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The *Drosophila* segment polarity gene *dishevelled* encodes a novel protein required for response to the *wingless* signal

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The *Drosophila* Wnt-1 homolog, *wingless* (*wg*), is involved in the signaling of patterning information in several contexts. In the embryonic epidermis, Wg protein is secreted and taken up by neighboring cells, in which it is required for maintenance of *engrailed* transcription and accumulation of Armadillo protein. The *dishevelled* (*dsh*) gene mediates these signaling events as well as *wg*-dependent induction across tissue layers in the embryonic midgut. *dsh* is also required for the developmental processes in which *wg* functions in adult development. Overall, cells lacking *dsh* are unable to adopt fates specified by Wg. *dsh* functions cell autonomously, indicating that it is involved in the response of target cells to the Wg signal. *dsh* is expressed uniformly in the embryo and encodes a novel protein with no known catalytic motifs, although it shares a domain of homology with several junction-associated proteins. Our results demonstrate that *dsh* encodes a specific component of Wg signaling and illustrate that Wnt proteins may utilize a novel mechanism of extracellular signal transduction.

By the time the *Drosophila* embryo becomes cellularized, the segmented body pattern has been established. Organization within the segment itself occurs via interactions among its nascent cells. Some 15 genes have been identified that are required for this initial intrasegmental patterning, known as segment polarity genes, many of which have maternal as well as zygotic contributions (for review, see Klingensmith and Perrimon 1991; Peifer and Bejsovec 1992).

The cuticle defects of segment polarity mutants are largely the result of abnormal cell interactions occurring much earlier. For example, the most posterior cells of the segment, which express *engrailed* (*en*) and secrete either denticle or naked cuticle, are instructed by signals from their neighbors to the anterior, which express *wingless* (*wg*) (Bejovec and Martinez-Arias 1991; Dougan and DiNardo 1992). *wg*, the *Drosophila* homolog of the secreted murine oncoprotein Wnt-1 (Rijsewijk et al. 1987), appears to directly promote the cell fate of naked cuticle secretion in that ubiquitously expressed *wg* results in an entirely naked cuticle (Noordermeer et al. 1992). This observation also reveals that not only the *en* domain but also cells in the anterior of the segment, which normally secrete denticles, can respond to the *wg*-dependent cell fate signal.

The earliest known cell interaction leading to segmental patterning also involves communication between the *wg* and *en* domains, but in this case the purpose is for mutual maintenance of expression. Although the initial expression of *wg* and *en* in adjacent stripes is directed by the pair–rule genes, maintenance of *wg* and *en* expression requires the function of the other gene in the adjacent cells (DiNardo et al. 1988; Martinez-Arias et al. 1988).

The signal from the *wg* domain that acts to maintain *en* expression is thought to be Wg itself. Electron microscopy of sections of embryos stained with anti-Wg antibodies reveals that Wg is secreted by those cells that express it and is taken up by surrounding cells. Double-labeling experiments indicate that Wg antigen can be detected inside *en*-expressing cells (van den Heuvel et al. 1989; Gonzalez et al. 1991). In addition, maintenance of *en* expression in purified, cultured cells of the *en* domain requires the presence of cocultured *wg* transfectant cells (Cumberledge and Krasnow 1993). These results are consistent with the nonautonomous behavior of *wg* mutant clones in adult structures (Morata and Lawrence 1977; Babu and Bhat 1986; Baker 1988a).

The molecular mechanism by which *wg* signals is unknown. Moreover, although *Wnt* homologs in many

other species and in cell culture systems appear to encode paracrine cell fate signals, in no case is there any information on the molecular pathway of *Wnt* signal transduction (for review, see Nusse and Varmus 1992). Candidates for genes involved in *wg* signaling in the embryonic epidermis are the other segment polarity genes, especially those resulting in a mutant cuticle phenotype like that of *wg*. Based on similarity of phenotypes and time course of En decay, the maternal-effect segment polarity genes *armadillo* (*arm*) (Klingensmith et al. 1989; Peifer et al. 1991), *disheveled* (*dsh*) and *porcupine* (Klingensmith and Perrimon 1991; van den Heuvel et al. 1993a) may encode components of the *wg* pathway. *arm*, a catenin-like junction protein (Peifer et al. 1992), is a target of *wg* signaling, in that *wg* directs post-transcriptional accumulation of Arm protein in cells including and flanking the *wg* stripe (Riggleman et al. 1990). This effect of *wg* was evaluated in various segment polarity mutant backgrounds, and *dsh* appears to be an essential requirement. Similarly, we have shown that *dsh* is necessary for the effects of ubiquitous *wg* expression on segmentation (Noordermeer et al. 1994). *dsh* therefore seems a likely candidate for a gene encoding a component of the *wg* signaling pathway.

We have examined the roles of *dsh* in patterning of the embryonic epidermis and in other contexts in which *wg* is known to function and conclude that *dsh* is required for proper cell fate in *wg*-responsive tissue. We have analyzed the cellular requirement, molecular structure, and expression of *dsh* and propose that *dsh* represents a novel signal transduction molecule generally required for the response of target cells to the Wg signal.

Results

dsh *mediates the effects of* wg *in the embryo*

Although *dsh* is expressed both maternally and zygotically, either the maternal or the zygotic component is sufficient to permit normal embryonic development (Perrimon and Mahowald 1987; Fig. 1A). When both components are lacking, *dsh* embryos display patterning defects identical to those of *wg* null mutants (Fig. 1B,C), from the earliest stage (11) at which morphological defects can be detected (Perrimon and Mahowald 1987). We refer to embryos lacking both maternal and zygotic contributions as *dsh* embryos.

Wg signaling in the ventral epidermis The timing of disappearance of En antigen in *dsh* embryos is identical to that observed in *wg* embryos (van den Heuvel et al. 1993a); however, the role of other genes in the maintenance of *en* expression by *wg* is difficult to study because *en* in turn is required for maintenance of *wg* expression (Martinez-Arias et al. 1988). Thus in *dsh* embryos, we have found that En and Wg staining patterns are initially normal but both antigens disappear during extended germ-band stages. To address directly which antigen disappears first, and thus what aspect of the loop *dsh* mutants affect most directly, we double-stained *dsh* em-
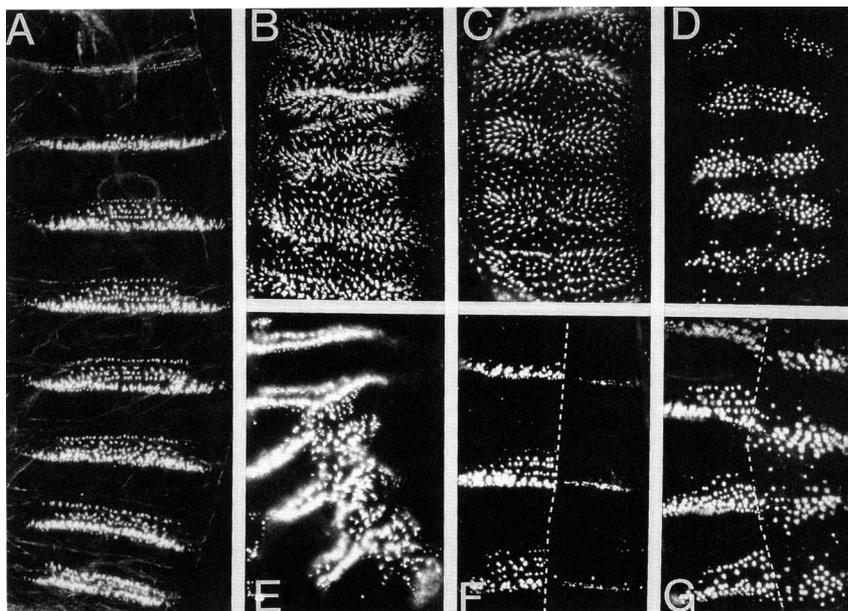
Figure 1. Embryonic phenotype and autonomy of *dsh*. Cuticles of terminally differentiated embryos are shown in darkfield (ventral views; anterior is *up*). (*A*) Wild-type (*dsh/ +*) larva, early first instar. A denticle belt spans the anterior portion of each segment, with the tips of denticles in most rows being aligned toward the posterior. Posterior cells secrete naked cuticle. Note that *wg* and *dsh* embryos are less than half the length of their wild-type siblings. (*B*) *dsh*^{v26} embryo. Denticles form an irregular lawn without naked cuticle, with many lateral denticles oriented toward the midline. Note that no segment borders form to define segments. These embryos also lack the filzkörper structure at the posterior and are missing all head structures. (*C*) *wg*^{CX4}/*wg*^{CX4} embryo. The embryo displays the same phenotypes as the *dsh* mutant. (*D*) *svb,dsh*^{v26} embryo. The *svb* mutation reduces the number and size of denticles, further revealing the metameric pattern of denticles persisting in this phenotype. (*E*) *dsh*^{M20}/*ring-X* mosaic embryo. Mosaicism is indicated by filzkörper material (not in focus), with a patch of denticles displaying the *dsh* phenotype (*lower right* quadrant). This pattern is sharply demarcated from the adjacent wild-type pattern, suggesting lack of rescue by adjacent wild-type tissue; however, the exact border cannot be determined because the mutant tissue is not marked. (*F*) *svb*/*ring-X* mosaic embryo. *svb* denticles occur in a wild-type pattern, with no effect on the adjacent wild-type denticles (*left* of dotted line). (*G*) *svb,dsh*^{v26}/*ring-X* mosaic embryo. The mutant side of the embryo (*right*) displays a segment polarity phenotype identical to that of *svb,dsh* alone (cf. *D*). Wild-type denticles (*left*) are unaffected.

bryos with Wg and En antibodies. We examined the expression of these antigens during stages 9–11 in which *en* expression is dependent on Wg (Heemskerk et al. 1991). In late stage 9 *dsh* embryos, En expression has largely deteriorated while Wg antigen appears wild type (data not shown). Wg antigen persists in *dsh* until mid-stage 10, at which time it also begins to disappear in *en* mutants (van den Heuvel et al. 1993a). Thus, *dsh* appears to mediate the role of *wg* in directing accumulation of Arm in *wg*-expressing and -flanking cells, as well as in maintenance of *en* expression in the posteriorly adjacent cells.

Wg-mediated induction across tissues of the midgut In addition to its role in epidermal development, *wg* is also required for development of a region of the midgut (Immergluck et al. 1990; Reuter et al. 1990). In late wild-type embryos, a deep second midgut constriction forms just posterior to a broad band of *labial* (*lab*) expression in the endodermal layer (Fig. 2A). *wg* and *dsh* embryos never form the second constriction (Fig. 2B,C). Although *wg* is expressed in the visceral mesoderm at the site where the second midgut constriction will form (Reuter et al. 1990), it is required for correct expression of *lab* in the adjoining endoderm (Immergluck et al. 1990). Normally, *lab* expression forms a gradient of nuclear staining such that the strongest staining is in the cells adjacent to the constriction, with lower levels toward the anterior (Fig. 2D; Immergluck et al. 1990). In *wg* and *dsh* mutants, this gradient is absent, with most cells in the *lab* domain expressing lower levels of antigen (Fig. 2E,F; Immergluck et al. 1990). Therefore, *dsh* appears to mediate *wg* function between cells in different germ layers.

## *dsh* and *wg* have similar phenotypes throughout development

We investigated the relationship between *wg* and *dsh* during imaginal development to examine their phenotypic correlation in other developmental contexts. Hemizygous *dsh* animals derived from heterozygous mothers usually die as late larvae or early pupae, but in the case of a hypomorphic lethal allele a few survive long enough to develop adult tissues. When these pharate pupae are dissected from their pupal cases, they exhibit defects primarily involving distal derivatives of the imaginal discs. Most notably, they lack antennal structures and have bulbous, truncated legs (Fig. 3B). The pupal phenotypes of *dsh* are very similar to those of *wg* pupal-lethal alleles, which also result in a lack of antennal structures and partial, malformed legs (Baker 1988b; Fig. 3C). Although the extent of defects in both mutants varies greatly from animal to animal, the sensitive tissues are affected in a similar way. The legs of *dsh* pupae typically show a deletion of ventral markers, replaced with a duplication of dorsal pattern elements (Fig. 3D). These defects are also seen in legs of *wg* pupae (Peifer et al. 1991; Couso et al. 1993). *wg* mutants usually lack one or both wings, replaced by a duplicated notum (Baker 1988b; Fig. 3F); *dsh* animals occasionally show an identical defect (~20%; Fig. 3E).

dsh and *wg* are required for ventral patterning of the leg Several experiments suggest that localized expres-
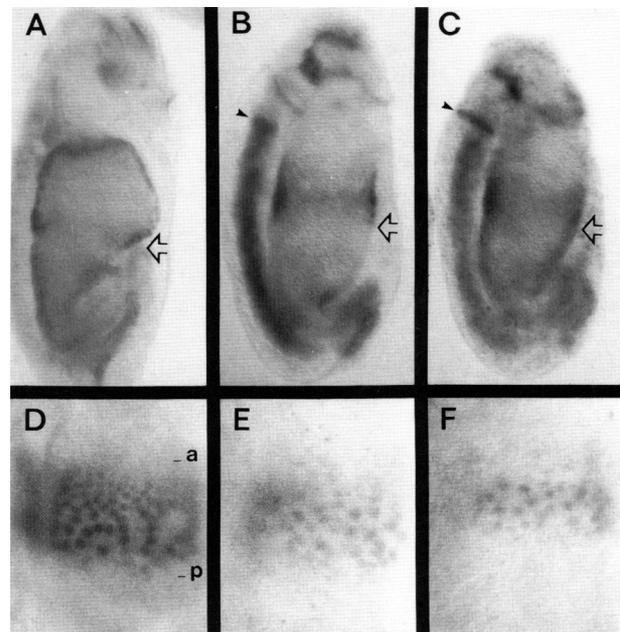
Figure 2. Midgut patterning is affected similarly in *dsh* and *wg* mutants. Whole-mount lateral, parasaggital views of embryos stained with anti-Lab with anterior *up*. (*A*) Wild-type embryo, stage 15. The second midgut constriction (SMC) forms a sharp indentation in the midgut (open arrow), reaching its narrowest point just posterior to the domain of endodermal cells expressing Lab antigen (dark staining). The first and third constrictions become pronounced somewhat later. (*B*) *wg^{IN67}/wg^{IN67}* embryo, stage 15. The SMC is completely absent (open arrow), although Lab antigen still occurs in its normal domain in the midgut (dark staining). Ectopic Lab staining also occurs in the CNS (arrowhead). (*C*) *dsh^{v26}* embryo, stage 15. Lack of *dsh* results in complete failure of the SMC (open arrow), despite Lab expression (dark staining). These embryos show identical defects to *wg* embryos, including the induction of ectopic Lab antigen in the CNS (arrowhead). (*D*) Wild-type embryo, stage 14. Lab antigen in the endodermal nuclei forms a graded pattern, with strongest staining at the posterior (p) of the domain where the SMC begins to invaginate. Toward the anterior (a) the nuclei look smaller and less heavily stained. (*E*) *wg^{IN67}/wg^{IN67}* embryo, stage 14. Lab staining is less intense overall and occurs in a nongraded manner. (*F*) *dsh^{v26}* embryo, stage 14. Lab staining is affected in the same way as in *wg* embryos.

sion of *wg* in early larvae acts to organize ventral patterning in the leg disc (Peifer et al. 1991; Couso et al. 1993; Struhl and Basler 1993). We have investigated the requirement for *dsh* in leg development by examining mosaic legs consisting of wild-type and *dsh* mutant cells. As in the body as a whole, the frequency of *dsh* clones in the leg is less than that of wild-type, control clones (Table 1; data not shown). However, clones of cells lacking *dsh* are recovered in even the most ventral bristle rows throughout the leg. Whereas dorsal and lateral *dsh* clones seem to have no effect on development (Fig. 4A), ventral clones are almost always associated with supernumerary appendages arising from the ventral face of the leg (Fig. 4B,C,D). Control clones, in which the *dsh* allele
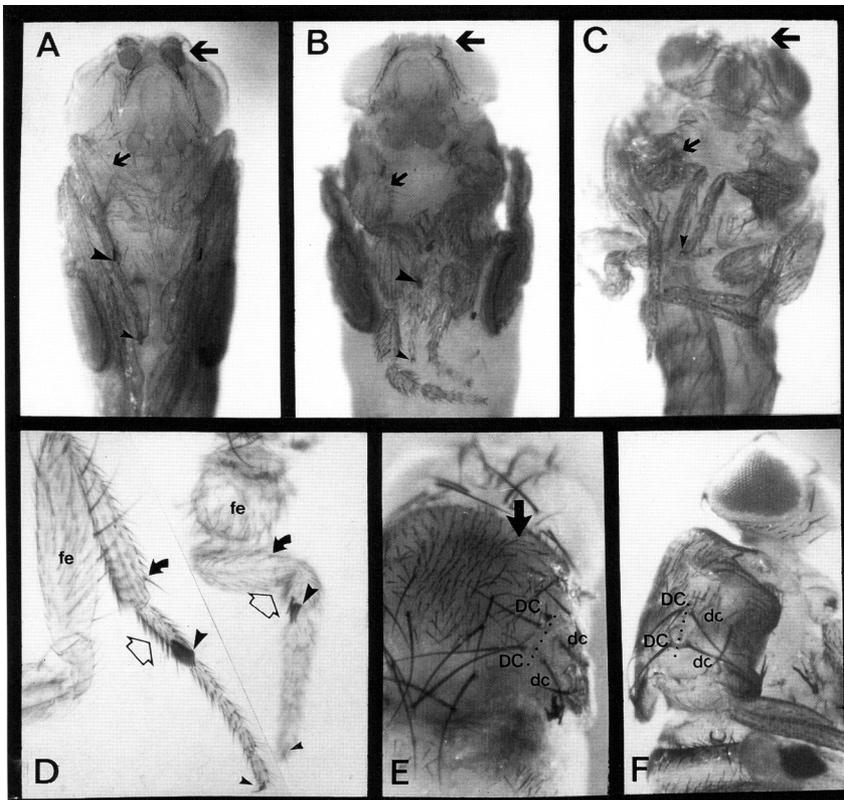
Figure 3. Phenotypes of *dsh* in imaginal development. (*A*) Wild-type pharate pupa, male (ventral aspect). Note the presence of antennae (large arrow) and the thin, tubular, and ordered appearance of the six legs. The femur of the first left leg is indicated (small arrow), and more distally the sex comb (large arrowhead) and claw (small arrowhead). (*B*) *dsh^{M20}/Y* male pupa (ventral aspect). Note lack of antennal structures (large arrow) and truncated, malformed legs. The femur of the first left leg (small arrow) is short and bulbous. Sex comb (large arrowhead) and claw (small arrowhead) are present, indicating presence of dorsal pattern. The distal-most tarsal segments of legs are often lacking. (*C*) *wg* pupa, female (ventral view, *wg^{CX3}/wg^{CX4}*). The defects are similar to those of *dsh*, with a lack of antennae (large arrow) and incomplete, malformed legs; e.g., the femur of the left first leg is truncated and bulbous (small arrow). Claws often form (small arrowhead). Leg development is typically less affected in *wg* than in *dsh*; wing development is usually affected more. (*D*) Prothoracic (first) male legs, wild-type (*left*) and *dsh* (*right*). The femur of both legs is indicated (fe)—note that the *dsh* femur lacks the long bristles characteristic of the wild-type ventral femur (*right*) and is much reduced in length. *dsh* legs differentiate dorsal structures, such as the tibial subapical bristle (curved arrow) and the claw (small arrowhead). The dorsal edge of the sexcomb (large arrowhead) is also normal. Ventral structures are absent, such as the peg-like bristles in the tarsal segments (open arrow). (*E*) *dsh^{M20}/Y* pupa, dorsolateral view. The wing has been replaced by a duplication of the notum (*right*). The axis of duplication (dotted line) is flanked by the normal dorsocentral bristles (DC) and ectopic dorsocentral bristles (dc) in mirror-image symmetry. Note also the dishevelled alignment of the bristles on the thorax (arrow), which in wild type all point posteriorly. (*F*) *wg^{CX3}/wg^{CX4}* pupa (lateral view). Axis of duplication is the same as in *E*.

was wildtype, were not associated with pattern irregularities in any compartment. The supernumerary appendages arising from *dsh* clones can be composed of mutant (Fig. 4B) or wildtype cells (Fig. 4C). We have never observed any supernumerary dorsal appendages associated with clones. Therefore, both *dsh* and *wg* are

Table 1. *Mosaic analysis of* dsh

Cross	Genotype	Phase	Total	Mosaic	Autonomy	Nonautonomy	Ambiguous
FM7/svb × ring-X/Y	*svb/ring-X*	E	62	9	6	0	3
GLC *svb dsh × ring-X/Y*	*svb dsh/ring-X*	E	161	22	16	1	5
y w f/y w f × X/Y	*y w f/X*	A	81	15[a]	15	0	0
FM7/y w dsh f × X/Y	*y w dsh f/X*	A	230	37	24	0	13[b]

Numbers reflect results from single experiments; other experiments yielding similar results have been done in each case. Except when *ring-X* instability was used to generate mosaics, progeny of crosses were irradiated in first to second instar to induce mitotic recombination. Females bearing germ-line clones (GLC) are homozygous for the genotype indicated. Numbers refer only to the progeny class of interest (genotype), scored for mosaicism as embryos (E) or adults (A). Animals showing marked and unmarked tissue (mosaic), were evaluated for autonomy or nonautonomousy of the *dsh*, *shavenbaby* (*svb*), *yellow* (*y*), *white* (*w*), or *forked* (*f*) loci. Some mosaics were ambiguous: in embryos, because mosaic borders could not be seen due to folding, and in adults, because of cell death or lack of scorable phenotype. Data shown are for *dsh^{v26}*, but similar ratios were obtained with other lethal alleles. Clones indicated are the results of a single experiment, in which both genotypes were irradiated simultaneously.
[a]The frequency of clones wild type for *dsh* was reproducibly higher than clones mutant for *dsh* (lethal alleles), in this case 27% vs. 16%. This is due at least in part to the absence of *dsh* clones in distal-most portions of legs and antennae, in which *dsh* is required for viability as judged from mutant phenotypes.
[b]All *dsh* clones were consistent with autonomy; however, large clones in the lower legs and antennae were usually associated with pattern regulation due to cell death or inappropriate fate (see text). In a separate analysis of clones in the leg, 0/47 control *y f* clones were associated with pattern defects. Among *y dsh^{v26} f* clones, 10/12 ventral clones and 0/14 dorsal clones were associated with pattern abnormalities, such as missing bristles and outgrowths from the plane of the leg.

Figure 4. Clonal analysis of *dsh* in imaginal development. Genetically marked clones of *dsh* mutant cells in heterozygous females. (*A*) *y dsh^{v26} f^{36a}* clone, first leg femur (lateral view). The clone of mutant cells (within broken line) is marked by *yellow* and *forked*, resulting in bent bristles of light pigmentation (small arrowhead). In lateral or dorsal (D) locations, clones of *dsh* mutant cells (large arrowhead) do not result in any abnormal patterning. A dorsal pattern element is indicated by the open triangle. The ventral (V) face is indicated. (*B*) *y dsh^{v26} f^{36a}* clone, first leg femur (lateral view). The ventral clone (large arrowhead; below broken line) of *dsh* mutant cells in the leg is shown, occurring on a duplicated appendage. The cells on the supernumerary tissue secrete only mutant bristles (small arrowhead). (△, ▲) Dorsal (D) and ventral (V) markers, respectively. (*C*) *y dsh^{v26} f^{36a}* clone, second leg tibia (lateral view). Triplication of tarsal segments resulting from clone (within broken line) at distal end of tibia. The axis of mutant tissue is marked by bristle markers (small arrowhead), forming a truncated appendage (large arrowhead). A second ectopic axis is indicated by the arrow and appears to consist entirely of wild-type cells with two ventral surfaces, indicated by the thick, peg-like bristles on either side. The normal basitarsus extends to the *left*. The ventral (V) face is characterized by thick, peg-like bristles. (▲) Indicative ventral pattern elements; (△) dorsal (D). (*D*) *y dsh^{v26} f^{36a}* clone, third leg basitarsus (ventral (V) view). A supernumerary appendage grows out of the ventral face, associated with a large ventral and ventrolateral *dsh* mutant clone (large arrowhead), as indicated by the distribution of marked bristles (small arrowhead). The clone and duplication (below broken line) lie immediately adjacent to the ventral-most cells, which secrete large, thick bristles (▲). (*E*) *y dsh^{v26} f^{36a}* clone, anterior wing margin (dorsal view). The mutant cells are revealed by their marked hairs (above broken line). Wild-type cells, with straight hairs (arrow), do not occur between the ventral (medium arrowheads) and medial (large arrowheads) bristles. These bristles normally abut each other, with no intervening hair cells, as seen in the more ventral view of a wild-type wing (inset). This clone has displaced the medial bristles to the level of the dorsal bristle row (small arrowheads). (*F*) *y dsh^{v26} f^{36a}* clone (dorsal thorax, anterior up). The *dsh* lesion disrupts the polarity of hairs, each of which is the product of a single cell. The *f^{36a}* mutation makes hairs slightly thin and bow-shaped but does not alter their polarity. The hairs in this clone, only 4–6 cells wide, show the *dsh* phenotype, in that their polarity is chaotic. Adjacent wild-type hairs are unaffected. The mutant region lies between the broken lines. The large structures are microchaetae. [The patterning defects shown were observed regardless of whether mutant clones were induced following X-ray irradiation or by site-specific recombination.]
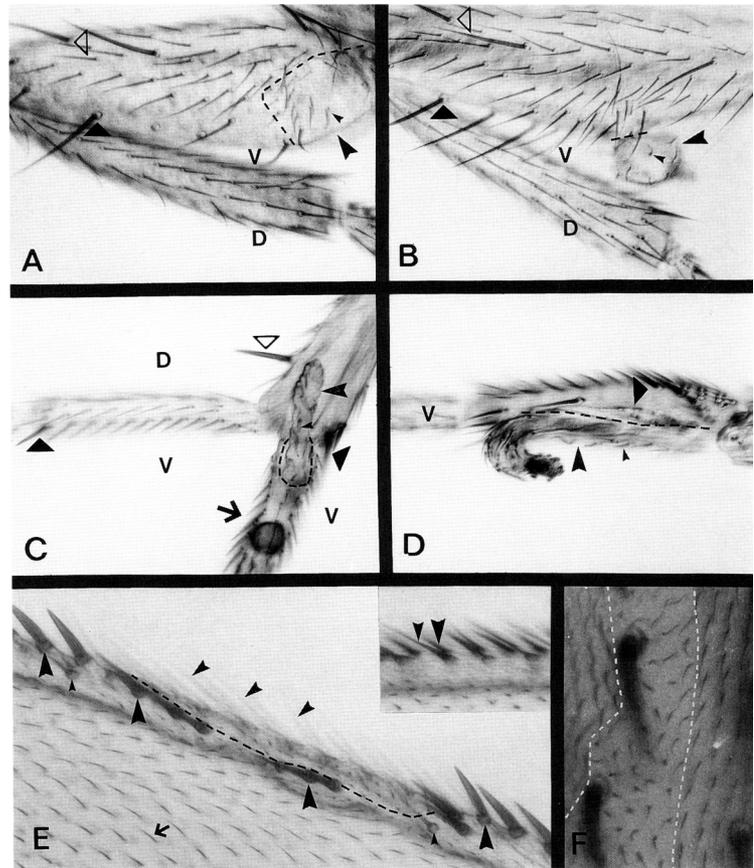
required in the leg by ventral cells, among which it is necessary for patterning but not viability.

dsh *and* wg *function in patterning of the wing margin* In the wing discs of late larvae, *wg* is expressed in a narrow strip of cells that form the wing margin (Baker 1988b; Phillips and Whittle 1993). A predifferentiation property of these cells is that they stop proliferating sooner than other cells in the disc, forming a "zone of non-cell proliferation" (O'Brochta and Bryant 1985). Experiments involving a temperature-sensitive allele of *wg* reveal that *wg* is required to make these cells quiescent (Phillips and Whittle 1993). We tested whether null alleles of *dsh* might result in wing margin defects by inducing clones of mutant cells during larval stages. When clones of cells lacking *dsh* occur at the margin of otherwise wild-type wings, the normal distribution of pattern elements at the wing margin is altered in a manner sug-

gestive of excess cell proliferation. For example, the anterior wing margin is decorated with a triple row of bristles (Hartenstein and Posakony 1989), with one or no hair-secreting cells occurring between the ventral and medial rows (Fig. 4E, inset). *dsh* clones in this region result in supernumerary cells at the margin. The extra cells are mutant, as indicated by a genetically linked hair morphology marker, whereas the surrounding bristle and hair cells are wild type (Fig. 4E). These experiments suggest that, like *wg*, *dsh* is required for normal proliferation and patterning of cells at the wing margin.

dsh *is cell autonomous*

The experiments described show that *dsh* is involved in *wg*-mediated patterning in multiple tissues throughout development. In each instance we studied, the defects of
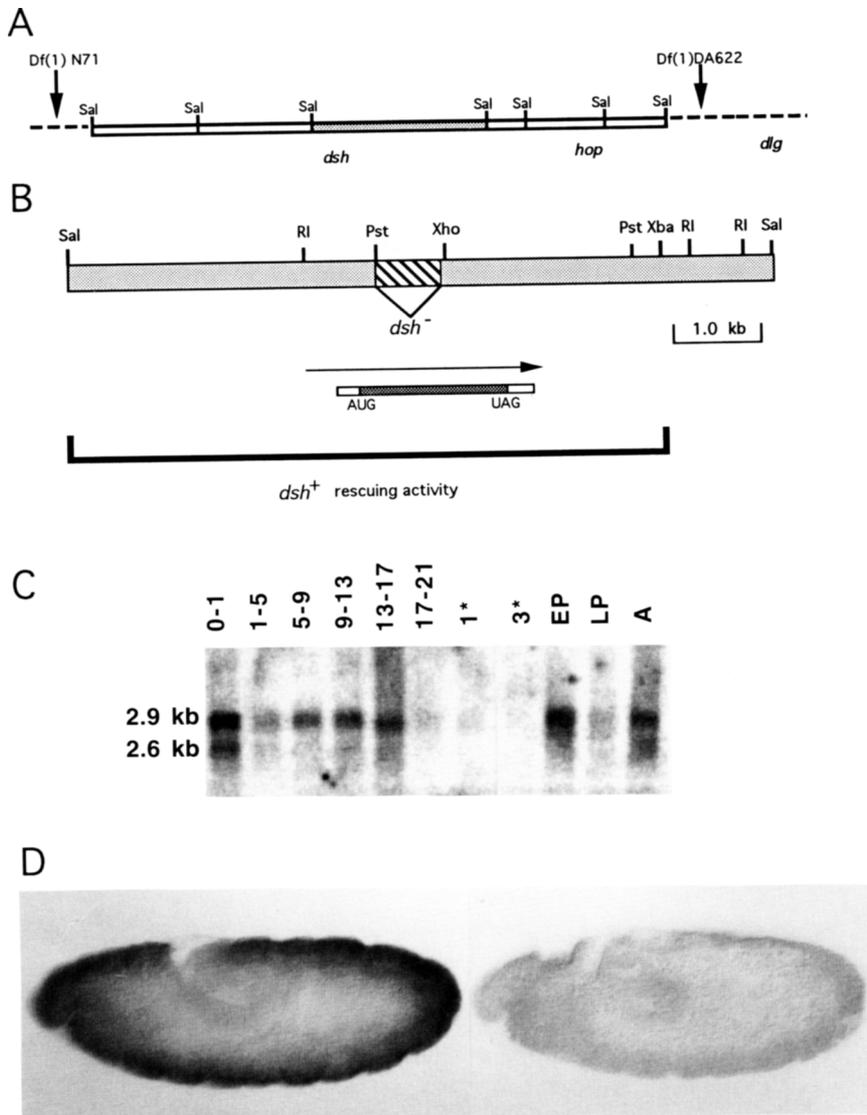
Figure 5. Molecular cloning and expression of *dsh*. (*A*) Genetic map of the *dsh* region. *dsh* maps to the 10B6 region of polytene chromosomes between the deficiency breakpoints of *Df(1)N71* and *Df(1)DA622*, just distal to *hopscotch* (Perrimon and Mahowald 1987). We used a series of *Sal*I fragments extending distally from *hop* to probe genomic DNAs of *dsh* alleles for rearrangements. (*B*) Structure of the *dsh* locus. A 7.9-kb *Sal*I fragment detected a change in *dsh^{v26}*, which proved to be a deletion of ~600 bp (hatched box). A 0.65-kb *Pst*I–*Xho*I fragment spanning the deletion was used to probe developmental Northern blots and to screen an embryonic cDNA library. The extents of the transcript detected, the corresponding largest cDNA, and the ORF within it are indicated. On the basis of this mapping data, we designed a P-element rescue construct that contained a 6.7-kb *Sal*I–*Xba*I fragment as shown below. All lines tested rescued all *dsh* phenotypes. (*C*) Developmental profile of *dsh* expression. The blot is loaded with poly(A$^+$)-selected RNA derived from animals of the indicated ages (in hr) and probed with the *dsh* cDNA (dc2.6). The messages detected are exactly those detected by the 0.65-kb genomic fragment used to screen the cDNA library. A 2.9-kb transcript is expressed throughout development, falling to low levels during larval stages. A 2.6-kb transcript is strictly maternal. Time points: (1*) first instar; (3*) third instar; (EP) early pupae; (LP) late pupae; (A) adults—both males and females. All lanes are approximately equally loaded except 13–17 hr, which is overloaded by about twofold. (*D*) Whole-mount in situ hybridizations with *dsh* (*left*) and vector (*right*) probes, stage 11 embryos. The *dsh* probe was a 650-bp *Pst*I–*Xho*I fragment, which hybridizes to a transcript uniformly distributed in the embryo, at highest levels in the epidermis. The control probe was a fragment derived from the vector pBSK, prepared and hybridized in parallel.

dsh are consistent with the interpretation that *dsh* mutant cells are unable to respond to the cell fate signals specified by *wg* and thus adopt an inappropriate fate. If so, *dsh* would be expected to behave cell-autonomously; that is, a given cell in a responsive tissue would require *dsh* to achieve its normal fate, regardless of its neighbors. We tested the cellular requirement for *dsh* via mosaic analysis.

To determine the cellular requirement for *dsh* in embryos we examined the phenotype of large patches of mutant tissue in otherwise wild-type embryos. Such mosaics are comprised of both denticle belts in wild-type pattern and a patch of cuticle covered with denticles in

disorganized array (Table 1; Fig. 1E), showing that the mutant tissue has not been rescued. For comparison, we made similar chimeric embryos for *arm* (data not shown), using an allele with the same developmental genetics and phenotype as *dsh* and that functions cell autonomously in embryos (Klingensmith et al. 1989). We observed the same kind of mosaics in both cases, suggesting that *dsh* is also cell autonomous. However, the clone boundary among denticle-secreting cells cannot be determined in these embryos. To make this determination, we marked mutant cells with the cell-autonomous cuticle marker *shavenbaby* (*svb*) (Table 1; Fig. 1D,F), which decreases the size and number of denticles (Ger-

gen and Wieschaus 1986). Examination of *svb,dsh* mosaics reveals that genotypically mutant tissue is phenotypically mutant as well (Table 1), in that *svb* denticles always occur in the pattern seen in nonmosaic *svb,dsh* mutants (Fig. 1G). Although not scorable at single-cell resolution, these mosaics strongly suggest that *dsh* is cell autonomous in its embryonic function.

To study the cellular requirement for *dsh* in imaginal development, we generated mosaics bearing small patches of *dsh* mutant tissue (Table 1). As described above, *dsh* pupae exhibit defects similar to those of *wg*; in addition, they have a tissue polarity defect in which the polarity of hairs and bristles is chaotic, seen most clearly on the dorsal thorax. Because a given hair is the product of a single epidermal cell (Poodry 1980; Mitchell et al. 1983), this feature can be used to determine *dsh* phenotype at the level of individual cells. Thoracic *dsh* clones show the tissue polarity defect, but adjacent wild-type cells have hairs of wild-type polarity (Fig. 4F). This holds true whether the clone spans hundreds of cells or a few cells, revealing that *dsh* acts cell autonomously in imaginal patterning.

The cells involved in abnormal pattern in the *dsh* clones in the wing margin and leg disc sometimes include wild-type cells, as described above. However, in both cases, the cause of the abnormality appears to be a cell-autonomous defect in the cells lacking *dsh*. In the wing margin, only *dsh* cells occur ectopically, resulting in a displacement of the bristle cells normally residing there. In the leg, pattern regulation seems to result from a failure of ventral *dsh* cells to behave in a manner consistent with their location (see Discussion).

Molecular identification of dsh

The most distal genomic phage clone from the *discs-large* (*dlg*; Woods and Bryant 1989, 1991; Fig. 5A) region provided a starting point for a chromosomal walk to clone the genomic DNA encoding *hopscotch* and *dsh* (Binari and Perrimon 1994). Restriction digests of the various alleles of *dsh* were probed with restriction fragments of genomic DNA extending from the *hop* region toward the distal *N71* breakpoint (Fig. 5A). One allele, *dsh*[v26], carries a deletion of ~600 bp relative to its parental chromosome (Fig. 5B; data not shown). A probe corresponding to the deleted region hybridizes to messages of 2.9 and 2.6 kb among RNA derived from very early embryos, before transcription of the zygotic genome. The larger transcript is detected also in RNA from later stages (Fig. 5C). Mapping of these transcripts allowed design of a P-element rescue construct (Fig. 5B). Each of the five independently derived transgenic lines tested rescued all *dsh* phenotypes to full viability and normal pattern.

A genomic restriction fragment encompassing the region deleted in *dsh*[v26] was used to screen an embryonic cDNA library (Materials and methods). On Northern blots, the cDNA inserts hybridized to the same transcripts detected by the genomic probe (Fig. 5C). Thus, the two transcripts encoded by the deleted region and encompassed by the rescuing construct are related. Although these are the only transcripts encoded entirely by the genomic fragment that rescues *dsh*, other transcripts hybridize to the outer ends of the fragment (data not shown). To demonstrate conclusively that *dsh* functions are encoded by the cDNAs isolated and not by truncated products from one of the other transcripts, we cloned the largest cDNA into a heat-inducible P-element expression vector (Materials and methods). Basal levels of expression from two separate transformants were sufficient to rescue all embryonic *dsh* phenotypes.

Whole-mount in situ hybridization experiments revealed that *dsh* message is distributed uniformly throughout the embryo through germ-band shortening (Fig. 5D). This is consistent with the observation that ubiquitous, low level expression of *dsh* permits completely wild-type embryonic development.

Sequence analysis of dsh *and structural implications* Sequencing of the rescuing cDNA revealed that it contains a single significant open reading frame (ORF; Fig. 6). The deduced amino acid sequence indicates a 623-amino-acid protein of 70 kD. This cDNA encodes a protein of ~70 kD when subjected to in vitro transcription and translation (J. Klingensmith, R. Nusse, and N. Perrimon, unpubl.).

The sequence implies several structural features of the protein (Fig. 6). A tract composed largely of glutamines corresponds to an *opa* repeat, as found in many *Drosophila* proteins (Wharton et al. 1985). Several regions conform to the criteria for PEST sequences, which are correlated with rapid turnover of proteins (Rogers et al. 1986). Three stretches of hydrophobic residues are sufficiently long to raise the issue of whether they span the membrane. However, their predicted structure and averaged hydrophobicity suggest that they probably do not (Kyte and Doolittle 1982; Rao and Argos 1986). In microsomal incorporation assays of the in vitro translation product, the Dsh protein does not appear to enter the secretory pathway (F. van Leeuwen and J. Klingensmith, unpubl.).

dsh *encodes a novel but conserved protein with limited homologies to junctional proteins* Searches of several data bases failed to find any reported sequence with extensive homology to the *dsh* ORF. However, genes highly homologous to *dsh* have been cloned in several vertebrates, including mouse (D. Sussman, J. Klingensmith, P. Salinas, R. Nusse, and N. Perrimon, in prep.), human (D. Beier, pers. comm.), and frog (S. Sokol, pers. comm.). Predicted structures of these homologous genes also fail to indicate what molecular function they might serve.

The only significant similarities detected in data base searches were to the *Drosophila* junctional protein Discs-large (Dlg) and its relatives. The block of homology is in the middle of the ORF, in the center of a long region of very high homology among all known Dsh cognates. The conserved motif is also found in postsynaptic density protein 95 (PSD-95) and erythrocyte membrane protein p55, which have one or more repeats of this domain, an SH3 domain, and a guanylate kinase domain

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   1 ATTCCGCTAGAGCGACCAGGGGGAGAAAAGGCAAAATCGAGTGAATTTTGTTTGTTGTAT   60
  61 CGGCTTTTCCCTGGCTTAATCCCGTTTTCCCTGTGATTTTTCCCCACCAACTTCCCACGC  120
 121 CACCTCCCCACCGCATCCATCGCATTTCGTTTTGATCGCGTTTTATCGCGTCGAGGAGTT  180
 181 TTCCCGTGGATTTCCGCAGTCGGCGCAGCTAAAATGGACGCGGACAGGGGCGGCGGGCAG  240
                                   M  D  A  D  R  G  G  G  Q
 241 GAGACGAAGGTGATATACCACATCGACGATGAGACGACGCCGTATCTGGTGAAGATCCCC  300
      E  T  K  V  I  Y  H  I  D  D  E  T  T  P  Y  L  V  K  I  P
 301 ATTCCATCCGCCCAGGTGACGCTGCGCGATTTCAAGCTGGTGCTGAACAAGCAGAACAAC  360
      I  P  S  A  Q  V  T  L  R  D  F  K  L  V  L  N  K  Q  N  N
 361 AACTACAAGTACTTCTTCAAGTCAATGGACGCCGATTTCGGTGTGGTCAAAGAGGAAATA  420
      N  Y  K  Y  F  F  K  S  M  D  A  D  F  G  V  V  K  E  E  I
 421 GCCGACGACTCCACCATACTGCCCTGCTTCAATGGGCGAGTGGTGTCCTGGCTGGTCTCC  480
      A  D  D  S  T  I  L  P  C  F  N  G  R  V  V  S  W  L  V  S
 481 GCCGATGGTACCAATCAGTCAGATAACTGCTCCGAGCTGCCAACCAGTGAGTGTGAATTG  540
      A  D  G  T  N  Q  S  D  N  C  S  E  L  P  T  S  E  C  E  L
 541 GGCATGGGTCTGACCAACAGGAAGCTACAGCAGCAACAGCAGCAGCATCAGCAGCAGCAA  600
      G  M  G  L  T  N  R  K  L  Q  Q  Q  Q  Q  Q  H  Q  Q  Q  Q
 601 CAACAACAGCAGCAGCAGCACCAGCAACAGCAACAACAACAGCAGCAGCAGGTGCAGCCT  660
      Q  Q  Q  Q  Q  H  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  V  Q  P
 661 GTCCAGCTGGCGCAGCAGCAACAGCAGCAGGTGCTCCATCACCAGAAGATGATGGGCAAT  720
      V  Q  L  A  Q  Q  Q  Q  Q  V  L  H  H  Q  K  M  M  G  N
 721 CCGCTGCTGCAGCCGCCACCGCTCACATATCAATCGGCATCCGTGCTCTCCAGCGATCTG  780
      P  L  L  Q  P  P  P  L  T  Y  Q  S  A  S  V  L  S  S  D  L
 781 GACTCGACCAGTCTCTTTGGCACCGAATCCGAATTGACGCTGGACCGCGACATGACCGAC  840
      D  S  T  S  L  F  G  T  E  S  E  L  T  L  D  R  D  M  T  D
 841 TATAGCAGCGTTCAGCGATTGCAGGTGCGCAAGAAGCCGCAGAGGCGCAAAAAACGGGCG  900
      Y  S  S  V  Q  R  L  Q  V  R  K  K  P  Q  R  R  K  K  R  A
 901 CCCAGCATGTCGCGCACCTCCTCGTACAGCTCCATAACCGACTCGACCATGTCCCTAAAT  960
      P  S  M  S  R  T  S  S  Y  S  S  I  T  D  S  T  M  S  L  N
 961 ATCATTACCGTCTCCATCAACATGGAGGCGGTCAACTTTCTGGGCATATCCATTGTCGGG 1020
      I  I  T  V  S  I  N  M  E  A  V  N  F  L  G  I  S  I  V  G
1021 CAATCGAATCGCGGCGGCGACGGCGGCATCTACGTGGGCAGCATCATGAAGGGCGGCGCA 1080
      Q  S  N  R  G  G  D  G  G  I  Y  V  G  S  I  M  K  G  G  A
1081 GTGGCCACTCGATGGACGCATCGAACCGGGCGACATGATCCTCCAAGTGACGATGTCTAAC 1140
      V  A  L  D  G  R  I  E  P  G  D  M  I  L  Q  V  N  D  V  N
1141 TTCGAGAACATGACCAACGACGAGGCGGTACGTGTGCTGAGAGAAGTCGTTCAGAAGCCG 1200
      F  E  N  M  T  N  D  E  A  V  R  V  L  R  E  V  V  Q  K  P
1201 GGACCCATCAAGCTGGTGGTGGCCAAGTGCTGGGACCCAAATCCCAAGGGCTACTTCACC 1260
      G  P  I  K  L  V  V  A  K  C  W  D  P  N  P  K  G  Y  F  T
1261 ATTCCGCGCACGGAGCCGGTGCGACCCATCGATCCCGGTGCTTGGGTGGCGCACACTCAG 1320
      I  P  R  T  E  P  V  R  P  I  D  P  G  A  W  V  A  H  T  Q
1321 GCCCTCACATCGCACGACAGTATTATTGCCGACATTGCGGAGCCGATTAAGGAGCGACTG 1380
      A  L  T  S  H  D  S  I  I  A  D  I  A  E  P  I  K  E  R  L
1381 GACCAGAATAACCTCGAGGAGATCGTTAAGGCGATGACGAAGCCGACAGCGGCCTGGAG 1440
      D  Q  N  N  L  E  E  I  V  K  A  M  T  K  P  D  S  G  L  E
1441 ATCAGGGATCGCATGTGGCTAAAGATAACGATTCCCAATGCATTTATCGGCGCCGATGCG 1500
      I  R  D  R  M  W  L  K  I  T  I  P  N  A  F  I  G  A  D  A
1501 GTCAATTGGGTGCTGGAGAATGTGGAGGATGTTCAGGATAGGCGGGAGGCGCGAAGGATC 1560
      V  N  W  V  L  E  N  V  E  D  V  Q  D  R  E  A  R  R  I
1561 GTCTCGGCGATGCTGCGCAGCAATTACATCAAGCATACGGTCAATAAGTTGACCTTCTCG 1620
      V  S  A  M  L  R  S  N  Y  I  K  H  T  V  N  K  L  T  F  S
1621 GAGCAGTGCTACTACGTGGTGAACGAGGAGCGCAATCCCAATCTGTTGGGCCGAGGACAT 1680
      E  Q  C  Y  Y  V  V  N  E  E  R  N  P  N  L  L  G  R  G  H
1681 CTGCATCCGCACCAGCTGCCGCACGGGCATGGCGGCCATGCGCTTAGCCATGCGGACACC 1740
      L  H  P  H  Q  L  P  H  G  H  G  G  H  A  L  S  H  A  D  T
1741 GAGAGCATCACCAGCGACATCGGGCCGCTACCGAACCCGCCCATTTATATGCCATATTCG 1800
      E  S  I  T  S  D  I  G  P  L  P  N  P  P  I  Y  M  P  Y  S
1801 GCCACGTACAATCCAAGTCACGGCTATCAGCCGATCCAGTACGGCATCGCCGAGCGACAC 1860
      A  T  Y  N  P  S  H  G  Y  Q  P  I  Q  Y  G  I  A  E  R  H
1861 ATCTCGTCGGGATCGAGTAGCAGCGATGTGCTGACCAGCAAGGATACCTCGGCGTCGCAG 1920
      I  S  S  G  S  S  S  D  V  L  T  S  K  D  T  S  A  S  Q
1921 AGCGACATCACCTCGGTGATCCATCAGGCCAACCAGCTGACCATCGCCGCTCATGGCTCT 1980
      S  D  I  T  S  V  I  H  Q  A  N  Q  L  T  I  A  A  H  G  S
1981 AACAAATCATCCGGCTCCTCCAATCGGGGCGGCGGCGGAGGCGGTGGCGGTGGCGGCAAC 2040
      N  K  S  S  G  S  S  N  R  G  G  G  G  G  G  G  G  G  N
2041 AATACCAACGATCAGGACGTATCCGTATCTAATTACGTATTGTAGAAACCTTTCTTTTTG 2100
      N  T  N  D  Q  D  V  S  V  S  N  Y  V  L  *
2101 CAATGTGTTTTTAATTAATTAAATTAAATATGTACCGGATTATTCTTTTTTATATACGAG 2160
2161 TATACAAGCAGCAACAGCAACCACAATATAATAATTATAATTTATAATAATAATAATAATA 2220
2221 ATAATTATAATAATAATAATAATACTTATGTATATGTATGGTATGTGTAAGTCATAGAAA 2280
2281 AGAGCTAACACCAGCGCACGAGTCAGGATCAGCGAGGCTCGTGAGCAGCAGAGATCAGCA 2340
2341 CAGGATATCGTGTCGTCGCAGCATCGTGTGACCGATCAGATCAGATCAACTGAGATATAT 2400
2401 GATATATGATATATATATATTATTATTATATTATTATATATATATATATATATGTGT 2460
2461 TTGAGTGCAGGACGGATGATGCGGAATGGAGCGGAAGAGCAGGACGGCAGCATGACGGGC 2520
2521 GAGAATGATTTTGAATAAAGTTCTAAGAATTTAGTAATCCAAAGTAAATAAATACAAATA 2580
2581 AAATGAAAAAAAAAAAAAAGGA  2602
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Figure 6. The deduced Dsh amino acid sequence. The nucleotide sequence of the *dsh* cDNA dc2.6 is shown, along with its only significant ORF. At the 3′ end are three potential polyadenylation signals (bold), followed by a string of 14 As (underlined). At the 5′ end, the first methionine of the ORF (and of the cDNA) is preceded by a good match (bold) to the *Drosophila* translational start site consensus (C/A A A C/A; Cavener 1987). The ORF encodes a putative protein of 623 residues. An Opa repeat extends from residues 118 to 159 (italics). Several stretches conform to the criteria for PEST sequences. Two long runs of hydrophobic residues occur near the middle of the protein. Within this region is a domain of homology to Dlg and its relatives (underlined), extending from residues 274–308. There are numerous potential post-translational modification sites, including those for certain serine–threonine kinases, for amino-linked glycosylation, and for myristilation. One region of basic residues (224–228) is similar to that of nuclear localization signals.

(Bryant and Woods 1992; Cho et al. 1992). Dsh and its homologs have neither of these other domains. A less conserved version of this domain is present in the gene encoding nitric oxide synthase (bNOS), which is otherwise unrelated to the other genes (Bredt et al. 1991). The alignment of these sequences is shown in figure 7 with a consensus sequence.

Discussion

dsh *functions in the response of cells to the* wg *signal*

Throughout development, lack of *dsh* function results in defects similar to those seen in *wg* mutants. Several lines of evidence strongly support the hypothesis that the role of *dsh* in *wg* signaling is to allow target cells to respond and that *dsh* therefore functions in the reception or interpretation of the *wg* signal. First, *dsh* functions cell autonomously; a cell devoid of *dsh* function has the mutant fate. Because *wg* function is nonautonomous (Morata and Lawrence 1977; Babu and Bhat 1986; Baker 1988a; Struhl and Basler 1993), genes that are involved in signal production would also be expected to be nonautonomous, whereas genes involved in responding to the signal are expected to be cell autonomous. The *zw3* gene has been shown genetically to function downstream of *wg* in regulating *en* expression (Siegfried et al. 1992) and is cell autonomous (Perrimon and Smouse 1989; Simpson et al. 1988).

We have presented several experiments demonstrating or supporting the cell-autonomy of *dsh*. The best demonstration is the tissue polarity phenotype of mutant *dsh* clones in the adult thorax. Even very small clones of only a few cells have abnormal polarity, indicating that *dsh* is cell autonomous. However, because this phenotype may be unrelated to *wg* function, we sought independent evidence that *dsh* functions cell autonomously in *wg*-dependent processes. To study the requirement for *dsh* in embryonic segmentation, we made large patches of mutant cells and studied their denticle pattern. Mutant cells exhibit the mutant pattern, and wild-type cells contribute to wild-type pattern. *dsh* cells appear to behave in a cell-autonomous manner in *wg*-mediated leg and wing development as well, but the defects of mutant clones most likely result in local pattern regulation (see below).

More data consistent with the idea that *dsh* functions in the target cells of *wg* come from immunohistochemical experiments. Wg protein is expressed in *dsh* embryos in an entirely normal pattern until after En has decayed. Under light microscopy, *dsh* mutants have no effect on distribution of Wg protein prior to its deterioration. The temporal and spatial pattern of En decay is identical in *dsh* and *wg* embryos. The level or distribution of Arm protein is also regulated by *wg* and *dsh* (Riggleman et al. 1990), and it in turn is necessary for the effects of *wg* on *en* expression and cuticle patterning (Klingensmith et al. 1989; Noordermeer et al. 1994). *dsh* is upstream of *zw3*, whereas *arm* is downstream (Siegfried et al. 1994). These genes are all cell autonomous, consistent with their apparent function in response to

Figure 7. Homology to the "undefined domain" of Dlg. Alignment of several sequences displaying homology to the undefined domain of Dlg, listed in order of decreasing similarity to Dsh, as indicated (*right*). The location of the first residue indicated for each sequence within its ORF is indicated at *left*. The mouse *dishevelled-1* homolog (Dvl) is virtually identical to Dsh in this region. For Dlg and its rat homologs, PSD-95, and erythrocyte major

		Similarity
dsh-275	GIYVGSIMKGGAVALDGRIEPGDMILQVNDVNFENMTNDEAVRVLREV	
Dvl-275	GIYIGSIMKGGAVAADGRIEPGDMLLQVNDVNFENMSNDDAVRVLREI	96%
dlg-508	GIYVSFILAGGPADLGSELKRGDQLLSVNNVNLTHATHEEAAQALKTS	67%
PSD95-188	SIYVTKIIEGGAAHKDGRLQIGDKILAVNSVGLEDVMHEDAVAALKNT	64%
bNOS-40	PVIISDLIRGGAAEQSGLIQAGDIILAVNDRPLVDLSYDSALEVLRGI	62%
p55-93	SCTVARILHGGMIHRQGSLHVGDEILEINGTNVTNHSVDQLQKAMKET	57%
consensus	G G iy V I GGa V G I GD I L VN vn FEN S D A V L R
	S I A L L LTD T E A K

palmitylated protein p55, only the most homologous of the three repeats of this domain in each sequence are shown. The domain is also found in bNOS. This region has been refered to as the "GLGF" domain by Cho et al. (1992), which occurs several amino acids prior to the region shown; however, the GLGF residues are not conserved in the Dsh, Dvl, or p55 proteins. A consensus sequence of residues conserved in five or more (uppercase letters) or a majority (lowercase) of the domains is indicated below. In some cases, either of two residues occurs in a given position. We indicate both in the consensus.

the Wg signal. Moreover, all three are expressed uniformly in the embryonic epidermis (Riggleman et al. 1990; Siegfried et al. 1990), as expected based on the ability of all such cells to respond to Wg (Noordermeer et al. 1992).

wg *and* dsh *in imaginal patterning*

The phenotypes of pupal lethality of *wg* and *dsh* hypomorphic alleles are very similar, in that the distal derivatives of most discs fail to form or are truncated. Analysis of the consequences of *wg* under- or overproduction in the leg disc suggests that *wg* acts to promote ventral cell fates during early larval development of the leg discs (Couso et al. 1993; Struhl and Basler 1993). Clones of cells lacking either *arm* (Peifer et al. 1991) or *dsh* result in abnormal ventral patterning in the leg. This suggests that the pathway mediating *wg* signaling in the embryonic epidermis may also function in the imaginal epidermis. However, unlike *arm*, *dsh* is not required for cell viability in the ventral compartment of leg discs; rather, *dsh* appears to be required specifically for normal ventral patterning.

Whereas dorsal *dsh* clones in the proximal leg have no adverse effect on patterning, ventral clones are invariably associated with pattern irregularities. Typically, supernumerary appendages consisting of mutant or wildtype cells arise from the ventral face of the leg. These outgrowths are similar to those that arise out of dorsal aspects of legs in association with dorsal clones of cells overexpressing *wg*, whereas ventral clones of *wg* overexpression are inconsequential (Struhl and Basler 1993). These workers noted that supernumerary appendages of this nature result from grafting experiments in which dorsal and ventral leg tissues are directly juxtaposed (e.g., French 1978). They reasoned that if *wg* induces ventral fates, cells overexpressing *wg* in dorsal tissue would cause such a juxtaposition. Similarly, if *dsh* is required by cells to respond to the Wg signal, thereby becoming ventralized, then clones of cells lacking *dsh* in the ventral compartment would juxtapose wild-type, ventral and mutant, nonventral cells. The predicted consequence is observed: ventral supernumerary appendages.

We have presented several results indicating that *dsh* functions in a cell-autonomous manner, yet these ventral outgrowths often involve wildtype cells. Similarly, although *arm* functions cell autonomously (Wieschaus and Riggleman 1987; Klingensmith et al. 1989), *arm* clones can also give rise to nonautonomous patterning defects in the leg (Peifer et al. 1991). One possibility is that these genes do function in an anomalous, nonautonomous manner in this particular context. However, we suspect that the apparent nonautonomy is a secondary consequence of cell-autonomous defects followed by pattern regulation (French et al. 1976). In the case of *dsh*, this would be a consequence of inappropriate juxtapositions as described above, whereas in *arm* mosaics pattern regulation would occur among the cells remaining after death of the mutant *arm* cells (Peifer et al. 1991).

In clones mutant for a null allele of *dsh* induced through mid-third instar, we observe a displacement or absence of bristles normally at the margin, replaced by mutant cells that do not make bristles, sometimes straddling the dorsal–ventral border. Examination of the genotype of these cells, using linked cuticular markers, shows that the cells between the normal margin elements and the displaced elements are always mutant while the displaced bristles are always wild type. The data suggest that these *dsh* clones result in a cell-autonomous overproliferation of non-neural cells along the wing margin. If Wg is required as a signal to slow division at the wing margin (Phillips and Whittle 1993), cells lacking Dsh may be unable to respond.

Dsh *represents a novel signal transduction molecule*

We have presented evidence that *dsh* functions in the reception or interpretation of the Wg signal. Dsh may feed into a kinase-mediated signal transduction pathway to the nucleus since it functions upstream of *zw3*, a serine–threonine kinase (Siegfried et al. 1992, 1994) in Wg signaling. However, how *dsh* might act to transduce the Wg signal is not revealed or suggested by its sequence. Because Dsh doesn't appear to be an integral membrane protein, it seems unlikely that it encodes a transmembrane receptor.

The only detected homologies to Dsh are found in junction proteins. Dsh has a domain homologous to a repeated sequence found in Dlg and PSD95. Dlg encodes a *Drosophila* tumor suppressor gene that localizes to septate junctions and is thought to be involved in transduction of proliferation signals (Woods and Bryant 1991). PSD-95 is a major component of postsynaptic densities, which are junctions involved in the reception and transduction of synaptic stimuli (Cho et al. 1992). A more divergent homolog of these genes is erythrocyte membrane protein p55, which is also peripherally associated with the plasma membrane (Ruff et al. 1991; Bryant and Woods 1992). The conserved sequence homologous to Dsh in these genes, termed the "undefined domain" by Bryant and Woods (1992), occurs in three repeats in the amino-terminal half of Dlg and relatives. Their function is unknown, but in Dlg it is speculated that this general part of the protein may associate with junctions (Woods and Bryant 1991). The only other reported sequence with this motif is bNOS (Bredt et al. 1991), which is otherwise unrelated to either Dsh or Dlg. Interestingly, some bNOS is associated with neuronal membranes (Bredt et al. 1990). Because none of these proteins has other features suggesting membrane localization, it is tempting to speculate that the undefined domain motif promotes peripheral association with the plasma membrane and that Dsh might be found there.

The results presented here suggest that Dsh is very specific to Wg-mediated patterning events until late in imaginal development and that it is involved in the specific response of cells to the cell fate instructions dictated by *wg* in many or all cases. In contrast, *zw3* is involved in many other patterning mechanisms. For example, *zw3* is involved in lateral inhibition in the wing (Blair 1992) and also appears to function downstream of Notch, a cell-autonomous receptor-like protein (Heitzler and Simpson 1991; Ruel et al. 1993). These considerations suggest to us that Dsh renders a more general signal transduction pathway specific for Wg signaling. Possibly Dsh is part of a receptor complex specific to the Wg signal, with binding of the ligand activating a more general transduction pathway. We have no evidence that Dsh is not part of a receptor complex, although the structural data we have presented suggest it is not a receptor in itself. If Dsh is downstream of the actual receptor, then its specificity suggests that the receptor also should be highly specific to the Wg signal. Alternatively, it is possible that Dsh gives specificity to a general signal reception–transduction pathway at the intracellular level. Wg signal would then be bound by a general receptor, and Dsh would channel transduction of this particular stimulus through the pathway mediated by Zw3.

Materials and methods

Fly stocks

The origins of dsh^{v26}, dsh^{M20}, dsh^{VA153}, and dsh^1 are described in Perrimon and Mahowald (1987). Isogenic control chromosomes for v26 (v24 and v145) are reported in Geer et al. (1983). wg^{IN67} behaves as a null allele but makes a protein that appears

not to be secreted in transfected cells or in embryos (van den Heuvel et al. 1993b). The markers *yellow* (y^1), *white* (w^1) and *forked* (f^{36a}) are described by Lindsley and Grell (1968). Embryonic cuticles were marked with svb^{YP17b} (Gergen and Wieschaus 1986). Germ-line clones were produced using either ovo^{D1} or ovo^{D1}, FRT^{101}; FLP^{38}/FLP^{38} (Chou and Perrimon 1992) as described previously. The ring-X chromosome (maintained with $In(1)dl-49$, $y\ w\ lz/Yy^+$) was obtained from J. Hall (Brandeis University, Waltham, MA). Embryos were mounted for cuticle inspection as described by Wieschaus and Nüsslein-Volhard (1986). Wild-type embryos were *Canton-S* or *Oregon-R* (*Ore-R*). All embryos and flies were grown at 25°C.

Whole-mount embryo stainings and genotype identification

Embryos aged 5–20 hr for anti-Lab stainings or 2–8 hr for other stainings were prepared as described in van den Heuvel et al. (1989). Immunostainings with polyclonal antibodies against Wg (van den Heuvel et al. 1989) and Lab (T. Kaufmann, Indiana University, Bloomington, IN) and monoclonal antibodies against En (Gay et al. 1988), Arm (Peifer et al. 1992), and β-galactosidase (Promega) were performed as described in Noordermeer et al. (1992). Stained embryos were dehydrated and mounted for photography in methylsalicylate. Embryos were staged according to Wieschaus and Nüsslein-Volhard (1986).

For double stainings of embryos with anti-Wg and anti-En, embryos were stained overnight with both antibodies and then stained with goat anti-rabbit (Vector Laboratories) and developed with DAB (Sigma) to visualize Wg expression. A second overnight incubation with anti-*en* was followed by staining with horse anti-mouse antibody (Vector), with nickel (1 mg/ml) added to the DAB solution to yield a dark precipitate in the *en*-expressing cells.

Identification of mutant embryos in stainings for the Lab antigen was by visual inspection of the epidermis, which is highly abnormal by stages 13–15. *wg* and *dsh* embryos are devoid of segment furrows and have abnormal head morphologies.

To identify *dsh* mutant embryos unambiguously, females bearing germ-line clones of *dsh* were crossed with males carrying on their balancer X-chromosome a *fushi tarazu* (*ftz*)–*lacZ* gene fusion (FM7, *ftz*–*lacZ* obtained from P. Gergen, SUNY, Stony Brook, NY). Staining with anti-β-gal antibody (Promega) identified the paternally rescued (wild-type) progeny by their *ftz* stripes. *wg* embryos were identified similarly by use of a *hunchback* (*hb*)–*β-gal* balancer in crosses among heterozygotes (van den Heuvel et al. 1993b).

For double stainings with anti-Wg and anti-En, embryos prior to stage 11 were identified genetically by the status of En antigen, which disappears by stage 10 in *wg* and *dsh* mutants but remains until much later in wild type. From stage 11, *wg* and *dsh* mutants can be recognized morphologically, as the labial lobe does not develop.

Mosaic analysis

Germ-line clones were produced as described by Klingensmith et al. (1989) and Chou and Perrimon (1992). Embryonic gynandromorphs were among the progeny of ring-X/Y males crossed to females bearing either *dsh* or *arm* germ-line clones. Mosaic embryos were indicated by the presence of mutant and nonmutant denticles (as indicated by the denticle marker *svb*) or by the presence of filzkörper material, which never develops in *dsh* mutants.

Clones in adult structures were induced by mitotic recombination between heterozygous X chromosomes. Females heterozygous for a marked (*y w f*) *dsh* allele were mated to males

carrying a wild-type X chromosome. Progeny were irradiated 30–54 hr after laying (first to second instar) at a constant dose of 1000 rads of X-rays. Control clones were induced in the progeny of matings between females homozygous for *y w f* and wild-type males. Clones were scored on the dorsal thorax, head, legs, and wings under the dissecting microscope in 70% ethanol. Interesting specimens other than wings were dissected, incubated for 10 min in 10% KOH at 60°C, dehydrated, and mounted in Faure's mountant for viewing at higher magnification. Wings were dissected, dehydrated further, and mounted directly in Accumount (Baxter). Except in the eye, only clones showing the presence of *y* and *f* were considered, because distal crossovers between these markers might have eliminated the *dsh* allele. In the mosaic analysis of the legs, we used topology along the dorsoventral axis and, when possible, bristle morphology to score for ventral or dorsal patterning. Compartments and bristle patterns in the leg are discussed in Struhl and Basler (1993) and references therein.

Structural analysis of dsh *genomic DNA*

Genomic DNA from adult females was purified as described by Finkelstein et al. (1990). We first screened *dsh* alleles for structural differences in the *dsh* region relative to each other and the control chromosome *FM7c*, with which the alleles were balanced. DNA was digested with *Sal*I, *Hind*III, or *Eco*RI, electrophoresed, and Southern blotted. We used as probes a contiguous series of *Sal*I fragments from the region of the chromosomal walk immediately distal to the *hop* gene. We found that *dsh*[v26] contained a small deletion relative to isogenic controls [*v24* and *v145* (Geer et al. 1983)] of ~600 bp within a 7.9-kb *Sal*I fragment, starting just distal to the *Pst*I site 3.2 kb from the distal end and extending nearly to the *Xho*I site 0.65 kb farther proximal. Molecular techniques followed the protocols of Sambrook et al. (1989).

cDNA isolation and analysis

We used the 0.65-kb *Pst*I–*Xho*I fragment largely deleted in *dsh*[v26] to screen a 9- to 12-hr size-selected embryonic cDNA library (kindly provided by K. Zinn) as described by Finkelstein et al. (1990). The longest of 12 independently derived, related cDNAs was subcloned into pBSK (Stratagene) for sequencing and further analysis (pdc2.6). For in situ hybridizations, *Ore-R* embryos were collected overnight, fixed, and hybridized as described Tautz and Pfeifle (1989). The probe used was a 0.65-kb *Pst*I–*Xho*I fragment derived from the *dsh* cDNA. It was labeled via random priming using the Genius kit (Boehringer Mannheim). A negative control probe was a fragment derived from the vector plasmid pBSK.

DNA sequencing and sequence analysis

For sequencing, nested deletions in both strands were made in pdc2.6 using exonuclease III (Erase-a-Base kit, Promega). Double-stranded plasmid was used as template DNA. We used the T3 and T7 primers for DNA synthesis with ^{35}S and Sequenase (U.S. Biochemical Corp.) per the manufacturer's protocol. Sequence analysis utilized the GCG sequence analysis package (Devereux et al. 1984). Homology searches were performed using the BLAST (Altschul et al. 1990) and FAST (Lipman and Pearson 1985) algorithms on the GenBank, EMBL, and PIR data bases.

P-element transformation and rescue

We generated germ-line transformants carrying an ectopic copy of the *dsh* region by cloning the 6.7-kb *Sal*I–*Xba*I fragment of pC5S8 (Fig. 6) into the P-element vector pCasper4 (Thummel et al. 1988) and injecting the plasmid into precellular blastoderm embryos using standard techniques (Spradling 1986). Twelve lines were obtained. Males from five lines in which the insert was autosomal were crossed to virgin females of the genotype *FM7/y w dsh*[v26] *f*[36a]. Each line rescued the mutant phenotypes of *dsh* .

Transformants carrying the *dsh* cDNA under the control of a heat-inducible promoter were generated in the same manner, using as the injected material an *Eco*RI–*Eco*RV fragment of pdc2.6 (which contains the entire ORF) cloned into pCasperHS (C. Thummel, pers. comm.). Male transformants were crossed to virgin females bearing germ-line clones of *dsh*[v26] marked with *svb*. The presence of *dsh*[+],*svb*[−] larvae indicated rescue of all embryonic *dsh* phenotypes.

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Note added in proof

The sequence data for *dishevelled* has been deposited in the GenBank data library under accession number LZ6974.

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