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The Segment Polarity Phenotype of *Drosophila* Involves Differential Tendencies toward Transformation and Cell Death

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The segment polarity genes of *Drosophila* are required for intrasegmental organization, as revealed by their abnormal cuticular morphology in mutant embryos. Lesions in most of these loci result in a similar cuticular phenotype, in which the normally naked, posterior region of the segment is covered to varying degrees by ectopic denticles. A temperature-sensitive allele of *armadillo*, which allows us to vary the level of arm^+ activity, generates this entire range of phenotypes, suggesting that these genes affect a common pathway. Previous work with a strong allele of *arm* revealed the locus to be cell-autonomous, in that small homozygous epidermal clones secreted denticles. We have conducted a similar clonal analysis at all levels of arm^+ activity. This shows a differential tendency toward cell transformation and cell death within the segment. Antibodies to segmentation gene-fusion products show that the cell death is primarily in the most posterior region of the segment. We suggest that differential cell respecification, resulting in transformation or death, is involved in generating the segment polarity phenotype.

INTRODUCTION

Within 6 hr of fertilization, the metameric pattern of the *Drosophila* embryo has been established. This process depends on at least 30 segmentation genes, which have been grouped into three classes (Nusslein-Volhard and Wieschaus, 1980; see reviews by Akam, 1987; Perrimon and Mahowald, 1988). Mutations in any of the gap genes affect entire regions of the embryo, while lesions of the pair-rule genes cause deletions in alternating segments. In embryos mutant for a segment polarity gene, the correct number of segments is formed, but within these segments organization is abnormal.

There are 12 segment polarity loci known to date. Mutations at three loci, *patched* (*ptc*), *naked* (*nkd*), and *engrailed* (*en*), are associated with unique phenotypes. The denticles of *ptc* mutants occur in thin, well-defined belts separated by double segment borders (Nusslein-Volhard and Wieschaus, 1980). Lethal alleles of *costal-2* can produce a similar phenotype (Grau and Simpson, 1987). In *nkd* embryos nearly all denticles are absent (Jurgens *et al.*, 1984; Martinez-Arias *et al.*, 1988). *en* embryos have extensive segment fusion organized in a pair-rule fashion (Nusslein-Volhard and Wieschaus, 1980).

The embryonic defects associated with lesions at the other eight segment polarity loci involve the distribution and polarity of denticles. Normally these structures occur in belts several rows wide in the anterior of each segmental unit. In this group of mutants, the naked cuticle of the posterior section is absent; instead, ectopic denticles appear, often of reverse polarity to those in the anterior region. *Cubitus-interruptus* (Orenic *et al.*, 1987) and *wingless* (Baker, 1987) are expressed only zygotically, while four others are required both maternally and zygotically: *fused* (Counce, 1956; Perrimon and Mahowald, 1987), *armadillo* (Wieschaus and Noell, 1986; Perrimon and Mahowald, 1987), *dishevelled* (Perrimon and Mahowald, 1987), and *porcupine* (Perrimon *et al.*, 1989; our unpublished results). The period of requirement of *hedgehog* and *gooseberry* is unknown.

The severity of the segment polarity defect varies greatly among these eight loci and sometimes among alleles of particular genes. Thus paternally rescued *fused* embryos have a few patches of ectopic denticles, while in *wingless* and *dishevelled* embryos the entire ventral cuticle is covered with a dense lawn of denticles (Nusslein-Volhard and Wieschaus, 1980; Perrimon and Mahowald, 1987). It is unclear to what extent the phenotypic variations reflect different functions or different levels of gene activity.

Analysis of the *in situ* expression patterns of several segment polarity genes in wild-type and mutant embryos has provided some insight into their functional relationships. In addition to regulation by pair-rule genes (Howard and Ingham, 1986; DiNardo and O'Farrell, 1987; Ingham *et al.*, 1988), the segment polarity genes also regulate each other (DiNardo *et al.*, 1988; Martinez-Arias *et al.*, 1988). Whereas *en* (Weir and Kornberg, 1985; Ingham *et al.*, 1985), *wg* (Baker, 1987) and *gsb* (Bopp *et al.*, 1986; Cote *et al.*, 1987) transcripts

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occur in narrow segmental stripes, *arm* is expressed throughout the embryo (Riggleman *et al.*, 1989). Nevertheless, *arm* is like the other loci in that lesions cause localized defects.

In this paper we describe experiments using a temperature-sensitive allele of *armadillo*. By modulating both maternal and zygotic expression of arm^+ we generate a broad range of segment polarity phenotypes, from a few scattered ectopic denticles to a dense lawn. Each phenotype reflects a particular level of arm^+ activity. Using techniques of clonal analysis, we have examined the effect of homozygous mutant cells on the differentiation of the embryonic epidermis. This study has revealed a differential capacity of homozygous cells to express the mutant phenotype, indicating that the fate of epidermal cells depends on their level of arm^+ activity. At relatively low levels of arm^+ activity, we find extensive cell death in the most posterior section of each segment, corresponding to the domain of *en* expression. We discuss how the differential tendency of cells toward transformation and cell death may effect the segment polarity phenotype and what these results imply for the genetic analysis of other segment polarity genes.

MATERIALS AND METHODS

Strains

Two alleles of *armadillo*, arm^{XK22} and arm^{XM19}, and a *shavenbaby* allele, svb^{YP17b}, were obtained from E. Wieschaus and maintained in *FM7* stocks. The *wingless* allele used (wg^{IG22}) was from the Bowling Green Stock Center and maintained in a *Cy0* stock. The duplication *Dp(1:Y)y²Y67g* (1A1 to 2B17-18 and 20A3 to the base; Y, Craymer and Roy (1980)) covers the *armadillo* locus (2B15-17) and was maintained as an attached-X stock: *C(1)DX, yf/Dp(1:Y)y²Y67g*. The X-linked dominant female sterile mutation *Fs(1)K1237* (Busson *et al.*, 1983; Perrimon, 1984) is maintained as an attached-X stock: *C(1)DX, yf*/Y females crossed to *Fs(1)K1237*, v^{24}/Y males.

The *en-lacZ* strain was obtained from C. Hama and T. Kornberg; and the *ftz-lacZ* strain from Y. Hiromi and W. Gehring. These chromosomes were introduced into the appropriate mutant background by mating males bearing the fusion construct to virgins heterozygous or homozygous (bearing germ line clones) for $arm^{H8.6}$.

Descriptions of balancer chromosomes and stocks, unless identified in the text, are in Lindsley and Grell (1968) or Lindsley and Zimm (1987). Except where noted, stocks and matings were maintained at 25°C on standard *Drosophila* medium.

Isolation and Mapping of $arm^{H8.6}$

The temperature-sensitive lethal allele of *armadillo* ($arm^{H8.6}$) was induced in a screen for X-linked larval-pupal lethal loci on a *yellow* (*y*) and *forked* (*f*) chromosome (Perrimon *et al.*, 1989). It was mapped meiotically between *yellow* (0.0) and *white* (1.5) and was complemented by *Dp(1:Y)y²Y67g*, a duplication covering the 1A1 to 2B17-18 region of the X-chromosome. $arm^{H8.6}$ failed to complement other *arm* alleles. Males of genotype *y* $arm^{H8.6}$/Dp(1:Y)y²*Y67g* are fertile and viable.

Germ Line Clonal Analysis

Germ line clones of $arm^{H8.6}$ were obtained by the dominant female sterile technique as previously described (Perrimon, 1984; Perrimon *et al.*, 1984), as shown in Fig. 2. Briefly, virgin females heterozygous for *FM7c/y* $arm^{H8.6}$ were mated to *Fs(1)K1237* v^{24}/Y males. At the end of the first larval instar stage, progeny were irradiated at a constant dose of 1000 rads (Torrex 120D X-ray machine; 100 kv, 5 mA, 3-mm aluminum filter). Mitotic recombination in the germ line of $arm^{H8.6}$/Fs(1)K1237 females was detected by individual inspection of ovary development. The frequency of females carrying germ line clones homozygous for $arm^{H8.6}$ varied from 5 to 8%.

Epidermal Clonal Analysis

Mitotic recombination during embryonic development was induced by a procedure similar to that of Wieschaus and Riggleman (1987), as shown in Fig. 2. Virgin females heterozygous for $arm^{H8.6}$, arm^{XK22}, or wg^{IG22} or carrying homozygous $arm^{H8.6}$ germ line clones were mated to wild-type *Oregon R* (*OreR P2*) males at the indicated temperatures. Eggs were collected hourly and incubated at the same temperature to an age of 3.5 ± 0.5 hr after egg laying at which time they were irradiated with 500 rads. This corresponds to shortly after the cellular blastoderm stage of embryonic development. Embryogenesis was continued at a second temperature, so that every combination of oogenic and embryogenic temperatures was tested. Embryos were allowed to develop for 36 ± 12 hr at the indicated temperatures before mounting. Larvae and unhatched eggs were prepared for microscopy and scored for hemizygous phenotype and frequency of ectopic denticle clones.

To provide an external marker for epidermal clones, recombinants were made between arm^{XK22} and *svb*. The *svb* mutation marks denticles in that most are eliminated and those remaining are very small (Wieschaus *et al.*, 1984). Because *arm svb* clones were scarce and hard

to detect (data not shown), the marker was not used further.

Analysis of Cuticular Phenotypes

All larvae and unhatched eggs were soaked in 50% Clorox bleach, heated in glycerol/acetic acid, and mounted in Hoyers mountant as described by van der Meer (1977). Cuticle preparations were scored for gross phenotype and incidence of ectopic denticles using phase-contrast and Nomarski optics at 200 to 400×. The size, polarity, and location of each clone were recorded. Lethal phase determination was performed as described by Perrimon *et al.* (1984).

Immunohistochemistry and Embryonic Sections

Vitelline membranes were removed using minor modifications of published procedures (Mitchison and Sedat, 1983) and histochemical localization of horseradish peroxidase was performed as published (Ghysen *et al.*, 1986).

Embryos were dechorionated in 50% Clorox bleach, fixed for 10 min in PBS-buffered 4% paraformaldehyde/heptane, and devitellinized with absolute methanol. All steps were performed at room temperature. Embryos were washed in PBS + 0.1% Tween 20 (PT) and incubated with mouse anti-β-galactosidase primary antibody (dilution, 1:1000) overnight at 4°C. Embryos were then washed in PT for 5 hr at room temperature and incubated overnight at 4°C with biotinylated horse anti-mouse IgG (dilution, 1:500). Embryos were washed in PT for 5 hr at room temperature. Visualization of horseradish peroxidase was performed using a Vectastain ABC kit. Silver intensification of the stain was performed in some preparations with an Amersham DAB enhancement kit. Embryos were washed briefly, dehydrated in ethanol, and cleared in methyl salicylate. Whole mounts were viewed using a Zeiss Axiophot microscope with Nomarski optics.

After staining and dehydration some embryos were embedded in JB4 plastic (Polysciences). Serial 4-μm sections were cut using a Leitz 1516 microtome and stained with methylene blue. Slides were dried and mounted in Aquamount.

RESULTS

The Armadillo Phenotypic Series

Embryos hemizygous for an allele of *armadillo* display cuticular defects in every segment, the severity of which depends on the strength of the allele (Gergen and Wieschaus, 1986; Wieschaus and Riggleman, 1987). We have isolated a temperature-sensitive allele of *armadillo* ($arm^{H8.6}$, see Materials and Methods) which shows a broad range of segment polarity phenotypes, encompassing those of other *arm* alleles (Table 1, Fig. 1). The lethal phase and cuticular phenotype of hemizygous males ($arm^{H8.6}$/Y) derived from heterozygous mothers ($arm^{H8.6}$/+) vary with temperature. At 18°C, males hemizygous for the allele survive to late larval stages (data not shown) and possess wild-type cuticle (Fig. 1A). When stocks are maintained at 22°C, 68% of mutant males die during embryogenesis (Table 1) and show occasional patches of ectopic denticles. These patches typically contain 4 to 12 denticles with their tips pointing posteriorly; this constitutes a very weak

TABLE 1
LETHAL PHASE AND GERM LINE CLONAL ANALYSIS

Maternal genotype	T°C	N	Nuf	%U	Embryonic phenotype (%)					
					WT	VW	W	M	S	VS
$arm^{H8.6}$/+	18	364	92	7	95	5	0	0	0	0
	22	374	26	17	14	69	16	1	0	0
	25	426	104	25	9	3	71	15	2	0
	29	594	55	34	21	0	3	69	7	0
GLC $arm^{H8.6}$	18	230	80	58	4	0	4	75	17	0
	22	191	43	46	15	0	1	12	62	10
	25	211	27	55	4	0	3	1	22	70

Note. Females heterozygous for $arm^{H8.6}$, or possessing homozygous germ line clones (GLC $arm^{H8.6}$), were crossed to wild-type, *Oregon R* males. Temperature (T°C) reflects that at which both mothers and embryos were incubated. The total number of progeny examined (N) and the number of unfertilized eggs (Nuf) for each cross are indicated. The percentage of unhatched embryos (%U) was calculated as N_e − Nuf/N − Nuf, where N_e represents the number of unhatched embryos. The embryos were grouped according to their segment polarity phenotype: wild-type (WT), very weak (VW), weak (W), medium (M), strong (S), and very strong (VS), as described in Fig. 1. At 18 and 22°C all hemizygous embryos which hatch will die during larval stages (occasionally a few pupate at 18°C). At 22°C a few larvae display a very weak phenotype.

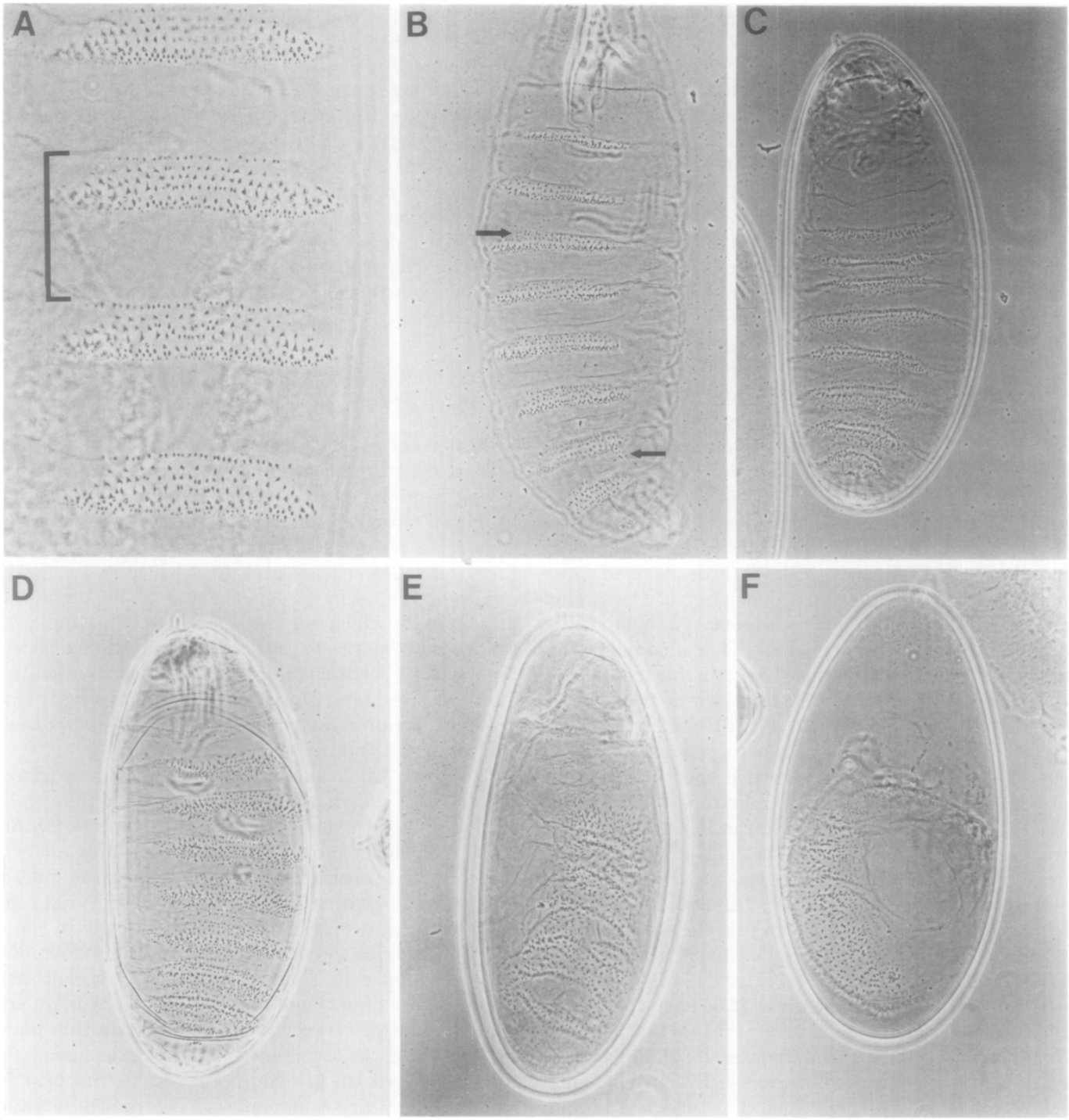


FIG. 1. The phenotypic series of embryos mutant for *armadillo*$^{H8.6}$. (A) In the wild-type pattern, as indicated by the bracket, the ventral cuticle of each abdominal segment consists of a belt of seven rows of denticles, five or six of which point posteriorly, followed by a posterior region of naked cuticle. Males hemizygous for *arm*$^{H8.6}$ exhibit the wild-type pattern when stocks are maintained at 18°C. (B) The very weak phenotype is nearly normal, except for a few patches of ectopic denticles, indicated here by arrows. These animals often hatch, as did this one, a hemizygous *arm*$^{H8.6}$ male incubated at 22°C. (C) In the weak phenotype, typically exhibited by hemizygous embryos at 25°C, ectopic denticles are scattered throughout the naked regions, and the posterior limits of the denticle belts are poorly defined. (D) The medium phenotype is characterized by ectopic fields of denticles in every segment, many of which are of reverse polarity to the normal denticles. The denticles are less dense in the posterior margin of the segment. This phenotype predominates at 18°C when hemizygotes are derived from mothers bearing homozygous germ line clones of *arm*$^{H8.6}$. (E) In the strong phenotype the ventral cuticle is covered with a lawn of denticles, the polarities of which are ambiguous due to extensive wrinkling of the cuticle. This *arm*$^{H8.6}$/*Y* embryo underwent embryogenesis at 22°C and was derived from a germ line clone. (F) Embryos displaying the very strong phenotype are roughly spherical, their ventral surfaces covered with denticles. They lack segment borders and filzkorpers. Such hemizygotes are seen at 25°C if obtained from germ line clones. Anterior is up in all panels.

phenotype (Fig. 1B). At 25°C, the cuticles of hemizygous embryos show continuous fields of sparse denticles, sometimes of reverse polarity, occupying the more anterior section of the normally naked region. This is a weak segment polarity phenotype. All these cuticular phenotypes are less severe than those of previously characterized *armadillo* alleles (Gergen and Wieschaus, 1986; Wieschaus and Riggleman, 1987). At 29°C, hemizygous mutant males exhibit a medium phenotype in that the posterior section of each segment is covered with denticles of reverse polarity. This pattern is identical to that produced by arm^{XM19} (Gergen and Wieschaus, 1986).

Mutations at the *armadillo* locus exhibit a maternal effect. Homozygous germ line clones of previously isolated *armadillo* alleles produce abnormal eggs which fail to develop, revealing an absolute requirement for *armadillo* gene product during oogenesis (Wieschaus and Noell, 1986; Perrimon and Mahowald, 1987). However, a maternal role of *armadillo* in the segmentation process was demonstrated by varying the maternal dosage. Mutant embryos derived from attached-X mothers (females with two wild-type copies of the arm^+ gene) have a weaker phenotype than those derived from mothers heterozygous for a deficiency of the region (Wieschaus and Noell, 1986). Thus embryos hemizygous even for possible amorphic alleles of *arm* do not reflect a true null phenotype, because the germ line from which they are derived has one wild-type copy of the gene.

Using the dominant female sterile technique (see Materials and Methods), we are able to generate germ line clones of the temperature-sensitive $arm^{H8.6}$ allele that produce eggs which undergo embryonic development. This maternal effect is fully paternally rescuable at all temperatures (Table 1). The cuticles of hemizygous males derived from mothers possessing such germ line clones are shown in Figs. 1D, 1E, and 1F. At 18°C hemizygotes derived from germ line clones exhibit the medium cuticular phenotype, like those derived from heterozygous mothers at 29°C (Fig. 1D). Male embryos derived from germ line clones at 22°C have the phenotype of strong alleles such as arm^{XK22} (Wieschaus and Noell, 1986; Perrimon and Mahowald, 1987). The ventral cuticle of these animals is covered with a lawn of denticles of ambiguous polarity. Head structures are missing or malformed (Fig. 1E). A very strong phenotype is observed at 25°C, with embryos lacking head structures, dorsal cuticle, and filzkorpers. The ventral cuticle is covered with a thick lawn of denticles (Fig. 1F). These embryos lack segmental boundaries, and sections through them reveal massive pockets of cell death (see below). These features are identical to the defects of *wingless* (*wg*) and *dishevelled* (*dsh*) embryos (Perrimon and Mahowald, 1987), but are more severe than previously described *arm* phenotypes.

In summary, reduction of maternal *armadillo* product via germ line clones of homozygously mutant tissue allows us to produce a series of segment polarity phenotypes by increasing incubation temperature. Hemizygous embryos derived from heterozygous mothers vary from wild-type at 18°C to a medium segment polarity phenotype at 29°C, while hemizygous embryos derived from germ line clones display segment polarity phenotypes ranging from medium at 18°C to very strong at 25°C. These defects encompass the entire range of segment polarity phenotypes seen among mutants of the segment polarity genes, with the exceptions of *ptc, cos-2, nkd,* and *en.*

Transformation Capacities Are Correlated with arm^+ Activity

In segment polarity embryos, all segments are present but their organization is abnormal, manifested by ectopic denticles in their posterior region. Using X-rays to induce mitotic recombination in embryos heterozygous for a strong allele of *armadillo,* Wieschaus and Riggleman (1987) were able to produce in the naked region of the segment small clones of homozygously mutant cells which secreted denticles. This indicates that cells giving rise to the naked cuticle, when deficient for the arm^+ product, can undergo transformation to a more anterior fate. Moreover, these data reveal that *armadillo* is cell-autonomous, in that the genotype of an epidermal cell determines its phenotype.

The $arm^{H8.6}$ allele allows us to uncouple maternal and zygotic arm^+ contributions and to modulate the zygotic arm^+ activity by varying incubation temperature. Because the gene is autonomous we can use this allele to examine the propensity of cells to express the mutant phenotype at different levels of arm^+ activity. The genetic manipulations are shown schematically in Fig. 2 and are described under Materials and Methods. Results are shown in Table 2. As control experiments, strong alleles of *arm* and *wg* were used as positive and negative controls, respectively. To ascertain that nothing prevented the occurrence of these clones under the conditions used, the procedure was tested using arm^{XK22} at all temperature combinations. The results revealed that the hemizygous phenotype was invariably strong and that clones were induced to approximately the same extent regardless of temperature (data not shown). An example is listed on the first line of Table 2. When flies were reared at 25°C and embryonic development was at 18°C, hemizygous embryos were of a strong segment polarity phenotype, and among the wild-type animals 45 clones were observed. One-third (153) of the progeny not showing a segment polarity phenotype were expected to be heterozygous for *arm;* therefore,

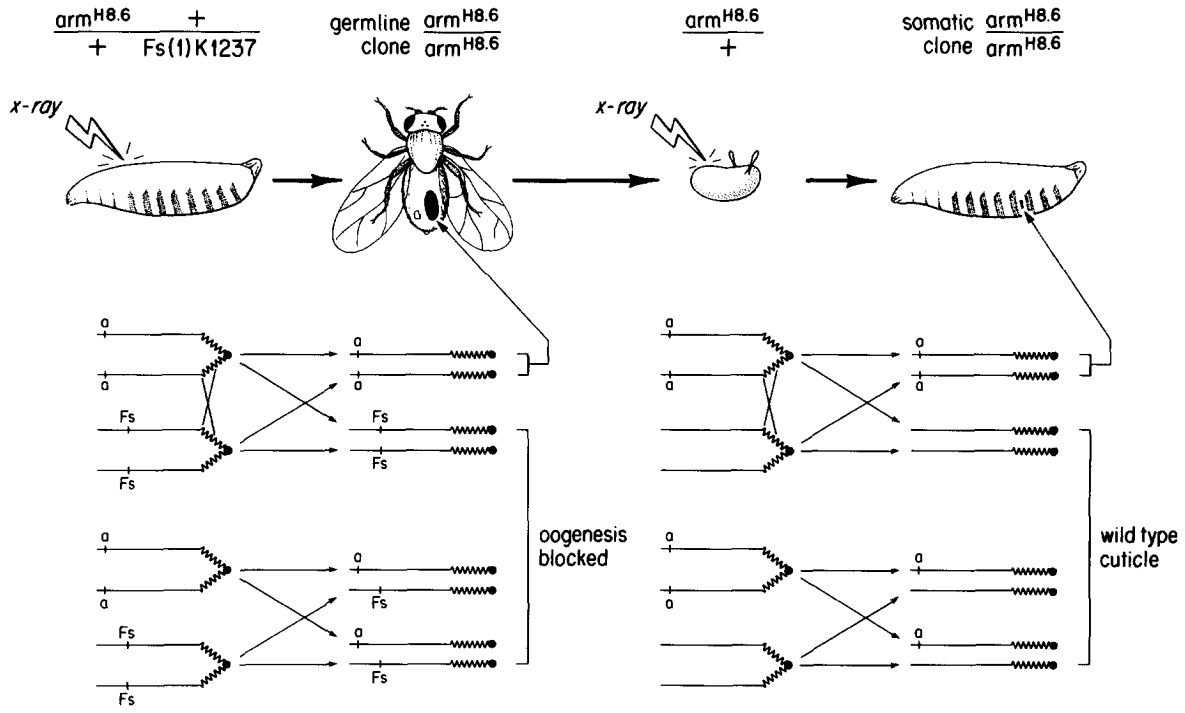


FIG. 2. Experimental scheme for clonal analysis of *armadillo*$^{H8.6}$. To generate homozygous maternal germ line clones of *arm*$^{H8.6}$, the dominant female sterile technique of Perrimon *et al.* (1984) was used. Females heterozygous for *arm*$^{H8.6}$ were mated to males carrying the dominant female sterile allele *Fs(1)K1237*. Both of these loci reside near the distal end of the X-chromosome. First instar larvae from this cross were X-irradiated to induce mitotic recombination between nonsister chromatids in the transheterozygotes, which usually occurs in the heterochromatic region near the centromere (indicated by wavy lines). Upon segregation at anaphase, any cell which receives a dominant female sterile allele will give rise to an abnormal ovarium blocked for oogenesis, while a cell receiving two *arm*$^{H8.6}$ alleles will give rise to a germ line clone. Epidermal clones of *arm*$^{H8.6}$ were induced by irradiating eggs shortly after the blastoderm stage, derived from heterozygous mothers or those with germ line clones mated to wild-type males. In embryos heterozygous for *arm*$^{H8.6}$, mitotic recombination in an epidermal precursor cell yields a cell homozygously mutant for *arm*$^{H8.6}$, which may give rise to a clone secreting denticles.

the frequency of these clones was 29%. When the progeny of the cross between females heterozygous for *wg*^{IG22} and wild-type males were irradiated, only one clone was found among over 100 heterozygous larvae. The same result was obtained by Wieschaus and Riggleman (1987) in an analogous cross and is used here as a negative control for the experiment. Because there is no means of marking individual cells in the larval cuticle, only those clones secreting denticles can be scored.

Among progeny heterozygous for *arm*$^{H8.6}$, occurrence of ectopic denticle clones was dependent on both temperature and maternal germ-cell genotype (Table 2). When derived from crosses in which normal heterozygous mothers were mated to wild-type males, very few animals bore clones, and the number of ectopic denticles in these clones was never more than three (data not shown). Temperature of oogenesis had little effect on embryonic development. However, the temperature of embryogenesis exerted a profound influence on the hemizygous phenotype, ranging from wild-type cuticle at 18°C to a medium segment polarity phenotype at 29°C.

When females bore germ line clones of the *arm*$^{H8.6}$ allele, the reduction in maternal *armadillo* product intensified the hemizygous defects by several classes at each combination of temperatures. Concomitantly, the incidence of denticle clones in heterozygous progeny rose dramatically. Clonal frequencies peaked at about 35% when embryogenesis was at 22°C and oogenesis was at 18°C or 22°C. In general, denticle clones were detected readily whenever the hemizygous phenotype was strong, like that of *arm*^{XK22}/Y control embryos. Some examples are shown in Figs. 3A-3C.

The average size of these epidermal clones is quite small; for example, the *arm*^{XK22} clones ranged from 1 to 11 denticles, with the average being 3.8. Since each differentiated (anterior) epidermal cell secretes about 2.8 denticles (Wieschaus and Riggleman, 1987), these clones typically contain one or two cells and never more than four. The small size of these clones allows their precise localization within the naked posterior region of the segment. Each clone was localized to one of nine subdivisions of the posterior region; finer delimitations of the cuticle made assignment rather subjective. A rep-

TABLE 2
DENTICLE CLONE ANALYSIS

Maternal genotype	Temperature		Hemizygous phenotype	Expected No. heterozygous progeny	No. of clones	% of clones
	$T°O$	$T°E$				
$arm^{XK22}/+$	25	18	Strong	153	45	29
$wg^{IG22}/+$	25	18	Very strong	107	1	1
$arm^{H8.6}/+$	18	18	Wild-type	46	1	2
$arm^{H8.6}/+$	18	22	Very weak	53	0	0
$arm^{H8.6}/+$	18	25	Weak	62	0	0
$arm^{H8.6}/+$	18	29	Weak	41	0	0
$arm^{H8.6}/+$	22	18	Wild-type	49	0	0
$arm^{H8.6}/+$	22	22	Very weak	60	0	0
$arm^{H8.6}/+$	22	25	Weak	71	0	0
$arm^{H8.6}/+$	22	29	Weak	48	1	2
$arm^{H8.6}/+$	25	18	Wild-type	56	0	0
$arm^{H8.6}/+$	25	22	Very weak	71	0	0
$arm^{H8.6}/+$	25	25	Weak	52	2	4
$arm^{H8.6}/+$	25	29	Medium	83	4	5
$arm^{H8.6}/+$	29	18	Very weak	44	0	0
$arm^{H8.6}/+$	29	22	Very weak	37	0	0
$arm^{H8.6}/+$	29	25	Weak	46	1	2
$arm^{H8.6}/+$	29	29	Medium	39	0	0
GL$arm^{H8.6}$	18	18	Medium	47	2	4
GL$arm^{H8.6}$	18	22	Strong	48	17	35
GL$arm^{H8.6}$	18	25	Strong	46	7	15
GL$arm^{H8.6}$	22	18	Medium	53	3	6
GL$arm^{H8.6}$	22	22	Strong	64	23	36
GL$arm^{H8.6}$	22	25	Strong	48	10	21
GL$arm^{H8.6}$	25	18	Medium	67	7	10
GL$arm^{H8.6}$	25	22	Strong	56	8	14
GL$arm^{H8.6}$	25	25	Very strong	71	2	3

Note. Heterozygous females of the various indicated genotypes, including those bearing homozygous germ line clones of $arm^{H8.6}$ (GLC $arm^{H8.6}$) were mated to wild-type males such that oogenesis occurred at a particular temperature ($T°O$). Eggs were collected and irradiated at 3.5 ± 0.5 hr after laying and allowed to complete embryogenesis at the indicated temperature ($T°E$). The expected number of heterozygous progeny was calculated as one-third of the animals failing to show a segment polarity phenotype from heterozygous crosses and all such animals when the mother bore a germ line clone. The frequency of clones expressing denticles (% of clones) in each cross was calculated as the number of clones observed divided by the number of heterozygous progeny, multiplied by 100.

resentative distribution of clones in animals heterozygous for arm^{XK22} is shown in Fig. 3D. Most clones occurred in the middle of the naked region, with very few in more posterior locations. Thirty percent of the clones displayed reverse polarity in that their denticles pointed anteriorly, and essentially all of these appeared in the middle sections (e.g., Fig. 3A). Those clones which appeared in the most anterior portion of the naked region resembled the denticles at the posterior margin of the denticle belt, in that the denticles were generally small (e.g., Fig. 3B). Clones occurred evenly throughout the abdominal segments (data not shown); a paucity of clones in segment A7 is attributed to the difficulty of visualizing this segment in mounted specimens. Overall, the distribution of clones in $arm^{H8.6}$ heterozygotes resembled that in the arm^{XK22} heterozygotes (data not shown).

Altogether, experiments on the induction of homozygous *armadillo* clones show that cells are transformed to the mutant phenotype whenever arm^+ activity is at low levels, corresponding to those giving rise to hemizygotes with a strong segment polarity phenotype. Few if any denticle clones are produced when arm^+ levels are higher (in the heterozygous siblings of embryos displaying weak and medium phenotypes) or lower (in those of very strong phenotypes). Very few clones appear in the posterior third of the naked region of each segment, coinciding approximately with the domain of cells expressing *engrailed* (see below).

Cell Death and Segmentation Gene Expression in Armadillo Embryos

An explanation for the absence of denticle clones in the heterozygous siblings of embryos with the very

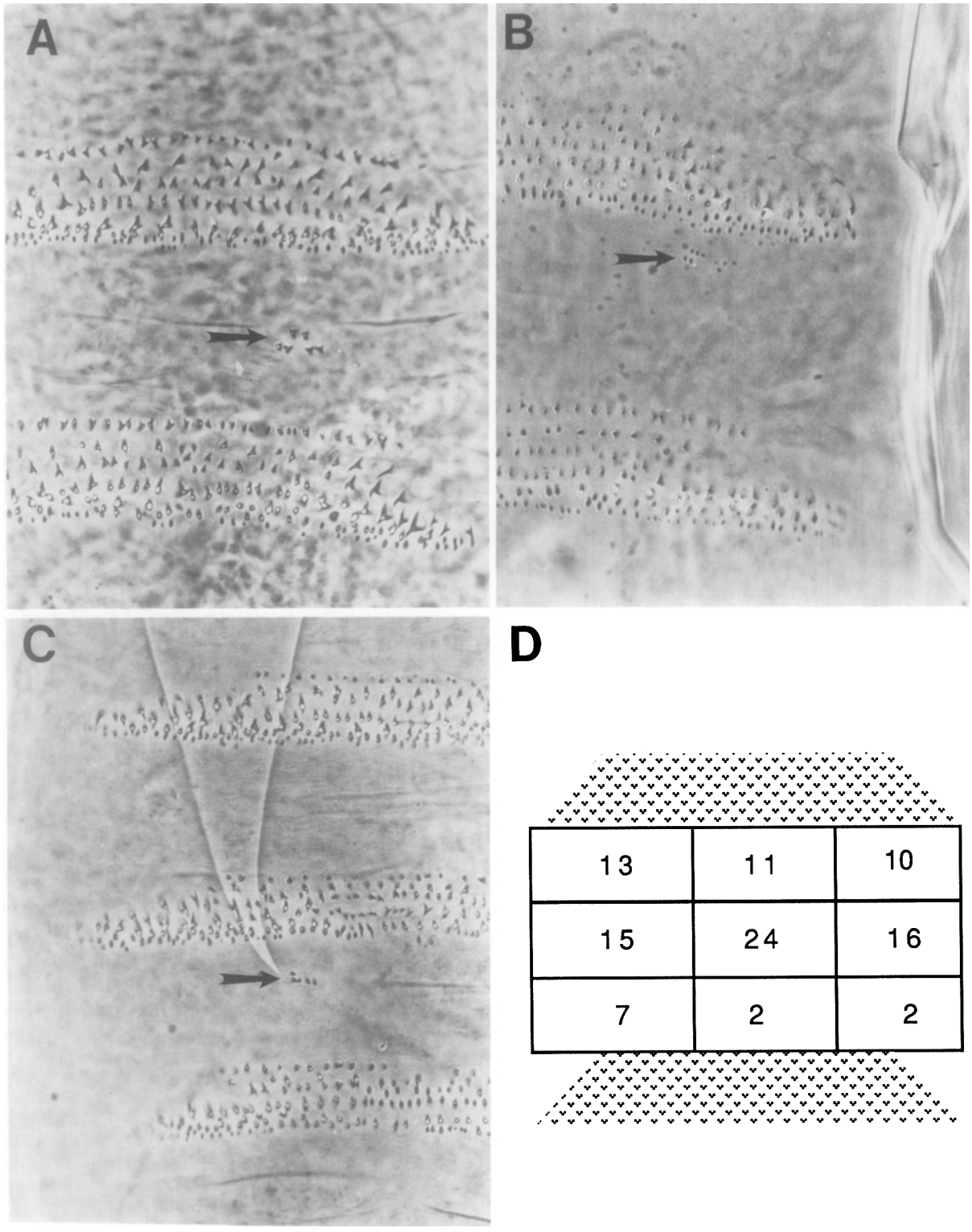


FIG. 3. Examples and distribution of denticle clones. Three examples are shown of denticle clones induced in animals heterozygous for $arm^{H8.6}$ and derived from mothers carrying germ line clones, with oogenesis and embryogenesis at 22°C. (A) Clones near the middle of the naked region of a segment, like this one, secreted large denticles. Unlike these, most denticles were of normal polarity. (B) Clones in the anterior part of the naked region secreted small denticles like those at the posterior margin of the denticle belt, which displayed normal or indeterminate polarity. (C) The typical clone contained four or five denticles pointed posteriorly. (D) The distribution of the 45 clones observed in arm^{XK22} heterozygotes at 18°C shows the tendency for most clones to occur in the anterior two-thirds of the naked region, with few in the posterior third. The distribution of clones was similar in $arm^{H8.6}$ heterozygotes (see text). Arrows indicate the positions of clones. Anterior is up in all panels.

strong phenotype is that homozygous cells in the posterior region cannot survive at very low levels of arm^+ activity. Cell death has been implicated in the establishment of many imaginal and embryonic phenotypes of *Drosophila* (Fristrom, 1969; Arking, 1975; Giorgi and Deri, 1976; White and Lehmann, 1986; Bryant, 1988), including the segment polarity defect (Martinez-Arias, 1985; Perrimon and Mahowald, 1987). In each case zones of cell death were identified by the presence of pycnotic and fragmented nuclei, as revealed by stains specific for chromatin, such as toluidine blue and methylene blue. However, a characteristic of developmental cell death is that dead cells are often pushed into extracellular spaces, making their origins difficult to determine (Wyllie *et al.*, 1980). To examine directly the pattern of cell death in various classes of *armadillo* embryos we followed the fates of domains of cells expressing particular segmentation gene fusions, visualized by antibody staining of embryonic whole mounts and sections stained with methylene blue.

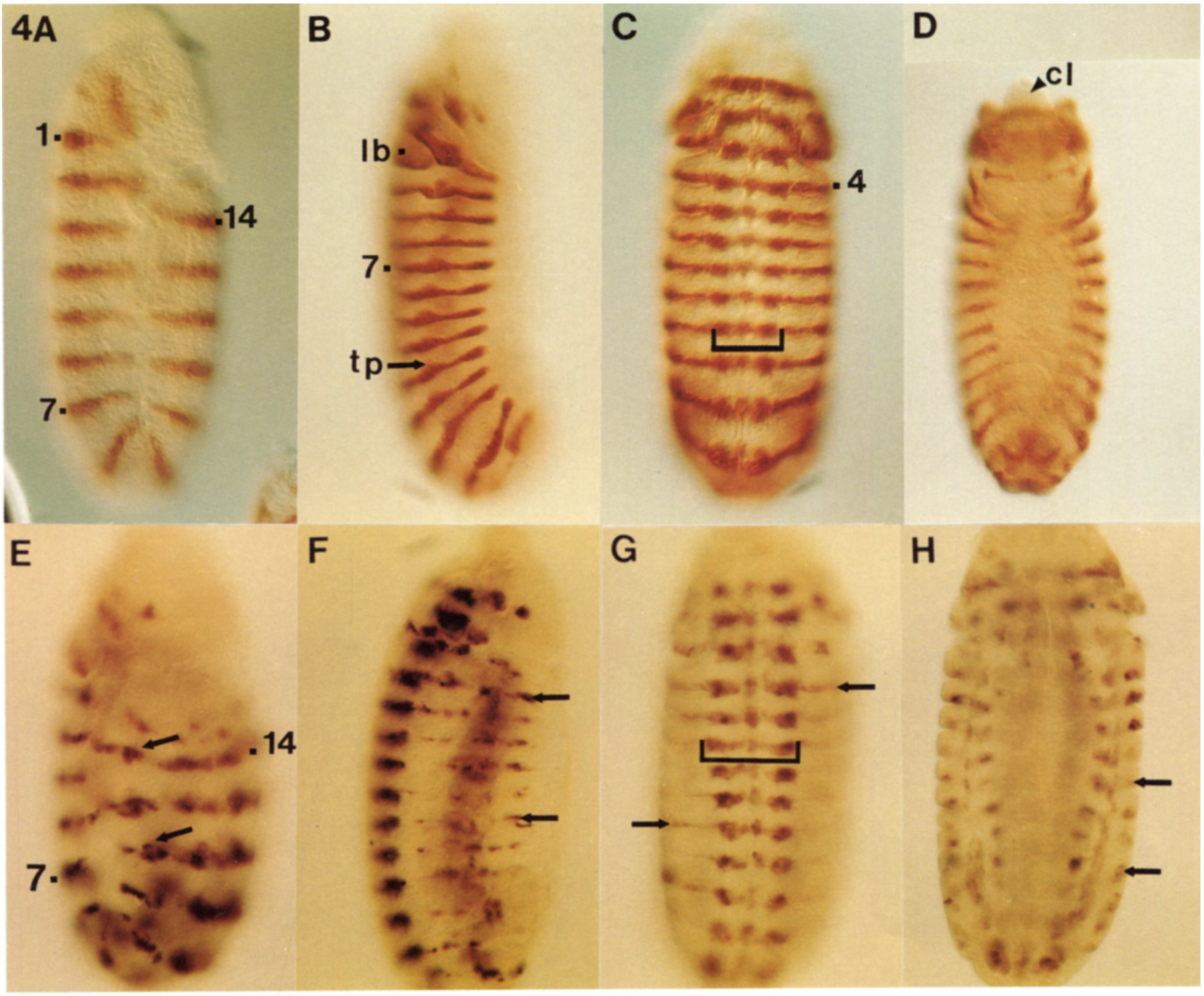
We examined the progeny of females heterozygous for $arm^{H8.6}$ or carrying homozygous germ line clones crossed with males expressing the β-galactosidase (*lacZ*) gene under the control of the *fushi tarazu* (*ftz*) or *engrailed* (*en*) regulatory regions (Hiromi and Gehring, 1987; C. Hama and T. Kornberg, personal communication). After the blastoderm stage, the *ftz-lacZ* and *en-lacZ* strains express the fusion proteins in patterns identical to the endogenous *en* protein (DiNardo *et al.*, 1985) and *ftz* protein (Carroll and Scott, 1985) expression patterns, as shown by staining wild-type embryos possessing these fusion genes with antibodies to β-galactosidase (Figs. 4A–4D, 5A, 5C, and 6A). However, these fusions produce a product which is more stable than the native protein, such that their expression patterns are persistent, enhancing their suitability as markers for particular domains of cells.

We examined the *ftz-lacZ* and *en-lacZ* expression patterns in $arm^{H8.6}$ hemizygotes corresponding to the medium, strong, and very strong segment polarity phenotypes (see Materials and Methods). In all three classes of embryos the early patterns of *en-lacZ* and *ftz-lacZ* expression are normal (data not shown), but gross abnormalities in the patterns of expression are detected after germ band extension (6 hr after fertilization). In the strong phenotype both *en-lacZ* and *ftz-lacZ* stripes of staining are abnormal at 6 hr of development (Figs. 4E and 5D). The epidermal staining becomes fainter and spottier as the embryo ages (Figs. 4F–4H). However, the *en-lacZ* staining does not completely disappear in older embryos (Fig. 4F). In the very strong phenotype no epidermal *en-lacZ* expression is detectable at 6 hr of development (Fig. 6B). However, staining is observed in the central nervous system, persisting in older embryos (Fig. 6C). The cause of disappearence of *en-lacZ* staining in the epidermis of the stronger *arm* mutant embryos cannot be determined unambiguously, in that either cell death or respecification in the *en* domain could generate the patterns we see. Late epidermal expression of *en* is positively regulated by *arm* and other segment polarity genes (DiNardo *et al.*, 1988). However, the abnormal persistence of the fusion peptide encoded by the *en-lacZ* construct used suggests it is the cells themselves that are disappearing. Moreover, the degenerated pattern of epidermal expression is not specific to *en-lacZ*, as shown by the spotty *ftz-lacZ* pattern (Figs. 5C and 5D). The cells most affected are those at the posterior of each segment, corresponding to the *en* domain.

Stained sections of embryos with segment polarity phenotypes have indicated that extensive cell death occurs as early as 6 hr after fertilization, demonstrated by toluidine blue staining of *fu* embryos (Martinez-Arias, 1985) and methylene blue staining of *dsh* and *wg*

FIG. 4. Expression of *en-lacZ* in the strong segment polarity phenotype. (A, B, C, D) The pattern of *en-lacZ* in wild-type embryos at various stages of development. (A) An embryo at 6.5 hr of development. Note the 14 stripes of *en-lacZ* expression; stripes 1, 7, and 14 have been labeled. (B, C, D) The pattern of *en-lacZ* in wild-type embryos at 11 hr. (B) Lateral view. Staining at the level of the tracheal pits is indicated. In A and B the ventral side of the embryo is on the left. (C) Ventral view. The central nervous system staining (CNS; indicated by the bracket) is superimposed on the epidermal staining. (D) Dorsal view. Dorsal closure is not yet complete, leaving the amnioserosa unstained. (E, F, G, H) The pattern of *en-lacZ* in embryos of genotype $arm^{H8.6}/Y$ derived from heterozygous females at 25°C (strong phenotype). (E) Ventrolateral view of an embryo at 7 hr. The epidermal pattern is spotty (indicated by arrows). (F, G, H) Embryos at 11 hr. (F) Lateral view with ventral on the left. (G) Ventral view. (H) Dorsal view. Note the intense staining in the CNS in F and G and the poor epidermal staining in F, G, and H (indicated by arrows). *Note.* In all figures anterior is up. The staining of embryos in E, F, and G has been silver intensified. Nomenclature: lb, labial; tp, tracheal pit; cl, clypeolabrum.

FIG. 6. Expression of *en-lacZ* in the very strong segment polarity phenotype. (A) The epidermal pattern of *en-lacZ* expression in a wild-type embryo at 7.5 hr of development. Note the 14 stripes of *en-lacZ* expression; stripe 14 and the staining at the level of the tracheal pits (arrow) have been labeled. (B, C) The expression pattern of *en-lacZ* in embryos of genotype $arm^{H8.6}/Y$ derived from females carrying a germ line clone homozygous for $arm^{H8.6}$ at 25°C. These embryos exhibit a very strong segment polarity phenotype. (B) Embryo at 6.5 hr. Note the staining in the CNS (arrow) and the absence of staining in the epidermis. (C) Embryo at 12 hr showing CNS expression and absence of epidermal expression. DiNardo *et al.* (1988) noted a decline in *en* antigen before detecting cell death in *wg* embryos. Possibly the product encoded by the fusion we use is more stable than theirs, preventing our detection of fine gradations in the temporal pattern of *en* expression. *Note.* In A and B ventral is on the left and anterior is up. In C anterior is on the left and dorsal is up, and staining of the embryo has been silver intensified. Nomenclature: lb, labial.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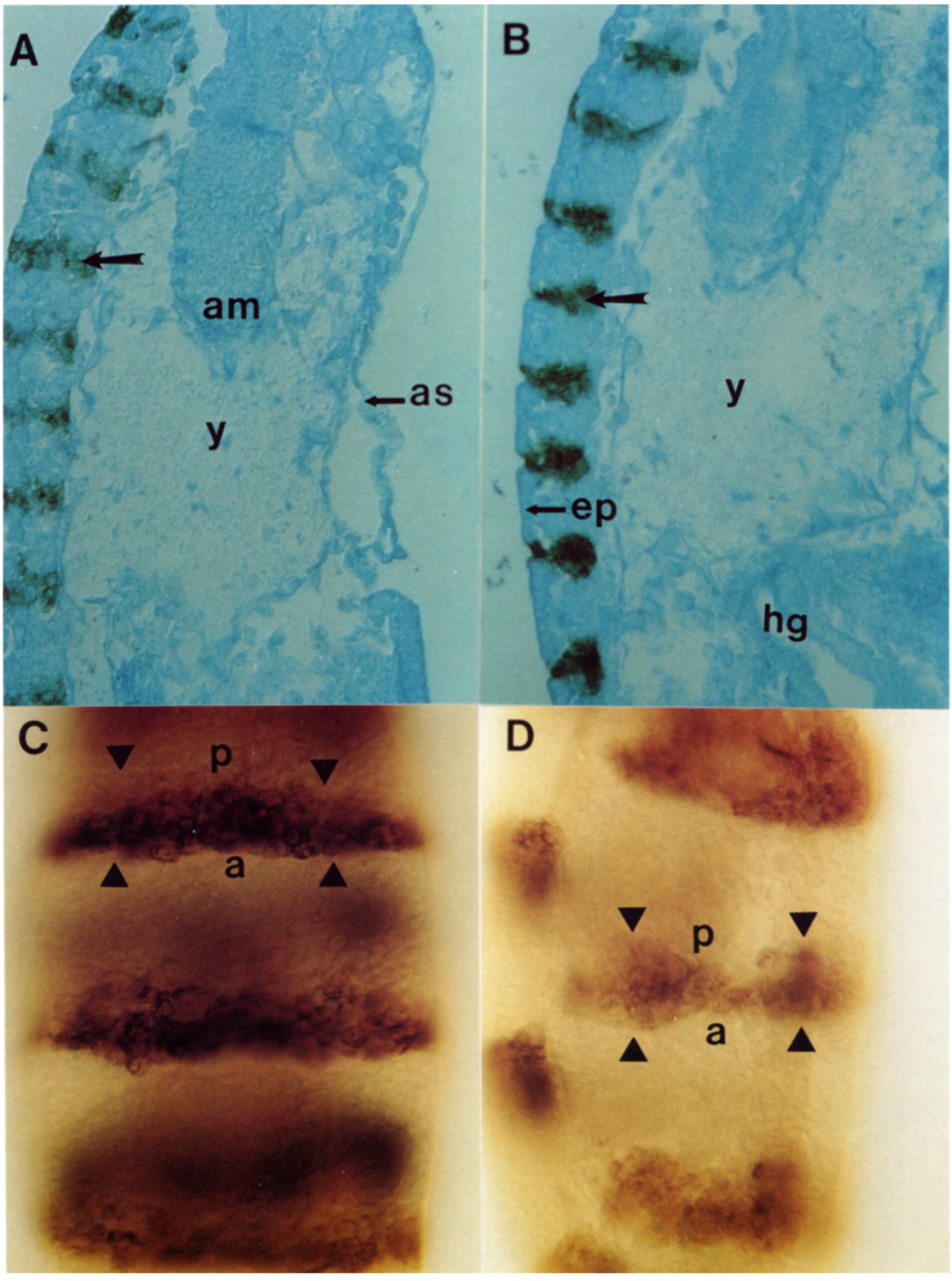


FIG. 5. Cell death in *armadillo* embryos. (A, B) The pattern of *en-lacZ* expression in longitudinal sections of wild-type (A) and $arm^{H8.6}/Y$ (B) embryos at 10 hr of development (ventral side is on the left). Note the normal stripes of staining visible in both the epidermis and the CNS. In the mutant embryo, corresponding to a medium segment polarity phenotype, the epidermal staining is thin and internal pockets of stained cells are visible. These pockets, which consist of the *en-lacZ* domain, contain dying cells. The arrows indicate one such pocket and the corresponding stripe in a wild-type embryo. (C, D) The pattern of *ftz-lacZ* expression in whole mounts of wild-type (C) and $arm^{H8.6}/Y$ (D) embryos at 5.5 hr of development. In the wild-type embryo parasegmental units are regularly stained. In D, which corresponds to an embryo with a strong segment polarity phenotype, the borders of the parasegmental units are irregular as a result of cell death, as seen by comparison of the staining within the triangles in C and D. The most anterior (a) and posterior (p) regions of the parasegments are shown. *Note.* The staining of all four embryos has been silver intensified. Nomenclature: y, yolk; am, anteromidgut; hg, hindgut; as, amnioserosa; ep, epidermis.

embryos (Perrimon and Mahowald, 1987). We also see extensive cell death in *arm* embryos with medium, strong, or very strong phenotypes (data not shown). In embryos mutant for any of these genes, this segmental pattern of cell death is detected by the presence of pockets of darkly stained cells under the epidermis. However, because the dying epidermal cells are internalized as they degenerate, their intrasegmental origin is difficult to assess. By anti-β-galactosidase staining of cells expressing *en-lacZ* it is possible to visualize the identity of these dead cells in the medium segment polarity phenotype. As shown in Fig. 5B, most (if not all) cells within these pockets are expressing *en-lacZ*, whereas other cells are not. Thus in the medium segment polarity phenotype, corresponding to low levels of arm^+ activity, the domain of cells expressing *engrailed* dies. In embryos of stronger phenotypes, *en-lacZ* expression is not detected during cell degeneration, perhaps because of respecification of the *en* domain in strong *arm* mutants (DiNardo *et al.*, 1988). Nevertheless, the domain of cell death is more extensive in these embryos (data not shown).

DISCUSSION

The temperature-sensitive *armadillo* allele ($arm^{H8.6}$) described in this paper has allowed us to manipulate the zygotic level of arm^+ activity. The segment polarity phenotype of *arm* embryos depends on the total arm^+ activity present, consisting of both maternal and zygotic contributions, which we have been able to manipulate by producing homozygous germ line clones and by varying the temperature of incubation. The cuticles of the resulting hemizygotes range from wild-type to a very strong segment polarity phenotype and directly reflect the level of arm^+ activity.

Because *arm* is cell-autonomous (Wieschaus and Riggleman, 1987), the ability of any epidermal cell to express the mutant phenotype is independent of the status of neighboring cells; however, we show that the phenotype of a mutant cell depends on its level of arm^+ activity, denticle secretion being only one alternative fate. When small clones of homozygously mutant cells were induced in heterozygotes at various temperatures, cells secreting ectopic denticles were observed only in a narrow range of arm^+ activity. Hemizygous embryos with this level of activity exhibit a strong phenotype, like that produced by arm^{XK22}, an apparent amorphic allele (Wieschaus and Riggleman, 1987). These results are explained schematically in Fig. 7. At relatively high levels of arm^+ activity, any clones of homozygously mutant cells cannot be detected because they are not transformed. At very low levels, clones cannot be detected because so little arm^+ product remains that the cells die. A narrow window of *armadillo* activity, corresponding to that which gives rise to the strong hemizygous phenotype, allows homozygous cells to be transformed and remain viable long enough to secrete cuticle. This clonal analysis of the *armadillo* phenotypic series demonstrates functionally the phenomenon of cell death. Additionally, by staining these embryos with antibodies to segmentation gene fusions, the extent of cell death can be correlated with the severity of the phenotype. Thus in embryos with a medium segment polarity defect, a broad, posterior band of cells (expressing *en*) rapidly deteriorates and dies.

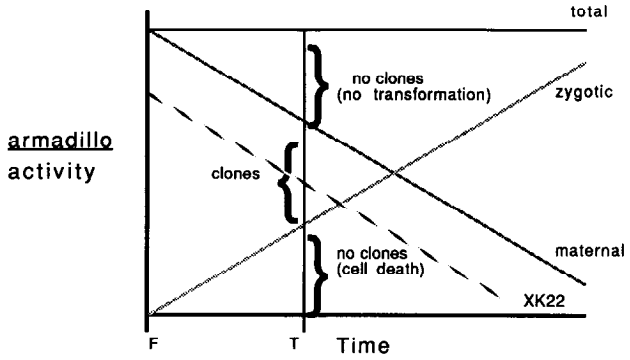


FIG. 7. The fate of cells homozygous for $arm^{H8.6}$ depends on their total level of *armadillo* activity. The total level of embryonic $armadillo^+$ activity is the sum of the maternal and zygotic components. The maternal component constitutes total activity at fertilization and subsequently decreases. The zygotic component begins shortly after fertilization and ultimately accounts for all $armadillo^+$ activity. The fate of cells made homozygous by mitotic recombination at Time *T* depends on the arm^+ activity in those cells. At high levels of activity clones of mutant cells induced in heterozygotes do not secrete denticles because sufficient activity remains that they are not transformed. At low levels of activity, homozygously mutant cells fail to secrete denticles because they die. At intermediate levels of activity, clones survive but express the mutant phenotype. This level of activity corresponds to that giving rise to the strong phenotype of hemizygous embryos, such as those mutant for arm^{XK22}, an apparent null allele. There is reduced maternal product and little if any zygotic activity in these animals, as indicated by the line labeled XK22.

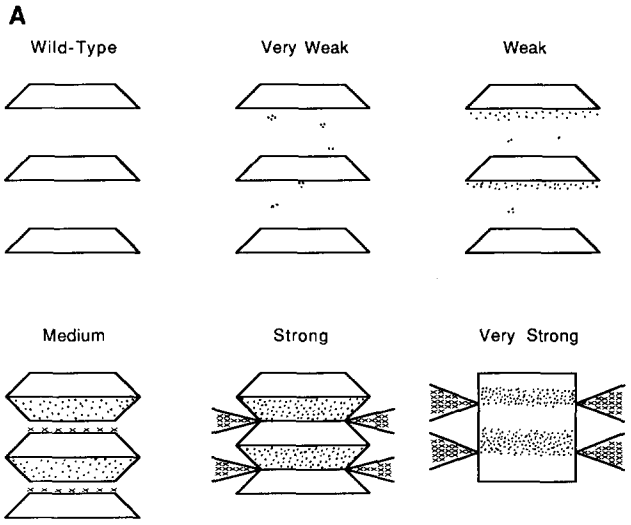
Generation of the Segment Polarity Phenotype via Cell Death and Respecification

The cuticular phenotypes of the segment polarity genes *nkd*, *ptc*, and *cos-2*, and *en* are unique, but those of the remaining eight loci are similar, as discussed earlier. The segment polarity phenotypes associated with these eight loci differ mainly in the extent of ectopic denticle production and remaining naked cuticle. The temperature sensitivity of $arm^{H8.6}$ allows us to generate embryos spanning this range of segment polarity phenotypes in a single genetic background. Many of these embryos are indistinguishable from embryos mutant

for other loci; their defects probably derive from the same mechanism. Thus we believe that $arm^{H8.6}$ shows that this phenotypic series results from disruptions of the same developmental processes and that it provides a means of studying the establishment of the segment polarity phenotype.

Our studies suggest that each segment polarity phenotypic class is the result of regional respecification in the segmental anlagen, manifested by transformation of cell fate or by cell death. The degree of respecification is inversely proportional to the level of arm^+ activity, and regions vary in their sensitivity to arm^+ reduction. Figure 8A diagrams the cuticular morphology of the six phenotypic classes derived from $arm^{H8.6}$ hemizygotes. At nearly wild-type levels of arm^+, the embryonic requirement for the gene product is fulfilled; these animals survive to late larval stages. A small decrease in activity results in a few scattered ectopic denticles, constituting a very weak segment polarity phenotype. We believe this pattern occurs because the level of arm^+ activity varies slightly within the cells. In a few cells it may cross a threshold beyond which the identity of cells is respecified (see below). Consequently these cells express a more anterior fate, the secretion of denticles. The weak phenotype is of similar origin, with a lower level of arm^+ causing more cells to be respecified. The confinement of ectopic denticles to primarily the most anterior portion of the naked cuticle suggests that these cells are most sensitive to arm^+ loss.

Low arm^+ activity in the segmental anlagen culminates in the respecification of most cells, many of which are transformed to secrete denticles but some of which die. In embryos displaying a medium segment polarity phenotype we detect a broad band of degenerating cells at the posterior margin of the segment. Most of the remaining cells secrete denticles, although a narrow strip of naked cuticle usually persists. Most of these ectopic denticles point anteriorly and are of inverse polarity to the normal belt of denticles. Although the mechanism of polarity determination is unknown, most likely it derives from the juxtapositions of domains of cells, with polarity defects arising from abnormal juxtapositions (French *et al.*, 1976; Wright and Lawrence, 1981). In the strong phenotype cell death seems to have more impact on cuticle morphology. The general polarity of the ectopic denticles remains inverted, but the



B

DOMAIN	wild-type		v. weak		weak		medium		strong		v. strong	
	T	D	T	D	T	D	T	D	T	D	T	D
naked	-	-	-	-	-	-	-	-	-	-	-	-
patched	-	-	(+)	-	+	-	+	-	+	-	+	-
wingless	-	-	(+)	-	(+)	-	+	-	++	-	-	++
engrailed	-	-	(+)	-	(+)	-	-	++	-	++	-	++

FIG. 8. Regional transformation and cell death resulting from respecification constitute the segment polarity phenotypes. (A) These diagrams show the regional distribution of ectopic denticles and cell death in the six classes of embryos hemizygous for $arm^{H8.6}$. The characteristics of each are described in the text. Two full segments are shown in each, with the denticle belt of a third at the bottom. The clear parallelograms represent the normal denticle belts, while dots denote ectopic denticles. An "x" represents a dead cell. (B) Results from blastoderm fate-mapping and *in situ* analysis of segmentation gene expression together have indicated that the segmental primordium is four cells wide along the anteroposterior axis. By ablating blastoderm cells and studying the effects on the larval epidermis, Lohs-Schardin *et al.* (1979) found that the segmental anlagen are equally spaced and composed of three to four cells. Studies on the spatial expression of segmentation genes have shown that at the end of the blastoderm stage the transverse stripes of *ftz* and *even-skipped* expression are three cells wide and separated by a single cell (Harding *et al.*, 1986; MacDonald *et al.*, 1986). Cells giving rise to the larval epidermis will normally undergo only two rounds of cell division before differentiation (Szabad *et al.*, 1979; Campos-Ortega and Hartenstein, 1985). The identity of each of the four rows is believed to be specified by the expression of or requirement for a unique set of segmentation genes (see text), which are indicated on the left. We can account for the patterns of ectopic denticles and cell death shown in A by assuming differential capacities for each cell type for respecification due to arm^+ loss, as described in the text. The phenotype is indicated in the top row, with "T" designating transformation to anterior fate and "D" denoting cell death. The occurrence of the phenomenon is shown by a plus, with two plusses meaning thorough change of the domain. (+) indicates variable occurrence of the phenomenon. A minus means the phenomenon is not occurring significantly in these cells.

polarity of individual denticles becomes variable. Possibly this is the result of random cell death among these anterior cells. Concomitantly, death of the posterior domain is more thorough, and no naked cuticle remains.

We suspect that embryos of the very strong phenotype reflect the absence of arm^+ activity, because this phenotype is identical to that caused by amorphic alleles of *wg* (Baker, 1987, 1988). The ventral cuticle of these embryos is covered with a dense lawn of denticles, such that the ectopic denticles can no longer be distinguished from those occurring normally. The surface is highly irregular, suggesting considerable cell death. The greatly reduced length of the embryo supports this notion. We do not see epidermal clones of homozygous cells at this level of activity, presumably because the cells in the domain in which the clones occur cannot survive without arm^+ activity.

Consideration of clonal, genetic, and molecular work has led to an understanding of the organization and specification of domains in the segmental anlagen (see Ingham, 1988). As described in the legend to Fig. 8, the segmental epidermis seems to arise from four cells. The identities of these cells are specified by at least four of the segment polarity genes, such that the cell giving rise to the most posterior domain expresses *en* (Weir and Kornberg, 1985; Ingham *et al.*, 1985), preceded anteriorly by one expressing *wg* (Baker, 1987). Molecular labels have not been found for the two anterior cells, but they require particular gene products for proper segmentation gene expression. Descendents of the most anterior cell require *nkd* (Martinez-Arias *et al.*, 1988), followed by a domain requiring *ptc* (DiNardo *et al.*, 1988; Martinez-Arias *et al.*, 1988). Although the origin of cells that make the denticle belts is not precisely understood, the basic pattern seems to be that cells derived the *nkd* domain and at least a fraction of those from that of *ptc* secrete denticles and that cells from the *wg* and *en* domains will generate the naked cuticle (Martinez-Arias *et al.*, 1988).

Because we see regional tendencies toward transformation and cell death within the segment, we believe that the segment polarity phenotype is the result of differential effects on rows of cells rather than an overall reorganization of fate within the segmental anlagen. Thus we can extrapolate from our results to explain the effects of arm^+ loss on particular domains of cells (Fig. 8B). We assume that the *nkd*-requiring domain does not require *arm* for its role in embryonic cuticle secretion. The *ptc*-requiring cells, however, are probably most sensitive to respecification since they are likely responsible for the rows of ectopic denticles in the weak phenotype. In the medium phenotype at least some of the *wg* cells are transformed also, while the *en* domain dies at this level of activity. Because we rarely observed clones of homozygous cells in the posterior region, we suspect that the *en* domain responds to arm^+ deficit by cell death rather than transformation. We speculate that the band of naked cuticle remaining arises from the *wg* domain. This is supported by our failure to see extensive transformation of homozygous clones at this level of arm^+ activity. However, at lower levels this band is transformed also. Finally, in the very strong embryos we believe that the *wg* domain dies also, because of the aberrant pattern of segmentation gene expression and because we see no clones at this lowest level of activity. The future availability of a *wg* antibody should allow direct demonstration of this idea.

In summary, *armadillo* activity seems to be required to prevent the secretion of denticles by the *wg* domain and at least part of the *ptc*-requiring domain and to allow survival of the *en* domain. DiNardo *et al.* (1988) have reported that *arm* function is required for late expression of *en;* perhaps this contributes to the death of these cells at low *arm* activity.

Structural and Functional Implications for Armadillo

Fertile germ line clones of previously characterized alleles of *armadillo* cannot be generated (Wieschaus and Noell, 1986; Perrimon and Mahowald, 1987), such that *arm* phenotypes were always viewed in the context of significant maternal contribution to embryonic development even for possible amorphic alleles (Wieschaus and Riggleman, 1987). In contrast, we have been able to generate fertile germ line clones with the temperature-sensitive allele $arm^{H8.6}$. Perhaps this allele gives a product which retains enough activity to fulfill the oogenic requirement; alternatively, the product could have separate domains of activity for oogenesis and embryogenesis, in which case the oogenic function might be unaffected by $arm^{H8.6}$. A further possibility is that the *armadillo* locus encodes multiple products. Molecular characterization of the locus reveals two transcripts differing by an exon (Riggleman *et al.*, 1989). The role of each transcript is unknown; perhaps $arm^{H8.6}$ affects only one of these.

Whatever the molecular explanation, sufficient arm^+ activity remains for successful oogenesis, but embryonic development is severely affected by reduced maternal product, resulting at the most nonpermissive temperature in a phenotype stronger than phenotypes seen in any hemizygotes derived from heterozygous mothers. The data suggest that cells respond to maternal and zygotic *arm* activity integrated over the course of oogenesis and zygotic development (Fig. 7). The requirement for strictly zygotic segment polarity genes is probably not until after cellularization of the blastoderm (Baker, 1988; Ingham, 1988). Perhaps maternal

effect segment polarity genes like *arm* will have a different period of requirement for their zygotic functions.

Implications for the Analysis of Other Segment Polarity Genes

We show that cells making up a segment have a differential requirement for *armadillo* product, corresponding to their position along the anteroposterior axis of the segment. Because the mutant phenotypes of *armadillo* are often indistinguishable from those of other segment polarity loci, it is likely that these genes are also involved in establishing or responding to differential cues along this axis. To dissect the elements of genetic control of intrasegmental organization, it will be necessary to conduct similar clonal analyses of mutations at these loci.

Wieschaus and Riggleman (1987) attempted to induce homozygous clones of *wingless* among the epidermal cells of heterozygous animals, but did not detect patches of ectopic denticles. We too are unable to induce epidermal clones homozygous for *wg*. These results are consistent with the nonautonomy observed for visible alleles of this locus (Morata and Lawrence, 1977; Babu and Bhat, 1986; Baker, 1988). However, our analysis with *arm* demonstrates that such a negative result does not imply nonautonomy since we have shown that cell death can prevent the appearance of epidermal clones. Thus we predict that clonal analysis of alleles of other segment polarity loci giving a very strong phenotype (such as *dishevelled* and *porcupine*) will fail to yield ectopic denticles, regardless of the cellular autonomy of these loci.

The phenotypic series generated by $arm^{H8.6}$ represents a broad range of segment polarity phenotypes: those in which the polarity of every segment is altered, as evidenced by abnormal occurrence of denticles. This suggests that many of the segment polarity genes may work via the same general process, with phenotypic differences the result of degree of activity retained by each allele.

The gene about which most is known is *wg* (Baker, 1987, 1988). Molecular data indicate that it is the *Drosophila* homolog of *int-1*, a proto-oncogene associated with mammary tumorigenesis in mice (Rijsewijk *et al.*, 1987; Cabrera *et al.*, 1987), whose product is presumably secreted (Papkoff *et al.*, 1987). Assuming that *wg* is nonautonomous in embryonic as well as adult development and that it is secreted, the very strong phenotype of *wg* null mutants may be explained by a key role as a signal for anteroposterior information within the segment. It is likely that at least some other members of the segment polarity class are of two types: those which regulate or process the signal, expected to be nonautonomous, and those which receive or transduce the signal, expected to be autonomous. The autonomy of *armadillo* prompts us to suggest that it is involved in the latter process, supported by our finding that at very low levels of arm^{+}, mutant embryos are indistinguishable from those of *wg* null alleles (Baker, 1988). Clonal analysis will be crucial in determining the general roles of other segment polarity genes to be defined more precisely by molecular characterization.

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