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

John Klingensmith, Roel Nusse, and Norbert Perrimon

1Howard Hughes Medical Institute, Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115 USA;
2Howard Hughes Medical Institute, Department of Developmental Biology, Stanford University School of Medicine, Stanford, California 94305 USA

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.

[Key Words: Drosophila; wingless; dishevelled; signal transduction]

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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) (Bejsovec and Martinez-Arias 1991; Dougan and DiNardo 1992). wg, the Drosophila homolog of the secreted murine oncprotein 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], dishevelled (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 dark-field (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 denticles are less than half the length of their wild-type siblings. (B) dshv26 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 filzk6rper structure at the posterior and are missing all head structures. (C) wgM20/wgM20 embryo. The embryo displays the same phenotypes as the dsh mutant. (D) svb,dshv96 embryo. The svb mutation reduces the number and size of denticles, further revealing the metameric pattern of denticles persisting in this phenotype. (E) dshM20/ring-X mosaic embryo. Mosaicism is indicated by filzk6rper 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,dshv26/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 Wg-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; 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 2. Midgut patterning is affected similarly in dsh and wg mutants. Whole-mount lateral, parasagittal 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) wglN67/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) dshv26 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) wglN67/wg IN67 embryo, stage 14. Lab staining is less intense overall and occurs in a nongraded manner. (F) dshv26 embryo, stage 14. Lab staining is affected in the same way as in wg embryos.

![Figure](image-url)
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) dshM2~ 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, wgCX3/wgCX4). 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 [small arrow] is short and bulbous. Sex comb [large arrowhead] and claw [small arrowhead] are present, indicating presence of dorsal pattern. 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 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 sex comb [large arrowhead] is also normal. Ventral structures are absent, such as the peg-like bristles in the tarsal segments [open arrow]. (E) dshM2~/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) wgCX3/wgCX4 pupa [lateral view]. Axis of duplication is the same as in E.

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 nonautonomously 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 re6, 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.

"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.

"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 re6 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\(^{c26}\) \(^{p56}\) 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\(^{c26}\) \(^{p56}\) 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). (Δ, ▲) Dorsal (D) and ventral (V) markers, respectively. (C) y dsh\(^{c26}\) \(^{p56}\) 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; (Δ) dorsal (D). (D) y dsh\(^{c26}\) \(^{p56}\) clone, third leg basitarsus (ventral [V] view). A supernumerary appendage grows out of the ventral face, associated with a large ventral and ventralateral 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\(^{c26}\) \(^{p56}\) 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\(^{c26}\) \(^{p56}\) clone (dorsal thorax, anterior up). The dsh lesion disrupts the polarity of hairs, each of which is the product of a single cell. The \(^{p56}\) 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 suggestive 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
**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 dentine-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 [Poody 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

A genomic restriction fragment encompassing the region deleted in dsh

**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.
Figure 6. The deduced Dsh amino acid sequence. The nucleotide sequence of the \textit{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 consensus sequence.

\textbf{Discussion}

d\textit{sh} functions in the response of cells to the \textit{wg} signal

Throughout development, lack of \textit{dsh} function results in defects similar to those seen in \textit{wg} mutants. Several lines of evidence strongly support the hypothesis that the role of \textit{dsh} in \textit{wg} signaling is to allow target cells to respond and that \textit{dsh} therefore functions in the reception or interpretation of the \textit{wg} signal. First, \textit{dsh} functions cell autonomously; a cell devoid of \textit{dsh} function has the mutant fate. Because \textit{wg} function is nonautonomous (\textit{Mora} and \textit{Lawrence} 1977; \textit{Babu} and \textit{Bhat} 1986; \textit{Baker} 1988a; \textit{Struhl} and \textit{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 \textit{wg} in regulating \textit{en} expression (\textit{Siegfried} et al. 1992) and is cell autonomous (\textit{Perrimon} and \textit{Smouse} 1989; \textit{Sim-

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

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

**Figure 7.** Homology to the “undefined domain” ofDlg. 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 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 domain has been referred 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 only detected homologies to Dsh are found in junction proteins. Dsh has a domain homologous to a repeated sequence found inDlg and PSD95. Dsh 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 ofDlg 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 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 dshvu26, dshvu20, dshV1A153, and dshb are described in Perrimon and Mahowald [1987]. Isogenic control chromosomes for v26 (v24 and v145) are reported in Geer et al. [1983]. wgDI67 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), white (w) and forked (fka) are described by Lindsay and Grell [1968]. Embryonic cuticles were marked with svbF7b [Gergen and Wieschaus 1986]. Germ-line clones were produced using either ovary1 or ovary2, FRT201, FLP93, FLP98 [Chou and Perrimon 1992] as described previously. The ring-X chromosome [maintained with int(1)dl-49, y,w,I/Y+] 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 Nüsslein-Volhard 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 non-mutant 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 fl 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. Inter-
esting 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 Ac-
cumount (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. Compartment 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 screen 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 SalI, HindIII, or EcoRI, electrophoresed, and Southern blotted. We used as probes a contiguous series of SalI fragments from the region of the chromosomal walk immediately distal to the hop gene. We found that dsh26 contained a small deletion relative to isogenic controls [v24 and v145] of ~600 bp within a 7.9-kb SalI fragment, starting just distal to the PstI site 3.2 kb from the distal end and extending nearly to the XhoI 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 PstI–XhoI fragment largely deleted in dsh26 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 by Tautz and Pfeifle [1989]. The probe used was a 0.65-kb PstI–XhoI 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 [3S] 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.
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130 GENES & DEVELOPMENT
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