P- or N-type channels trigger release less efficiently than facilitation Ca^{2+} channels; about five times the Ca^{2+} influx per vesicle is required (Fig. 3*D*, *a*). Figure 3*D*, *b* plots the rate of capacitance change as a function of time ($\Delta C/\Delta T$); although N- and P-type Ca^{2+} currents were larger in comparison to facilitation Ca^{2+} currents, the rate of capacitance change was smaller. In addition, P- or N-type Ca^{2+} channels showed a markedly increased latency for release when compared to facilitation channels.

From these results, one possibility is that the Ca^{2+} channels in chromaffin cells are not randomly distributed; facilitation Ca^{2+} channels may be closer to the docking and release sites than either of the other two channels. Indeed, a physical association of Ca^{2+} channels with components of the exocytotic apparatus has already been proposed[2,15,16]. If all three channel types are randomly distributed, they might support secretion equally efficiently. The fact that this is not the case suggests that the precise localization of Ca^{2+} entry may be as important as its magnitude in determining its role in secretion. Nonetheless, physical association between Ca^{2+} channels and release sites alone cannot explain all our results. A remote priming site[17] or different pools of vesicles in various stages of availability[14] could account for the delay observed between depolarizing the cell and the onset of secretion. The large secretion observed in the 500 ms between depolarizations cannot be explained exclusively by a sustained increase in $[Ca^{2+}]_i$ (ref. 2), which will be similar for all three types of channels[18]; a maintained elevation of $[Ca^{2+}]_i$ and a local Ca^{2+}-dependent priming site together would better explain the observation. Other explanations for the difference in efficiency between distinct Ca^{2+} channel types and secretion are possible. Our results suggest that different Ca^{2+} channels in chromaffin cells regulate secretion depending on the level of electrical activity. Under conditions when few action potentials are generated, only N- and P-type channels would be expected to activate. Because channels are inefficiently coupled to exocytosis, little secretion occurs. Conditions of elevated electrical activity recruit facilitation. Because facilitation Ca^{2+} channels are coupled more efficiently to secretion, catecholamine release can increase dramatically. The novel gating properties of facilitation Ca^{2+} channels, that is, recruitment by trains of depolarizations or by agents that increase $[cAMP]_i$, and their possible proximity to release sites suggests that these channels may be responsible for the dramatically augmented catecholamine secretion from the adrenal medulla that occurs during conditions of stress or danger. As most neurons and other secretory cells have more than one type of Ca^{2+} channel, the concept of differential coupling of specific channels to different rates of transmitter or hormone release may be generally applicable[19]. □

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Components of *wingless* signalling in *Drosophila*

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THE determination of specific cell fates and polarity within each segmental unit of the *Drosophila* embryo involves the products of the segment polarity genes[1]. One of these, *wingless* (*wg*), encodes a secreted protein[2,3] that is homologous to the mammalian proto-oncogene Wnt-1 (refs 4, 5). In the embryonic epidermis, *wg* is expressed in a single row of cells within each segmental unit, although its activity is required for the correct patterning of most of the epidermis[4,6]. Initially Wg signals to adjacent posterior cells, maintaining *engrailed* (*en*) expression[7,8]. Later during embryogenesis, *wg* specifies the differentiation of naked cuticle[9]. Wg signalling functions by inactivating or antagonizing the activity of *zeste-white* 3 (*zw3*)[10]. We have investigated the requirement in the Wg signal transduction pathway for the three genes *armadillo* (*arm*)[11,12], *dishevelled* (*dsh*) and *porcupine* (*porc*)[13], all of which have embryonic mutant phenotypes similar to *wg*. Our results indicate that *dsh* and *porc* act upstream of *zw3*, and *arm* acts downstream of *zw3*.

Embryos that lack *wg* activity (referred to as *wg* embryos) are completely covered with denticles on the ventral cuticle, in contrast to wild-type embryos, in which the ventral cuticle is an alternating pattern of naked cuticle and denticle belts (Fig. 1*a*)[14]. The wild-type cuticular pattern depends on the expression of both *wg* and *en*. These genes are expressed in adjacent stripes within each segmental unit and their expression is mutually dependent; in *wg* embryos the striped pattern of En in the epidermis decays and in *en* embryos the epidermal pattern of Wg decays[7,8,15]. Although Wg signalling is essential[6], its mechanism is unclear. *arm* is a member of a multigene family that includes the vertebrate plakoglobin and β-catenin proteins, involved in cell–cell adhesion[16–18]. *dsh* encodes a novel protein of unknown biochemical function[19] and *porc* has not been characterized. Genetic epistasis can be used to understand the function of these genes in Wg signalling. However, analysis of embryos doubly mutant for *wg* and any single gene are uninformative because of the similarity of their phenotypes. Double-mutant combinations with *zw3* are revealing, because loss of *zw3* activity results in an embryonic phenotype opposite to that of *wg* embryos; *zw3* embryos lack denticles on the ventral cuticle (Fig. 1*e*) and each of the epidermal En stripes are expanded (Fig. 2*e*)[20]. *zw3*, another component of Wg signalling, encodes the *Drosophila* homologue of the mammalian serine/threonine protein kinase glycogen synthase kinase 3 (refs 21–23).

Embryos that are doubly mutant for *zw3* and *dsh* resemble *zw3* embryos; they lack denticles on the ventral cuticle (Fig. 1*f*), as observed in *zw3* embryos (Fig. 1*e*) and in contrast to *dsh* embryos (Fig. 1*b*). In *zw3 dsh* embryos, the pattern of En expression is expanded in a manner identical that seen in *zw3* embryos (compare Fig. 2*e* with *f*). After En expression expands, ectopic stripes of Wg appear in *zw3* and *zw3 dsh* embryos (compare Fig. 3*f* with *g*)[10,20]. This is in contrast to *dsh* embryos, where Wg is

not detected in the epidermis at this stage of embryogenesis (Fig. 3*b*)[19]. The similarity of the phenotypes of *zw3* and *zw3 dsh* embryos suggests that *dsh* functions upstream of *zw3* in Wg signalling.

Conversely, *arm zw3* double-mutant embryos exhibit the *arm* cuticle phenotype, the ventral cuticle is covered with denticles (compare Fig. 1*c* with *g*). But in the double-mutant embryos, En and Wg are detected in the epidermis at a stage where they are no longer expressed in *arm* embryos (compare Fig. 2*c* with *g*, and Fig. 3*c* with *h*)[11,12]. The stripes of En staining in *arm zw3* embryos are not as broad as in *zw3* embryos (Fig. 2*e*) and they eventually fade (data not shown). The cuticular phenotype of *arm zw3* embryos suggests that *arm* function is downstream of *zw3* in Wg signalling. The epistasis is not entirely clear because the patterns of En and Wg staining in the doubly mutant embryos do not resemble the patterns observed in *arm* embryos. The phenotype we observe in *arm zw3* embryos may reflect residual activity of the temperature-sensitive mutation, $arm^{H8.6}$, which we used. Possibly different levels of Arm activity are needed for the expression of En versus the specification of naked cuticle. In an independent study, using *arm* alleles that are not temperature sensitive, *arm zw3* embryos were found to resemble *arm* embryos for the cuticular phenotype, as well as the pattern of En expression[24]. These results suggest that *zw3* function is mediated through Arm.

We have also examined the relationship between *zw3* and *arm* in another fashion. *wg* regulates *arm* post-transcriptionally, resulting in the appearance of alternating stripes and interstripes of Arm protein in the epidermis of wild-type embryos (Fig. 4*Aa*)[25]. To determine whether the *wg*-dependent accumulation of Arm is regulated by *zw3* activity, we examined the distribution of Arm in *zw3* embryos. The pattern of stripes and interstripes of Arm is lost in *zw3* embryos, and the protein is uniformly distributed at high levels (Fig. 4*Ab*). The accumulation of Arm into stripes, relative to interstripes, reflects both increased levels and distinct subcellular localization of the protein[24]. Furthermore these authors demonstrate that both the levels as well as the subcellular localization of Arm protein are altered in *zw3* embryos.

Finally we examined embryos lacking both *zw3* and *porc* activity. *zw3 porc* embryos exhibit a cuticular phenotype similar to *zw3* embryos: the ventral cuticle is devoid of denticles (compare Fig. 1*e* with *h*). The pattern of En and Wg stripes are identical in *zw3 porc* embryos and *zw3* embryos (Figs 2*e*, *h* and 3*f*, *i*). These results suggest that *porc* functions upstream of *zw3* in the transduction of the Wg signal.

Although embryos that are mutant for *dsh*, *arm or porc* exhibit similar phenotypes, they differ in the pattern of Wg staining. Epidermal Wg staining can be detected in *porc* embryos at a developmental stage where it has faded in *dsh* and *arm* embryos.

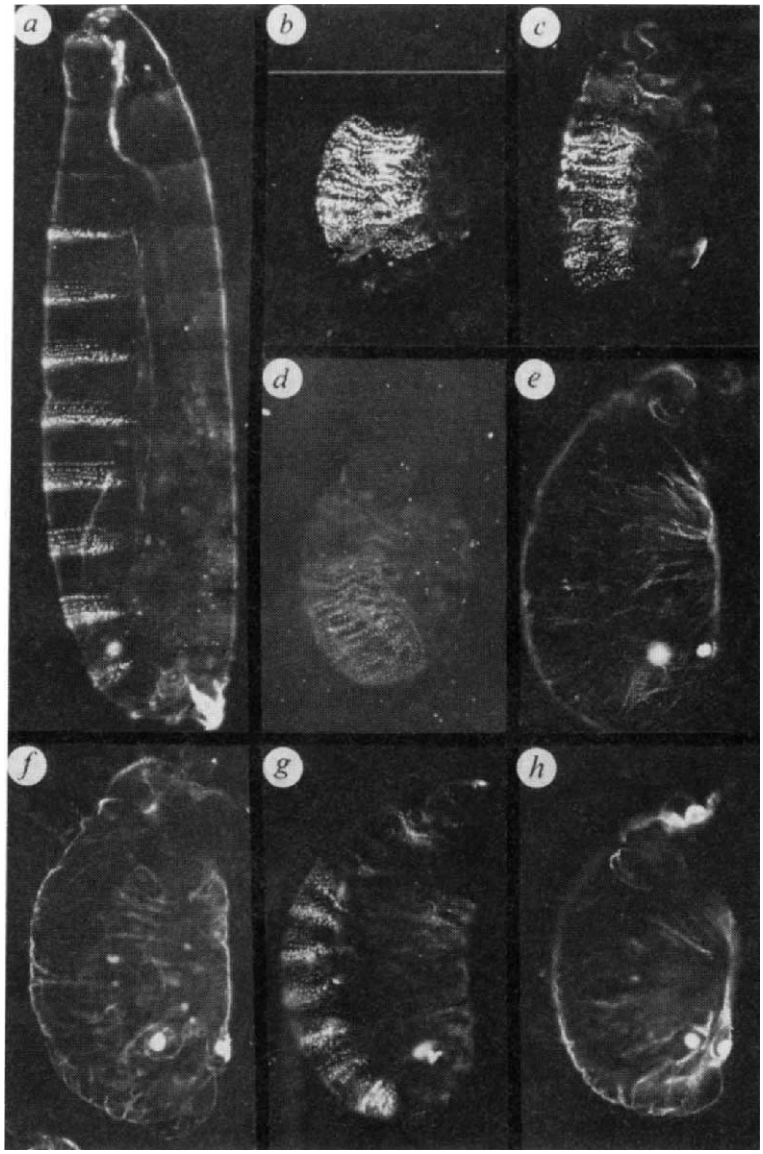


FIG. 1 The cuticle phenotype of wild-type (*a*), dsh^{V26} (*b*), $arm^{H8.6}$ (*c*), $porc^{PB16}$ (*d*), $zw3^{M11-1}$ (*e*), $zw3^{M11-1}$ dsh^{V26} (*f*), $arm^{H8.6}$ $zw3^{M11-1}$ (*g*) and $zw3^{M11-1}$ $porc^{PB16}$ (*h*) mutant embryos. All the mutant embryos shown are derived from homozygous mutant germ lines and have received neither maternal or zygotic wild-type product. All experiments were at 25 °C. In general the phenotype of *arm zw3* embryos is not as severe as that of *arm* embryos. Embryos are shown anterior up and ventral to the left.

METHODS. Cuticles were prepared and mounted in Hoyer's medium[28] mixed 1:1 with lactic acid, viewed and photographed under dark field. Mutant embryos are derived from females with mosaic germ lines generated by the 'FLP-DFS' technique[29]. Females with germ-line clones were of the genotypes $zw3^{M11-1}$ FRT^{101}/ovo^{D1} FRT^{101}; FLP^{F38}/+, dsh^{V26} FRT^{101}/ovo^{D1} FRT^{101}; FLP^{F38}/+, $arm^{H8.6}$ FRT^{101}/ovo^{D1} FRT^{101}; FLP^{F38}/+, $porc^{PB16}$ FRT^{9-2}/ovo^{D2} FRT^{9-2}; FLP^{F38}/+, $zw3^{M11-1}$ dsh^{V26} FRT^{101}/ovo^{D1} FRT^{101}; FLP^{F38}/+, $arm^{H8.6}$ $zw3^{M11-1}$ FRT^{101}/ovo^{D1} FRT^{101}; FLP^{F38}/+, $zw3^{M11-1}$ $porc^{PB16}$ FRT^{9-2}/ovo^{D2} FRT^{9-2}; FLP^{F38}/+. Descriptions of ovo^{D1} FRT^{101}, FLP^{F38} and the production of germ line clones, with the exception of *porc* and *zw3 porc*, have been given previously[29]. FRT^{9-2} chromosome has an insertion of an FRT element at position 18E on the polytene chromosome map (T. B. Chou, E. Noll and N.P., unpublished data). ovo^{D2} FRT^{9-2} is a recombinant chromosome between the female sterile mutation ovo^{D2} (ref. 30) and FRT^{9-2}, provided by E. Wieschaus. To generate germ-line clones, larvae of the genotype $porc^{PB16}$ FRT^{9-2}/ovo^{D2} FRT^{9-2}; FLP^{F38}/+ and $zw3^{M11-1}$ $porc^{PB16}$ FRT^{9-2}/ovo^{D2} FRT^{9-2}; FLP^{F38}/+ were heat-shocked at 37 °C three times during larval development, for 2 h each time. *dsh*, *arm* and *porc* are completely paternally rescued, such that germ-line clone-derived embryos which receive one wild-type copy of the gene paternally are wild-type. *zw3* is only partially paternally rescued[10].

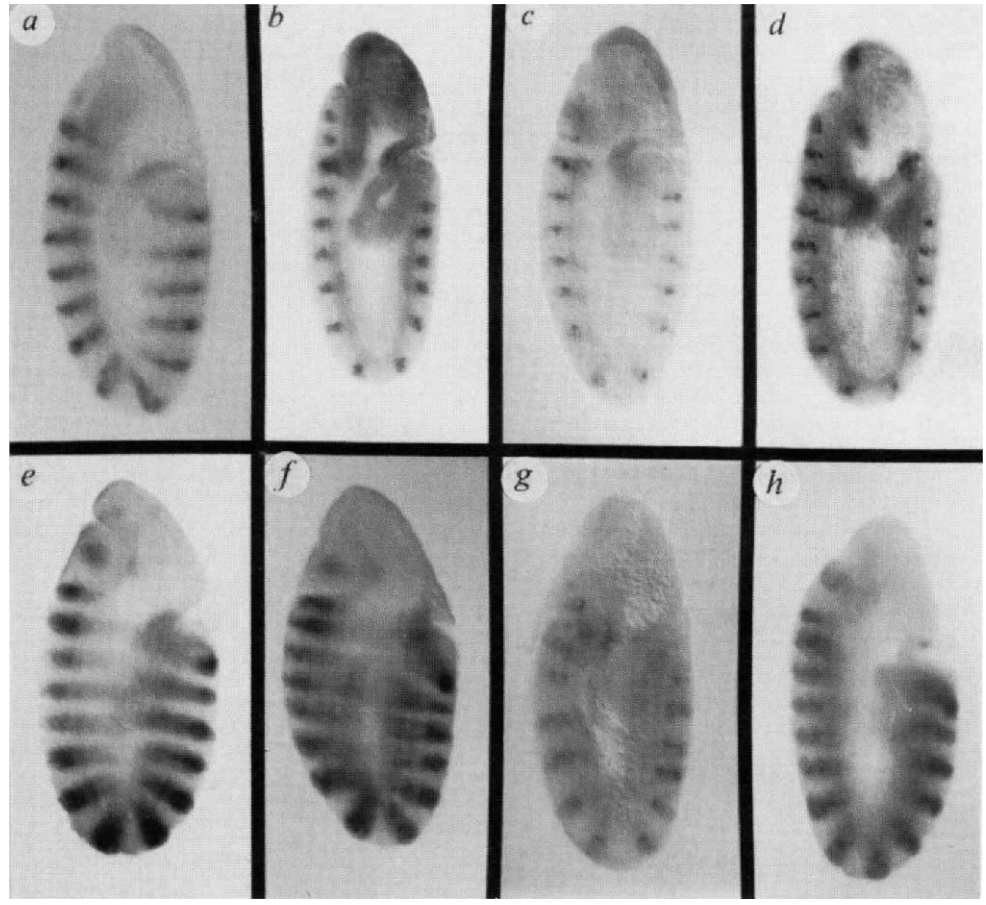


FIG. 2 Engrailed immuostaining in stage 10 wild-type (*a*), dsh^{V26} (*b*), $arm^{H8.6}$ (*c*), $porc^{PB16}$ (*d*), $zw3^{M11\ 1}$ (e), $zw3^{M11-1}$ dsh^{V26} (*f*), $arm^{H8.6}$ $zw3^{M11-1}$ (*g*) and $zw3^{M11-1}$ $porc^{PB16}$ (*h*) mutant embryos. En expression has disappeared from the epidermis of *dsh*, *arm* and *porc* embryos but can still be detected in the nervous system. In contrast, En can be detected in the epidermis of *zw3 dsh* and *zw3 porc* embryos as observed in *zw3* embryos. In *arm zw3* embryos the En stripe is not as wide as in *zw3* embryos and does eventually fade (data not shown). Embryos are shown anterior up and ventral to the left. All experiments were at 25 °C.

METHODS. Females of the genotypes described in Fig. 1 were mated to *FM7*, *ftz-lacZ /Y* males[31]; this *FM7* chromosome carries a transposon that contains the *fushi tarazu* promoter fused to the *Escherichia* coli LacZ gene. This allowed us to distinguish the genotypes of embryos derived from germ-line clones. Paternally rescued embryos that have received one wild-type copy of the gene from the father stained for β-galactosidase. Null embryos that received neither maternal or paternal wild-type gene product do not stain for β-galactosidase. The embryos were collected, dechorionated in 50% bleach and fixed for 10–15 min in 4% formaldehyde in PBS. Embryos were first stained for X-gal to detect the paternally rescued embryos and then immunostained using a mouse monoclonal antibody directed against En[32], diluted 1:1, as previously described[10]. Embryos were mounted and viewed in methyl salicylate. Only the null mutant embryos are shown. All staging of embryos is according to previous descriptions[33].

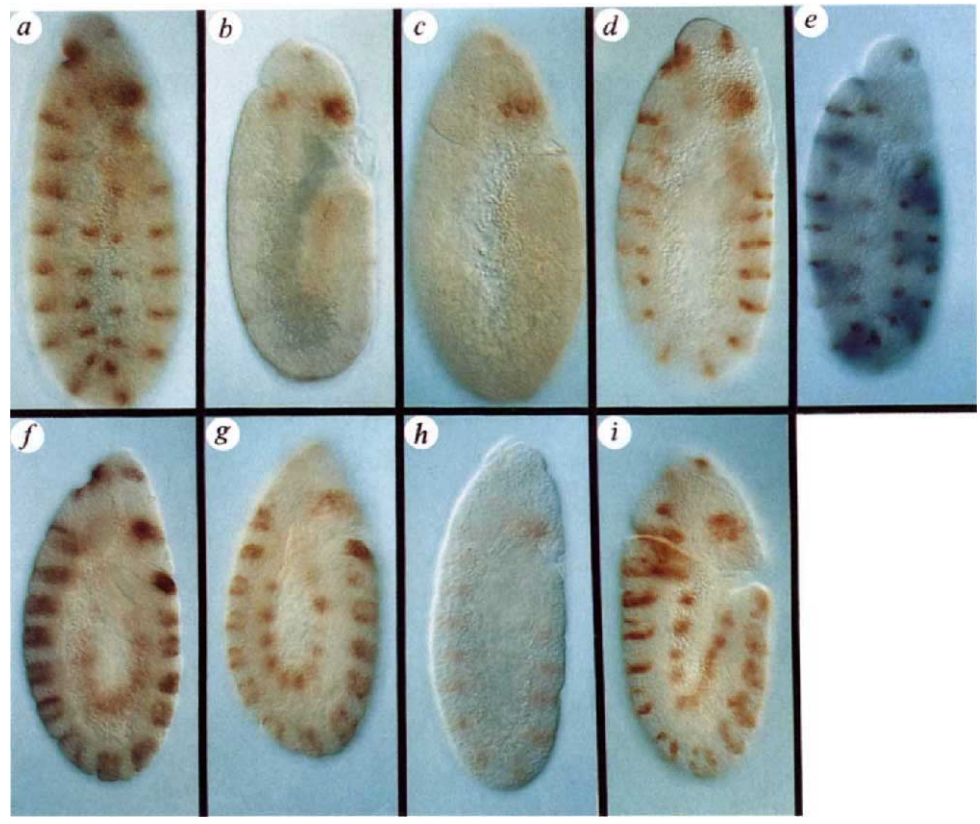


FIG. 3 Wingless immunostaining in stage 11 wild-type (*a*), dsh^{V26} (*b*), $arm^{H8.6}$ (*c*), $porc^{PB16}$ (*d*), $porc^{PB16}$ rescued (e), $zw3^{M11-1}$ (*f*), $zw3^{M11-1}$ dsh^{V26} (*g*), $arm^{H8.6}$ $zw3^{M11-1}$ (*h*) and $zw3^{M11-1}$ $porc^{PB16}$ (*i*) mutant embryos. Embryos are shown anterior up and ventral to the left. All experiments were at 25 °C. Wg staining decays in *dsh* and *arm* embryos, but can still be detected in *porc* embryos. In *arm zw3* embryos only the wild-type stripe of Wg can be detected. In *porc* embryos, as well as *porc*-rescued embryos, Wg staining is discrete, the protein appears to be retained by the cells that synthesize it. *zw3 dsh* and *zw3 porc* embryos show an ectopic stripe of Wg, as observed in *zw3* embryos.

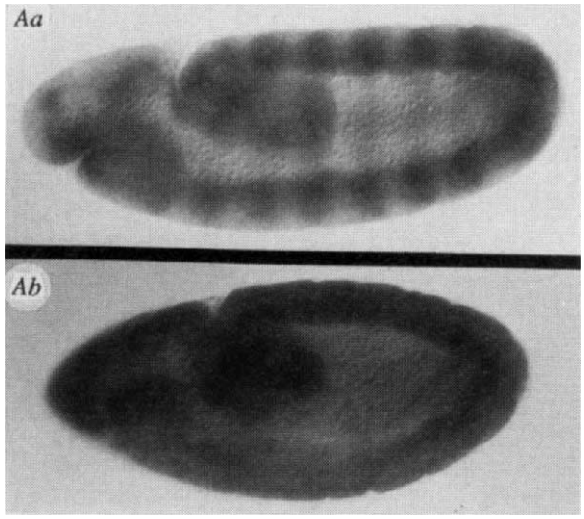
METHODS. Females of the genotypes described in Fig. 1 were mated to *FM7*, *ftz-lacZ/Y* males. The embryos were collected, dechorionated in 50% Chlorox and fixed for 35 min in 4% formaldehyde in PBS. Polyclonal serum directed against Wg[2] was used at a 1:500 dilution and immunostaining was detected with peroxidase-conjugated goat anti-rabbit secondary antibody (diluted 1:500). Rescued and null mutant embryos were distinguished by staining using mouse monoclonal antibody against β-galactosidase, 1:1,000 dilution, and detected with alkaline phosphatase conjugated goat anti-mouse secondary antibody (diluted 1:500). Embryos were mounted and viewed in 70% glycerol. Wg immunostaining is brown and β-galactosidase immunostaining is blue.

In addition, in *porc* embryos Wg protein appears confined to the *wg*-expressing cells, leading to the suggestion that in *porc* embryos Wg is retained by the cells that synthesize it (Fig. 3*d*)[26]. The *porc* embryonic segment polarity phenotype, which results from the lack of both maternal and zygotic product, can be completely rescued to adulthood by one wild-type copy of the zygotic gene[13]. We were therefore surprised to observe that *porc*-rescued embryos also exhibit confined Wg staining, indicative of protein retention (Fig. 3*e*). Later in development (stage 13), Wg does appear to be secreted in *porc*-rescued embryos (data not shown). These results suggest that early zygotic expression of *porc* enables low levels of Wg to be secreted or that it allows Wg to reach the cell surface, where it is sufficient to signal to adjacent cells and consequently maintain En expression.

We conclude that both *porc* and *dsh* activities are required upstream of *zw3* in Wg signalling. Our data are consistent with a proposed role for Porc in the secretion of Wg protein and a role for Dsh in the reception or transduction of the Wg signal. *arm* activity is required downstream of *zw3* and our results, as well as others, suggest that *zw3* activity is mediated through a redistribution of Arm protein. We propose that these gene products interact in a linear pathway based on the epistatic relationships we have determined. But it is possible that these genes function in parallel pathways. This seems unlikely in the case of *zw3* and *arm* because Arm protein distribution is altered in *zw3* embryos. Our conclusions are also consistent with recent work that indicates that both *dsh* and *arm*, but not *porc*, are required for the gain-of-function phenotype generated by ubiquitous embryonic expression of *wg* (ref. 27).

We propose the following model for Dsh, Arm and Porc function in the Wg signal transduction pathway (Fig. 4*B*). Porc is necessary for the correct distribution of Wg protein. Wg protein is apparently secreted by cells that synthesize it and received by neighbouring cells[2,3], where a signal transduction cascade is initiated. Once received by cells, the Wg signal is transduced through Dsh, which results in the inactivation of Zw3 kinase, which in turn modulates the level of active Arm protein. The correct epidermal En expression, as well as the specification of naked cuticle, is achieved by the modulation of Arm. Zw3 kinase may directly phosphorylate Arm, thereby reducing the levels of active protein, whereas Wg signalling can abrogate this effect by inactivating Zw3 kinase, resulting in high levels of active Arm. This hypothesis is consistent with the observation that Arm is a phosphoprotein and that Zw3 kinase is required for its phosphorylation (M. Peifer, personal communication).

But how might Arm, a molecule involved in cell adhesion, regulate the expression of *en*? Simplistically, high levels of active Arm protein may directly transduce the Wg signal from the membrane to the nucleus. Alternatively, Arm accumulation may be required to make specific intercellular contacts, and these contacts are a prerequisite for a second process that would regulate *en* expression and cell fate determination. □



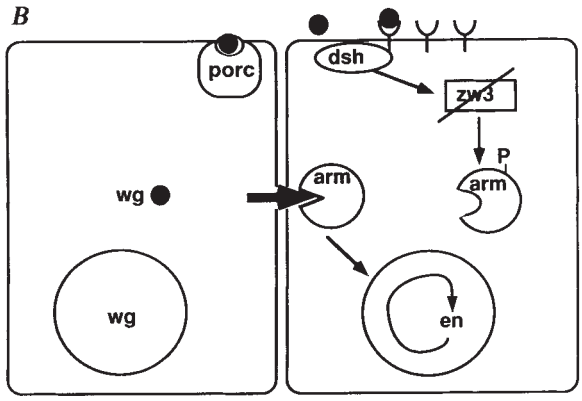


FIG. 4 *A*, Armadillo immunostaining in stage 10 wild-type (*A*, *a*) and *zw3* mutant (*A*, *b*) embryos. Embryos are shown anterior to the left and dorsal up. In wild-type embryos a low level of Arm is detected throughout the epidermis, in addition to stripes of high levels of staining positioned over the parasegmental border[25]. In *zw3* embryos a high level of Arm staining is detected throughout the epidermis. *B*, Model for the mechanism of Wg signalling. The maintenance of *en* expression requires input from Wg (shown as black dots). The distribution of Wg protein is regulated by Porc. Dsh is required for the transduction of the Wg signal in adjacent cells, perhaps in association with the unidentified Wg receptor. Transduction of the Wg signal inactivates or antagonizes Zw3 kinase, which functions to modulate the levels of active Arm. Zw3-dependent phosphorylation of Arm results in low levels of active protein, whereas Wg signalling can revert this effect and result in accumulation of active Arm. Arm either directly, or through the formation of a second signalling complex (large arrow), results in the maintenance of En expression.

METHODS. Embryos derived from *zw3* mutant germ lines were generated as described for Fig. 2. Embryos were collected, dechorionated and fixed as described for Fig. 3. Rabbit polyclonal antibody against Arm[25], kindly provided by M. Peifer, was used at a 1:500 dilution. Embryos were then incubated with a 1:500 dilution of biotinylated goat anti-rabbit secondary, followed by incubation with Vectastain. Embryos were mounted and viewed in methyl salicylate.

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dishevelled and *armadillo* act in the Wingless signalling pathway in *Drosophila*

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THE *Wnt* genes encode conserved secreted proteins that play a role in normal development and tumorigenesis[1,2]. Little is known about the signal transduction pathways of *Wnt* gene products. One of the best characterized *Wnt* family members is the *Drosophila* segment polarity gene *wingless*[3–6]. We have investigated whether segment polarity genes with a *wingless*-like phenotype mediate the *wingless* signal. We used a *wingless* transgene controlled by a heat-shock promoter for genetic epistasis experiments. We show that *wingless* acts through *dishevelled* and *armadillo* to affect the expression of the homeobox gene *engrailed* and cuticle differentiation.

During germ-band extension, *wingless* (*wg*) is expressed in stripes flanking a parasegmental border[3]. The Wg protein is secreted[7,8] and taken up by adjacent cells for maintenance of expression of the homeobox gene *engrailed* (*en*)[9–12]. Later, *wg* is necessary for the generation of smooth cuticle[13]. No naked cuticle is formed in the absence of *wg*; instead the ventral cuticle consists of a lawn of denticles.

Genes that mediate the *wg* signal are probably among the segment polarity mutants with a *wg*-like phenotype. Absence of functional products of the genes *dishevelled* (*dsh*), *armadillo* (*arm*), *porcupine* (*porc*) and *hedgehog* (*hh*) lead to cuticle defects similar to that of *wg* mutant embryos and also to a loss of en expression[9,14 16]. It has been difficult to order these mutations in a genetic pathway because their phenotypes are nearly indistinguishable and because expression of *wg* depends on *en* expression. But the order of action of two genes in a common pathway can be found by examining epistasis between a dominant gain-of-function mutant for one gene in combination with a loss-of-function mutant for the other. Dominant *wg* alleles have not been identified, but we previously established a transgenic *Drosophila* strain that expresses *wg* under the control of a heat-shock promoter[17]. Heat-shock during particular stages of development causes expansion of the En expression domain and generation of a completely naked ventral cuticle[17] (Figs 1*b* and 2*b*). We used the HS-*wg* allele to determine which of several segment polarity genes with a *wg*-like phenotype are required for *wg* action.

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We first investigated whether the effects of HS-*wg* on expansion of En expression require endogenous *wg* and *en* genes. The en^{CX1} allele produces a cytoplasmic, non-functional protein, in contrast to the nuclear localization of the En protein in wild type. In en^{CX1} embryos, En expression is not maintained[11]. The heat-shocked double mutant en^{CX1};HS-*wg* embryos can be distinguished from the single mutants by their unique pattern of En protein expression, which is ectopically induced at early stage 10 (ref. 18) as in HS-*wg* embryos, but localized in the cytoplasm as in en^{CX1} (Fig. 1*i*, *j*). Normal or ectopic En domains are not maintained: at stage 11 most En protein has disappeared from the ectoderm (data not shown).

In HS-*wg* embryos, an ectopic domain of Wg protein made from the normal *wg* gene is formed just posterior to the expanded En domain[17]. In the heat-shocked double mutant *wg*;HS-*wg* embryos, the ectopic Wg domain does not appear, yet the expanded domain of En expression is induced (compare Fig. 1*a* with *h*) and maintained (data not shown)[20]. Thus neither functional En nor Wg protein made from the endogenous *wg* gene are required to mediate the effects of HS-*wg* on induction of ectopic En.

To examine whether genes with mutant phenotypes similar to *wg* (*dsh*, *arm*, *porc and hh*)[14–16,19] are required for the effects of HS-*wg* on En expression, we made double mutants between HS-*wg* and loss-of-function mutations in these genes. As Dsh, Arm and Porc products are provided both maternally and zygotically, germ-line mosaic females were derived to remove completely the gene product from the developing embryo. In all four single mutants, En expression decays as in *wg* embryos (Fig. 1*c*), but there are some differences in pattern and timing of En decay[9,14]. The En patterns in the double mutant embryos are shown in Fig. 1*d–g*. In *dsh*;HS-*wg* embryos (Fig. 1*d*), the En expression pattern is very similar to *dsh* and *wg* embryos, although dorsally the En protein disappears from the ectoderm slightly later in development than in *dsh* embryos. Likewise, *arm*;HS-*wg* embryos (Fig. 1*e*) show a pattern of En distribution similar to *arm* embryos. In contrast, *porc*;HS-*wg* (Fig. 1*f*) and *hh*, HS-*wg* embryos (Fig. 1*g*) show ectopic En expression as seen in HS-*wg* embryos. Thus *dsh* and *arm* are required for induction of ectopic En in HS-*wg*, whereas *hh* and *porc* are not.

We then studied the effects of the absence of *dsh*, *arm*, *porc* or *hh* on cuticle pattern formation in HS-*wg*. The cuticle patterns of *dsh*;HS-*wg* (Fig. 2*d*) and *arm*;HS-*wg* (Fig. 2*e*) embryos are indistinguishable from the germ-line clone-derived *dsh* or *arm* embryos and from *wg* embryos (Fig. 2*c*) and develop a continuous lawn of denticles. In contrast, *porc*;HS-*wg* embryos show some restoration of the segmental denticle pattern (Fig. 2*f*). *hh*;HS-*wg* embryos (Fig. 2*g*) have a similar but not identical cuticle pattern to HS-*wg* (Fig. 2*b*)[20]: naked throughout most of the ventral cuticle, with dispersed patches of non-polarized denticles mostly present at the lateral sides. Thus *dsh* and *arm*, but not *porc* and *hh*, are epistatic to and presumably downstream of HS-*wg*, not only in the effect on En expression but also in the generation of naked cuticle. The cuticle of *wg*;HS-*wg* double-mutant embryos is shown in Fig. 2*h*. After a 20-min heat shock during germ-band extension, an embryo of almost wild-type size is formed with partially restored head and tail structures (filzkörper) and a segmental pattern. This unexpected result suggests that differential levels of *wg* are not essential for at least some of its functions[20]. It is possibe that *wg* normally regulates pattern together with other determinants that function in a spatially restricted manner. For example, not all cells may be equally sensitive to *wg* activity, because of differences in concentrations of interpreting molecules.

In conclusion, *dsh* and *arm* are essential components of the *wg* signalling pathway and probably act downstream of *wg*. In contrast to the non-autonomous behaviour of *wg* in mutant cell clones, *dsh* and *arm* act autonomously, suggesting a role in reception of the *wg* signal[16,21]. In addition, *wg* is required for post-transcriptional regulation of *arm*, resulting in an accumulation