MATERNAL-ZYGOTIC GENE INTERACTIONS DURING FORMATION OF THE DORSOVENTRAL PATTERN IN DROSOPHILA EMBRYOS

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ABSTRACT

Maternal-zygotic interactions involving the three genes dorsal (*dl*), twist (*twi*) and snail (*sna*) are described. The results suggest that all three are involved in the process by which the dorsoventral pattern of the Drosophila embryo is established. First, the lethal embryonic mutant phenotypes are rather similar. In homozygous *twi* or *sna* embryos invagination of the ventral presumptive mesodermal cells fails to occur, and the resulting embryos are devoid of internal organs. This is very similar to the dominant phenotype described for *dl*; in the case of *dl*, however, the effect is a maternal one dependent on the mutant genotype of the female. Second, a synergistic interaction has been found whereby dominant lethality of *twi*- or *sna*-bearing zygotes is observed in embryos derived from heterozygous *dl* females at high temperature. The temperature sensitivity of this interaction permitted definition of a temperature-sensitive period which is probably that of *dl*. This was found to extend from approximately 12 hr prior to oviposition to 2–3 hr of embryogenesis. A zygotic action for the *dl* gene in addition to the maternal effect was revealed by the finding that extra doses of dl^+ in the zygotes can partially rescue the dominant lethality of heterozygous *twi* embryos derived from heterozygous *dl* females. Two possible interpretations of the synergism are considered: (1) *twi* and *sna* are activated in the embryos as a result of positional signals placed in the egg as a consequence of the functioning of the *dl* gene during oogenesis and, thus, play a role in embryonic determination. (2) The gene products of dl^+ and twi^+ (or sna^+) combine to produce a functional molecule that is involved in the specification of dorsoventral pattern in the early embryo.

DROSOPHILA embryos develop from eggs that possess distinct anteroposterior and dorsoventral asymmetries. Development along the anteroposterior axis leads to the setting up of the general body plan and metameric segmentation. Development along the dorsoventral axis leads essentially to the formation of ectoderm from the dorsal and lateral sides and mesoderm from the ventral side of the embryo. The endoderm derives from an anteroventral and a posterior position, and together these three tissues form the germ layers of the embryo (POULSON 1950; LOHS-SCHARDIN, CREMER and NÜSSLEIN-VOLHARD 1979). It is probable that two classes of genes are involved in the process by which the dorsoventral pattern of the embryo is established: maternally

acting genes, which function during oogenesis and specify positional information in the egg, generally considered to be in the form of a gradient of unknown nature (SANDER 1959, 1976; SCHUBIGER 1976; NÜSSLEIN-VOLHARD 1979), and zygotically acting genes, which are responsible for the interpretation of the maternally deposited positional signals and lead to the activation of genes that bring about the determination of different embryonic tissues (GARCIA-BELLIDO 1975). It is convenient to view embryonic development in terms of these two classes of genes, but it must be noted that a number of genes involved in embryonic patterning have been found to function both maternally and zygotically: trithorax (INGHAM and WHITTLE 1980; INGHAM 1981), *fs(1)h* (FORQUIGNON 1981), extra sex combs (STRUHL 1981) and Notch (JIMENEZ and CAMPOS-ORTEGA 1982).

Recently a number of mutations affecting development along the dorsoventral axis have been described. The mutations dorsal (NÜSSLEIN-VOLHARD 1979), *fs(1) K10* (WIESCHAUS 1980), easter and gastrula-defective (ANDERSON and NÜSSLEIN-VOLHARD 1983) have maternal effects that cause a partial to complete dorsalization of the embryos such that structures normally made only on the dorsal side are formed in all regions of the embryo, whereas the dominant maternal effect mutation Toll causes ventralization of the embryos. Two zygotic lethals, twist and snail (C. NÜSSLEIN-VOLHARD, E. WIESCHAUS and H. KLUDING, unpublished results) cause a partial dorsalization of the embryos, such that only the ventral part is abnormal and no mesoderm differentiates. Other zygotic lethals, big brain, mastermind, neuralizer, Delta (LEHMAN *et al.* 1981) and Notch (POULSON 1940; WRIGHT 1970), affect a later step, that of the separation of the ectoderm into epidermis and neurectoderm.

This report describes a synergism between the maternal action of dorsal and the zygotic action of twist and snail. The results enable us to define the time of functioning of the dorsal gene more clearly and strongly suggest that the three genes act on the same embryonic process.

MATERIALS AND METHODS

Stocks: The mutations and rearrangements used for the main part of this study are listed in Table 1 together with their sources. Additional stocks used were: *cn bw sp* and *b pr cn bw* chromosomes that had been used to isolate the *twi* and *sna* mutations. Delta-*IL*, mastermind-*IB*, big brain-*ID*, *K10*, neuralized-*IF*, paired-*IIB* and a lethal allele of engrailed were obtained from C. NÜSSLEIN-VOLHARD. Notch-*264.47* was obtained from the Bowling Green stock center. The Oregon R strain came from M. ASHBURNER. The gastrula-defective-*14-473* stock came from A. MAHOWALD. *Df(3R)red*^{P52} and *Df(3R)P9* came from E. LEWIS and trithorax^{2} from P. INGHAM. For a description of other mutations mentioned in the text, see LINDSLEY and GRELL (1968). Throughout the text, the *dl*-bearing chromosomes will be referred to simply as *dl* and the *twi*-bearing or *sna*-bearing chromosomes simply as *twi* or *sna*, respectively. For details concerning other mutations on these chromosomes see the tables. *dl*, *twi* or *sna* chromosomes were maintained over either *CyO*: *In(2LR)O,Cy dp*^{hvl}*pr cn*^{2} or *CyO, bw*: *(In (2LR)O, Cy dp*^{hvl} *pr cn*^{2} *bw* or *In(2L)Cy, In(2R)Cy, al*^{2} *Cy pr Bl cn*^{2} *sp*^{2}.

Mutagenesis: New mutations or rearrangements reported here were generated with either 0.024 M EMS according to the method of LEWIS and BACHER (1968) or X rays at a dose of 4000 r (100 kV, 10 mA given for 5 min, 1.5 mm Aluminium filter, Philips Constant Potential X-ray system MG 102, Beryllium window).

Culture media: Flies were raised on a corn meal, yeast, agar, sugar medium.

TABLE 1

List of mutations and rearrangements used, together with their source and cytology where known

Chromosome	Origin	Cytology	Cytology according to:	Discoverer
twi^{1D96}	EMS			NÜSSLEIN-VOLHARD, WIESCHAUS and KLUDING
twi^{1IH07}	EMS			NÜSSLEIN-VOLHARD, WIESCHAUS and KLUDING
twi^{1IE27}	EMS			NÜSSLEIN-VOLHARD, WIESCHAUS and KLUDING
twi^{1G23}	EMS			NÜSSLEIN-VOLHARD, WIESCHAUS and KLUDING
twi^{ry50}	X ray	No visible abnormality	G. RICHARDS	This report
twi^{ry63}	EMS	No visible abnormality	G. RICHARDS	This report
twi^{ry51}	X ray	No visible abnormality	G. RICHARDS	This report
Df(2R)twi^{S60}	X ray	59C3.4; 59D1.2	G. RICHARDS	This report
Df(2R)bw^{DR+31}	X ray	59B6.8; 60A8.16	G. RICHARDS	This report
Df(2R)bw^{S46}	X ray	59D8.11; 60A7	G. RICHARDS	This report
Df(2R)bw^{DR+23}	X ray	59D4.5; 60A1.2	G. RICHARDS	This report
Dp(2;Y)bw^{+}*Y*	X ray	58 F to tip of *2R*	G. RICHARDS	Dempster (LINDSLEY and GRELL 1968)
Df(2L)dl^{H20}	X ray	36A12.14; 36E1.2	G. RICHARDS	C. NÜSSLEIN-VOLHARD
Df(2L)119	X ray	Not visibly deficient	WRIGHT, HODGETTS and SHERALD (1976)	WRIGHT, HODGETTS and SHERALD (1976)
dl^{1}	EMS			NÜSSLEIN-VOLHARD (1979)
dl^{3}	EMS			JURGENS, WIESCHAUS and NÜSSLEIN-VOLHARD
dl^{4}	EMS			JURGENS, WIESCHAUS and NÜSSLEIN-VOLHARD
sna^{IIG05}	EMS	No visible abnormality	G. RICHARDS	NÜSSLEIN-VOLHARD, WIESCHAUS and KLUDING
sna^{S1}	X ray	No visible abnormality	G. RICHARDS	This report
sna^{r1}	X ray	No visible abnormality	G. RICHARDS	This report
sna^{ry1}	X ray	No visible abnormality	G. RICHARDS	This report
sna^{HG31}	EMS	No visible abnormality	M. ASHBURNER	ASHBURNER, TSUBOTA and WOODRUFF (1982)
Df(2L)75c	X ray	35A1.2; 35D4.7	WOODRUFF and ASHBURNER (1979)	WOODRUFF and ASHBURNER (1979)
Dp(2;3)osp^{3}	γ ray	35B3.4; 36C11	M. ASHBURNER	C. DETWILER
Df(2L)PA4	γ ray	35D1.2; 36A1.2	M. ASHBURNER	P. ANGEL
Df(2L)osp^{18}	γ ray	35B1.2; 35C4.5	M. ASHBURNER	C. DETWILER
Df(2L)b80e4	γ ray	34C4; 35D1.2	M. ASHBURNER	S. TSUBOTA
Df(2L)A376	X ray	34E3; 35C4.5	ASHBURNER, AARON and TSUBOTA (1982)	AARON (1979)

Temperature-sensitive period (TSP): *dl*^{1} *cn sca/CyO, bw* females, aged between 0 and 5 days, raised at either 18° or 25° were mated to *cn twi*^{1D96} *bw sp/CyO* males and placed in egg-laying chambers at their respective temperatures. Two hr egg collections were made for shifts up or down of

embryos and larvae. Five days later the two sets of females were exchanged between 18° and 25°, and the eggs were collected every 12 hr.

Preparation of embryos: For examination of embryonic phenotypes, living embryos were observed by transmitted light under 3S Voltaleff oil (NÜSSLEIN-VOLHARD (1977). Late stages were mounted in Hoyers medium (VAN DER MEER 1977) for cuticle observation using the compound microscope. Some embryos were fixed with heptane/glutaraldehyde and stained with fuchsin according to the method of ZALOKAR and ERK (1977).

RESULTS

Embryonic phenotypes

In normal embryos a defined sequence of tissues develop along the dorsoventral axis: amnio-serosa, dorsal hypoderm, ventral hypoderm, nervous system and mesoderm. At gastrulation, the presumptive mesodermal cells invaginate along a ventral furrow and later form the musculature, fat bodies and gonads. The endoderm also invaginates as an anterioventral and posterior prolongation of the mesoderm and subsequently makes the gut. Germ band elongation takes place. Soon after gastrulation, the neuroblasts, precursors of the central nervous system, separate from the rest of the ectoderm which mainly makes the hypoderm or outer skin of the animal. [See POULSON (1950) for a detailed description of these processes.]

The mutation *dl* has been fully described by NÜSSLEIN-VOLHARD (1979). A brief description is included here for comparison with *twi* and *sna*. Embryos derived from homozygous *dl/dl* females develop as though the entire dorsoventral polarity is absent, and all of the cells from a normal blastoderm differentiate into dorsal hypoderm (NÜSSLEIN-VOLHARD 1979). This phenotype is entirely dependent upon the maternal genotype, and the eggs cannot be rescued even if fertilized by a dl^+ sperm. At 29° *dl* becomes dominant; eggs laid by *dl*/+ females at this temperature hatch to produce morphologically normal larvae that fail to develop to adulthood. At 29° and in the presence of certain enhancer chromosomes, heterozygous *dl* females produce embryos that display a specific embryonic phenotype described by NÜSSLEIN-VOLHARD as the dorsal-dominant phenotype: the ventral furrow does not form, the cells situated ventrally, therefore, do not invaginate and the mesodermal derivatives are not made. Larval hypoderm develops from the ventral cells and a normal larval cuticle is produced in some cases.

The mutants *twi* and *sna* were first isolated by C. NÜSSLEIN-VOLHARD, E. WIESCHAUS and H. KLUDING (unpublished results) on the basis of their lethal embryonic phenotype. They are recessive zygotic lethals and both show a phenotype very similar to that of *dl*-dominant embryos. Normal blastoderms are formed, but at gastrulation no ventral furrow is visible, although other gastrulation events are apparent: the endoderm invaginates, the cephalic furrow is formed and germ band elongation takes place. The resulting embryos have few if any mesodermally derived internal tissues. Figure 1a and b show gastrulating mutant *twi* embryos that can be compared with the wild-type embryos seen in Figure 1d and e. These two genes must, therefore, be very early acting.

Many *twi* and *sna* embryos (between 40 and 100% depending upon the allele), nevertheless, make normal ectodermal derivatives: the larval hypoderm displays normal or reduced segmental denticle belts, mouth-hooks and spiracles form, but tracheae are seen only in weaker alleles. The head segments appear everted, due to abnormal head involution, and the anterior end of the embryo is twisted in the egg because of its extra length (see Figure 1c). Some embryos, however, fail to make a properly differentiated cuticle. When dissected from the egg case such embryos are seen to be composed of a wide, continuous folded tube of featureless cuticle, filled with yolk. Although all are completely lethal, more variability in embryonic phenotype is apparent between different alleles of *sna* than between different alleles of *twi* (a detailed comparison of the different alleles will be described elsewhere).

Cytological localization of twist and snail

The two genes have been mapped with respect to their specific embryonic phenotype. Meiotic mapping showed *twi* to be located close to *bw*. Deficiencies of *bw* were generated by X rays either as revertants of bw^D or as lethal *bw* alleles. An approximate localization of the locus to between 59B6.8 and 59D4.5 has been deduced from the fact that it is uncovered by $Df(2R)bw^{DR+31}$ but not by $Df(2R)bw^{DR+23}$ or $Df(2R)bw^{S46}$, and that it is covered by $Dp(2;Y)bw^+$ (see Figure 2 and Table 1). The gene may, however, be more precisely located between 59C3.4 and 59D1.2, the limits of a small deficiency, $Df(2R)twi^{S60}$, that was obtained in a screen for X-ray-induced alleles of *twi* (see also Figure 2 and Table 1).

Mutations at the *sna* locus are uncovered by *Df(2L)75c* and covered by $Dp(2;3)osp^3$ (see Figure 3 and Table 1). Complementation tests performed in collaboration with M. ASHBURNER have revealed that $l(2L)br28^{HG31}$ (described in ASHBURNER, TSUBOTA and WOODRUFF 1982) is allelic to the mutant alleles of *sna* described here. *l(2L)br28* is uncovered by *Df(2L)PA4* and *Df(2L)b80e4* but not by $Df(2L)osp^{18}$ nor by *Df(2L)A376* (M. ASHBURNER, personal communication; see Figure 3 and Table 1). The estimated location of *sna* is, thus, at 35D1.2.

Maternal-zygotic interactions between dorsal and twist (or snail)

As pointed out by ANDERSON and NÜSSLEIN-VOLHARD (1983), the similarity in phenotypes of *dl*, *twi* and *sna* suggests that these genes all act upon the same embryonic process. Informative tests of this hypothesis are limited by the facts that *dl* is a nonrescuable maternal effect lethal and *twi* and *sna* are zygotic lethals. One possibility, however, is to test for synergistic interactions in the double heterozygotes. In some systems double heterozygosity for recessive mutations affecting the same pathway causes one or both of them to display a dominant phenotype (CLINE 1980; GANS, FORQUIGNON and MASSON 1980; ROBBINS 1980). Since the recessiveness of *dl* is known to be a function of temperature, crosses generating heterozygous *twi* or *sna* zygotes from heterozygous *dl* females were performed at 18°, 25° and 28°. The results, presented in Table 2, show that there is an interaction and that it is temperature sensitive. When *dl/Cy* females are crossed to twi/bw^D males, the resulting *twi*-bearing

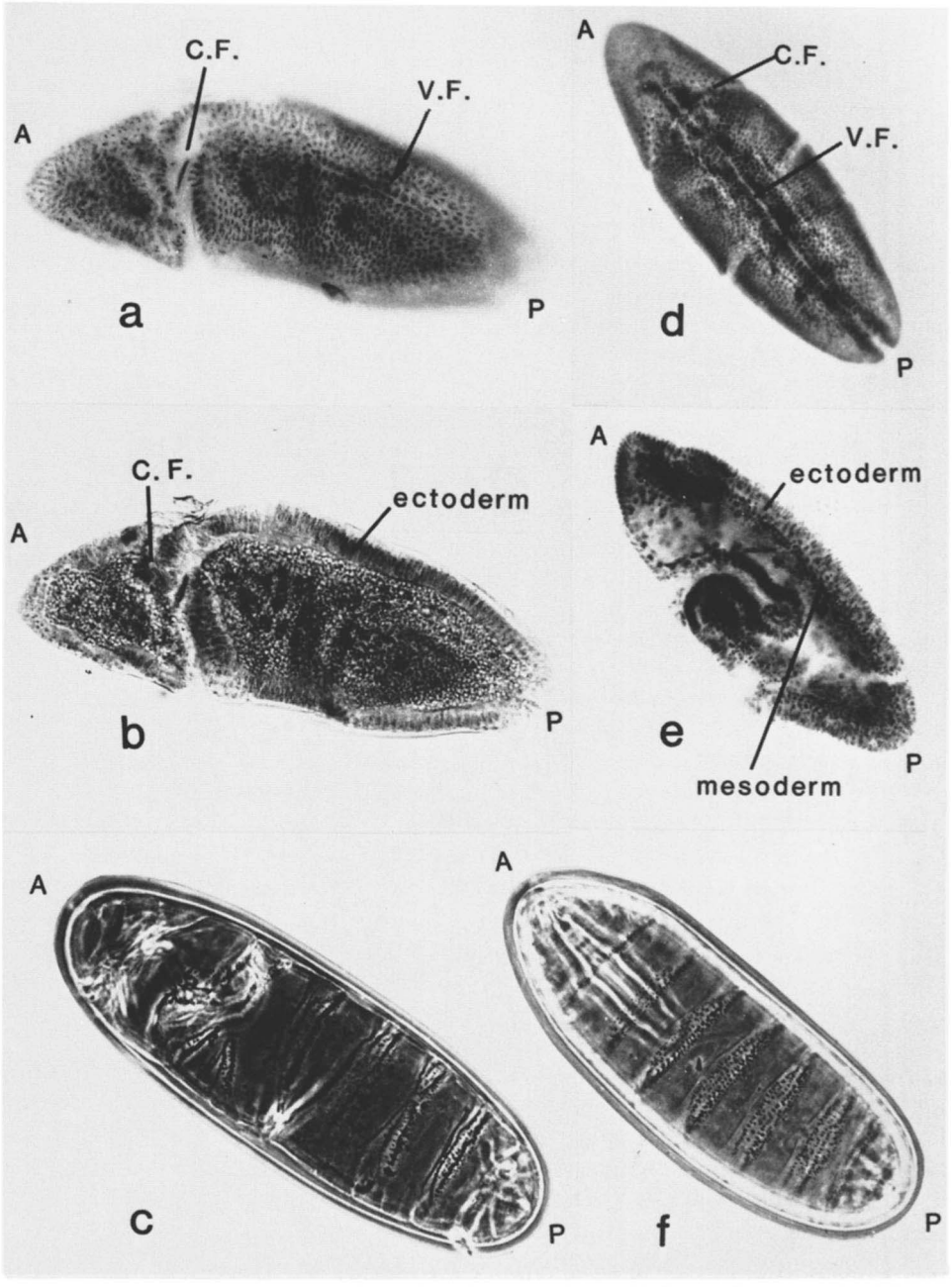

FIGURE 1.—Whole mounts of twist and wild-type embryos. Embryos shown on the left are homozygous *cn twi*^{ID96}*bw sp*; embryos on the right are wild type (Ore R). a and b, ventrolateral and sagittal view of the same mutant embryo; germ band elongation is nearly complete. The arrow in a points to a very slight lining up of nuclei in a position where the ventral furrow should have been. No invagination of ventral (presumptive mesoderm) cells is apparent, however, as seen by the absence of the inner layer in b; c, a 24-hr-old mutant embryo; the number of segmental cuticle belts is normal, but the head end is twisted in the egg case; d, gastrulating wild-type embryo,

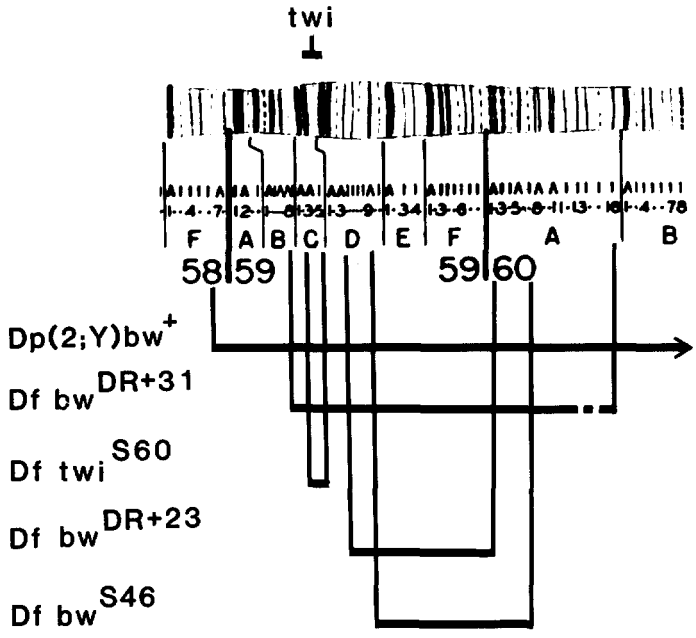

FIGURE 2.—Chromosomal rearrangements in *2R* used to localize twist (see Table 1).

zygotes (both *dl/twi* and *twi/Cy*) are viable at 18°, severely reduced at 25° and virtually absent at 28°. This is also true when females carrying a deficiency of *dl* are used. Similar crosses involving *sna* lead to a decrease but never a complete loss of *sna*-bearing progeny. A chromosome mutant for both *sna* and *twi*, however, leads to interactive dominant lethality even at 18°, a temperature that is permissive for interactive viability of zygotes heterozygous for either *sna* or *twi* alone. A series of further crosses was performed to test whether (1) the effect of *dl* must be maternal and that of *twi* and *sna* zygotic, (2) the interaction is a result of particular mutant alleles or of hypodosage of the wild-type allele, (3) the lethality of the non-*dl* progeny depends upon the homologue and (4) genes other than *dl*, *twi* or *sna* affect the interactions.

Maternal hypodosage of dorsal: Three independant EMS-induced alleles of *dl* and two deficiencies uncovering the *dl* locus (Table 1 and Figure 3) were tested for interaction with *sna* and *twi*. Table 3 shows the survival at 26° of heterozygous *twi* flies resulting from crosses between *twi/CyO* males and females bearing different *dl* chromosomes. All crosses lead to a dramatic loss of the

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ventral surface view; the ventral furrow extends almost the entire length of the embryo; germ band elongation nearly complete; e, a slightly older wild-type embryo, sagittal view; germ band elongation is complete. Both the outer epithelial layer (ectoderm) and the darker staining inner layer (mesoderm) of more rounded cells can be seen; f, a fully formed but as yet unhatched wild-type larva. a, b, d and e: embryos fixed with heptane/glutaraldehyde and stained with fuchsin (ZALOKAR and ERK 1977); c and f embryos cleared and mounted in Hoyers medium (VAN DER MEER 1977). Magnification, × 200. A, anterior end; P, posterior end; C.F., cephalic furrow; V.F., ventral furrow.

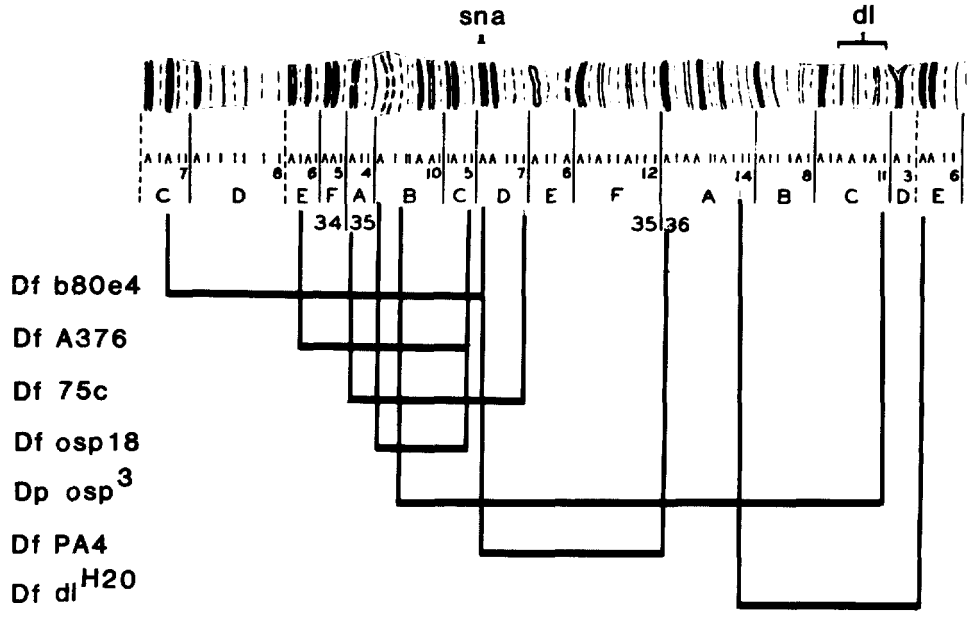

FIGURE 3.—Chromosomal rearrangements in *2L* used to localize snail (see also Table 1).

twi-bearing progeny class that was scored (Cy^+). That the effect of *dl* is a maternal one is shown by the reciprocal cross in which survival is normal. The reciprocal cross also eliminates the possibilities of a zygotic interaction between *dl* and *twi* (or *sna*) and of temperature sensitivity of *twi* or *sna* heterozygotes *per se*. The first four rows of Table 3 show the effect of outcrossing the *dl/CyO* stock to a wild-type strain. There is only a slight difference between *dl/CyO* and *dl/+* females on the survival of *twi*-bearing zygotes. In addition, very little variation is seen between repeats of the same cross at intervals of several months. These results show that the *dl-twi* interaction is independant of the genetic background, *i.e.*, that there are no other genes present in the *dl* stocks that affect the synergism. Similar crosses involving *sna* were also performed (at 28°), and the results, although less dramatic, are comparable to those obtained with *twi*.

The fact that females carrying a deficiency for *dl* lead to a similar synergism with *twi* as do females carrying mutant alleles of *dl* suggests that the primary cause is hypodosage of dl^+ in the female and not an effect of a mutant gene product. This leads to the prediction that an increase in the dosage of dl^+ in the female will rescue the heterozygous *twi* progeny. Therefore, crosses between $dl/dl^+/dl^+$ females and *twi/Cy* males were undertaken. The results are shown in Table 4, where it can be seen that heterozygous (Cy^+) *twi* flies derived from $dl/dl^+/dl^+$ females are completely viable since they represent half (48%) of the total progeny. A zygotic effect of *Dp* osp^3, dl^+ is not sufficient to account for this viability since only half of the *twi*-bearing zygotes receive the duplication.

Zygotic action of dorsal: It is possible that an increase in the zygotic dosage of dl^+ would also rescue heterozygous *twi* progeny derived from heterozygous *dl*

TABLE 2

The effect of temperature on the survival of twist- or snail-bearing zygotes from dl[4]/CyO *or* Bl Df(2L)dl[H20] pr cn sca/CyO *females*

Genotype			Zygotic genotype				
Maternal	Paternal	Temperature	*dl/twi* or *sna*	*Cy/twi* or *sna*	*dl/bw*	*Cy/bw*	% *twi*- or *sna*-bearing
dl[4]/CyO	*twi*	18°	148	131	156	138	49
	sna		113	107	109	122	49
	sna twi		1	7	544	512	<1
	twi	25°	25	58	273	255	9
	sna		156	156	210	198	43
	sna twi		0	3	209	221	0
	twi	28°	0	1	451	420	<1
	sna		94	107	428	451	19
	sna twi		1	4	259	238	1
dl⁻/CyO	*twi*	18°	100	108	92	112	50
	sna		246	232	244	225	50
	twi	25°	9	38	323	332	7
	sna		149	171	171	191	47
	twi	28°	1	1	214	189	≤1
	sna		100	104	394	381	20

Females were crossed to *cn twi[ID96] bw sp/bw[D]*, *sna[IIG05]* cn bw sp/bw[D] or *sna[IIG05] cn twi[IIH07] bw sp/bw[D]* males.

TABLE 3

The maternal effect of different second chromosomes on the survival of heterozygous twist- or snail-bearing zygotes

	Paternal genotype: *twi/Cy*			Paternal genotype: *sna/Cy*		
	No. of progeny			No. of progeny		
Maternal genotype	*Cy*	*Cy⁺*	% *Cy⁺*	*Cy*	*Cy⁺*	% *Cy⁺*
dl[1] cn sca/CyO	992	20	2	582	100	15
dl[1] cn sca/+ -Ore-R	1213	10	<1	651	105	15
+ -Ore-R/CyO	455	230	34	608	281	32
+ -Ore-R/+ -Ore-R	314	251	44	318	236	43
Df(2L)119, cn bw/+ -Ore-R	904	16	2	643	112	15
Df(2L)dl[H20], pr cn sca/+ -Ore-R	310	0	0	265	43	14
dl[3]/CyO	278	1	<1	599	112	16
dl[4]/CyO	546	1	<1	526	93	15

	Paternal genotype: *dl[1] cn sca/CyO*		
	No. of progeny		
	Cy	*Cy⁺*	% *Cy⁺*
cn twi[ID96] bw sp/CyO	488	267	35
sna[IIG05] cn bw sp/CyO	605	245	29

Females were crossed to *cn twi[ID96] bw sp/CyO* males, at 26°, or *sna[IIG05] cn bw sp/CyO* males, at 28°.

TABLE 4

The effect of maternal dosage of dl *on the survival of twist-bearing progeny at 26°*

Maternal genotype	No. of maternal doses dl^+	No. of Cy^+ progeny	Total progeny	% Cy^+ progeny
L^2 dl^1 *cn/CyO;* +/+	1	4	429	1
L^2 dl^1 *cn*/+; *Dp (2;3)* osp^3, sna^+ dl^+/+	2	349	720	48

Females were crossed to *cn* twi^{ID96} *bw sp/CyO males.* + refers to wild-type chromosomes.

females. Some indication that this is the case is already apparent from results in Table 2, where from the cross of *dl/Cy* females by *twi/Cy* males the *twi/Cy* progeny are seen to be more viable than the *dl/twi* progeny. The effect of adding additional doses of dl^+ to *twi*-bearing zygotes derived from heterozygous *dl* females is shown in Table 5. Survival of embryos that receive two or three doses of dl^+ increases considerably. Nevertheless, even for flies carrying three doses of dl^+ viability is not complete: 44% *twi*-bearing flies (*twi/Cy;Dp/+*) were recovered relative to their corresponding control sibs (+/*Cy;Dp/+*). It should be noted that *Dp* osp^3 also carries sna^+, and the possibility exists that extra doses of sna^+ might be able to rescue the *twi* heterozygotes. That this is unlikely can be seen by the fact that survival of *twi* is directly proportional to dl^+ dosage but is not proportional to sna^+ dosage.

Zygotic hypodosage of twist and snail: Although mutant alleles at both the *twi* and the *sna* loci show similar interactions with *dl*, it is possible that these interactions are due to other mutations on the mutagenized chromosomes. To eliminate this possibility, several independently induced alleles of both *twi* and *sna* were studied. Five EMS-induced alleles of *twi*, one X-ray-induced allele and one *twi* deficiency were employed, as well as two EMS-induced alleles of *sna*, three X-ray-induced alleles and one *sna* deficiency. Each allele at the *twi* locus is lethal when homozygous, and all combinations of *trans*-heterozygotes are lethal. The *sna* alleles that were used here are all lethal when homozygous and also fail to complement for viability in *trans*-heterozygotes. Complete complementation is observed for alleles of *twi* over alleles of *sna*, *i.e.*, no synergism is observed in double heterozygotes for *twi* and *sna*. The results of crossing these different alleles to *dl/Cy* females are shown in Table 6. The results are essentially the same for all alleles at the *twi* locus and somewhat variable for the different alleles at the *sna* locus. It is, therefore, likely that the interaction is due solely to *twi* and *sna*.

Deficiencies of either *twi* or *sna* behave in a fashion similar to the mutant alleles, suggesting that lethality is due to zygotic hypodosage of twi^+ or sna^+. To test this, the survival of *sna*/sna^+/sna^+ animals derived from heterozygous *dl* females was tested using *Dp(2;3)*osp^3, sna^+, dl^+. The results are reported in Table 7 where it can be seen that *sna*/sna^+/sna^+ flies are recovered in the same proportion as sna^+/sna^+ *flies.*

Phenotype of twist-bearing embryos derived from heterozygous dorsal females: If the dominant lethality of heterozygous *twi* progeny from heterozygous *dl* females is in fact due to an interaction between these two genes, then one may expect

TABLE 5

The effect of adding additional doses of dl^+ *to twist-bearing zygotes developing from heterozygous* dl *females*

Genotype of zygote	No. of zygotic doses		No. of adults	
	dl^+	sna^+	A	B
dl/twi; Ki/+	1	2	25	43
dl/twi; Dp/+	2	3	56	103
twi/Cy; Ki/+	2	2	62	111
twi/Cy; Dp/+	3	3	176	288
dl/+; Ki/+	1	2	407	
dl/+; Dp/+	2	3	423	
+/Cy; Ki/+	2	2	420	
+/Cy; Dp/+	3	3	401	

dl^1*cn sca/CyO, bw* females were crossed to *cn* twi^{ID96} *bw sp/+;* $Dp(2;3)$ osp^9*/Ki* males at 26°. In series A all categories of flies were counted, whereas in series B only the first four genotypes were recorded.

TABLE 6

The survival of zygotes bearing different paternally inherited alleles of twist (experiment performed at 26°) or snail (experiment performed at 28°) from dl^1 cn sca/CyO, bw *mothers*

Paternal genotype	No. of Cy^+ progeny	Total progeny	% Cy^+ progeny
cn twi^{ID96} *bw sp/CyO*	1	717	<1
cn twi^{IIH07} *bw sp/CyO*	1	569	<1
cn twi^{IIE27} *bw sp/CyO*	15	1012	2
cn twi^{IG23} *bw sp/CyO*	0	630	0
b pr cn twi^{ry50}*bw/CyO*	2	800	<1
b pr cn twi^{ry63}*bw/CyO*	0	509	0
$Df(2R)bw^{DR+31}$*/CyO, bw*	16	605	3
sna^{IIG05} *cn bw sp/CyO*	226	1506	15
sna^{HG31} *cn/CyO*	113	594	19
b sna^{ry1} *pr cn bw/CyO*	239	997	24
b sna^{S1} *pr cn bw/CyO*	336	1459	23
b sna^{r1} *pr cn bw/CyO*	537	1989	27
$Df(2L)75c/In(2L+2R)Cy$	115	718	16
cn bw sp/cn bw sp[a]	444	889	50
b pr cn bw/b pr cn bw[a]	285	548	52

[a] Chromosomes on which the different alleles of *twi* and *sna* were induced.

the lethal animals to show a phenotype similar to that seen for either of the two mutants. Accordingly, the phenotype of the living embryos from *dl/Cy* ♀ × *twi/Cy* ♂ at 28° was studied. Two hundred blastoderms were selected for observation. At gastrulation these were divided into three classes: those that made an apparently normal ventral furrow, those with an abnormal one and those with no visible furrow (see Table 8). Animals that were classified as having no abnormal ventral furrow were of two types: either the ventral furrow was seen as a much thinner band than is usual or else it was not visible

TABLE 7

The survival of sna*-bearing progeny at 28° from the cross* L^2 dl^1 cn/CyO ♀ × b sna^{ry1} pr cn bw/CyO, Dp(2;3) osp^3, sna^+ dl^+/TM3 ♂

	No. of zygotic doses			
	dl^+	sna^+	No.	%
L dl/sna; TM3/+	1	1	64	6
sna/Cy; TM3/+	2	1	44	4
L dl/Cy; TM3/+	1	2	248	22
L dl/sna; Dp/+	2	2	268	23
sna/Cy; Dp/+	3	2	260	23
L dl/Cy; *Dp/+*	2	3	262	23

TABLE 8

Ventral furrow formation at 28° in 200 embryos from the cross dl^1cn sca/CyO ♀ × cn twi^{1D96} bw sp/CyO ♂

		Ventral furrow		
	Died before gastrulation	Not formed	Partially formed	Normal
No. of embryos in each class	2	65	25	108
No. of embryos in each class that hatched as larvae	0	0	11	96

over the entire length of the embryo. All of the embryos that failed to hatch (47% of the total) produced a normal larval cuticle with segmental denticle belts. Mouth parts, spiracles and sometimes tracheae were also visible, but the anterior end was clearly abnormal and often twisted, and the embryos were inert and bore few internal tissues. This phenotype cannot be distinguished from that seen in embryos homozygous for a weak *twi* allele nor from that seen in embryos displaying the dorsal-dominant phenotype. It is reasonable to assume that the abnormal embryos observed here (approximately half of the total) represent the *twi*-bearing progeny: *CyO/CyO* embryos develop to normal first instar larvae, and dominance of the *dl* maternal effect is not observed at 28° (200 eggs from *dl/Cy* females crossed to Ore-R males at 28° gave rise to 187 adults and, even at 29° *dl/Cy* females, when crossed to Ore-R males, produce eggs that hatch as larvae (91 of 100), although none of them develop to adults). The embryos described here may, therefore, be said to display a weak *twi* phenotype or, conversely, *twi* can be considered as a zygotic enhancer of the maternal *dl*-dominant phenotype.

TSP

The temperature sensitivity of the *dl-twi* interaction (Table 2) permits determination of the TSP. Heterozygous *dl/Cy* females were crossed to *twi/Cy* males. Since the interaction involves both a maternal and a zygotic component, temperature shifts were performed on both timed embryos (zygotic: postoviposition) and on the females themselves (maternal: preoviposition). The results, shown in Figure 4, show that the TSP appears to finish at 2–3 hr after egg

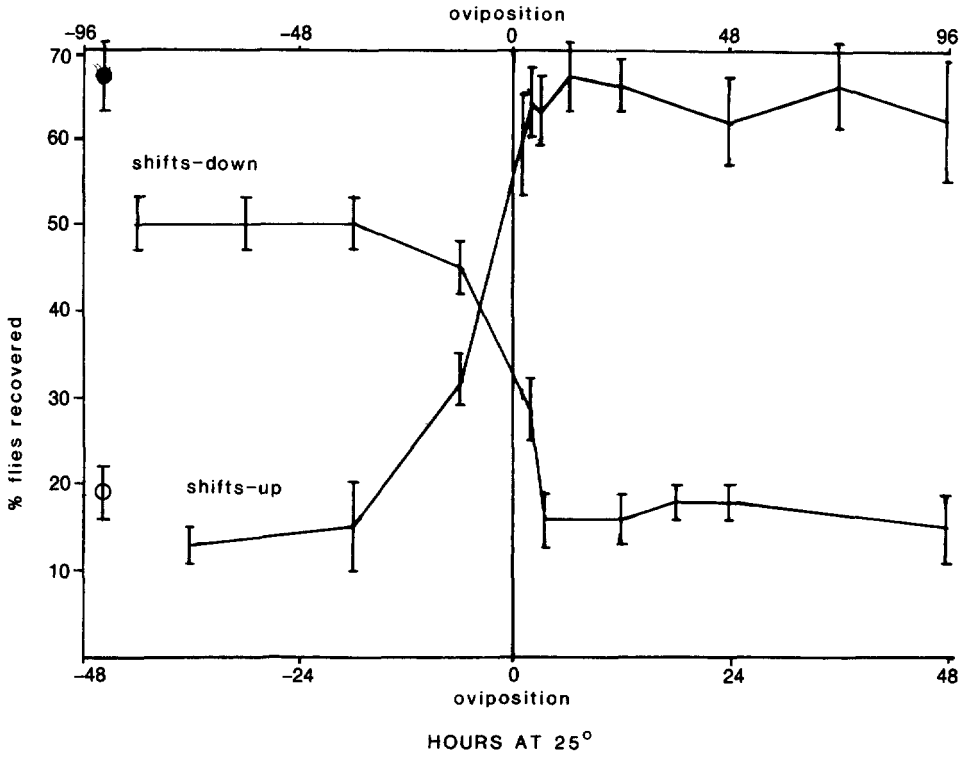

FIGURE 4.—The TSP of the *dl-twi* interaction as determined by shifting embryos from 2-hr egg collections from 18° to 25° and vice versa and by shifting females from one temperature to the other and collecting the eggs every 12 hr. *dl¹cn sca/CyO, bw* females were crossed to *cn twi*^{ID96} *bw sp/CyO* males. The number of heterozygous *twi* flies (*dl/twi* and *twi/Cy*) is plotted. These are expected to represent two-thirds of the total number of flies if viability is complete as is the case for cultures grown continuously at the permissive temperature of 18° (●); see also Table 2. At the restrictive temperature, 25°, they represent less than 20% of the flies recovered (○, cultures grown continuously at 25°). Each point is based on a minimum of 300 flies, and in a few cases animals from two consecutive collections have been pooled.

laying: after this time *twi*-bearing embryos that are shifted from 18° to 25° still survive, whereas those shifted from 25° to 18° still die. The TSP begins during vitellogenesis, at about 12 hr before oviposition: prior to this time the *twi*-bearing progeny of females shifted from 18° to 25° die, whereas those of females shifted from 25° to 18° survive. One unexpected result, however, is that, whereas the survival of *twi*-bearing progeny from females shifted from 18° to 25° rapidly decreases to the level of control cultures grown continuously at 25°, survival of the same progeny from females shifted from 25° to 18° rapidly increases but never attains the same level as that of the progeny of control females grown continuously at 18°. Females that had been shifted from 25° to 18° were followed for 10 days; throughout that time survival of the *twi*-bearing progeny never surpassed 50% (*cf.* 67% for the controls).

The dorsal-twist and dorsal-snail interactions are quite specific

To obtain some idea of how specific the interactions between *dl* and *twi* (or *sna*) are, *Df(2R)vg*^{D} and also five mutations that affect development of the

nervous system, Notch, big brain, neuralizer, mastermind and Delta, were tested for a similar dominant lethality in animals derived from heterozygous *dl* females at 28°. These mutants are defective at a slightly later stage of development and affect the size of the neurogenic region; large amounts of nervous system develop at the expense of the hypoderm (POULSON 1940; WRIGHT 1970; LEHMAN *et al.* 1981). A minimum of 500 flies were counted for each cross; only Notch was found to interact weakly with *dl*: 399 $N/+$ females were recovered compared with 789 w^+Y males from a cross between $dl^1cn\ sca/CyO$ females and $y\ N^{264.47}/w^+Y$ males at 28°. A cross between Ore-R females and $y\ N^{264.47}/w^+Y$ males yielded approximately equal numbers of males and females.

Several embryonic lethals whose primary effect is upon segmentation and which are, therefore, assumed to be involved in determination of the anterior-posterior axis of the embryo were also tested: *Df(3R)P9*, deficient for the bithorax complex (LEWIS 1978); *Df(3R)red*, deficient for trithorax; trithorax-*2* (INGHAM 1981); paired and a lethal allele of engrailed (NÜSSLEIN-VOLHARD and WIESCHAUS 1980). None of these was found to interact with *dl* ($n > 450$ for each cross).

In addition, *twi/Cy and sna/Cy* males were crossed to females heterozygous for two other maternal effect mutations whose phenotypes is similar to that of *dl*; these were *K10* and gastrula defective. Both *twi*- and *sna*- carrying progeny survived in the expected proportions ($n > 600$) at both 18° and 28°.

DISCUSSION

The results presented here support the hypothesis that the three genes, dorsal, twist and snail, all act upon the process of patterning along the dorsoventral axis of the embryo.

First, the phenotypes are very similar. In embryos from homozygous *dl* mothers the entire blastoderm including the ventral part is transformed into dorsal hypoderm (NÜSSLEIN-VOLHARD 1979). The dl-dominant phenotype, however, is visible only in the cells situated ventrally (in the area of the presumptive mesoderm) that fail to invaginate and lead to embryos devoid of internal organs (NÜSSLEIN-VOLHARD 1979). It has been shown that the fate of these ventral cells is altered and that they produce a part of the hypoderm (NÜSSLEIN-VOLHARD *et al.* 1980). The phenotype of homozygous *twi* or *sna* embryos is very similar to that of *dl*-dominant. The fate of the ventral cells in these embryos is not yet known.

Second, these normally recessive mutants interact to cause lethality. The crosses performed here show clearly that the synergistic interactions between *dl* and *twi* or *sna* are due entirely to these three loci and are independant of the genetic background. Such strong synergistic interactions are generally interpreted as an indication that the genes involved have a common functional relationship. Maternal-zygotic interactions have also been observed when both the mother and the zygote have only one copy of the same gene (GARCIA-BELLIDO and MOSCOSO DEL PRADO 1979). A number of other mutations affecting the development of either the nervous system or the pattern of seg-

mentation did not show any synergism with *dl*; Notch was the only exception. It seems possible that searching for mutations that cause this kind of synergism may provide a means for identifying genes with related functions. Accordingly, several thousand EMS-treated second chromosomes have been tested for dominant lethality when crossed to heterozygous *dl* females; so far, apart from additional *sna* and *twi* alleles, only one other putative interacting locus has been identified (P. SIMPSON, unpublished results). Furthermore, neither *twi* nor *sna* show any synergism with *K10* or gastrula-defective, maternal effect mutations causing dorsalization of the embryos. The interactions described here appear, therefore, to be very specific.

Third, zygotic action of twi^+ rescues the dominant maternal action of dorsal at 25°. In the interactions described here the effect of *dl* is essentially a maternal one, since all classes of progeny are viable in the reciprocal cross, heterozygous *twi* or heterozygous *sna* females crossed to *dl* males, and also since in the interacting cross (*dl/Cy* ♀ × *twi/Cy* ♂ or *sna/Cy* ♂) both classes of *twi*- or *sna*-bearing zygotes, those with and those without the *dl* chromosome, have decreased viability. Furthermore, at high temperature haplo-insufficiency of the *dl* gene is observed: heterozygous *dl* females at 29° lay eggs that fail to develop to the adult stage. This dominance indicates that heterozygous *dl* females produce abnormal eggs and further suggests that the amount of positional information in eggs laid by heterozygous *dl* females at less than 29° is sufficient for development if such eggs are fertilized by twi^+, sna^+ sperm. Thus, from a cross between *dl/Cy* females and *twi/Cy* males, progeny of the genotypes *dl/Cy* and *dl/twi* each have only one dose of dl^+; however, *dl/Cy* zygotes have two doses of twi^+ and are viable, whereas *dl/twi* zygotes have only one dose of twi^+ and they are lethal. In other words, the presence of two doses of twi^+ (and sna^+) in the embryo is sufficient to rescue the maternal haplo-insufficiency of *dl*, once more underlining the similar action of these genes.

Fourth, dorsal has both a maternal and a zygotic action, since additional doses of dl^+ brought in via the male parent can partially rescue heterozygous *twi* progeny born of heterozygous *dl* females. (A similar rescue experiment involving *sna* has not been attempted due to the lack of a suitable duplication.) This must mean that the *dl* gene is active in the zygote. Such a zygotic role of *dl* is neither necessary nor sufficient for normal development: eggs laid by *dl/dl* females cannot be rescued by dl^+ sperm, and *dl/dl* zygotes from heterozygous mothers are viable. The zygotic action of *dl* can, however, either restore or substitute for the twi^+ function that is otherwise reduced in heterozygous *twi* embryos born of heterozygous *dl* females.

Interpretations of the dorsal-twist synergism

The temperature sensitivity of the *dl-twi* and the *dl-sna* interactions is somewhat unusual but is not without precedent. It presumably cannot involve a mutant gene product since the interaction involving females carrying a deficiency of *dl* or zygotes bearing a deficiency of *twi* or *sna* is similarly temperature sensitive. An analogous interaction is that between heterozygous daughterless females and the presence of Sex-lethal; the interaction is temperature sensitive for both the mutant and the deficiency-bearing females (CLINE 1980).

Such a temperature-sensitive effect is best interpreted in terms of a temperature-sensitive wild-type gene product. It is difficult, however, to ascertain which component of the system is temperature sensitive; it could be the wild-type gene product of *dl* or *twi* (or *sna*) or both, or even some other gene with which they interact. Furthermore, zygotes heterozygous for both *twi* and *sna* are lethal at all temperatures when derived from *dl*/+ females. The simplest interpretation is that it is the TSP of the *dl* gene product, revealed by means of the interaction with *twi*. The TSP is restricted to a period extending for about 12 hr prior to oviposition to between 2 and 3 hr of embryogenesis. One result that is difficult to explain is the fact that females that have been exposed to 25° for several days and then placed at the permissive temperature of 18° never completely recover; they continue to lay eggs that result in reduced viability when fertilized by a *twi*-carrying sperm.

It seems reasonable to assume that hypodosage of the dl^+ gene in the females leads to the deposition of a reduced amount of positional signals in the egg. Females heterozygous for either a mutant allele of *dl* or a deficiency for *dl* show haplo-insufficiency at 29° and interact with *twi* at temperatures between 25° and 28°. Two possible interpretations of this will be considered here.

Twist and snail affect embryonic determination: The *twi* and *sna* genes are activated in the embryo by means of positional signals placed in the egg as a result of dl^+ action during oogenesis. In the context of this hypothesis, *twi* and *sna* would correspond to selector genes (GARCIA-BELLIDO 1975), and their activation would lead to a determinative decision between alternate developmental pathways. This model leads to the prediction that the genes will be active in only some cells of the embryo, perhaps those situated ventrally.

Twist and snail are involved in the specification of dorsoventral information in the early embryo: The gene products of *dl* and *twi* (or *sna*) might combine to produce a functional molecule. An insufficiency of either the *dl* product made during oogenesis or the *twi* product made in the zygote can be overcome but not an insufficiency of both. The combined *dl-twi* molecule might then serve to specify dorsoventral position in the embryo. This model predicts that the *twi* and *sna* genes are active in all cells and that their absence in the zygote might cause a shift in the fate map comparable with that shown for *dl*-dominant embryos.

Closer examination of the embryonic phenotypes and analysis of mosaic individuals will hopefully provide information as to the tissue specificity of the functioning of *twi* and *sna* and may enable a distinction to be made between these two hypotheses.

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