snail expression. Our attention was drawn to the elbow to no-ocelli region by the study that ASHBURNER, TSUBOTA and WOODRUFF (1982) made of the Scutoid chromosome. These authors showed that on this chromosome the no-ocelli gene has been transposed to a position immediately distal to snail. They obtained evidence that suggested an interaction between snail and the elbow-no ocelli region. We present further data in support of this suggestion and report the discovery of another interacting gene(s) just proximal to snail.

MATERIALS AND METHODS

The mutations and rearrangements used in this study are listed in Table 1 together with their sources. They were balanced over either *CyO: In(2LR)O, Cy* dp^{lvl} *pr* cn^2, or *CyO, bw: In(2LR)O, Cy* dp^{lvl} *pr* cn^2 *bw* or *In(2L)Cy* + *In(2R)Cy,* al^2 *Cy pr Bl* cn^2 *vg c* sp^2. Viability data are obtained from crosses between stocks carrying mutant chromosomes balanced over *Cy* and are expressed as the number of Cy^+ progeny over total progeny. For a description of other mutations used see LINDSLEY and GRELL (1968). All crosses were done at 25°.

New mutations reported here were generated with 0.025 M EMS according to the method of LEWIS and BACHER (1968), or 4000-r X rays generated by a Philips MG102 constant potential X ray machine (100 kV, 10 mA).

Flies were raised on a corn meal, soy bean flour, yeast, malt extract, molasses and sugar medium.

Living embryos were observed by transmitted light under 3s Voltaleff oil (NÜSSLEIN-VOLHARD 1977). Crosses were made between heterozygous stocks balanced over *CyO*. *CyO* homozygotes die as first instar larvae, and unhatched eggs can, therefore, be presumed to be mutant embryos. Late embryos were mounted in Hoyers medium (VAN DER MEER 1977).

RESULTS

Identification and complementation patterns of snail alleles: The first allele of snail, sna^{IIG05}, was obtained by C. NÜSSLEIN-VOLHARD, E. WIESCHAUS and I. KLUDING (unpublished results) in a screen for mutations affecting embryonic pattern. We have subsequently obtained by mutagenesis a number of chromosomes that are noncomplementing with sna^{IIG05}. Five were obtained by X rays from a total of 13,700 treated chromosomes. Three of these, sna^{S1}, sna^{R1} and sna^{RY1}, are studied here (the interaction between these alleles and dorsal was described in SIMPSON 1983). Four EMS-induced mutations were isolated from 6300 treated chromosomes, and three of these are studied here: sna^{EY1}, sna^{EY2} and sna^{EY3}. Two additional alleles were supplied by M. ASHBURNER: sna^{HG31} was obtained in an EMS screen over a deficiency of the *sna* region, and Sco^{R+15} is an EMS-induced revertant of Scutoid (*Sco*), which is mutant only for *sna* (ASHBURNER *et al.* 1983).

ASHBURNER *et al.* (1983) assigned *sna* (they called it *l(2)br28*) to a small deficiency, *Df(2L)TE36-GW3* (see Table 1 and Figure 1), and described two flanking genes: *l(2)br35* distally and *l(2)br36* proximally. We have attempted to saturate this region and have screened 9000 EMS-treated chromosomes for a visible phenotype and 5000 EMS-treated chromosomes for a lethal phenotype over *Df(2L)TE36-GW3*. No new genes were discovered between *l(2)br35* and *l(2)br36*. For the purposes of this paper, therefore, we shall consider *sna* alleles to be "point" mutations if they complement both *l(2)br35* and *l(2)br36*. All of

the mutations we isolated complement the flanking mutants (data shown in Table 2) except sna^{S1} which is a deletion. *Df(2L)*sna^{S1} is mutant for *rd, l(2)br34, l(2)br35, sna, l(2)br36* and *l(2)br37* (see Figure 1) as determined independently by M. ASHBURNER and ourselves. Cytologically, however, it is not visible.

The results of crossing mutant *sna* alleles *inter se* are shown in Table 2. They clearly fall into one complementation group. Escapers are associated only with combinations of sna^{R1}, sna^{EY2} and sna^{EY3}; these will later be shown to have the weakest embryonic phenotype. The escapers very occasionally have one haltere missing. None of the alleles, not even the weaker ones, gave homozygous escapers, but no attempt has been made to remove extraneous lethals from the mutagenized chromosomes.

Complementation patterns of snail alleles with deletions and Scutoid revertants: Five deletion-bearing chromosomes have been studied (see Figure 1 and Table 1). In four of these the *sna* gene is deleted: *Df(2L)TE36-GW3, Df(2L)75c, Df(2L)75c Dp(2;2)GYS* and *Df(2L)*sna^{S1}, and one, *Df(2L)fn2,* is a deletion of the elbow to no-ocelli *(el-noc)* region. As can be seen in Table 3, all *sna* alleles are lethal over the *sna* deletions and viable over *Df(2L)fn2,* even though the viability is slightly reduced in some cases.

All of the *Sco* revertants used here were discovered and described by ASHBURNER *et al.* (1983). These authors have deduced that the *Sco* chromosome is a small reciprocal transposition wherein *noc osp* and *Adh* have been exchanged with at least three genes immediately distal to *sna*; as shown in Figure 1, the *Sco* chromosome is not mutant for *sna* (ASHBURNER, TSUBOTA and WOODRUFF 1982). These authors obtained indirect evidence suggesting that *el, l(2)br22, l(2)br29* and *noc* form some sort of complex or are under some sort of common control and possibly interact with the *sna* region. Experiments to be described later in this paper reveal a further interaction between the *el-noc* region and the *sna* locus. It should be borne in mind that the *Sco* revertants that are studied here are on a chromosome in which the normal order of the four genes in the *el-noc* region is perturbed.

ASHBURNER *et al.* (1983) determined that Sco^{R+25}, Sco^{R+19}, Sco^{R+7}, Sco^{R+16} and Sco^{R+10}, although not cytologically deficient, are genetically deficient for at least *sna* and *noc* (see Figure 1), whereas Sco^{R+15} is mutant only for *sna.* The authors based their conclusions on data obtained with sna^{HG31}. We have crossed seven snail alleles to these revertants and find that complete lethality is the rule (Table 3).

From crosses between *sna*-bearing females and *Dp(2;1)*Sco^{R+23}; *Df(2L)*Sco^{R+23}/*CyO* males some female escapers were found. They display an unexpected phenotype: one or both halteres are frequently missing and occasionally they are hemithorax [as described in ASHBURNER *et al.* (1983) for flies of the genotype *Dp(2;1)*Sco^{R+23}/+; *Df(2L)*Sco^{R+23}/sna^{HG31}]. This phenotype is somewhat similar to that of *el*; homozygous *el* flies have missing capitella and alar lobes. The *Sco* chromosome is not mutant for *el,* however, and all of the revertant/*sna* combinations studied here have two doses of el^+. *Dp(2;1)*Sco^{R+23} is situated proximally on the *X* chromosome, and, although it does not carry a normally functional sna^+ gene, it is possible that position effect variegation could lead to a partially functional sna^+ allele. We, therefore, tested the ability of the dupli-

TABLE 1

List of mutations and chromosomal rearrangements together with their cytology where known

Allele or rearrangement	Origin	Cytology	Cytology according to:	Genotype
sna^{IIG05}	EMS	No visible abnormality	G. RICHARDS	*sna*^{IIG05} *cn bw sp*
sna^{R1}	X ray	No visible abnormality	G. RICHARDS	*sna*^{R1}
sna^{RY1}	X ray	No visible abnormality	G. RICHARDS	*b sna*^{RY1} *pr cn bw*
sna^{EY1}	EMS			*b sna*^{EY1} *pr cn bw*
sna^{EY2}	EMS			*b sna*^{EY2} *pr cn bw*
sna^{EY3}	EMS			*b sna*^{EY3} *pr cn bw*
sna^{HG31}	EMS	No visible abnormality	M. ASHBURNER	*Adh*^{n7} *sna*^{HG31} *cn vg*
Df(2L)sna^{S1}	X ray	Not visibly deficient	G. RICHARDS	*Df(2L)sna*^{S1}
Df(2L)TE36-GW3	γ ray	35C1; 35D2	ASHBURNER *et al.* (1983)	*b Df(2L)TE36-GW3 pr pk cn sp*
Df(2L)75c	X ray	35A1-2; 35D4-7	WOODRUFF and ASHBURNER (1979)	*Df(2L)75c*
Df(2L)fn1	Formaldehyde	34F4-A1; 35D5-7	ASHBURNER, TSUBOTA and WOODRUFF (1982)	*Df(2L)fn1 pr cn*
Df(2L)fn2	Formaldehyde	35A3; 35B2-4	ASHBURNER, TSUBOTA and WOODRUFF (1982)	*Df(2L)fn2 pr cn*
Df(2L)osp^{29}	γ ray	35B1-2; 35E6	ASHBURNER, TSUBOTA and WOODRUFF (1982)	*Df(2L)osp*^{29}, *osp*^{29} *Adn*$^{n/3}$ *pr cn*
Df(2L)A48	X ray	35B3; 35D5-7	ASHBURNER, AARON and TSUBOTA (1982)	*b Df(2L)A48 cn bw*
Sco	X ray	Synapsis disturbed in 35	ASHBURNER, TSUBOTA and WOODRUFF (1982)	*Sco*
T(2;3)Sco^{R+7}	X ray	T(2;3)35D1-2;93F11-14 on *Sco*	M. ASHBURNER and G. HARRINGTON (unpublished data)	*T(2;3)Sco*$^{R+7}$
Df(2L)Sco^{R+10}	X ray	Cytologically like *Sco*	M. ASHBURNER and G. HARRINGTON (unpublished data)	*Df(2L)Sco*$^{R+10}$
Df(2L)Sco^{R+25}	X ray	Cytologically like *Sco*	M. ASHBURNER and G. HARRINGTON (unpublished data)	*b Df(2L)Sco*$^{R+25}$ *pr*
Df(2L)Sco^{R+16}	X ray	Cytologically like *Sco*	M. ASHBURNER and G. HARRINGTON (unpublished data)	*b Df(2L)Sco*$^{R+16}$ *pr*

Df(2L)Sco^{R+19}	X ray	Cytologically like *Sco*	M. ASHBURNER and G. HARRINGTON (unpublished data)	*b Df(2L)Sco*$^{R+19}$ *pr*
Df(2L)Sco^{R+23}	X ray	34F1-2;35D3-4	M. ASHBURNER and G. HARRINGTON (unpublished data)	*b Df(2L)Sco*$^{R+23}$ *pr*
Dp(2;1)Sco^{R+23}	X ray	34F1-2;35C1 into 20	M. ASHBURNER and G. HARRINGTON (unpublished data)	*Dp(2;1)Sco*$^{R+23}$
Sco^{R+15}	EMS	Cytologically like *Sco*	M. ASHBURNER and G. HARRINGTON (unpublished data)	*Sco*$^{R+15}$
l(2)br36	EMS			*Adh*^{n7} *l(2)br36*^{HG34} *cn (vg)*
l(2)br35	EMS			*Adn*^{n7} *l(2)br35*^{HG35} *cn (vg)*
Dp(2;2)GYS	Spontaneous	34D1-2;35C1-3 into 50A4-B5	ASHBURNER (1982)	*Df(2L)75c Dp(2;2)GYS bw*

TABLE 2

Viability of transheterozygous combinations of alleles of snail, l(2)br36 *and* l(2)br35

	sna^{R1}	*sna*^{RY1}	*sna*^{IIG05}	*sna*^{HG31}	*sna*^{EY1}	*sna*^{EY2}	*sna*^{EY3}	*Sco*$^{R+15}$	*sna*^{S1}	*l(2)br36*^{HG34}	*l(2)br35*^{HG35}
sna^{R1}	0/100									58/201	60/198
sna^{RY1}	0/703	0/1000								39/102	63/171
sna^{IIG05}	0/993	0/1038	0/1000							38/100	41/100
sna^{HG31}	0/752	0/611	0/597	0/700						83/400	103/456
sna^{EY1}	0/561	0/427	0/564	0/376	0/415					171/525	150/476
sna^{EY2}	4/762	0/510	0/570	0/625	0/345	0/480				186/618	173/562
sna^{EY3}	24/420[a]	0/535	0/570	0/513	0/344	0/256	0/477			201/656	232/728
Sco^{R+15}	0/423	0/616	0/445	0/305	0/406	0/1472	0/463	0/201		228/649	183/448
sna^{S1}	0/631	0/546	0/477	0/494	0/194	0/201	0/219	0/237	0/1000	0/301	0/298

Crosses are mutant/*Cy* × mutant/*Cy*; data are number of *Cy*$^{+}$ adults/total adult progeny. Some of the data concerning *sna*^{HG31} and *Sco*$^{R+15}$ were kindly supplied by M. ASHBURNER.

[a] Flies occasionally have missing halteres.

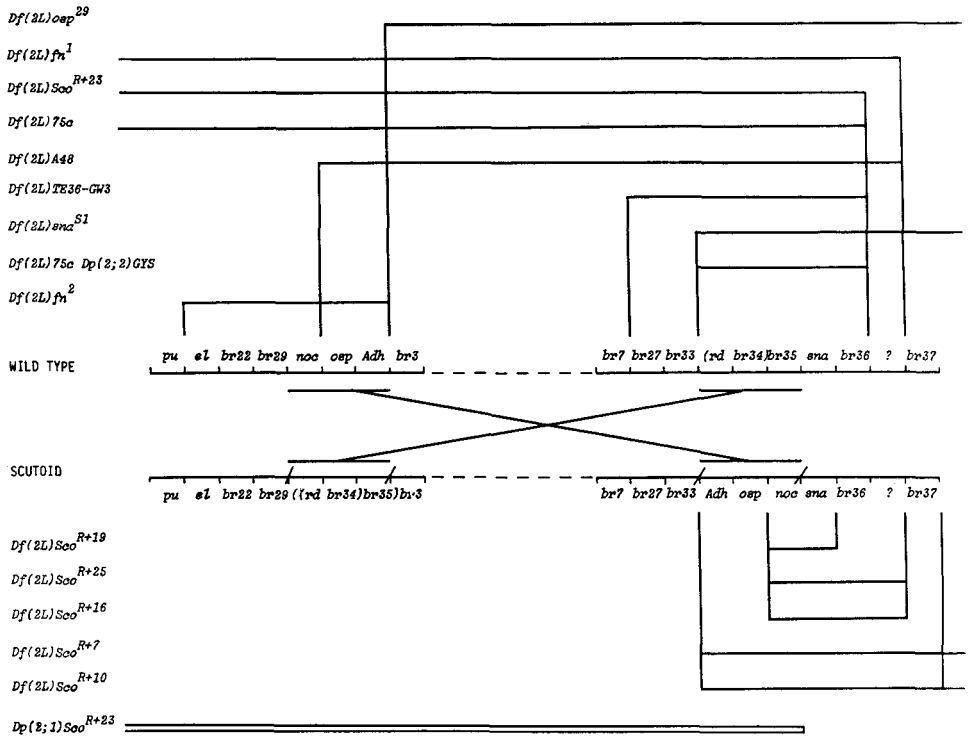

FIGURE 1.—Genetic map of region 35 in wild-type and *Sco* chromosomes taken from ASHBURNER, TSUBOTA and WOODRUFF (1983). The genetic limits of the deficiencies used in this study are shown. ? refers to a gene(s) located between *l(2)br36* and *l(2)br37* that is shown to enhance snail. The limits of deficiencies that are assumed to delete this region are inferred from data presented in Tables 5 and 6.

cation to rescue homozygotes and transheterozygotes of snail alleles. As shown in Table 4, most escapers are associated with sna^{R1}, sna^{EY2} and sna^{EY3}, which may be considered to be hypomorphic. Escapers frequently have missing halteres and are sometimes hemithorax.

Embryonic phenotypes: *Ventral furrow formation*: The foregoing observations revealed complete lethality for all of the *sna* point mutants over sna^{-} and suggested that sna^{R1}, sna^{EY2} and sna^{EY3} are somewhat weaker than the others. In an attempt to place the alleles in a phenotypic series, a comparative study of embryonic phenotypes was undertaken. The first phenotype studied was the presence or absence of the ventral furrow, *i.e.*, the long ventral invagination of presumptive mesoderm cells that takes place at gastrulation. Crosses were made between heterozygous *sna/CyO* parents, and only 25% of the embryos are expected to show the mutant phenotype. The results, presented in Table 5, show that homozygous sna^{RY1}, sna^{IIG05}, sna^{HG31}, sna^{EY1} and Sco^{R+15} and homozygous sna^{-} embryos [*Df(2L)Sco*$^{R+19}$, *Df(2L)TE36-GW3* and *Df(2L)75c Dp (2;2) GYS*; these deletions were chosen because they do not extend as far as the *el-noc* region distally, nor beyond *l(2)br36* proximally] fail to make the ventral furrow. Other events that occur at gastrulation, such as the cephalic furrow, invagination of the endoderm and germ band elongation, take place. sna^{R1} and

TABLE 3

Viability of snail alleles over deletions and Scutoid revertants.

	75c GYS	*75c*	*TE36-GW3*	*sna*^{S1}	*fn2*	*R+25*	*R+19*	*R+7*	*R+16*	*R+10*	*R+23*[a]
sna^{R1}	0/288	0/250	0/646	0/1165	249/833	1/505	0/313	0/313	0/381	0/413	11/434[b]
sna^{RY1}	0/252	0/444	0/219	0/1185	260/740	0/331	0/916	0/300	0/499	2/609	1/514[b]
sna^{IIG05}	0/276	0/280	0/301	0/1034	217/630	0/460	0/424	0/301	0/419	0/571	0/532
sna^{HG31}	0/203	0/173	0/358	0/494	160/470	0/188	0/620	0/185	0/187	0/199	7/376[b]
sna^{EY1}	0/233	0/425	0/569	0/434	208/713	0/424	0/585	0/315	0/476	0/513	2/450[b]
sna^{EY2}	0/253	0/587	0/740	0/475	135/540	0/612	0/591	0/375	0/515	0/607	28/536[b]
sna^{EY3}	0/214	0/475	0/642	0/494	162/556	0/592	0/765	0/416	0/680	0/749	83/592[b]

Crosses are *sna/Cy* × deletion/*Cy*; data are *Cy*$^{+}$ adult progeny over total adult progeny. Some of the data concerning *sna*^{HG31} were kindly supplied by M. ASHBURNER.

[a] *Dp(2;1)Sco*$^{R+23}$; *b Df(2L)Sco*$^{R+23}$ *pr/CyO* ♂ were crossed to *sna/Cy* ♀. Therefore, only the female progeny received the duplication, and all escapers from these crosses were female.

[b] Flies often have missing halteres or are hemithorax.

TABLE 4

Viability of homozygotes and transheterozygotes of snail alleles in the presence of Dp(2;1)Sco^{R+23}

	sna^{R1}	*sna*^{RY1}	*sna*^{IIG05}	*sna*^{HG31}	*sna*^{EY1}	*sna*^{EY2}	*sna*^{EY3}
sna^{RY1}	7/691[a]	0/596					
sna^{IIG05}	4/397	2/1156[a]	0/1051				
sna^{HG31}	21/433[a]	2/874[a]	7/406[a]				
sna^{EY1}	70/889	3/543[a]	0/471	12/624[a]	0/541		
sna^{EY2}	46/432	12/492	3/466[a]	15/608	5/490[a]	36/545	
sna^{EY3}	58/553	25/612	5/423[a]	20/620	56/426[a]	64/777	0/489

sna/Cy females were crossed to *Dp(2;1)Sco*$^{R+23}$; *sna/b el*^{1} *rd*^{s} *pr cn* males. Data are *Cy*$^{+}$*sna/sna* females over total progeny. In all cases (except homozygous *sna*^{R1} and *sna*^{HG31} which are omitted) eye color markers permitted distinction between *sna/sna* and *sna/el* flies.

[a] Flies often have missing halteres or are hemithorax.

TABLE 5

Phenotypes of different mutant alleles or deficiencies of snail when homozygous

Genotype	Ventral furrowless		Segmental denticle belts			% unhatched[a]
	No.	Frequency	None	Some	Normal	
sna^{R1}	0/150	0	2	13	35	24
sna^{R1}*/DfTE36-GW3*	5/99	0.05	0	18	56	27
sna^{R1}*/DfSco*$^{R+19}$	6.5/137[b]	0.05	1	22	48	25
sna^{R1}*/Df75c DpGYS*	7/138	0.05	1	20	36	26
sna^{RY1}	9/50	0.18	11	60	28	30
sna^{IIG05}	117/500	0.23	9	32	9	25
sna^{HG31}	26/104	0.25	7	30	29	26
sna^{EY1}	36/154	0.23	19	52	30	23
sna^{EY2}	18/186	0.1	5	21	75	25
sna^{EY3}	0/102	0	2	11	78	24
Sco	0/105	0	1	0	58	24
Sco^{R+15}	21/98	0.21	2	70[c]	6	25
DfSco^{R+19}	18/68	0.26	2	71	11	28
DfTE36-GW3	20/100	0.2	6	52	20	25
Df75c DpGYS	112/500	0.22	4	40	20	27

Crosses were made between heterozygous stocks balanced over *CyO*. From a first experiment the number of embryos with no visible ventral furrow at gastrulation is expressed over total number of embryos observed (columns 2 and 3). In a second experiment the phenotypes of unhatched embryos were examined 24 hr after egg laying (columns 4, 5, 6 and 7). Unhatched but developed embryos represent approximately 25% of total embryos in all cases (column 7).

[a] The percentage of unhatched, but developed, embryos was calculated after subtraction of nondeveloped, presumably unfertilized, eggs.

[b] Partial furrows were scored as one half.

[c] A number of *Sco*$^{R+15}$ embryos show a bicaudal phenotype.

sna^{EY3} (and also *sna*$^{+}$, *Sco*) embryos generally do make ventral furrows. *sna*^{EY2} appears to be intermediate in that fewer than the expected proportion of 25% embryos failed to make the furrow.

Embryonic phenotypes: cuticular differentiation: Most homozygous *sna* embryos die toward the end of embryogenesis, and all secrete a larval cuticle. The detailed cuticular structures vary from one allele to another. For the purposes of classifying cuticular differentiation, embryos were scored into three classes:

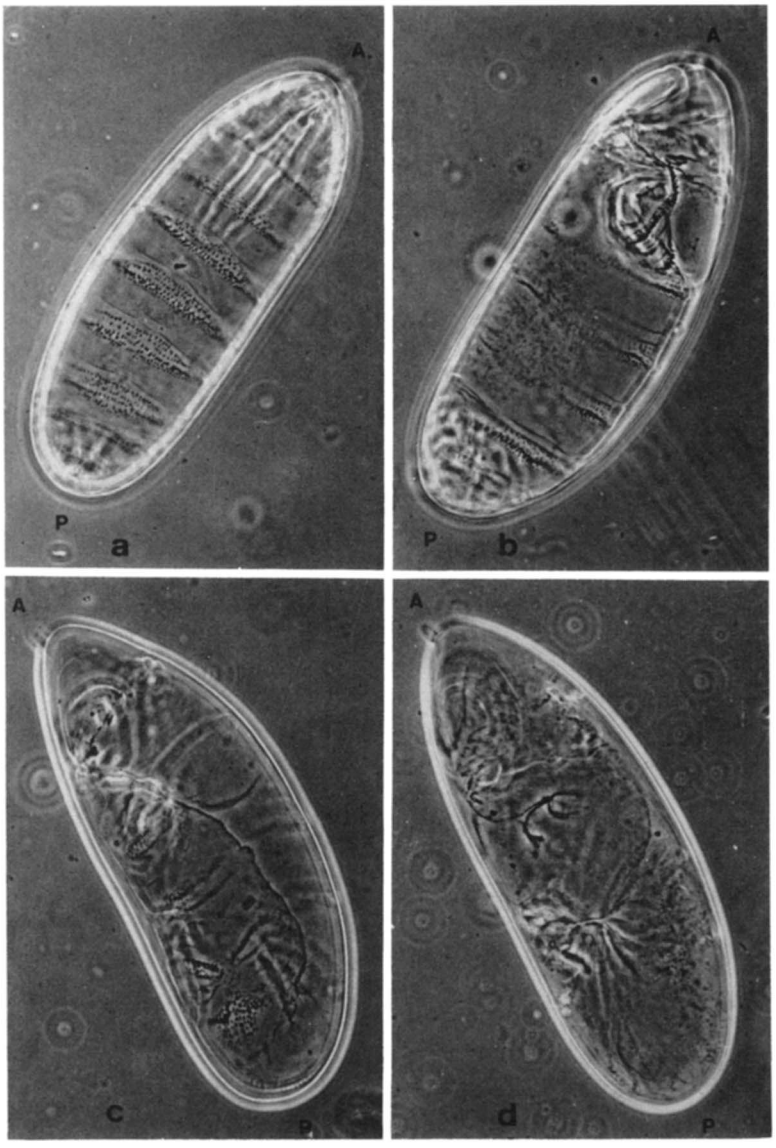

FIGURE 2.—Cuticular phenotypes of late embryos. a, a heterozygous sna^{IIG05} *cn bw sp/CyO* showing the normal pattern of segmental denticle belts. b, c and d, homozygous sna^{IIG05} *cn bw sp* embryos showing various degrees of cuticular differentiation: b, embryo with normal segmentation but with a twisted anterior end; c, embryo with partially formed segmental denticle belts; d, embryo composed of a clear featureless cuticle with no visible signs of segmentation.

the first included only those embryos with a clear featureless cuticle, the second, those embryos with partially formed ventral segmental denticle belts (either fewer, or thinner, or abnormally fused) and the third group included embryos bearing a morphologically normal larval cuticle (even though the embryos were often twisted in the egg case). Examples of these phenotypes are shown in Figure 2. These three groupings are of no particular significance

TABLE 6

Enhancement of the phenotype of sna^{R1} *at gastrulation by haploidy of the elbow-no-ocelli region and the* l(2)br36-l(2)br37 *interval*

Genotype	No. of furrowless embryos over total no. observed	Frequency
sna^{R1}/sna^{R1}	0/150	0
$sna^{R1}/Df\ TE36\text{-}GW3$	5/99	0.06
$sna^{R1}/Df(fn2)sna^{R1}$	16/108	0.15
$Df(fn2)sna^{R1}/Df(fn2)sna^{R1}$	19/90	0.21
$Df(fn2)sna^{R1}/Df\ TE36\text{-}GW3$	34/129	0.26
$sna^{R1}/Df\ 75c$	21/89	0.24
$Df(fn2)/Df(fn2)$	0/109	0
$sna^{R1}/Df\ sna^{S1}$	28/120	0.23
$sna^{R1}/Df\ osp^{29}$	18/74	0.24
$sna^{R1}/Df\ A48$	25/105	0.24
$sna^{R1}/Df\ fn1$	22/94	0.23

Egg collections were made between heterozygous stocks balanced over *CyO*. sna^{R1} was always inherited from the female parent. $Df(2L)fn2\ sna^{R1}$ was recovered from a cross of $Df(2L)fn2\ pr\ cn/sna^{R1}$ ♀ × *b* $el^1 rd^s\ pr\ cn$ ♂, as an *el* $pr^+\ cn^+$ fly; its genotype was confirmed as *sna* by lethality over sna^{IIG05}.

in themselves (the first category may reflect an earlier death), but they permit comparison of the strengths of different allelic combinations. It can be seen from Table 5 that, in the stronger mutant alleles, such as sna^{RY1}, and in the sna^- deletions, the cuticle of many embryos is poorly differentiated, whereas in the weaker ones, *e.g.*, sna^{R1}, a greater number of embryos produce a normal larval cuticle. This is in accordance with the observations on the gastrulating embryos, but it also shows that the ectoderm develops abnormally, and that the mutant effect is not confined to the ventral, presumptive mesodermal cells of the embryo.

Effects of other regions of the genome upon the snail phenotype: In order to explore the effects of other regions of the genome upon the snail mutant phenotype, a weak allele of snail was chosen for further study. A comparison between sna^{R1} homozygotes and hemizygotes can be drawn from the data presented in Table 5. The phenotype of sna^{R1} is only very slightly increased over deletions of snail as seen by the fact that less than 5% of the embryos now fail to make a visible ventral furrow. Very little difference in cuticular phenotype is detectable. These data will serve as a control for the experiments summarized in Table 6.

The phenotype of gastrulating sna^{R1} homozygous or hemizygous embryos is greatly enhanced by haploidy or absence of the *el-noc* region (Table 6). Thus, $sna^{R1}/Df(2L)75c$ embryos all fail to make a ventral furrow, whereas $sna^{R1}/Df(2L)75c\ Dp(2;2)GYS$ embryos generally gastrulate normally as was seen in Table 5. Homozygous sna^{R1} embryos that are also either heterozygous or homozygous for $Df(2L)fn2$ also fail to make ventral furrows. In addition to the

TABLE 7

Extent of cuticular differentiation of embryos of different snail alleles in trans *with either* Df(2L)TE36-GW3, sna$^-$ *or* Df(2L)75c, el-noc$^-$, sna$^-$

	Mutant/*Df(2L)TE36-GW3*			Mutant/*Df(2L)75c*		
	Segmental denticle belts			Segmental denticle belts		
Allele	None	Some	Normal	None	Some	Normal
sna^{R1}	2	20	59	8	27	2
sna^{RY1}	0	26	29	10	92	0
sna^{HG05}	0	17	15	8	75	2
sna^{HG31}	0	27	16	2	42	5
sna^{EY1}	3	37	15	13	68	16
sna^{EY2}	4	19	28	3	68	22
sna^{EY3}	1	10	42	14	32	7

Data refer to numbers of unhatched embryos examined 24 hr after egg laying. They represent approximately 25% of total eggs laid in all cases.

el-noc genes, *Df(2L)fn2* deletes only two other genes, outspread and Alcohol dehydrogenase, both nonvital (ASHBURNER, TSUBOTA and WOODRUFF 1982). Homozygous *Df(2L)fn2* embryos gastrulate normally, and the majority appear morphologically normal at the end of embryogenesis; a few show weak pigmentation. The cuticular phenotypes of all seven point alleles of snail over a *sna*$^-$ deletion, *Df(2L)TE36-GW3,* and an *el-noc*$^-$, *sna*$^-$ deletion, *Df(2L)75c,* are shown in Table 7. It can be seen that the phenotype of all of the alleles is stronger over *Df(2L)75c* but not over *Df(2L)TE36-GW3* when compared with the homozygous mutant embryos (Table 5).

No attempt has been made to subdivide the *el-noc* region in order to determine whether the enhancement is due to a discrete gene rather than an effect of the entire region, because the available deficiencies with breakpoints within *el-noc* all extend beyond *l(2)br36* to include another gene(s) that also enhances snail when haploid.

Snail is enhanced by haploidy of a gene(s) situated in the interval between *l(2)br36* and *l(2)br37.* The phenotype of sna^{R1}, when in *trans* over *sna*$^-$ deletions that extend proximally as far as *l(2)br37,* is enhanced, and the embryos fail to make ventral furrows (Table 6): $Df(2L)osp^{29}$ and $Df(2L)sna^{S1}$ have breakpoints proximal to *l(2)br37. Df(2L)fn1* and *Df(2L)A48* have breakpoints between *l(2)br36* and *l(2)br37.* They enhance sna^{R1}, and so we infer that they include a gene(s) situated in the interval between *l(2)br36* and *l(2)br37* that interacts with snail. Similarly, we reason that *Df(2L)TE36-GW3* and *Df(2L)75c, Dp(2;2)GYS,* which also have breakpoints between *l(2)br36* and *l(2)br37* but do not enhance snail (Tables 5 and 7), do not delete the hypothetical gene. Examination of the cuticular phenotype of embryos homozygous for these deletions supports this hypothesis. As shown in Table 8, $Df(2L)sna^{S1}$, $Df(2L)osp^{29}$, *Df(2L)A48* and also $Df(2L)Sco^{R+10}$, $Df(2L)Sco^{R+7}$, $Df(2L)Sco^{R+25}$ and $Df(2L)Sco^{R+16}$ all have an extreme phenotype. A much weaker cuticular phenotype was seen for

TABLE 8

Extent of cuticular differentiation of embryos homozygous for different snail deficiencies with breakpoints in the interval between l(2)br36 *and* l(2)br37

Genotype	Segmental denticle belts		
	None	Some	Normal
Df sna^{S1}	99	2	0
Df osp^{29}	98	4	0
Df A48	34	6	0
Df Sco^{R+10}	89	3	0
Df Sco^{R+7}	41	23	14
Df Sco^{R+25}	37	56	5
Df Sco^{R+16}	64	17[a]	16

Figures refer to number of unhatched embryos examined, 24 hr after egg laying. They represent approximately 25% of total eggs laid in all cases.

[a] Some *Df(2L)Sco*$^{R+16}$ embryos displayed a bicaudal phenotype.

Df(2L)Sco^{R+19}, *Df(2L)TE36-GW3* and *Df(2L)75c Dp(2;2)GYS* (Table 4). Embryos lacking the *l(2)br36-l(2)br37* interval are often completely devoid of any visible cuticular differentiation, being made up of a twisted, sac-like featureless cuticle. Unfortunately, as yet no deletion of this region is available that does not simultaneously delete snail.

DISCUSSION

Mutations at the snail locus form a single complementation group and have similar phenotypes that vary in intensity. Embryos of the weak alleles, such as *sna*^{R1} and *sna*^{EY3}, gastrulate normally, and ventral presumptive mesodermal cells invaginate. Nevertheless, they die most often as late embryos, and many of them do not differentiate normal internal tissues. The larval cuticle is generally well differentiated, although the embryos are twisted. These alleles are probably hypomorphic since, when over a *sna*$^{-}$ deletion, the embryonic phenotype is more extreme. Infrequently, escaper adults survive from transheterozygote combinations of the weak alleles; they occasionally lack halteres. Stronger alleles, such as *sna*^{RY1}, *sna*^{IIG05}, *sna*^{HG31} and *sna*^{EY1}, never survive to adulthood; the twisted larval cuticle is less well differentiated, the embryos fail to make a ventral furrow and they are usually devoid of internal tissues. These may be close to the amorphic condition since their phenotype resembles that of the homozygous *sna*$^{-}$ embryos seen for *Df(2L)TE36-GW3, Df(2L)75c Dp(2;2)GYS* and *Df(2L)Sco*$^{R+19}$.

The snail gene, therefore, appears to be necessary for the normal development of all tissues that are formed along the dorsoventral axis; the strongest mutant expression results in abnormal development of the dorsally derived ectoderm as well as the ventrally derived mesoderm; although the weaker alleles mainly affect development of the mesoderm, many embryos bear normal cuticle, an ectodermally derived tissue. Escaper adults have an unexpected phenotype; the ectodermally derived halteres are sometimes absent. This is

frequent among the transheterozygotes that survive in the presence of *Dp(2;1)Sco*$^{R+23}$. This phenotype could be attributable to a position effect of *sna* mutations upon a neighboring gene. Of the 9000 EMS-treated chromosomes tested for a visible phenotype over *Df(2L)TE36-GW3,* however, no mutations were recovered that caused missing halteres.

Embryos homozygous for *Df(2L)fn2, el-noc*$^{-}$ make ventral furrows; therefore, a complete absence of the *el-noc* region does not visibly affect gastrulation. Nevertheless, hypodosage of the *el-noc* region enhances *sna.* This dominant enhancement is visible as early as gastrulation when homozygous or hemizygous *sna*^{R1} embryos, which normally make ventral furrows, fail to do so in the presence of only one maternally derived copy of the *el-noc* region. Enhancement of the early ventral furrowless phenotype of *sna* must mean that the interacting gene(s) in this region are also active that early. An interaction between *el-noc* and snail was suspected by ASHBURNER, TSUBOTA and WOODRUFF (1982) from their studies of the *Sco* chromosome. The only similarity between these two regions that is apparent at the present time is the elbow-like phenotype of *sna* escapers from some combinations; *el* causes an absence of the capitella (distal haltere) and reduction of the posterior wing. Further experiments should reveal whether *el* is the gene responsible. The fact that *Df(2L)Sco*$^{R+19}$ does not enhance *sna* may allow us to eliminate *noc* since this chromosome is mutant for *noc.*

Hypodosage of a gene (s) situated between *l(2)br36* and *l(2)br37* also enhanced the *sna* phenotype. This dominant enhancement is also effective as early as gastrulation and, therefore, presumably reflects an early activity of the gene(s) involved. Embryos homozygous for deletions both of this region and of snail show a very extreme phenotype: they have no internal organs and few ectodermally derived structures. They are composed of a folded tube of clear featureless cuticle enclosing unconsumed yolk.

The enhancing effect of haploidy of the *el-noc* and the *l(2)br36-l(2)br37* regions on ventral furrow formation of *sna*^{R1} is zygotic since the deficiencies were inherited via the male parent (Table 6). In all crosses between heterozygotes balanced over *CyO,* 25% of the embryos displayed the mutant phenotype and it is reasonable to infer that they are homozygous or transheterozygous for the mutations. Traditionally, such phenotypes are attributed to zygotic gene activity. Some maternal perdurance of gene products placed in the egg during oogenesis cannot be ruled out, however, since the females used in these crosses are perforce heterozygous. Probably the phenotypes result from both maternal and zygotic gene activity.

Snail, and also twist, a zygotic lethal with a similar phenotype, have been shown to be sensitive to maternal hypodosage of dorsal (SIMPSON 1983). The dominant lethality of heterozygous twist embryos derived from heterozygous dorsal females can be rescued by extra doses of *dl*$^{+}$ which suggests that the products of these different genes can compensate for and replace one another to some extent. It is possible that the increased survival of *sna* transheterozygotes in the presence of *Dp(2,1)Sco*$^{R+23}$ is due not to variegation for *sna*$^{+}$ but to an extra zygotic copy of the *el-noc* genes. Further experiments will allow us to identify the interacting genes present in *Df(2L)fn2* and the *l(2)br36-l(2)br37*

interval. It remains to be seen how all of these genes function together in the process of specification of pattern in the embryo.

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