Development 1993 Supplement, 105-114 (1993)
Printed in Great Britain © The Company of Biologists Limited 1993

Cell patterning in the *Drosophila* segment: *engrailed* and *wingless* antigen distributions in segment polarity mutant embryos

Marcel van den Heuvel[1,*], John Klingensmith[1,2], Norbert Perrimon[2] and Roel Nusse[1]

[1]Howard Hughes Medical Institute and Department of Developmental Biology, Stanford University School of Medicine, Stanford, CA, 94305, USA
[2]Howard Hughes Medical Institute, Department of Genetics, Harvard Medical School, 25 Shattuck Street, Boston, MA, 02115, USA

*Present address: ICRF Developmental Biology Unit, Department of Zoology, University of Oxford, South Parks Road, Oxford, OX1 3PS, UK

SUMMARY

By a complex and little understood mechanism, segment polarity genes control patterning in each segment of the *Drosophila* embryo. During this process, cell to cell communication plays a pivotal role and is under direct control of the products of segment polarity genes. Many of the cloned segment polarity genes have been found to be highly conserved in evolution, providing a model system for cellular interactions in other organisms. In *Drosophila*, two of these genes, *engrailed* and *wingless*, are expressed on either side of the parasegment border. *wingless* encodes a secreted molecule and *engrailed* a nuclear protein with a homeobox. Maintenance of *engrailed* expression is dependent on *wingless* and vice versa. To investigate the role of other segment polarity genes in the mutual control between these two genes, we have examined *wingless* and *engrailed* protein distribution in embryos mutant for each of the segment polarity genes.

In embryos mutant for *armadillo*, *dishevelled* and *porcupine*, the changes in *engrailed* expression are identical to those in *wingless* mutant embryos, suggesting that their gene products act in the *wingless* pathway. In embryos mutant for *hedgehog*, *fused*, *cubitus interruptus Dominant* and *gooseberry*, expression of *engrailed* is affected to varying degrees. However *wingless* expression in the latter group decays in a similar way earlier than *engrailed* expression, indicating that these gene products might function in the maintenance of *wingless* expression. Using double mutant embryos, epistatic relationships between some segment polarity genes have been established. We present a model showing a current view of segment polarity gene interactions.

Key words: *Drosophila*, segmentation, segment polarity, *engrailed*, *wingless*

INTRODUCTION

The process of segmentation in *Drosophila melanogaster* embryos is coordinated by a cascade of genes dividing the embryo into 15 segments. Phenotypically three classes of zygotic segmentation genes can be defined (Nüsslein-Volhard and Wieschaus, 1980): the gap, pair rule and segment polarity genes. Of these three groups, the segment polarity genes are the last to act and are thought to define internal organization within each segment. The onset of expression of the zygotic segment polarity genes coincides temporally with cellularization of the embryo (for reviews see Ingham, 1988; Hooper and Scott, 1992). Cell-cell interactions and intracellular signal transduction are presumably important for the coordination of gene expression. Some of the cloned segment polarity genes encode molecules that would appear to be involved in signalling pathways. The *patched* (*ptc*) and *hedgehog* (*hh*) genes encode putative transmembrane proteins (Nakano et al., 1989; Hooper and Scott, 1989; Lee et al., 1992; Mohler and Vani, 1992; Tabata et al., 1992). Serine-threonine kinases are encoded by the genes for *zeste white-3* (*zw-3*) (Siegfried et al., 1990; Bourouis et al., 1990) and *fused* (*fu*) (Preat et al., 1990), while *wingless* (*wg*) encodes a secreted molecule (Rijsewijk et al., 1987). Since many of the cloned segment polarity genes have been shown to be highly conserved in evolution, the mechanism by which they control pattern may have implications for patterning in other animals. It is of particular importance to understand the way segment polarity genes interact with each other in *Drosophila* because of its unique accessibility for gene interaction studies.

After the initial activation of some segment polarity genes by the pair rule genes (Howard and Ingham, 1986; DiNardo and O'Farrell, 1987; Ingham et al., 1988), expression of the segment polarity genes becomes interdependent. Loss of function of one gene causes misexpression or loss of expression of others. The best known example of such regulation is the mutual dependence between *wg* and *engrailed* (*en*; DiNardo et al., 1988; Martinez-Arias et al., 1988). *wg* is expressed in the cells just anterior to the

parasegment border (Baker, 1987; van den Heuvel et al., 1989) and *en* is found expressed immediately next to the cells expressing *wg*, in all cells of the posterior compartment (Ingham et al., 1985; Kornberg et al., 1985; DiNardo et al., 1985). *wg* protein can be found outside the cells producing it and occasionally in neighbouring cells, including those expressing *en* (van den Heuvel et al., 1989; González et al., 1991). The maintenance of *en* by *wg* therefore might be a direct effect of the *wg* protein travelling between these cells. The expression of *wg* in the cells just anterior to the parasegment border is in turn dependent on *en* function (Martinez-Arias et al., 1988; Bejsovec and Martinez Arias, 1991). *en* encodes a nuclear homeobox protein that acts as a transcription factor (Jaynes and O'Farrell, 1988). Maintenance of *wg* expression in adjacent cells is therefore likely to depend on an extracellular signalling pathway originating from the *en* cell. It has been postulated that an interaction between *hh* and *ptc* is responsible for this regulation (Ingham et al., 1991).

Other segment polarity genes are likely to be required to mediate the maintenance of *wg* and *en*. To investigate possible functions of these genes we have surveyed the expression patterns of the *wg* and *en* proteins in all known segment polarity mutants and in some double mutant combinations. We present these data here, in the context of other studies on gene expression in segment polarity mutants.

RESULTS AND CONCLUSIONS

To examine the cross-regulation between the segment polarity genes, we chose to investigate the protein expression patterns of the *wingless* (*wg*) and *engrailed* (*en*) proteins. The maintenance of *wg* and *en* is of crucial importance for subsequent development, which is evident from the strong pattern aberrations in mutants of either gene. Based on our results, we divide the known segment polarity genes in three groups. (A) Genes that seem to be involved in *wg* signalling. In mutant embryos, *en* expression disappears before *wg* expression. (B) Genes that seem to be involved in *wg* regulation. In mutant embryos, *wg* expression disappears before *en* expression, and (C) genes that when mutant result in misexpression of *wg* and *en*. Some genes in this latter group have been shown to be involved in *en* or *wg* suppression (Ingham et al., 1991; Siegfried et al., 1992).

In most cases, we analyzed several mutant alleles, including the strongest available (see Table 1). We used mutant strains with balancer chromosomes carrying a *LacZ* fusion gene to mark non-homozygous mutant embryos. In the case of maternally acting genes (*fused, armadillo, dishevelled, porcupine, zeste-white 3*), we have generated germ line clones using the dominant female sterile technique, to remove both the maternal and the zygotic gene products; here the paternally contributed wild-type X chromosome was also marked by a *LacZ* fusion gene.

Cell death, prominent in some of these mutants at later stages, is a confounding factor in interpreting the results (Klingensmith et al., 1989 and N. P., unpublished observations). However, in most of the mutants the initial aberrations from wild-type staining patterns occur earlier in development than cell death is detectable.

Table 1. List of known segment polarity mutations

Class I: deletion of most of the denticle belts

naked	? [*7H16, 7E89*]
zeste white 3 (*shaggy*)	serine-threonin kinase (1,2) [*K22*]

Class II: deletion of part of the denticle belt and naked cuticle; duplication of segment boundaries (in *ptc*)

patched	putative transmembrane protein (3,4), [*P78, IN108*]
costal-2	?

Class III: anterior margin of each segment affected

engrailed	homeobox protein (5, 6, 7) [*DfenB, CX1, IO, Dfen11*]
lines	? [*IIF103, IIU35* *]

Class IVA: deletion of naked cuticle and mirror image duplication of denticle belt (some segmentation left)

cubitus interruptus D	zinc finger protein (8) [†]
Cell	allelic to *ciD* [2]
fused	serine-threonine kinase (9) [*IPP2, MH63* *]
gooseberry	homeobox/Pax box protein (10) [*Df(2R)IIX62*]
hedgehog	transmembrane/secreted protein (11, 12, 13) [*IJ35, G51* *]
smooth	? [*IIX43*]

Class IVB: deletion of naked cuticle and mirror image duplication of denticle belt, virtually any sign of segmentation lost

armadillo	*Drosophila* plakoglobin/B-catenin homologue (14, 15) [*H8.6*]
dishevelled	? (‡) [*M20, V26* *]
porcupine	? [*PB16, I8* *]
wingless	putative secreted factor (16) [*IG22, CX4* *]

Mutations that cause embryonic lethality with a segment polarity cuticle phenotype. The cuticle phenotypes are used to order the mutations in four classes. If known, the putative protein structure cq. function for each gene are added. The alleles we investigated for each mutation are given in brackets; the underlined allele was found to be the strongest; when marked with an asterisk no difference between alleles was observed; the stronger alleles were used in the double mutant combinations. (References: (1) Bourouis et al. (1990), (2) Siegfried et al. (1990), (3) Hooper and Scott (1989), (4) Nakano et al. (1989), (5) Poole et al. (1985), (6) Fjose et al. (1985), (7) Kuner et al. (1985), (8) Orenic et al. (1990), (9) Preat et al. (1990), (10) Bopp et al. (1986), (11) Lee et al. (1992), (12) Mohler and Vani (1992), (13) Tabata et al. (1992), (14) Peifer and Wieschaus (1990), (15) McCrea et al. (1991), (16) Rijsewijk et al. (1987).† at the time these experiments were done, ci^D revertants were not available. ‡ novel protein of unknown structure, J. K. and N. P., in preparation.

At embryonic stage 10, *wg* protein is detected in a discontinuous stripe one to two cells wide, along the parasegment border. For a description of *wg* and *en* protein patterns in wild-type embryos, see van den Heuvel et al. (1989); González et al. (1991), and DiNardo et al. (1985). The *en* stripe is continuous, two cells wide just posterior to the parasegment border. In all mutants, the early segmental patterns of *wg* and *en* protein expression are identical to what is seen in wild-type embryos, arguing that the initial expression of these genes is independent of the other segment polarity genes and presumably totally regulated by

Table 2. Results of immunolocalization of *wingless* and *engrailed* proteins in segment polarity mutant embryos

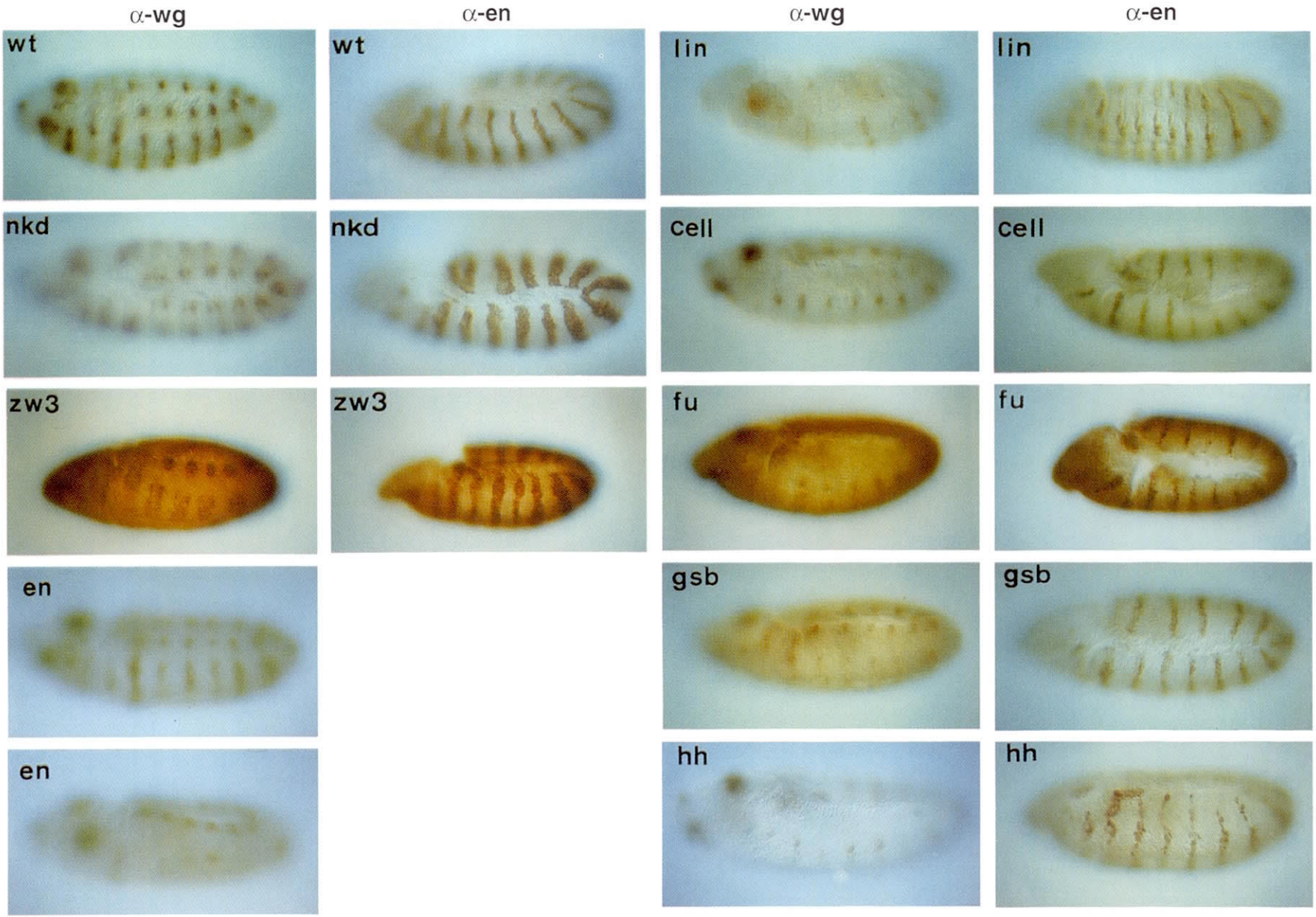
Mutant	*wingless* mRNA	*wingless* protein	*engrailed* protein	comments
CLASS A				
armadillo	id. to protein	stage 10, staining in epidermis gradually lost	stage 10, most protein gone	ectopic *wg* at dorsal side (stage 12/13); *en* as in *wg* embryo
dishevelled	id. to protein	stage 10, staining in epidermis gradually lost	stage 10, most protein gone	identical to *arm*, ectopic *wg* at dorsal side; *en* as in *wg* embryo
porcupine	mRNA as in *dsh* or *arm*	cells present express as in wildtype	stage 10, most protein gone	*wg* protein present while *wg* mRNA gone; *en* as in *wg* embryo
wingless	no mRNA	no protein	stage 10, most protein gone	ventral neuroblasts and gnathal and thoracic cells remain for *en*
CLASS B				
engrailed	id. to protein	disappears in 7-stripe pattern at stage 10/11, almost all expression gone at stage 12	see[1]	ventral neuroblasts positive for *wg*
lines	id. to protein	disappears in 7-stripe pattern at stage 12	small gaps in stripes form at stage 11/12	–
ciD/Cell	id. to protein	dorsal first affected (stage 10) ventral some cells left	stage 11, stripes show gaps	*Cell* and *ciD* are similar
fused	id. to protein	stage 10, staining in epidermis gradually lost	stage 11, stripes show gaps; at later stages, large gaps	ectopic *wg* at dorsal side (stage 12/13)
gooseberry	id. to protein	stage 10, ventral staining lost; later all locations affected	single cells no expression at stage11, later larger gaps	–
hedgehog	id. to protein	stage 10, staining in epidermis gradually lost	during stage 10 stripes become interrupted	ectopic *wg* at dorsal side (stage 12/13)
smooth	id. to protein	some ventral epidermal cells lost (stage 11)	small gaps in stripes during stage 11	the alleles used are not null alleles†
CLASS C				
naked	almost id. to protein*	extra stripe anterior to wildtype domain	stripe broader towards posterior	deep groove forms at new *wg/en* apposition;
patched	id. to protein	stripe broader towards anterior	extra stripe posterior to wildtype domain	new groove appears at new *wg/en* opposition
zeste-white 3	almost id. to protein*	extra stripe anterior to wildtype domain	stripe broader towards posterior	deep groove forms at new *wg/en* opposition
DOUBLE MUTANTS				
nkd,hh	id. to protein	stage 10, staining in epidermis gradually lost	initial broadening of stripes later gaps appear	as in *hh*$^-$ for *wg*; for *en* patterns are superimposed
en;hh	id. to protein	stage 11, staining in epidermis gradually lost	–	as in *hh*$^-$
en;nkd	id. to protein	disappears in 7-stripe pattern at stage 10/11	–	as in *en*$^-$
wg;hh	–	–	stage 10 most protein gone	as in *wg*$^-$
wg;nkd	–	–	stage 10 most protein gone	as in *wg*$^-$

The mutations are ordered based on our results. Only results of the effects on the staining in the trunk region of the embryos are presented. [1]Heemskerk et al. (1991), † the available smo alleles are all cold sensitive and probably not lack of function. **wingless* protein is seen in between two ventral stripes, while no mRNA is detected there.

earlier acting segmentation genes (Howard and Ingham, 1986; DiNardo and O'Farrell, 1987; Ingham et al., 1988). *wg* and *en* protein localizations are presented in Fig. 1 and the findings are summarized in Table 2.

Class A, *wingless* (*wg*), *dishevelled* (*dsh*), *armadillo* (*arm*) and *porcupine* (*porc*)

In embryos mutant for the *wg* allele used here, no *wg* protein is found at any time during embryogenesis. *wg* transcription


α-wg
α-en
α-wg
α-en
wt
wt
lin
lin
nkd
nkd
cell
cell
zw3
zw3
fu
fu
en
gsb
gsb
en
hh
hh

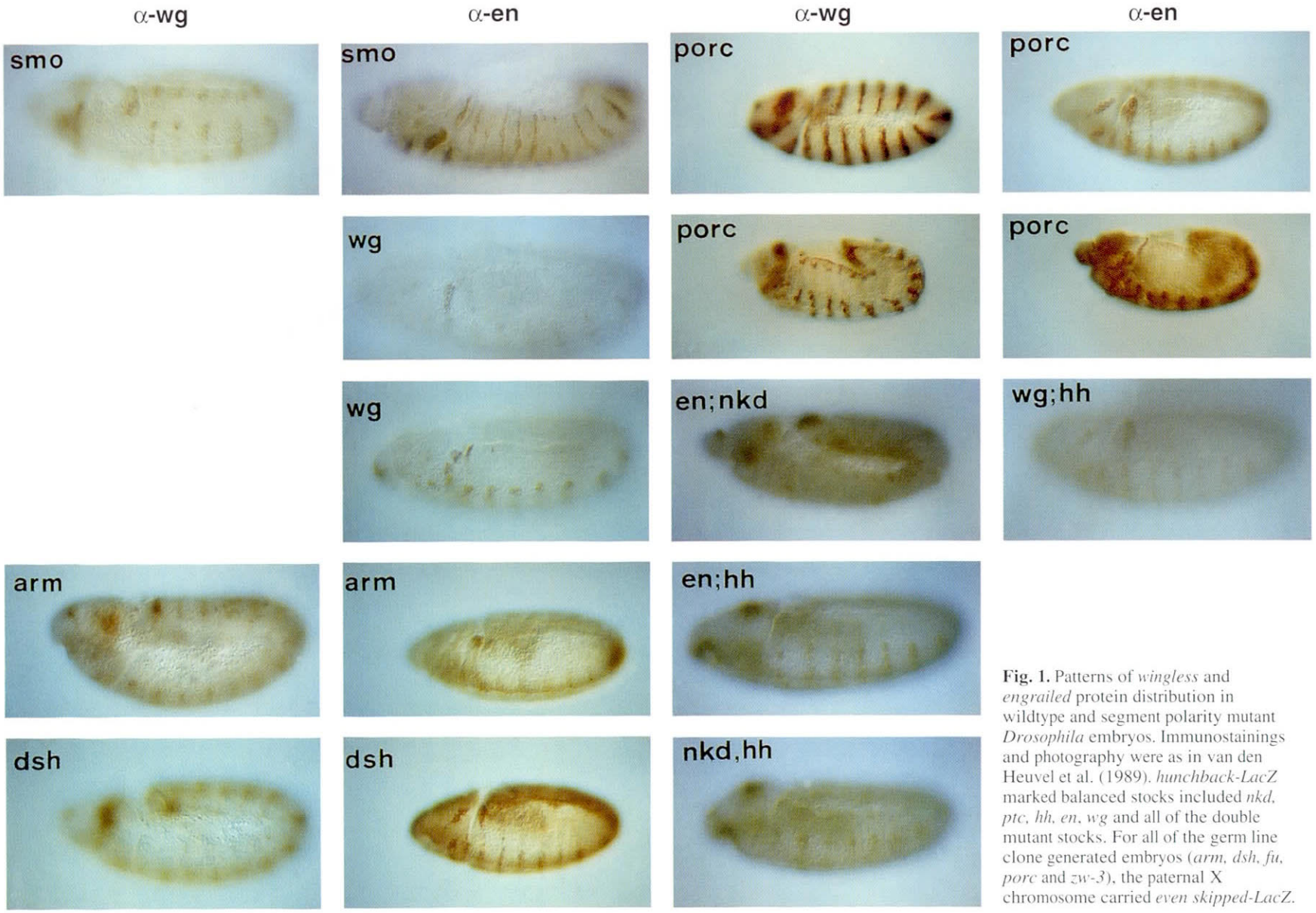


Fig. 1. Patterns of *wingless* and *engrailed* protein distribution in wildtype and segment polarity mutant *Drosophila* embryos. Immunostainings and photography were as in van den Heuvel et al. (1989). *hunchback-LacZ* marked balanced stocks included *nkd, ptc, hh, en, wg* and all of the double mutant stocks. For all of the germ line clone generated embryos (*arm, dsh, fu, porc* and *zw-3*), the paternal X chromosome carried *even skipped-LacZ*.

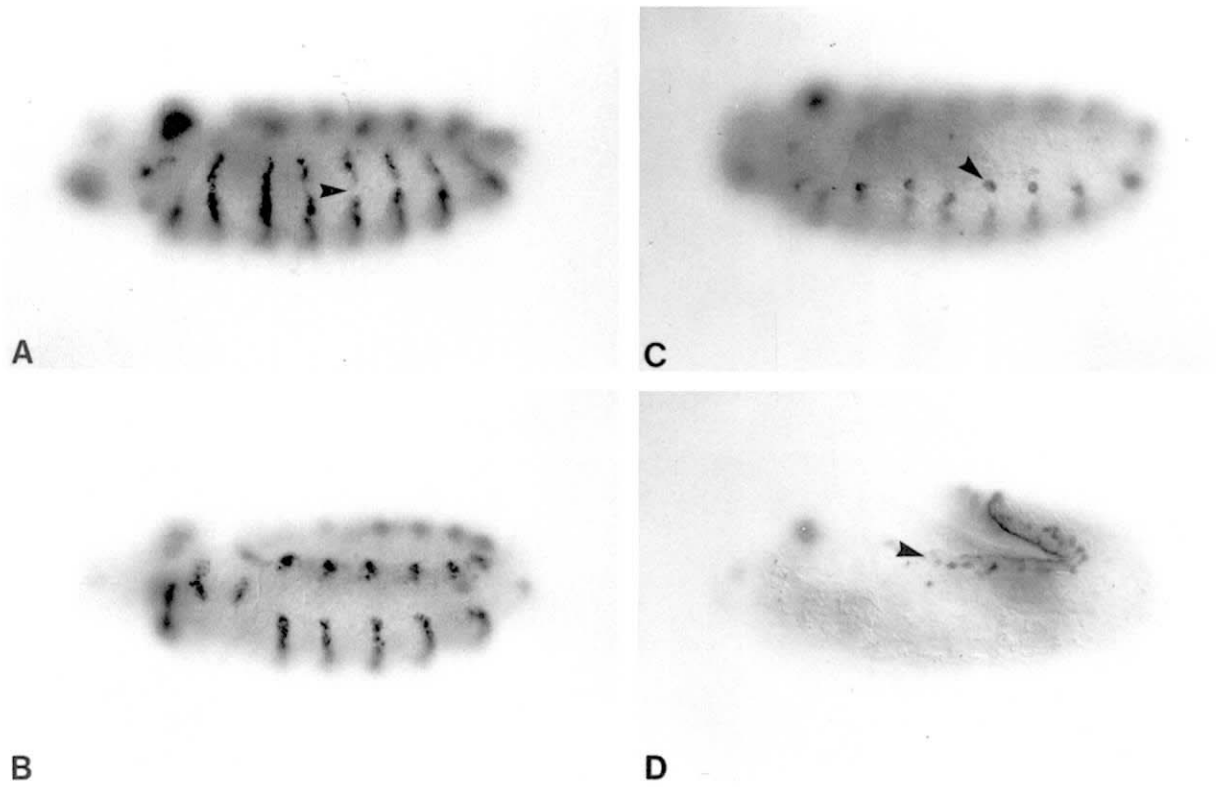


Fig. 2. Localizations of *wingless* mRNA in wildtype and mutant embryos. Whole mount in situ hybridisations were performed as described in Tautz and Pfeifle (1989), using a full length digoxigenin-labeled *wingless* cDNA. (A) Wildtype embryo, stage 10. Arrowhead indicates the formation of the lateral gap. (B) Wildtype embryo, stage 11. (C) Mutant (wg^{IN67}) embryo, stage 10. Note the absence of dorsal expression. Arrowhead points to large non-epidermal cell. (D) mutant (wg^{IL114} at 29°C) embryo, stage 11. Arrowhead indicates new dorsal expression, also noted by Ingham and Hidalgo (1993).

is initiated normally in most *wg* alleles and disappears from the germband during stage 10 (Fig. 2; for a full description of *wg* patterns in *wg* alleles, see van den Heuvel et al., 1993).

wg protein fades from the epidermis (dorsal first) during stage 10 in *arm* and *dsh* embryos; no more *wg* staining is observed by the end of stage 11. In contrast, in embryos lacking *porc*, the *wg* protein is found throughout most of embryogenesis but the subcellular localization appears altered. *porc* embryos show a retention of the *wg* protein in producing cells (van den Heuvel et al., 1993). Interestingly, whereas *wg* protein in *porc* embryos is present throughout stage 13, its mRNA can no longer be detected at the end of stage 11, as in *arm*, *dsh* and *wg* embryos (not shown).

en protein in *wg* embryos disappears from the epidermal cell layer at late stage 9 (see DiNardo et al., 1988; Martinez-Arias et al., 1988; Bejsovec and Martinez Arias, 1991). Only neuroblast cells on the ventral side are then positive for the *en* antigen and a distinct pattern in the gnathal and first thoracic segments in the epidermal cell layer persists. Identical effects on *en* expression are seen in *arm*, *dsh* and *porc* embryos and represent the earliest sign of segment polarity interregulation (for *arm* see also Peifer et al., 1991). In *arm*, *dsh* and *porc* embryos, it is thus possible to detect *wg* protein at certain stages while the epidermal *en* pattern is already completely disrupted. Apparently, the *wg* signalling pathway to *en* is impaired in these mutants. How might these gene products interact in a *wg* signalling pathway? Both *arm* and *dsh* function autonomously (Wieschaus and Riggleman, 1987; J. K. and N. P., unpublished data), consistent with a role in reception of the *wg* signal. Since *arm* is homologous to the intracellular vertebrate proteins, plakoglobin/β-catenin (Peifer and Wieschaus, 1990; McCrea et al., 1991), a proposed function for *arm* as a receptor for *wg* (Peifer et al., 1991) seems unlikely. An observation by Riggleman et al. (1990) indicates that the intracellular location of the *arm* protein is dependent on *wg* but also on *dsh*. Perhaps, *arm* protein becomes associated with different proteins upon activation by the *wg* signal and this reassociation is required for *wg* function. *dsh* is necessary for both aspects of *wg* activity: maintenance of *en* and the relocalization of *arm* protein. *dsh* seems therefore a good candidate for a protein involved in the reception of the *wg* signal. However the molecular cloning of *dsh* does not clarify what its function is (J. K. and N. P., unpublished data).

Since *wg* protein is present and accumulates in *porc* embryos (see also van den Heuvel et al., 1993), *porc* may be involved in processing of the *wg* protein. Consistent with a role in processing the *wg* protein is the non-autonomous function of *porc* (J. K. and N. P., unpublished observations). At what stage of the processing of the *wg* protein *porc* might act is not known, but it has been reported that the *wg*-

dependent relocalization of the *arm* protein in *porc* embryos is restricted to the cells that express *wg* (Riggleman et al., 1990). This suggests that the *wg* protein can still function intracellularly.

In *arm*, *dsh* and *porc* mutants, transcription of *wg* is lost (in *porc* embryos the *wg* mRNA is lost) in a pattern very similar to that seen in *wg* mutants, indicating that the *wg* signalling pathway might also regulate *wg* expression. This will be discussed later.

Class B, *engrailed* (*en*), *lines* (*lin*), *Cell*, *cubitus interruptus Dominant* (ci^D), *fused* (*fu*), *gooseberry* (*gsb*), *hedgehog* (*hh*) and *smooth* (*smo*)

The *wg* protein in *en* embryos disappears from the odd parasegmental stripes during stage 10. Staining in the even stripes persists but at stage 12 no more *wg* protein is detected in the germband (see also Martinez-Arias et al., 1988; Bejsovec and Martinez Arias, 1991). The transient seven stripe pattern of remaining *wg* expression is noteworthy since the cuticle phenotype of *en* also shows a paired-segment pattern. Indeed the most aberrant segments in the cuticle (Kornberg, 1981) correspond to the weak *wg* bands in the embryo.

In *lin* embryos, a paired-segment pattern for *wg* protein is seen, similar to the pattern in *en* mutants, although it arises later (stage 12) and is never as well defined. The expression of *en* is hardly affected in *lin* mutants; only some cells lose expression.

As argued by Orenic (Orenic et al., 1987; Orenic et al., 1990), *Cell* and ci^D could be allelic. In both mutations, dorsal *wg* expression is lost during stage 10, while ventrally *wg* protein persists longer. In *gsb* embryos, *wg* expression is lost from the ventral epidermis during stage 10 (see also Hidalgo and Ingham, 1990; Hidalgo, 1991), while dorsally, protein is present longer. In both ci^D/*Cell* and *gsb*, small gaps in the *en* domains are formed by stage 11. The patchy nature of the expression domain of *en* becomes clearer later in development.

In *fu* and in *hh* embryos, the *wg* protein fades from the dorsal epidermis by stage 10. By the end of stage 11, all staining has disappeared from the segmented region (see also Limbourg-Bouchon et al., 1991; Hidalgo and Ingham, 1990). In *fu* and *hh* embryos, gaps appear in the *en* stripes during stage 11 (see also DiNardo et al., 1988; Limbourg-Bouchon et al., 1991). The discontinuity of the *en* stripes becomes more obvious in later stages.

Embryos mutant for *smo* display normal patterns of expression of *wg* until stage 10. Most, but not all *wg* protein disappears from the ventral epidermis during subsequent development. During stage 11, small gaps appear in the *en* stripes which become clearer during subsequent development.

In contrast to class A embryos, *wg* expression is lost before *en* expression in all of the class B mutants. This happens during stage 10 of development, as is seen for the odd stripes of *wg* expression in *en* mutants. These gene products might therefore act in a pathway that maintains *wg* expression downstream of *en* activity. In *gsb* and ci^D embryos, loss of *wg* expression is seen, initially more or less confined to the ventral and the dorsal side of the embryo, respectively. ci^D is expressed in all cells expressing *wg* (Orenic et al., 1990) and gsb^d expression becomes restricted to the ventral epidermal cells overlapping the (ventral) *wg* and *en* domains (Baumgartner et al., 1987). Since both ci^D and *gsb* encode putative transcription factors, they could directly regulate *wg* expression. The *fu* kinase most likely also acts in this pathway. However, it is not clear what the substrate of *fu* is and the ubiquitous expression does not clarify in which cell *fu* works. *hh* has been implicated in the maintenance of *wg* expression as a possible signalling molecule (Hidalgo and Ingham, 1990; Ingham et al., 1991), consistent with its apparent non-autonomy (Mohler, 1988), its molecular structure and its expression in the cells marked by *en* (Lee et al., 1992; Mohler and Vani, 1992; Tabata et al., 1992). We have investigated and extended this proposed function of *hh* in several double mutant combinations.

(1) If *en* strictly regulates *hh* activity, the double mutant *en;hh* should display a *wg* expression pattern as in *en* mutants. We observe, however, a pattern as in *hh* mutants, indicating that *hh* activity is not regulated solely by *en*. Possibly pair rule genes are involved in the early regulation of *hh* (see also Lee et al., 1992; Tabata et al., 1992). Such an influence could explain the pair rule pattern of disappearance of *wg* expression in *en* mutants and thereby the cuticle phenotype of *en* mutant embryos. In the even parasegments, expression of *hh*, and thereby expression of *wg*, is maintained by pair rule gene activity. In the odd stripes, *wg* expression would be regulated by *en* via *hh*. On the other hand, maintenance of *hh* in the *en* cells is thought to be also dependent on *wg* signalling (Lee et al., 1992; Tabata et al., 1992); in *wg* mutants *hh* expression disappears as *en* expression. This indicates that an unknown gene acts downstream of *wg* signalling to regulate *hh* expression.

(2) If *hh* acts as a signal to maintain *wg* expression, it might also function to induce the ectopic *wg* expression seen in *nkd* embryos (see below). In double mutant *nkd,hh* embryos, we found no ectopic expression of *wg*, consistent with a role for *hh* as a signal from *en* cells to induce or maintain *wg* expression in neighbouring cells.

The pattern of disappearance of *wg* expression in these mutants can be directly correlated to the pattern that is seen in the mutants that are thought to function in the *wg* signalling pathway and in *wg* mutants. These results indicate that *wg* regulates its own transcription in a paracrine fashion (see also Ingham and Hidalgo, 1993). A *wg* signal is transduced which maintains *en* and *hh* activity in the neighbouring cell. *hh* then might function as a signal to maintain *wg* expression in the cell originally expressing *wg*.

Class C, *naked* (*nkd*), *zeste white-3* (*zw-3*) and *patched* (*ptc*)

In the thoracic and abdominal segments of *nkd* embryos, *wg* becomes expressed in a row of cells anterior to the normal expression domain (see also Martinez-Arias et al., 1988; Limbourg-Bouchon et al., 1991), resulting in two stripes of *wg* per segment by stage 11. The *en* protein domain expands into the cells posterior to the wildtype expression pattern (see also Martinez-Arias et al., 1988), resulting in a domain twice the normal width at stage 10.

In embryos lacking *zw-3* function, the *wg* protein is observed in the normal and in an ectopic domain, and the *en* protein domain enlarges, both in exactly the same manner

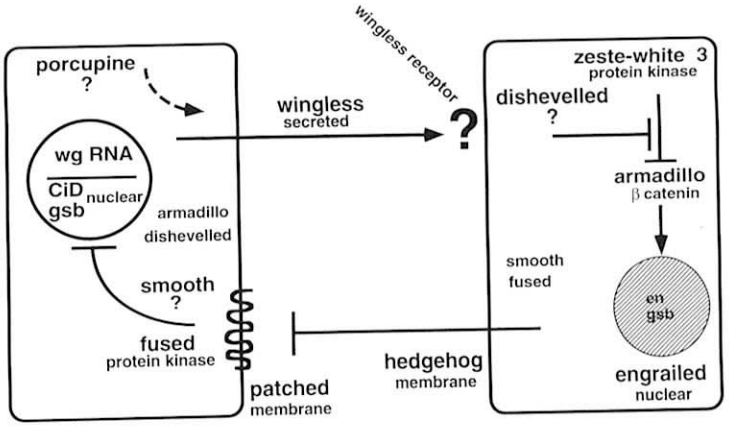


Fig. 3. Schematic view of the interactions between segment polarity genes. The figure shows two cells, a *wingless*- and an *engrailed*-expressing cell, with their nuclei depicted as large circles. In the embryo, however, these interactions take place between 4-12 cells per segment, depending on the stage of embryogenesis. For many of the segment polarity genes, it is not known in which cell they are required. It is assumed that *dishevelled*, *armadillo* and *zeste-white 3* act within the "*engrailed*" cell as part of the *wingless* signalling pathway, but they could be required in the "*wingless*" cell. Conversely, *fused* and *smooth* presumably act within the "*wingless*" cell but may also operate in the "*engrailed*" cell. For the genes that have been cloned, biochemical functions have been proposed (such as membrane proteins) but direct evidence for such functions is often lacking. It is assumed but not proven that *wingless* acts through a cell surface receptor (question mark). See the text for further explanations and discussions.

as in *nkd* embryos. The expansion of the *wg/en* patterns in *zw-3* and *nkd* embryos indicates that these genes might function in the same pathway; that is repression of *wg/en* in the anterior compartment. Recently, a model has been proposed in which *zw-3* functions as an antagonist of *en* autoregulation. The *wg* signal would repress *zw-3* activity, and thereby maintain *en* expression in its appropriate position. This model is inferred in part from the broadening of the *en* domain in the *zw-3;wg* double mutant combination (Siegfried et al., 1992). If *nkd* functions in this pathway, a similar pattern for *en* should be seen in the mutant combination *wg;nkd*. However, *wg;nkd* mutants show the loss of *en* expression as in *wg* mutants, a result that does not corroborate function of *zw-3* and *nkd* in the same pathway. On the other hand, it is not known if the existing *nkd* alleles are amorphic and residual activity of *nkd* might result in the observations we present.

In *ptc* embryos, a broadened stripe of *wg* is observed (see Martinez-Arias et al., 1988; DiNardo et al., 1988), consistent with its proposed role as a repressor of *wg* expression. An ectopic *en* stripe is observed slightly later than the broad *wg* stripe is generated (not shown).

nkd, *ptc* and *zw-3* appear to be involved in repression of *en* and *wg* in the anterior part of the segment, because of the ectopic expression found in these mutants. Interestingly, the patterns are established in two temporal stages. In *nkd* mutants, broadened expression of *en* is seen first and subsequently ectopic *wg* is detected. In *ptc* embryos, the stripe of *wg* is broadened and then an ectopic *en* stripe is induced. Expression of the second antigen might depend on the functional expression of the first, since it is known that *wg* and *en* are dependent on each other for continual expression. This possibility has been investigated in the double mutant *en;nkd*. Indeed no induction of ectopic *wg* is seen in these embryos. In a *ptc,wg* double mutant no ectopic *en* is induced, as previously observed (DiNardo et al., 1988).

GENERAL CONCLUSIONS

Once the initial expression domains of some of the segment polarity genes are established by pair rule gene activity, most of the segment polarity genes appear to function in two regulatory pathways, controlling maintenance and correct localization of *wg* and *en* expression on either side of the parasegment border. These pathways can be distinguished in time: first *wg* acts to stabilize *en* expression and subsequently *wg* expression is maintained by a signalling pathway originating from the *en* cell. Both act within a short time window (stage 9-10/11), although some of these genes have been shown to have later embryonic functions as well (Bejsovec and Martinez Arias, 1991; Heemskerk et al., 1991). Fig. 3 shows a simplified scheme of both pathways. The presentation or secretion of the *wg* protein is regulated by the *porc* gene product. The *wg* protein is secreted and interacts with the neighbouring (and perhaps also the producing) cell possibly via a putative transmembrane receptor. The *dsh* protein might be associated with or be downstream of the receptor. The interaction between *wg* and *dsh* and other putative molecules possibly leads to the inactivation of the protein kinase *zw-3*, which by itself is a negative regulator of *en* activity. *arm* functions upstream of *en*. Recent genetic epistasis experiments using a heat-shock *wg* transgene combined with loss of functions mutations in other segment polarity genes have shown that *arm* and *dsh* are both required for ectopic *en* expression induced by HS-*wg* (Noordermeer et al., 1993). In another series of double mutant embryos, Siegfried et al. (1993) have obtained evidence that *zw-3* acts downstream of *dsh* and upstream of *arm*. The *hh* transcript is only expressed in cells that express *en* and its activity is maintained by both *en* and possibly other genes, in conjunction with *wg*. *hh* activity controls *wg* expression, perhaps by relieving the negative action of *ptc*. *hh* protein is seen inside the *en*-expressing cells and also in neighbouring cells (Taylor et al., 1993), consistent with a role as a signal. The protein kinase *fu* and the product of *smo* are involved in this pathway, either in the presentation or in the interpretation of the *hh* signal (thus either in the *hh*-producing or in the *hh*-receiving cell). The transcription factors ci^D and *gsb* ultimately control *wg* expression. The precise role of *nkd* in the repression of *wg/en* is not clear for the moment. *lin* appears to be acting late in development, resulting in cuticle defects but in minor defects in *wg* and *en* expression.

Some of the gene products that are thought to function intracellularly in these pathways (e.g. *arm, fu, zw-3*) are maternally provided. The genes are located on the X chromosome of *Drosophila*. Techniques to remove all activity, including maternal, for X-chromosome located genes have been used to screen for mutants as the ones discussed here (Perrimon et al., 1986). Similar techniques for the other chromosomes have become available only recently (Chou et al., 1993) and it is likely that more mutants with a segment polarity phenotype will be isolated, hopefully to advance our understanding of patterning within the *Drosophila* segment.

We thank Claudie Lamour-Isnard, Christiane Nüsslein-Volhard, Trish Wilson, Phil Ingham, Thomas Kornberg, Peter Lawrence, Alfonso Martinez-Arias, Jym Mohler, Matthew Scott and Gary Struhl for fly stocks. The members of the Nusse Lab are acknowledged for their continuous interest. Many thanks to Phil Ingham for the advice. J. K. was in part supported by a grant from the National Institutes of Health. R. N. and N. P. are investigators of the Howard Hughes Medical Institute.

REFERENCES

Baker, N. E. (1987). Molecular cloning of sequences from wingless, a segment polarity gene in Drosophila: the spatial distribution of a transcript in embryos. *EMBO J.* **6**, 1765-1773.

Baumgartner, S., Bopp, D., Burri, M. and Noll, M. (1987). Structure of two genes at the gooseberry locus related to the paired gene and their spatial expression during Drosophila embryogenesis. *Genes Dev.* **1**, 1247-1267.

Bejsovec, A. and Martinez Arias, A. (1991). Roles of wingless in patterning the larval epidermis of *Drosophila*. *Development* **113**, 471-487.

Bopp, D., Burri, M., Baumgartner, S., Frigerio, G. and Noll, M. (1986). Conservation of a large protein domain in the segmentation gene *paired* and in functionally related genes of *Drosophila*. *Cell* **47**, 1033-1040.

Bourouis, M., Moore, P., Ruel, L., Grau, Y., Heitzler, P. and Simpson, P. (1990). An early embryonic product of the gene shaggy encodes a serine/threonine protein kinase related to the CDC28/cdc2+ subfamily. *EMBO J.* **9**, 2877-2884.

Chou, T., Noll, E. and Perrimon, N. (1993). Autosomal *P[ovo^{D1}]* dominant female sterile insertions in *Drosophila* and their use in generating germline chimeras. *Development* **119**, (in press).

DiNardo, S., Kuner, J. M., Theis, J. and O'Farrell, P. H. (1985). Development of embryonic pattern in D. melanogaster as revealed by accumulation of the nuclear engrailed protein. *Cell* **43**, 59-69.

DiNardo, S. and O'Farrell, P. H. (1987). Establishment and refinement of segmental pattern in the Drosophila embryo: spatial control of engrailed expression by pair-rule genes. *Genes Dev.* **1**, 1212-1225.

DiNardo, S., Sher, E., Heemskerk, J. J., Kassis, J. A. and O'Farrell, P. H. (1988). Two-tiered regulation of spatially patterned engrailed gene expression during Drosophila embryogenesis. *Nature* **332**, 604-609.

Fjose, A., McGinnis, W. J. and Gehring, W. J. (1985). Isolation of a homeobox containing gene form the *engrailed* region of *Drosophila* and the spatial distribution of its transcripts. *Nature* **313**, 284-289.

González, F., Swales, L., Bejsovec, A., Skaer, H. and Martinez-Arias, A. (1991). Secretion and movement of wingless protein in the epidermis of the Drosophila embryo. *Mech. Dev.* **35**, 43-54.

Heemskerk, J., DiNardo, S., Kostriken, R. and O'Farrell, P. H. (1991). Multiple modes of engrailed regulation in the progression towards cell fate determination. *Nature* **352**, 404-410.

Hidalgo, A. (1991). Interactions between segment polarity genes and the generation of the segmental pattern in Drosophila. *Mech. Dev.* **35**, 77-87.

Hidalgo, A. and Ingham, P. (1990). Cell patterning in the *Drosophila* segment: spatial regulation of the segment polarity gene *patched*. *Development* **110**, 291-301.

Hooper, J. and Scott, M. (1992). The molecular genetic basis of positional information in insect segments. In *Results and Problems in Cell Differentiation*, (ed. Wolfgang Hennig). Berlin: Springer-Verlag.

Hooper, J. E. and Scott, M. P. (1989). The Drosophila patched gene encodes a putative membrane protein required for segmental patterning. *Cell* **59**, 751-765.

Howard, K. and Ingham, P. (1986). Regulatory interactions between the segmentation genes *fushi tarazu, hairy*, and *engrailed* in the *Drosophila* blastoderm. *Cell* **44**, 949-957.

Ingham, P., Martinez-Arias, A., Lawrence, P. A. and Howard, K. (1985). Expression of engrailed in the parasegment of Drosophila. *Nature* **317**, 634-636.

Ingham, P. W. (1988). The molecular genetics of embryonic pattern formation in Drosophila. *Nature* **335**, 25-34.

Ingham, P. W., Baker, N. E. and Martinez, A. A. (1988). Regulation of segment polarity genes in the Drosophila blastoderm by fushi tarazu and even skipped. *Nature* **331**, 73-75.

Ingham, P. W. and Hidalgo, A. (1993). Regulation of *wingless* transcription in the *Drosophila* embryo. *Development* **117**, 283-291.

Ingham, P. W., Taylor, A. M. and Nakano, Y. (1991). Role of the *Drosophila patched* gene in positional signalling. *Nature* **353**, 184-188.

Jaynes, J. B. and O'Farrell, P. H. (1988). Activation and repression of transcription by homeodomain-containing proteins that bind to a common site. *Nature* **336**, 744-749.

Klingensmith, J., Noll, E. and Perrimon, N. (1989). The segment polarity phenotype of Drosophila involves differential tendencies toward transformation and cell death. *Dev. Biol.* **134**, 130-145.

Kornberg, T. (1981). *engrailed*: a gene controlling compartment and segment formation in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **78**, 1095-1099.

Kornberg, T., Siden, I., O'Farrell, P. and Simon, M. (1985). The engrailed locus of Drosophila: in situ localization of transcripts reveals compartment-specific expression. *Cell* **40**, 45-53.

Kuner, J. M., Nakanishi, M., Ali, Z., Drees, B., Gustavson, E., Theis, J., Kauvar, L., Kornberg, T. and O'Farrell, P. H. (1985). Molecular cloning of *engrailed*: a gene involved in the development of pattern in *Drosophila melanogaster*. *Cell* **42**, 309-316.

Lee, J. L., von Kessler, D. P., Parks, S. and Beachy, P. A. (1992). Secretion and localized transcription suggest a role in positional signaling for products of the segmentation gene *hedgehog*. *Cell* **71**, 33-50.

Limbourg-Bouchon, B., Busson, D. and Lamour-Isnard, C. (1991). Interactions between fused, a segment polarity gene in Drosophila, and other segmentation genes. *Development* **112**, 417-429.

Martinez-Arias, A., Baker, N. E. and Ingham, P. W. (1988). Role of segment polarity genes in the definition and maintenance of cell states in the Drosophila embryo. *Development* **103**, 157-170.

McCrea, P. D., Turck, C. W. and Gumbiner, B. (1991). A homolog of the *armadillo* protein in *Drosophila* (plakoglobin) associated with E-Cadherin. *Science* **254**, 1359-1361.

Mohler, J. (1988). Requirements for hedgehog, a segmental polarity gene, in patterning larval and adult cuticle of Drosophila. *Genetics* **120**, 1061-1072.

Mohler, J. and Vani, K. (1992). Molecular organization and embryonic expression of the *hedgehog* gene involved in cell-cell communication in segmental patterning of *Drosophila*. *Development* **115**, 957-971.

Nakano, Y., Guerrero, I., Hidalgo, A., Taylor, A., Whittle, J. R. and Ingham, P. W. (1989). A protein with several possible membrane-spanning domains encoded by the Drosophila segment polarity gene patched. *Nature* **341**, 508-513.

Noordermeer, J., Klingensmith, J., Perrimon, N. and Nusse, R. (1993). *dishevelled* and *armadillo* are essential components of the *wingless* signaling pathway in *Drosophila*. *Nature* (in press).

Nüsslein-Volhard, C. and Wieschaus, E. (1980). Mutations affecting segment number and polarity in *Drosophila*. *Nature* **287**, 795-801.

Orenic, T., Chidsey, J. and Holmgren, R. (1987). Cell and cubitus interruptus dominant: two segment polarity genes on the fourth chromosome in Drosophila. *Dev. Biol.* **124**, 50-56.

Orenic, T. V., Slusarski, D. C., Kroll, K. L. and Holmgren, R. A. (1990). Cloning and characterization of the segment polarity gene cubitus interruptus Dominant of Drosophila. *Genes Dev.* **4**, 1053-1067.

Peifer, M., Rauskolb, C., Williams, M., Riggleman, R. and Wieschaus, E. (1991). The segment polarity gene armadillo interacts with the wingless signalling pathway in both embryonic and adult pattern formation. *Development* **111**, 1029-1044.

Peifer, M. and Wieschaus, E. (1990). The segment polarity gene armadillo encodes a functionally modular protein that is the Drosophila homolog of human plakoglobin. *Cell* **63**, 1167-1176.

Perrimon, N., Mohler, J. D., Engstrom, L. and Mahowald, A. P. (1986). X-linked female sterile loci in *Drosophila melanogaster*. *Genetics* **113**, 695-712.

Poole, S. J., Kauvar, L.M., Drees, B. and Kornberg, T. (1985). The *engrailed* locus of *Drosophila*: structural analysis of an embryonic transcript. *Cell* **40**, 37-43.

Preat, T., Therond, P., Lamour, I. C., Limbourg, B. B., Tricoire, H., Erk, I., Mariol, M. C. and Busson, D. (1990). A putative serine/threonine protein kinase encoded by the segment-polarity fused gene of Drosophila. *Nature* **347**, 87-89.

Riggleman, B., Schedl, P. and Wieschaus, E. (1990). Spatial expression of the Drosophila segment polarity gene armadillo is posttranscriptionally regulated by wingless. *Cell* **63**, 549-560.

Rijsewijk, F., Schuermann, M., Wagenaar, E., Parren, P., Weigel, D. and Nusse, R. (1987). The Drosophila homolog of the mouse mammary oncogene int-1 is identical to the segment polarity gene wingless. *Cell* **50**, 649-657.

Siegfried, E., Chou, T. and Perrimon, N. (1992). *wingless* signaling acts through *zeste-white 3*, the *Drosophila* homolog of *glycogen synthase kinase-3*, to regulate *engrailed* and establish cell fate. *Cell* **71**, 1167-1179.

Siegfried, E., Perkins, L. A., Capaci, T. M. and Perrimon, N. (1990). Putative protein kinase product of the Drosophila segment-polarity gene zeste-white3. *Nature* **345**, 825-829.

Siegfried, E., Wilder, E. and Perrimon, N. (1993). Components of *wingless* signaling in *Drosophila*. *Nature* (in press).

Tabata, T., Eaton, S. and Kornberg, T. B. (1992). The *Drosophila hedgehog* gene is expressed specifically in posterior compartment cells and is a target of *engrailed* regulation. *Genes Dev.* **6**, 2635-2645.

Taylor, A. M., Nakano, Y., Mohler, J. and Ingham, P. W. (1993). Contrasting distributions of patched and hedgehog proteins in the *Drosophila* embryo. *Mech. Dev.* **42**, 89-96.

van den Heuvel, M., Nusse, R., Johnston, P. and Lawrence, P. A. (1989). Distribution of the wingless gene product in Drosophila embryos: a protein involved in cell-cell communication. *Cell* **59**, 739-749.

van den Heuvel, M., Harryman-Samos, C., Klingensmith, J., Perrimon, N. and Nusse, R. (1993). Mutations in the segment polarity genes *wingless* and *porcupine* impair secretion of the *wingless* protein. *EMBO J.* (in press).

Wieschaus, E. and Riggleman, R. (1987). Autonomous requirements for the segment polarity gene armadillo during Drosophila embryogenesis. *Cell* **49**, 177-184.