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dishevelled is required during *wingless* signaling to establish both cell polarity and cell identity

Heidi Theisen, Judith Purcell, Mary Bennett, Dipul Kansagara, Adeela Syed and J. Lawrence Marsh*

Developmental Biology Center and Department of Developmental and Cell Biology, University of California Irvine, Irvine, CA 92717, USA

*Author for correspondence

SUMMARY

The *dishevelled* gene of *Drosophila* is required to establish coherent arrays of polarized cells and is also required to establish segments in the embryo. Here, we show that loss of *dishevelled* function in clones, in double heterozygotes with *wingless* mutants and in flies bearing a weak *dishevelled* transgene leads to patterning defects which phenocopy defects observed in *wingless* mutants alone. Further, polarized cells in all body segments require *dishevelled* function to establish planar cell polarity, and some *wingless* alleles and *dishevelled*; *wingless* double heterozygotes exhibit bristle polarity defects identical to those seen in *dishevelled* alone. The requirement for *dishevelled* in establishing polarity is cell autonomous. The *dishevelled* gene encodes a novel intracellular protein that shares an amino acid motif with several other proteins that are found associated with cell junctions. Clonal analysis of *dishevelled* in leg discs provides a unique opportunity to test the hypothesis that the *wingless dishevelled* interaction specifies at least one of the circumferential positional values predicted by the polar coordinate model. We propose that *dishevelled* encodes an intracellular protein required to respond to a *wingless* signal and that this interaction is essential for establishing both cell polarity and cell identity.

Key words: *Drosophila* development, *dishevelled* gene, cell polarity, *wingless* signaling, pattern formation, polar coordinate model, *armadillo* gene, segmentation

INTRODUCTION

The *dishevelled* gene (*dsh*) is required for at least two functions in *Drosophila* development, namely, establishment of coherent arrays of polarized cells (Fahmy and Fahmy, 1959; Gubb and Garcia-Bellido, 1982) and establishment of embryonic segments (Perrimon and Mahowald, 1987). Polarity, as used in this communication, refers not to apical basal polarity but to the planar polarity exhibited by many cells of an epithelium. Planar polarity is evident in cells producing bristles and hairs which, in *Drosophila*, are cytoskeletal extensions of individual cells (Mitchell et al., 1983) and thus reflect the polarity of the cells that extend them (Piepho, 1955). In every region of every body segment, bristles and hairs are arranged in coherent arrays of defined polarity (Piepho, 1955; Gubb and Garcia-Bellido, 1982). Establishment of this polarity appears to involve a cell signaling event requiring at least the *dsh*, *frizzled* (*fz*) and *spiny legs* (*sple*) genes (Fahmy and Fahmy, 1959; Gubb and Garcia-Bellido, 1982; Adler et al., 1990; Vinson et al., 1989; Wong and Adler, 1993).

Complete loss of *dsh* (both maternal and zygotic) also produces a segment polarity defect indistinguishable from that seen in *wingless* (*wg*) embryos (Perrimon and Mahowald, 1987). Other genes that affect the same domain in each segment include the *wingless, dishevelled, armadillo (arm), porcupine (porc), gooseberry, hedgehog, cubitis interruptus*^{D} and *fused* genes (reviewed by Peifer and Bejsovec, 1992; Hooper and Scott, 1992). The *wingless* gene encodes a secreted protein (WG) (Rijsewijk et al., 1987; van den Heuvel et al., 1989), which provides a key signal during establishment of embryonic segment polarity (Nusslein-Volhard and Wieschaus, 1980; van den Heuvel et al., 1989; Bejsovec and Martinez Arias, 1991; Gonzalez et al., 1991; Peifer et al., 1991) and in patterning adult structures (Morata and Lawrence, 1977; Baker, 1988a; Struhl and Basler, 1993). Since the embryonic phenotypes of *wingless, armadillo, dishevelled* and *porcupine* are indistinguishable, it is postulated that these genes may be involved in *wingless* signal transduction in the embryo (Wieschaus et al., 1984; Perrimon et al., 1989; Klingensmith et al., 1989; Peifer and Bejsovec, 1992; Peifer et al., 1991). Although *porcupine* has not been described molecularly, activity of the *porc* gene is non-autonomous in mosaics and mutations prevent release of *wingless* from the *wingless*-producing cells; thus, *porc* acts 'upstream' of *wingless* (Klingensmith and Perrimon, personal communication). The *arm* gene encodes a β-catenin homologue and is expressed in all cells within the embryonic segment and expressed widely in discs (Peifer and Wieschaus, 1990; McCrea et al., 1991; Peifer et al., 1992) (Morata and Lawrence, 1977; Rijsewijk et al., 1987; Baker, 1988a; Struhl and Basler, 1993). It is required in

a cell autonomous manner for response to the *wingless* signal during adult patterning and thus is placed downstream of the *wingless* signal (Peifer et al., 1991).

The extent to which pattern formation and the emergence of tissue polarity share genetic elements is not clear, nor is the nature of the patterning information transmitted by the WG signal although the latter topic has received considerable discussion (Wilkins and Gubb, 1991; Hooper and Scott, 1992; Bryant, 1993; Cohen, 1993). We have cloned and sequenced the *dsh* gene and investigated the developmental requirements of *dsh* function. Clones of *dsh*, *dsh; wg* double heterozygotes, and *wg*/+ heterozygotes in combination with a reduced function dsh^{w} transgene, all cause adult pattern abnormalities that mimic those seen in *wingless* mutations. The patterning abnormalities can be understood in terms of a polar coordinate system of pattern formation where loss of *dsh* (and/or *wg*) function leads to loss or mis-specification of circumferential positional values followed by regulative growth (French et al., 1976; Bryant et al., 1981). We propose that *dsh* encodes an intracellular protein essential for interpretation of the *wingless* signal(s) and that this signal is required to establish cell polarity and cell identity.

MATERIALS AND METHODS

Fly strains and manipulation

Characteristics of the various genetic markers used are described in Lindsley and Zimm, 1992. Adult flies, *ras v* dsh^{1} *dy*/*Df(1)* GA118, were prepared for SEM by dehydration in alcohol followed by critical point drying and gold palladium shadowing in the UCI EM facility. Organization of photoreceptors was determined by cutting heads off of flies and mounting the heads on a spot of Vaseline for viewing in the compound microscope by antidromic illumination (Franceschini and Kirschfeld, 1971; Franceschini, 1975). Adult cuticle elements were dissected in 70% EtOH, dehydrated briefly in isopropanol and placed in Gary's magic mountant (Struhl and Basler, 1993) and photographed using a Nikon Optiphot.

Cloning

Since *dsh* is the second gene distal to the *discs-large-1* gene *(dlg-1)* (Lefevre, 1981; Zhimulev et al., 1981; Geer et al., 1983; Voelker et al., 1985), a chromosome walk was conducted from the *discs-large* gene region (Woods and Bryant, 1989) by screening a genomic library in lambda dash (Stratagene) (a kind gift of D. Woods) using standard methods (Maniatis et al., 1978, 1982). A restriction map was generated and the *hopscotch* gene (*hop*) (Perrimon and Mahowald, 1986), which maps between *dlg-1*and *dsh*, was located by mapping a p-element insert in the hop^{air} allele (Watson et al., 1991; J.L. Marsh, unpublished observations). Transcribed regions were first identified by probing Southern blots of cloned genomic DNA with hydrolyzed, kinased RNA (Cox et al., 1984) from various embryonic stages and late 3rd instar larval RNA (L3) (not shown). Individual transcripts were mapped by cloning cDNAs from embryonic cDNA libraries using the genomic clones as probes (Poole et al., 1985; Brown and Kafatos, 1988). Four transcripts, which are present in early embryos and are distal to *hop*, were identified. Northern blots were performed to determine whether multiple transcripts were evident and to determine the size of the transcripts relative to the cDNA clones. At the level of northern blotting, the longest cDNA from the *dsh* gene (identified below) was similar in size to the single transcript detected.

Germ-line transformation

Genomic fragments containing one or more of the transcripts (see Fig. 7A) were cloned into either the Carnegie 20B [ry^{+}], PW8 [w^{+}] or CasPer [w^{+}] transformation vectors, transformants recovered and linkage determined by segregation of markers. Complementation tests demonstrated that constructs that contained the 2.5 kb transcript (see Fig. 7A) rescued both the viable *dsh* and two lethal *dsh* alleles thus identifying the *dsh* gene. Construction of transformation plasmids was as follows: For Car 7E, a 7.5 kb genomic *Sal*I fragment was ligated to *Sal*I cut Carnegie 20B with the left-most *Sal*I site adjacent to the *rosy* gene. The left-most *Sal*I site shown in Fig. 7A in parentheses is an artificial linker site from the phage and Car 20B is a derivative of Car 20 (Rubin and Spradling, 1983) in which the *Hpa*I site has been replaced by *Xba*I. To eliminate one of the transcription units, Car 7E was cut with *Xba*I and religated to delete the *Xba*→*Sal* region thus leaving only one transcription unit intact (Car 7EX). For construct pW8 7EXB, the same *Sal*I fragment was cloned into the *Xho*I site of pW8 (Klemenz et al., 1987) and then recut at the *Bam*HI site of pW8 and the natural *Xho*I site in the genomic DNA, the ends filled in and blunt end ligated. This truncation contains only the 2 kb transcription unit intact. CasPer W was constructed by ligating an *Eco*RI; *Bgl*II genomic fragment into *Bam*HI/*Eco*RI cut CasPer (Pirrotta, 1986). Relative to the other vectors, we experienced a low frequency of insertion using the pW8 constructs.

Helper plasmid π25.7wc (Rubin and Spradling, 1983) and test plasmid were prepared as described (Rubin and Spradling, 1982; Spradling and Rubin, 1982) and coinjected into either *white* mutant embryos (CasPer and pW8 constructs), or $rosy^{506}$ mutant embryos (Car 20 constructs). Surviving adults were mated to appropriately marked strains and linkage determined by segregation analysis from balancer chromosomes. Transformants on the second or third chromosomes were tested for complementation of *dsh* mutations by crossing dsh^{x}/*FM7c* females to +/*Y;* transformant/*CyO* or /*TM3* depending upon the linkage of the transformant (x=v26, VA153 or 1). The presence of non-*FM7* non-*CyO* or non-*FM7*, non-*TM3* males indicated that a particular construct complemented *dsh*.

Sequencing

The DNA sequence was determined by sequencing selected subclones and a series of nested deletions generated from both ends of the cDNA clones using the Erase-a-Base digestion kit from Promega. Sequencing was performed by the dideoxy chain termination method (Sanger et al., 1977) adapted for use with fluorescently labeled primers. All subclone boundaries were crossed and both strands were sequenced. Actual sequence was determined using the ALF sequencing system (Pharmacia). Sequence output from the computer algorithm was checked by visual inspection of the raw data output. Analysis of the DNA sequence was performed using the Align and MacVector programs (IBI/Kodak). Homology searches of the SwissProt +PIR +GenPept +GPUpdate non-redundant databases (Bilofsky and Burks, 1988) were performed using the BLAST algorithm (Altschul et al., 1990).

Mitotic clone induction

To assess the autonomy of the *dsh* requirement in cell polarity, clones were produced using two lethal alleles of *dsh* both with and without *forked* (*f*) as a marker (i.e., *y w* dsh^{VA153}/f^{36a}, *y w* dsh^{v26}/f^{36a}, *y w* dsh^{v26}/w^{+}, *y w* dsh^{VA153}/w^{+}). In one experiment, irradiated animals were heterozygous for the bristle marker *forked* and only twin spots, where patches of *forked* bristles could be found adjacent to patches of *yellow* tissue, were scored. In the second experiment, *forked* was eliminated in order to better score putative polarity defects in normal cells when adjacent to mutant cells. The w^{+} insertion just proximal to *dsh* in the second set of animals is immaterial in this experiment. Although there is no independent check for distal recombinants in the second experiment, the frequency of such recombinants has been determined to be less than 14% (Becker, 1976). For clone induction, eggs were collected from the appropriate cross and aged to 24-48 or 70-74 hours and irradiated for 1.5 or 3 minutes respectively at 750 rad/minute in a gammator with a ^{137}Cs source. The irradiated animals were placed in fresh bottles of food and analyzed for clones upon

eclosion. For analysis of patterning defects, sibs from the *y w* dsh^{v26}/w^{+} and *y w* dsh^{VA153}/w^{+} animals were analyzed.

RESULTS

All cells that exhibit planar polarity require *dsh* function

The original dsh^{1} mutation was described as a viable mutation with deranged hairs on the thorax, divergent and blistered wings, and ellipsoid eyes (Fahmy and Fahmy, 1959; Lindsley and Zimm, 1992). To document the extent of the *dsh* requirement in polarized cells, we examined the effect of the dsh^{1} mutation on planar cell polarity using light microscopy and SEM. All cells in which polarity is evident are affected by *dsh* mutations (examples of the thorax, abdomen and legs are shown in Fig. 1). Note the abnormal polarity of hairs as well as bristles on the thorax in Fig. 1A-C. In the sternites (Fig. 1D,E), complete reversals of bristles are seen and hairs are also affected. On the leg, bracts normally arise on the proximal side of each bristle by a polarized induction from the bristle cells (Tobler et al., 1973). The dsh^{1} mutation leads to the induction of bracts in the wrong position relative to the bristle (Fig. 1F) (Held et al., 1986). In addition, there are extra, mirror-image duplications of tarsal joints in the legs (Fig. 1G). The rough eye phenotype is seen by SEM to actually reflect abnormal facet packing and incorrect placement of the sensilla (Fig. 2A,B). In addition, polarity of cuticular hairs adjacent to the eye is disrupted.

We used antidromic illumination (Franceschini and Kirschfeld, 1971; Franceschini, 1975) to examine the internal organization of the eyes of *dsh* mutant flies. In *dsh* mutants, (i.e., *Df* (1)*GA112*/dsh^{1}), the photoreceptors are correctly organized into the characteristic trapezoidal array and all pattern elements are present, but the photoreceptor clusters are misoriented reflecting an abnormal polarity of the whole ommatidium (Fig. 2C,D). Two classes of abnormalities are evident. Some ommatidia are rotated from their normal orientation but otherwise exhibit normal handedness. Others are reversed in either the A/P or D/V axis (relative to the axes of the adult) with varying degrees of rotation from these axes (Fig. 2D). In mutants (i.e., *Df* (1)*GA112*/dsh^{1}), about 41% of the ommatidia show polarity reversals while approximately 10% of the ommatidia are rotated but exhibit the correct handedness. By examining eyes at different planes of focus (not shown), it is evident that the location of the bristle sensilla in the anterior equatorial vertices is altered in a manner consistent with the polarity reversals and rotations (note placement of sensilla in Fig. 2B). We do not observe incorrect placement or orientation

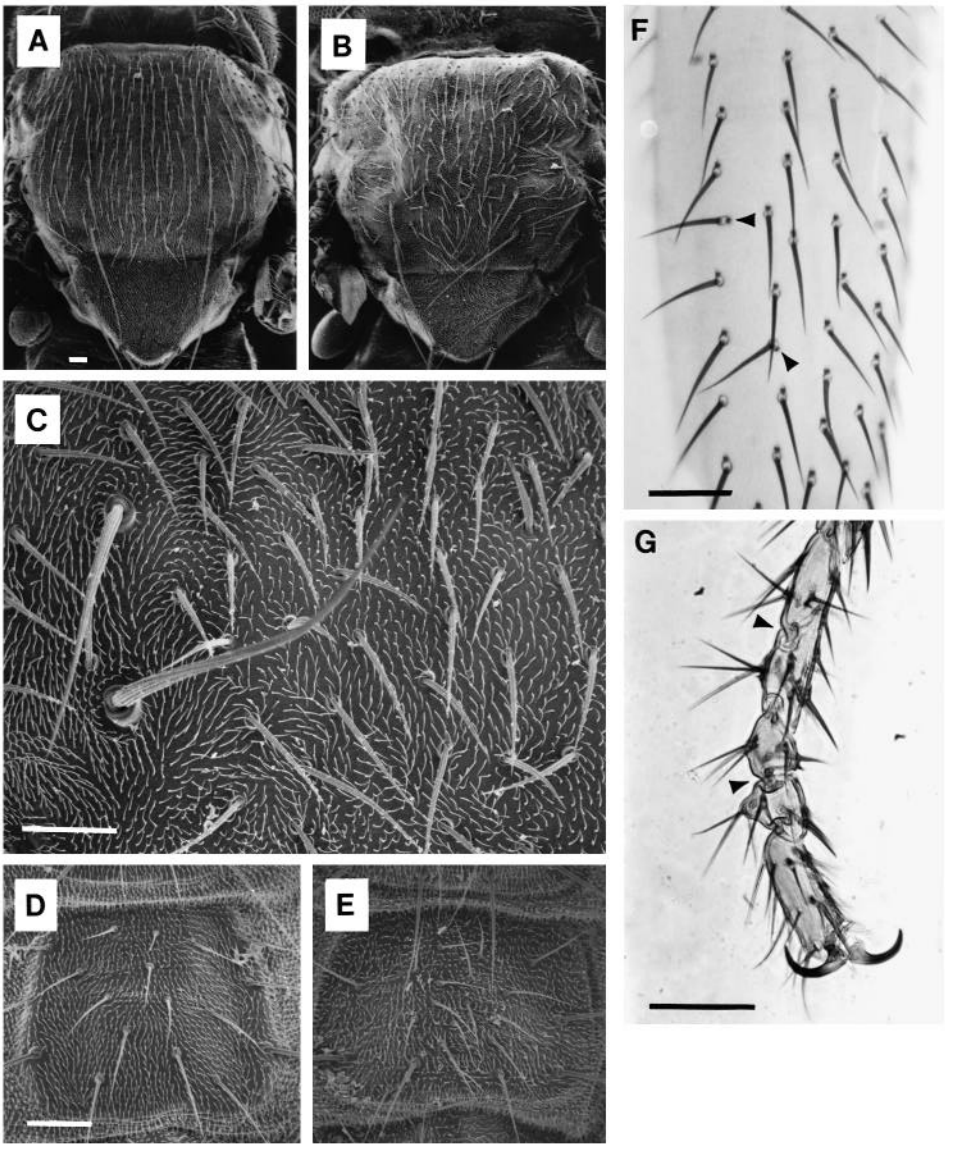


Fig. 1. *dsh* is required in all polarized cells. Scanning electron micrographs of various polarized structures in normal and dsh^{1}/*Df(1)*GA112 adults are shown. (A,B) A dorsal view of the thorax of a normal and a *dsh* mutant adult. Note the proper positional specification of pattern elements (e.g., the large macrochetes), but the abnormal polarity of all structures from bristles to hairs in *dsh* mutants. (C) A higher magnification view of another *dsh* thorax showing the effect on hairs as well as bristles. (D,E) Compare polarity in the sternites (ventral surface of abdomen) of a normal and a *dsh* mutant adult. Again, note the correct placement of pattern elements, but their abnormal polarity. (F,G) The polarity and pattern defects observed in the legs of *dsh* mutants. The femur in F shows that bristles do not all point distally as in normal legs and further, the short bract that is always induced by the developing bristle cell on the proximal side of the bristle (seen as a small dark wedge) is now induced in inappropriate locations (arrowheads). (G) The typical reversed polarity ectopic joints (arrowheads) and segments seen on the tarsi of *dsh* mutants. Note also the reversed bristle bract orientations. Scale bar, 50 μm.

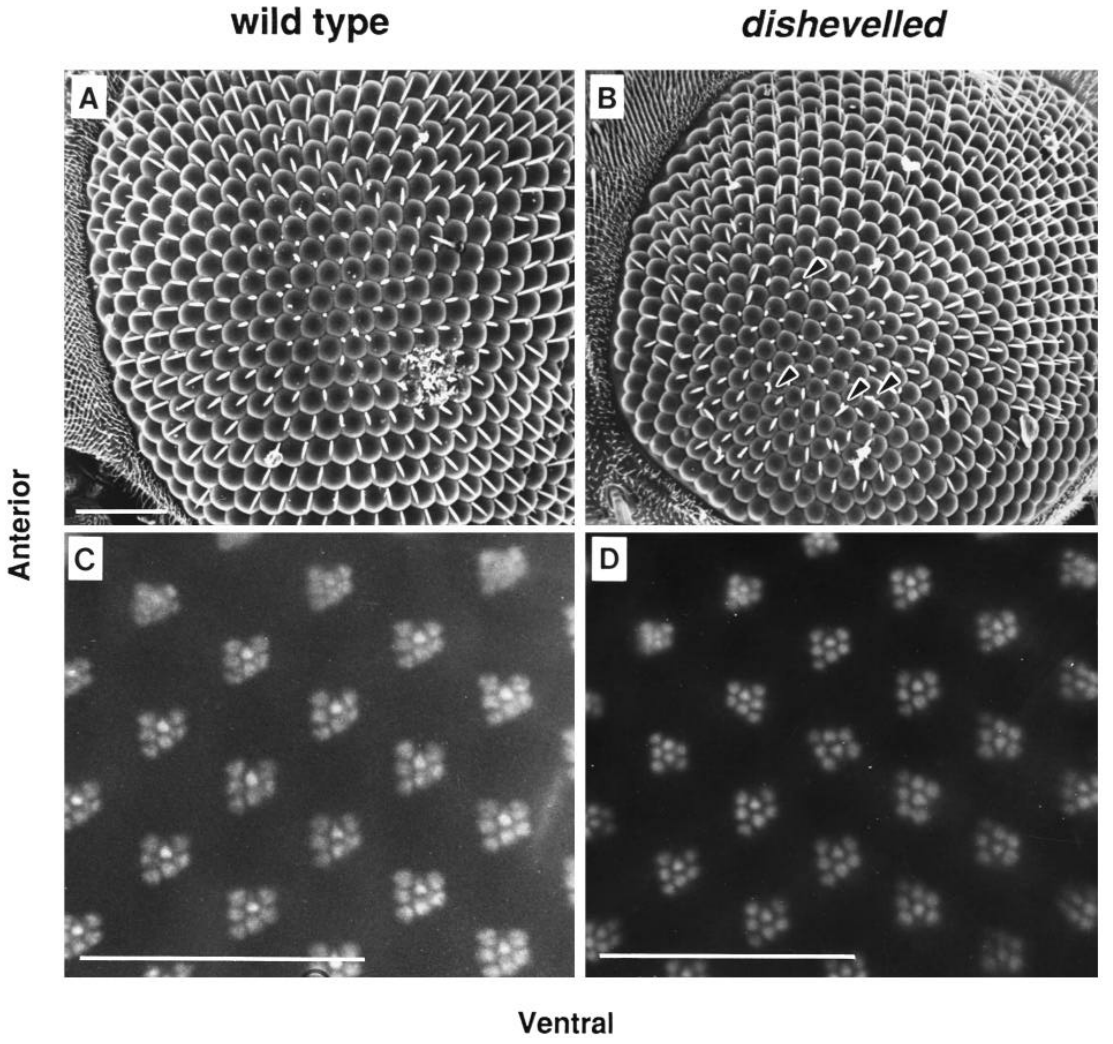


Fig. 2. *dsh* affects polarity of ommatidia. Scanning electron micrographs of normal (A) and *dsh* mutant (B) eyes. Note the abnormal packing of facets and placement of sensilla (arrowheads) in the mutant eye. Organization of the ommatidia in the left ventral regions of the eyes is revealed in normal (C) and *dsh* (D) eyes by shining a light through the back of the decapitated head and focusing on the rhabdomeres (the light-gathering organs of the photoreceptor cells). In *dsh* eyes, the characteristic trapezoidal array is maintained but the ommatidia display a number of abnormal orientations, namely rotations from the midline and reversals of polarity in both the D/V and A/P axes. Scale bar, 50 μm.

of individual photoreceptor cells within an ommatidium. Thus, the orientation defects appear to affect ommatidia as a whole.

dishevelled is required autonomously for cell polarity

Since only a single viable allele of *dsh* exists, it was important to determine whether the cell polarity phenotype is a unique property of the *dsh* viable allele versus the lethal alleles. X-ray induced somatic recombination was used to generate mosaics of the *dsh* lethal alleles dsh^{v26} and dsh^{VA153}, marked with *yellow* in two separate experiments. Clones of both lethal alleles were recovered in all body segments and in both cases cells exhibited polarity defects indistinguishable from those seen in dsh^{1} homozygotes (e.g., Fig. 3A). Thus, the cell polarity defect is not a novel effect of a single unusual allele and lethal alleles of *dsh* are not cell lethals. Clones of all sizes were observed from a few bristles to almost half the notum (Fig. 3A). Even in the smallest clones, *dsh* mutant bristles adopted abnormal orientations despite being intimately surrounded by normal cells. Along the edges of large clones and around the perimeter of smaller mutant patches of tissue, correctly oriented normal bristles were located immediately adjacent to incorrectly oriented mutant bristles. Thus, normal cells are unable to correctly orient *dsh* mutant cells and mutant cells do not adversely affect neighboring normal cells indicating that *dsh* functions autonomously in establishing cell polarity.

wingless-like phenotypes are produced by *dsh* clone induction

Combinations of *wingless* alleles that die as pupae produce a characteristic set of abnormalities including leg defects, loss of head structures and duplicated nota in place of wings (Sharma and Chopra, 1976; Morata and Lawrence, 1977; Baker, 1988b; Peifer et al., 1991). Leg defects in *wg* pupae can be formally described by the polar coordinate model as including both convergent and divergent duplications (Girton, 1982) (Fig. 4C,D). Similar leg abnormalities are observed when *dsh* clones are induced by irradiation (e.g., Fig. 4G,H). The ectopic leg associated with the *dsh* clone in Fig. 4G converges distally as seen by the symmetry of duplicated ventral elements, (except where the *dsh* clone occupies the location of a putative ventral element). The ectopic leg in Fig. 4H appears to be diverging as it is asymmetric and forms dorsal elements (e.g., the claw); however, as it extends distally, the *dsh* clone occupies the complete circumference and prevents ventral structures from forming. It is worth noting that, unlike previous studies of pattern regulation which always involved marked normal cells

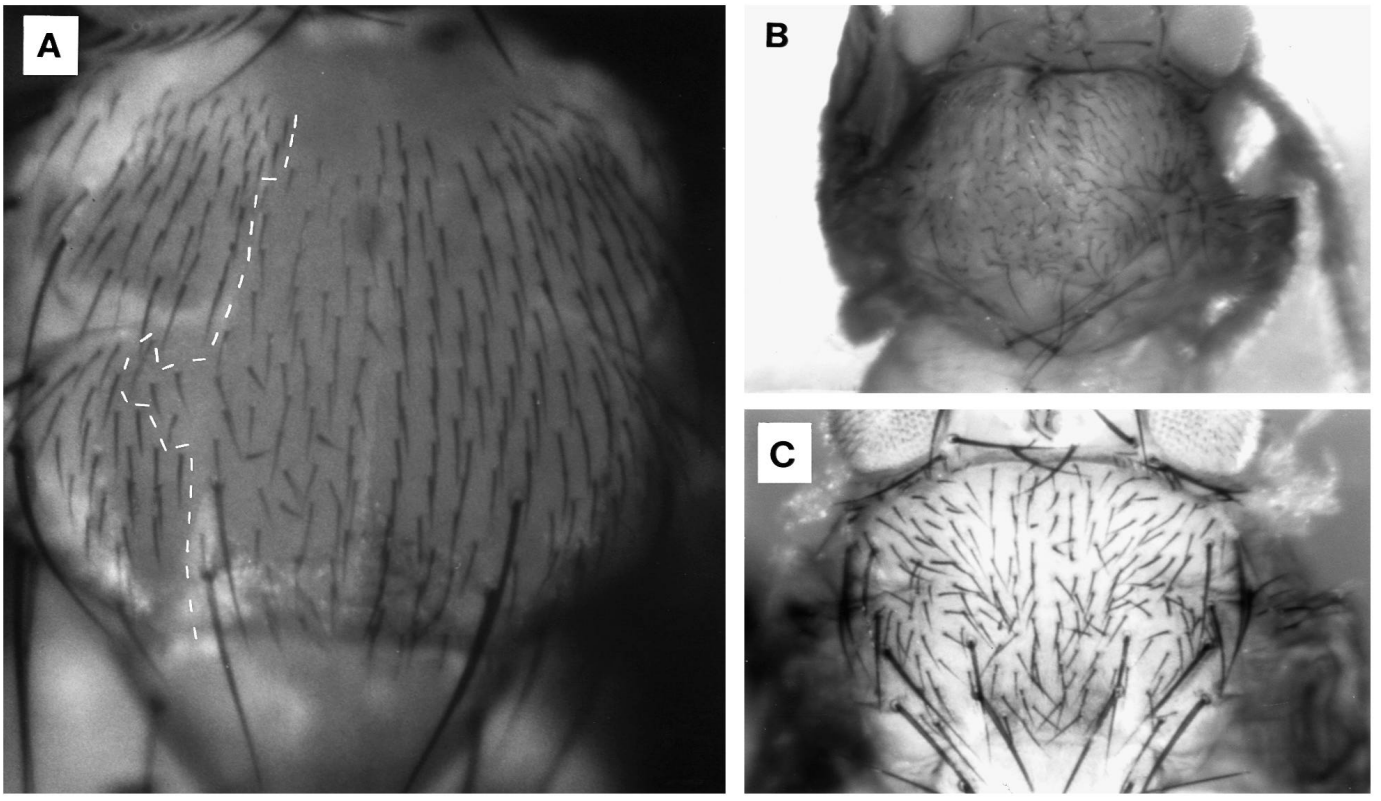


Fig. 3. Genetic requirements of cell polarity. (A) *dsh* function is cell autonomous. Mitotic clones of *dsh* were induced by irradiating first instar larvae bearing two different lethal *dsh* alleles marked with *yellow* both with and without *forked* as a marker for the twin spot (other genetic details are described in methods). Only clones that were not associated with patterning defects were scored for polarity. 31 of 37 clones made with *forked* exhibited polarity defects while 29 of 36 made without the marker exhibited polarity defects. All non-yellow tissue exhibited normal polarity in both experiments. The female shown is *y w* dsh^{V26}/+. Note the abnormal polarity of *yellow dishevelled* bristles within the clone while the polarity of the surrounding non mutant tissue is unaffected. (B,C) *wg* mutants affect bristle polarity. Pharate adults mutant for *wg* (B, wg^{CX4}/wg^{P}) or *dsh* (C, dsh^{1}/dsh^{1}) were dissected from the pupal case and photographed. In both cases, the polarity defects in the notum were evident before the animal was removed from the pupal cuticle and thus did not result from mechanical disruption while photographing. Similar polarity disruptions can be seen in a number of other published photographs of various *wg* alleles (referenced in text).

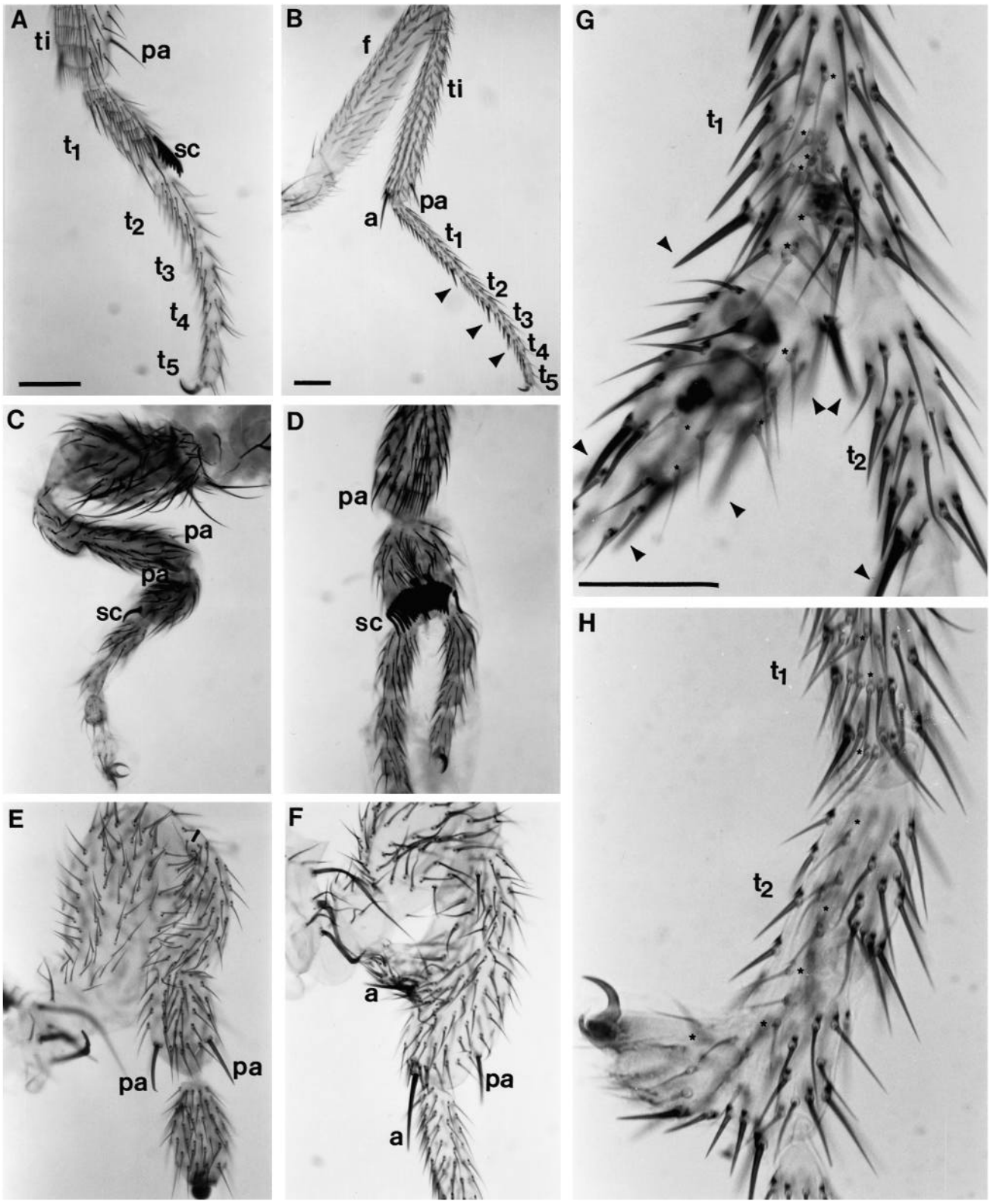
that were fully capable of responding to patterning signals (Girton, 1982), the *dsh* clones shown here are different in that the mutant clonal tissue is unable to respond to patterning cues during outgrowth and is incapable of producing ventral pattern elements. The location of *dsh* clones is shown on the fate map of the leg disc in Fig. 5. Clones that result in patterning defects are always located anteroventrally or ventrally on the fate map and mutant cells are often included in the abnormal leg but never in the normal one. Clones of *dsh* in the posterior and dorsal regions of the leg do not result in patterning defects. Clones of *dsh* also exhibit defects when they occur in the dorsal-medial region of the head, a region that exhibits defects in *wg* mutants (Peifer et al., 1991). Defects associated with *dsh* clones in the head and antenna include loss, ectopic location and duplication of pattern elements (not shown).

dsh and *wg* double heterozygotes produce synthetic *wingless*-like phenotypes

To explore the possibility that *dsh* and *wg* participate in a common patterning signal in imaginal tissue, we tested for genetic interactions between *dsh* and *wg* alleles. Phenotypes observed in animals that are heterozygous for more than one recessive mutation (i.e., synthetic phenotypes) often indicate that the two genes function in a common pathway (e.g., Simon et al., 1991). Several *dsh* and *wg* alleles were crossed to produce F_1 daughters which are doubly heterozygous for *dsh* and *wg* (Table 1). These flies were examined for pattern abnormalities and scored for lethality. Controls included scoring the sibs and scoring flies from the individual mutants used. A small percentage of doubly heterozygous flies exhibited abnormalities that mimic those seen in *wg* pharate adults including converging duplications (compare Fig. 4E to C) and bifurcations of leg segments (compare Fig. 4F to D). The abnormalities also mimic those seen in *dsh* clones (Fig. 4G,H), and those observed in temperature-sensitive *wg* heteroallelic heterozygotes exposed to restrictive temperature during late embryogenesis (Sharma and Chopra, 1976; Morata and Lawrence, 1977; Baker, 1988b; Peifer et al., 1991; Couso et al., 1993). The rate of spontaneous defects observed in the *wg* alleles used is rare (i.e., ~0.35%) while, in the double heterozygotes, the nature of defects observed is more severe and the frequency is increased approximately 10-fold (e.g., to 4.4%) (Table 1). The synthetic interaction appears to be allele specific since the frequency of defects is significantly higher than controls when wg^{IG} is in combination with either *dsh* allele but the frequency of defects with wg^{CX4} is only elevated in combination with dsh^{v26}. Thus, *dsh* and *wg* interact to give patterning defects similar to those seen in *wg* alone.

A *dsh* transgene partially rescues lethal alleles of *dsh* to give a *wg* phenotype

A transformant line carrying a *dsh* transgene with 184 bp of 5′ flanking and 240 bp of 3′ flanking DNA exhibits reduced *dsh* activity (P[dsh^w], described in Fig. 7A) such that flies carrying lethal alleles of *dsh* and one copy of the transgene survive to adulthood but they exhibit cell polarity defects (e.g., dsh^{VA153}/dsh^{VA153}; *P*[dsh^w/+]). 8% of these flies also have missing wings and duplicated nota and other defects typical of *wg* mutants (Fig. 6A,B). If these flies are also made heterozygous for *wg* (i.e., *dsh*/*dsh; wg*/ +;*P*[dsh^w/+]), the frequency of *wingless*-like defects increases to 100% (Fig. 6C). The defects



include missing and reduced wings, duplicated nota, head defects and leg abnormalities. The fact that reduced *wingless* exacerbates the defects seen in the reduced *dsh* background from 8% to 100% provides further evidence that *dsh* and *wg* interact in a common pathway to specify cell fate.

wg mutations exhibit cell polarity defects that mimic those seen in *dsh*

Examination of wg^{CX4}/wg^{P} pharate adults revealed a small fraction of animals with misoriented bristles on the notum, head and abdomen (Fig. 3B). The polarity defects could be observed through the pupal case or through the pupal cuticle after dissection and, thus, were not the consequence of mechanical disturbance. The polarity defects seen in these *wg* pharate adults are indistinguishable from those seen in *dsh* pharate adults (Fig. 3B,C). Further, some *dsh*/+*; wg*/+ double heterozygotes described above exhibited defects of the distal wing blade and those animals also exhibited bristle polarity defects but only on one side of the notum; namely, the same side as the wing defect. The concordance of the developmental defect in the wing blade and the polarity defect in the same disc derivative suggests a common cause for the two defects. Thus, loss of *wg* function alone or simultaneous reduction of *dsh* and *wg* activities can lead to polarity defects similar to those seen in *dsh*.

Fig. 4. *dsh* and *wg* function in the same pathway to pattern leg imaginal discs. Similar leg phenotypes are produced by *dsh* clones, *dsh; wg* double heterozygotes and *wg* mutants. All legs are oriented with ventral to left. Abbreviations are femur (f), tibia (ti), tarsal segments (t1-t5), ventral apical bristle (a), dorsal preapical bristle (pa) and anteroventral sex combs (sc). Arrowheads are used to mark the ventral-most peg-like bristles of the tarsal segments. The claw is a dorsal element bisected by the anterior/posterior compartment boundary. (A,B) Anterior aspects of normal first and second legs respectively for reference. (C,D) Legs of wg^{P}/wg^{CX4} mutants. (C) A male first leg with converging duplication showing characteristic reduction of ventral elements (note the single sex comb bristle) and duplication of dorsal elements (Note duplicated preapical bristles and duplicated claws). (D) A diverging duplication. The femur (not shown) is symmetrical about the dorsal axis and diverges distally (note the duplicated preapical bristles (one out of plane of focus) and more distally the increased size of the sex comb followed by bifurcation with complete circumferential pattern). (E) A converging duplication seen in a dsh^{v26}/+; wg^{CX4}/+ double heterozygote. Note the symmetrical duplication of dorsal elements (e.g., duplicated preapical bristles) and the absence of ventral elements (no preapical bristle). (F) A bifurcation of a second leg seen in dsh^{VA153}/+; wg^{IG}/+ double heterozygote. This leg appears symmetrical in the femur with missing ventral pattern elements (naked cuticle) and the outgrowth on the tibia appears asymmetric similar to the clonal leg in H (Note the duplicated apical bristle). (G,H) *dsh* clones associated with a converging triplication and diverging duplication, respectively. Some mutant bristles down the approximate middle of the clone are marked with an asterix for ease of recognizing the clones. (G) Anterior aspect of a second leg with a clone beginning in bristle row 6-7 and spreading distally. Note three sets of ventral elements (arrowheads). The normally paired ventral bristles occur singly when the expected location of one of the bristles is occupied by mutant tissue (asterix). (H) Anterior aspect of a first leg with a *dsh* clone. This clone begins in the transverse row (bristle row 8) at the proximal end of the first tarsal segment and extends through the second tarsal segment into the ectopic leg. The clonal tissue extends around the complete distal circumference of the extopic leg thus apparently preventing further regulative growth. Scale bar = 50 μm. (A,C-F); (G,H), same scale.

Structure of the *dsh* gene

The *dsh* gene was cloned and identified by transformation complementation (see Fig. 7A and Methods). A *dsh* cDNA clone that is the same length as the mRNA as measured by northern blots (not shown) and thus likely to be near full length was sequenced. The transcription unit represented by this cDNA is completely contained within the fragments that rescue *dsh* mutants (Fig. 7A). The sequence revealed a single long open reading frame beginning with the first AUG in the sequence (Figs 7B, 8). The cDNA contains 250 bp of 5′ UT and 529 bp of 3′ UT and terminates in a 42 nt poly(A) tail which is preceded by a poly(A) addition signal (AATAAA). In situ hybridization reveals widespread expression of *dsh* throughout development. As expected from the almost ubiquitous requirement for *dsh* function throughout development, *dsh* mRNA is found in egg chambers of the ovary and essentially ubiquitously throughout embryogenesis and in discs (not shown). Expression is not seen in salivary glands, muscles or ventral ganglia but is observed in brain lobes.

Conceptual translation of the *dsh* cDNA predicts a 68.9×10^3 M_r protein with a pI of 5.9. No hydrophobic regions were found that might correspond to either a secretory leader sequence or a membrane spanning or anchoring region (Kyte and Doolittle, 1982). Thus, the *dsh* protein exhibits the structural characteristics of an intracellular protein. The amino terminal half of the protein contains a stretch of 34 glutamine (Q) residues interrupted by 2 histidines and 5 other amino acids near the end of the stretch (Figs 7B, 8). The carboxy terminal half of the protein contains a string of 10 contiguous glycine (G) residues. The 530 bp 3′ untranslated region following the ORF is extremely A/T rich (70%) and repetitive. The motif TAA is repeated 15 times broken only by 3 TTA triplets and at position 2456, 50 of the next 56 bp are AT repeats. The 3′ untranslated region contains multiple stop codons in all frames.

dsh encodes a novel protein which shares a motif with seven other proteins

A BLAST search (Altschul et al., 1990) identified no proteins with extensive structural homology to *dsh*; thus, *dsh* appears to encode a novel protein. However, seven proteins, most of which are found or predicted to be found in association with cell junctions or junctional complexes, showed significant similarity in a shared motif (Fig. 7C) originally called the GLGF repeat (Cho et al., 1992) and now referred to as the DHR repeat (Discs - large Homology Region) (Bryant et al., 1993). Proteins containing this motif include the rat postsynaptic density protein (PSD-95) (Cho et al., 1992), the *discs large* tumor suppressor protein (DLG (Woods and Bryant, 1991), nitric oxide synthetase from rat brain (NOS) (Bredt et al., 1991), the human tight junction protein ZO-1 (Willott et al., 1992), erythrocyte membrane protein p55 (Ruff et al., 1991), an intracellular protein tyrosine phosphatase (PTP-meg) (Gu et al., 1991), and the putative Friedriech ataxia gene, x11 (Duclos et al., 1993). PSD-95 and DLG each have three copies of this motif, while DSH and the other proteins contain a single copy. The *dsh* DHR/GLGF motif, aligned with all the DHR/GLGF motifs in Fig. 7C, is most closely related to the first two repeats

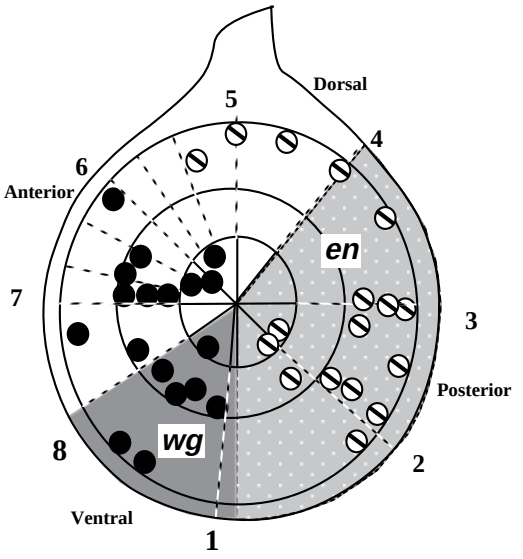


Fig. 5. Distribution of *dsh* clones in leg discs. The location of *dsh* clones on a generic leg disc fate map was mapped relative to the leg bristles. Filled circles represent clones that produced a pattern defect such as bifurcations of legs. Slashed circles represent clones that exhibited no patterning defect suggesting that *dsh* function is not required in those cells for cell fate determination. The location of the circles indicate the proximal-most location of the clonal tissue. Clones that originated proximal to the femur were observed but they are not placed on this map due to the lack of definitive markers in the coxa and trochanter to place them reliably on circumference of the fate map. Clones from all three legs are mapped on this generic disc. The fate map is taken from Bryant (1980). The concentric rings on the disc denote (from the center) the tarsi as a group, tibia and femur. The radiating lines indicate tarsal bristle rows and the extension of those rows onto the tibia and femur is indicated by continuing as dashed lines with bristle row numbers around the circumference while other bristle rows in tibia and femur are indicated by dashed lines (numbering according to Hannah-Alava, 1958; Held, 1993). The location of *wg* and *en* gene expression is from Hama et al. (1990), Couso et al. (1993) and Struhl and Basler (1993) and indicated by dark and light shading respectively. The posterior compartment defined by *en* expression is slightly less than half of the disc while *wg* expression is located in an anterior ventral wedge abutting the *en* domain. Fate map studies show that more than half of the positional values are located in the anterior compartment (Bryant, 1980). Patterning defects are associated with *dsh* clones in the anterior ventral approx. one third of the disc.

of PSD-95 and DLG and the single repeat of ZO-1 exhibiting 41 or 40 similar amino acids out of 80 with these 5 repeats.

DISCUSSION

In this report, we present evidence that (1) planar cell polarity requires the widespread action of the *dishevelled* gene, (2) a *dsh*-mediated *wg* signal is required for both cell fate choice and establishment of planar cell polarity and (3) *dishevelled* is required autonomously for response to the *wingless* signal. We also report the cloning and sequence of the *dishevelled* gene which coupled with autonomy studies suggests that (4) *dishevelled* encodes an intracellular protein necessary for reception of the *wingless* signal. We argue that this signal specifies some

Table 1. Patterning defects in *dsh/+; wg/+* double heterozygotes

Alleles crossed	wg^{CX4}	wg^{IG}	Controls (stock alone)
y w dsh^{v26}	15/513 2.92%	8/182 4.40%	1/748 0.13%
y w dsh^{VA153}	1/407 0.25%	10/363 2.75%	0/486 0%
Df(1)GA112, dsh^{-}	2/409 0.49%	10/370 2.70%	2/564 0.35%
Controls (stock alone)	6/1807 0.33%	2/898 0.22%	

Double heterozygotes were produced by crossing females heterozygous for *dsh* mutations to males heterozygous for *wg* mutations, i.e. *y w dsh*/*FM7c*; +/+ females to +/*Y*; *wg*/*CyO* males. The double heterozygous daughters were scored for patterning defects compared to their sibs (approximately 3600 sibs from the three wg^{CX4} crosses and 3100 from the three wg^{IG} crosses were scored with one defect from wg^{IG} observed). The defects observed in legs occurred in approximately equal numbers in the meso and metathoracic legs with only one in the prothoracic leg. The differences are judged significant by Fisher's Exact Test. The smallest *P* value for significance of the wg^{IG} crosses is $P=1.5\times10^{-4}$ and for the wg^{cx4} dsh^{v26} flies $P\cong2\times10^{-6}$. The frequency of defects in wg^{CX4} with dsh^{VA153} or *Df* is not different from controls.

of the circumferential positional values predicted by the polar coordinate model.

dsh is required wherever polarity is evident

In dsh^{1} mutants, bristle and hair polarity is disrupted in all cells in all body segments (Fig. 1). In addition, two polarized structures, which arise by inductive events (bracts and ommatidia), exhibit abnormal polarity in *dsh* mutants. Bracts on the legs are induced in inappropriate locations rather than proximal to the bristle that induces them (Fig. 1F) (Tobler et al., 1973; Held et al., 1986). The photoreceptor cells of the ommatidia are neurons that elaborate rhabdomeres. Since the polarity of individual photoreceptor cells is always consistent with the other members of that ommatidium and *dsh* mutations cause misorientation of entire ommatidia (Fig. 2D), it appears that ommatidia are independently developing units and that the orientation of the unit may be controlled by a primary organizing cell. R8 is the first cell of emerging photoreceptor clusters to exhibit differentiation and by implication is suggested to be the 'founder' cell (Tomlinson, 1987, 1988; Banerjee and Zipursky, 1990). Mosaic studies are underway to determine whether *dsh* function is required in a particular organizing cell, possibly R8. The independent development of individual ommatidia suggests that the ommatidia represent tertiary morphogenetic fields in *Drosophila* (the primary fields being segments and secondary fields being discs (Williams et al., 1993)). The range of polarized structures affected indicates that *dsh* is required both for polarity of individual cells as well as for polarized interactions between cells.

dsh is required autonomously for establishment of cell polarity

In mosaics, we frequently found abnormally oriented mutant bristle cells adjacent to correctly oriented normal cells indicating that dsh^{+} activity in a nearby cell cannot serve to orient correctly a mutant cell. This analysis is limited by the fact that

the *yellow* marker cannot be detected in hairs. Thus, normal and mutant cells along clone edges are separated by a variable number of hair-producing cells of unknown genotype. The closest apposition of normal and misoriented bristles that we observed has about four cells separating a normal and misoriented mutant bristle. Since we cannot determine which, if any, misoriented bristle cell are actually in direct contact with a normal cell, we cannot rule out the possibility of very short range interactions over 2 or 3 cell diameters. However, if *dsh* were capable of influencing neighboring cells, we would expect to see a ring of correctly oriented *yellow* bristles around the perimeter of *dsh* clones and this is not seen. Thus, within the limits of this technique, we conclude that *dsh* is required autonomously for the reception or interpretation of a cell polarity signal.

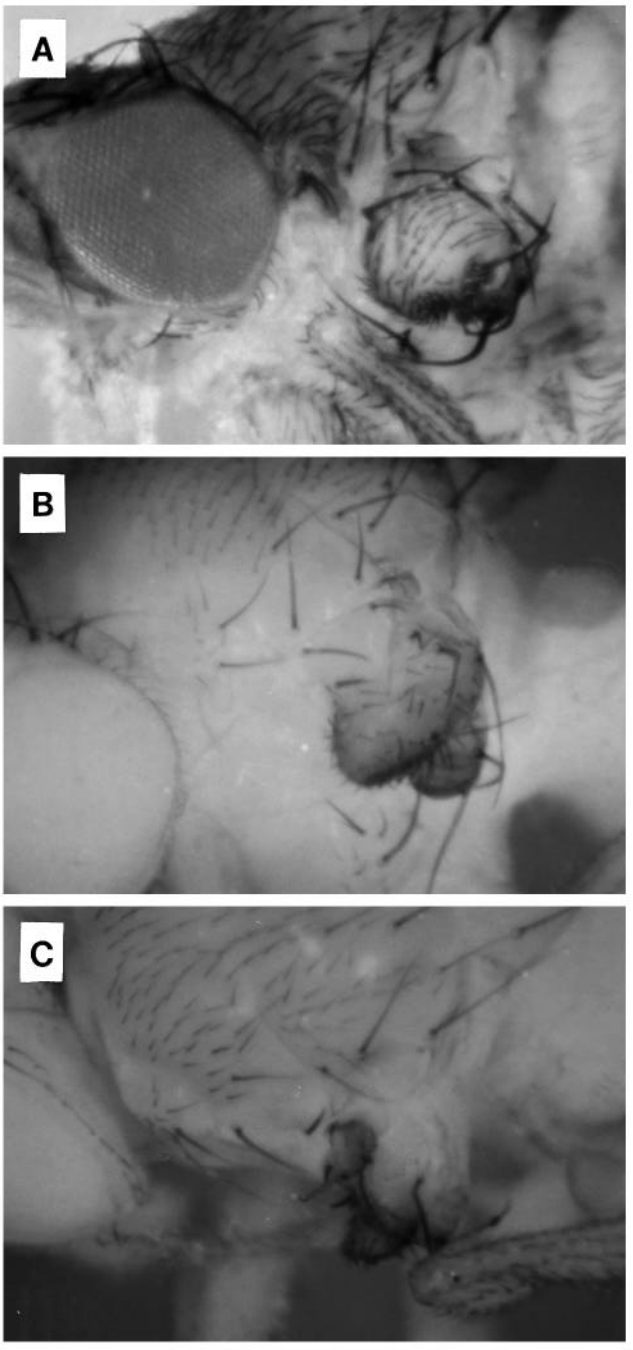


Fig. 6. A *dsh* transgene mimics the *wg* phenotype and interacts with *wg*. (A) Loss of wing blade and duplication of notum is seen in *wingless* mutants, wg^{CX4}/wg^{P}. (B) Approximately 8% of flies bearing a reduced function *dsh* transgene exhibit the same defect as seen in *wg* mutants, *y w* dsh^{VA153}*/Y; P[*w^{+}*; dsh*^{w}*]/+*. (C) If flies with reduced *dsh* function (B) are also made heterozygous for a recessive allele of *wg*, 100% of the animals exhibit defects such as the wing to notum transformation shown here in a *y w* dsh^{VA153}*/Y; CyO, wg/+; P[*w^{+}*; dsh*^{w}*]/+* fly. The *wg* allele used here is a lethal insert of a *LacZ* enhancer into the *wg* gene of a *CyO* balancer chromosome.

Loss of *dsh* function (in mutants or clones) does not specify a new axis or orientation but rather it removes an apparent bias leaving the cell 'undecided' as to which orientation to adopt. This is similar to other handed situations such as left/right handedness bias in mammals in which mutation removes a bias toward right handed but does not 'specify' left handedness (Annett, 1978, 1979). An alternative view is that all polarity reversals are the consequence of local pattern disruptions followed by regulation giving reversed tissues (a micro scale version of what happens with duplicated, reversed legs). Although the fine structure regulation of pattern in discs is not understood, this alternative seems unlikely since polarity disruptions are apparently not associated with extra, symmetrical or misplaced pattern elements as might be expected from a regulative cause of polarity disruptions.

wg is also required to establish cell polarity

Significantly, two observations implicate *wingless* in the establishment of cell polarity. Foremost is the observation that a small percentage of wg^{P}/wg^{CX4} pharate adults exhibit bristle polarity defects identical to those observed in *dsh* pupae and adults (Fig. 3B). Similar polarity defects in *wingless* mutant combinations can be seen in several figures of other publications, although polarity was not addressed (Morata and Lawrence, 1977; Baker, 1988a,b; Couso et al., 1993). Secondly, we also observed polarity defects on the nota of *dsh*;*wg* double heterozygotes. In each case, the polarity defects were restricted to one half of the notum and were associated with a defective wing blade. The concordance of wing and polarity defects to derivatives of a single disc under conditions of reduced *wg* signaling also suggests that a *wg* signal influences cell polarity.

Although acquisition of cell polarity and cell fate share some genes in common, other genes are clearly unique to each process. For example, unlike *dsh*, the *sple* and *fz* genes are required only for cell polarity since complete loss of function of either gene allows a viable animal with polarity defects but no patterning defects (Gubb and Garcia-Bellido, 1982; Adler et al., 1990). The *fz* gene encodes a novel protein with seven transmembrane domains reminiscent of G-protein-linked membrane receptors (Vinson et al., 1989) while the molecular nature of the *sple* gene is unknown. It is possible that WG interacts with two sets of signal reception/transduction molecules and that the two sets share some common elements (e.g., *dsh*) but otherwise lead to separate events within the cell. Indeed, the *fz* gene appears to carry out two functions, one being a cell autonomous transduction of an intercellular signal to the actin cytoskeleton and the other being a directionally non-autonomous transmission of an intercellular polarity signal (Vinson and Adler, 1987; Wong and Adler, 1993). Interaction with genes such as *fz* and *sple* may provide the distinc-

tion between the cell polarity and positional value functions mediated by *dsh*.

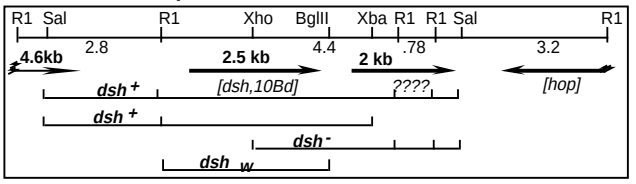
dsh and *wg* are required for patterning similar regions in discs

Mutations that reduce or eliminate *wg* function cause loss of structures from regions of the discs that extend beyond the regions of *wg* expression. The same structures are also affected by mutations that eliminate *dsh* or *arm* function (Peifer et al., 1991). Expression of *wingless* in discs is highly localized while expression of *dsh* (this report) and *arm* (Peifer et al., 1991) is widespread. In the leg disc, *wg* is expressed in an anterior/ventral wedge (Baker, 1988b; Peifer et al., 1991; Couso et al., 1993; Struhl and Basler, 1993) adjacent to the *engrailed* expression domain, which occupies the posterior compartment of the leg disc (Fig. 5) (Hannah-Alava, 1958; Steiner, 1976; Brower, 1986; Hama et al., 1990). *wingless* is not expressed in wing discs until the second larval instar when it initially appears as a wedge that refines to a focus of *wg* expression in the anlage of the notum and a stripe along the margin of the wing blade (Couso et al., 1993). In the eye antennal disc, a peak of *wg* expression occurs in the region fated to develop the dorsal portion of the head, and in a wedge in the antennal region (Baker, 1988b; Peifer et al., 1991; Couso et al., 1993; Struhl and Basler, 1993). The defective structures seen in *dsh* (this report) and *arm* mutants (Peifer et al., 1991) originate from regions in or near regions of high *wingless* expression in discs and are concordant with structures eliminated by *wg* mutations.

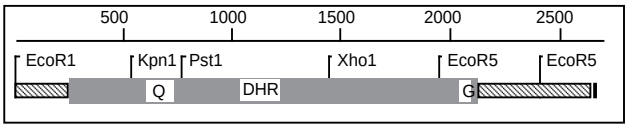
One of the earliest regions of *wg* expression in discs is the anterior/ventral wedge in leg discs (Couso et al., 1993). In *dsh; wg* double heterozygotes, anterior ventral structures of the leg are lost and dorsal structures are duplicated (Note the duplicated preapical bristles, Fig. 4E,F). Other defects include ectopic outgrowths (e.g., Fig. 4F), which exhibit the same circumferential restriction as those seen in *wg* mutants and *dsh* clones and mimic the abnormalities seen in *wg* pharate adults.

One dsh^+ transgene (P[dsh^w]) rescues lethal alleles of *dsh* but the rescued animals exhibit polarity defects similar to dsh^1 animals. When the sole source of dsh^+ activity comes from the transgene (i.e., *dsh*/*dsh*; P[dsh^w]/+), 8% of the flies exhibit defects that phenocopy *wg* defects (Fig. 6B) and *arm* defects (Baker, 1988b; Peifer et al., 1991). When the same genetic background is made heterozygous for a *wg* lethal allele (i.e., *dsh*/*dsh*; *wg*/+; P[dsh^w]/+), the frequency of defects increases to 100% with the same range of *wingless*-like defects including loss of wing, duplication of notum, defects in the legs and in the dorsocentral region of the head and antennal defects (Fig. 6C). The phenocopies of *wg* seen under conditions of reduced

A. Genomic map



B. *dsh* map



C. alignment of DHR/GLGF motifs

```
          1                                                  45
DSH       MEAVnFl.GiSIVGqSnRGGN........gGIYVgsIMKGGAVAL.
PSD1      eEItler.GnSgLGfSiaGGtdnphigddpsIfItkIipGGAAAq.
PSD2      MEIklik.GpkgLGFSiaGGvgnqhipgdnsIYVtkIieGGAAhk.
DLG1      eDIqler.GnsgLGFSiaGGtdnphigtdtsIYItkLisGGAAAA.
DLG2      iEIdlvk.GgkgLGfSiaGGignqhipgdnGIYVtkLtdGGrAqV.
ZO1HS     MkLVkFr kgdsVGlrlaGGNd.......vGIfVagVledspAAk.
PSD3      rrIVihr.GsTgLGfnivGGedge......GIfIsfIlaGGpAdL.
DLG3      rtItiqk.GpqgLGfnivGGedgq......GIYVsfIlaGGpAdL.
NOS       isVrlFkrkvggLGflvKervskp......pViIsdLiRGGAAeq.
PTP       vlIrmkpdengrfGfnvKGGydqk.....mpViVsrVapGtpAdLc
p55       rlIqfekvteepmGitlKlnekqs.......ctVarIlhGGmIhr.
x11       kDVfiekqkgeILGvvivesgwgsilp...tViIammMhGGpAek.

          46                                                 96
DSH       DGRIEpGDmILqVNDVN....FENmTnDEAVrVLREVvqkpGpIKLVVAK
PSD1      DGRLrvnDsILfVNEVd....vrevThsaAVeALKEA...gsiVRLyVmR
PSD2      DGRLq.GDkILaVNsVg....lEdvmhEDAVaALKnty...dVVyLkVAK
DLG1      DGRLsinDiIVsVNDVs....vvdvphasAVdALKkA...gnVKLhVkR
DLG2      DGRLsiGDkLIaVrtngseknlENvThElAVatLKsI...tdkVtLIIgK
ZO1HS     EG.LEeGDqILrVNnVd....FtNiirEEAVlfLlDLp kgeeVtILAqK
PSD3      sGeLrkGDqILsVNgVd....lrNashEqAAiALKnA...gqtVtIIAqy
DLG3      gseLkrGDqLLsVNnVN....lthaThEEAAqALKts...gGvVtLLAqy
NOS       sGLIqaGDiILaVNDrp....lvdlSyDsALeVLRgIasethVVLILrgp
PTP       vprLneGDqVVlINgrd....iaehThDqVVlfIKascerhsgelmLLVR
p55       qGsLhvGDeILeINgtN....vtNhSvDqLqkAmKEtkgmislkvIpnqq
x11       sGKLniGDqImsINgts....lvglplstcqsIIKgLenqsrvklnIVrc
```

Fig. 7. Molecular identification of *dsh.* (A) The genomic map in the *dsh* region is shown with transcription units (defined by cDNAs and northern blotting) indicated below and sizes of *Eco*RI restriction fragments indicated in kb below the line. The regions contained in the four transformation constructs were. Car 7E, (*Sal*)→*Sal* containing two transcripts; Car 7EX, (*Sal*)→*Xba* with only the 2.5 kb transcript intact; pW8 7EXB, *Xho*→*Sal* with only the 2 kb transcript intact and CasPerW, *Eco*RI→*Bgl*II which contains the 2.5 kb transcript and only 184 bp of 5′ flanking and 240 bp of 3′ flanking DNA. The Car 7E and Car 7EX constructs (*Sal* and *Sal*/*Xba* fragments, respectively) completely rescued the *dsh* viable and the *dsh* lethal alleles as did three lines of CasPer W (RI/*Bgl*II fragment); thus, providing positive identification of the 2.5 kb transcript as the *dsh* gene (10Bd is a locus designation for *dsh*). One line of of CasPer W, designated P[dsh^w], only partially rescued the polarity defects of dsh^1 and partially rescued the *dsh* lethal alleles but exhibited weak polarity defects in all the flies and patterning defects in ~8% of the rescued animals. Mapping and construction details are described in methods. The 2 kb transcript between *dsh* and hop does not appear to have been identified by mutation yet. (B) A map of the structure of the *dsh* gene as deduced from the DNA sequence. Untranslated 5′ and 3′ sequences are shown by cross-hatching while coding sequence is indicated by shading. Scale is in bp. The approximate location of 3 notable amino acid motifs is indicated: 34 Q refers to a stretch of 34 glutamine residues, DHR refers to a ~96 amino acid motif (DHR/GLGF motif) shared by *dsh* and seven other genes (aligned in panel C) and G indicates a stretch of 10 glycine residues. (C) The DHR/GLGF motif of DSH is aligned with the similar motifs of seven other genes, namely rat postsynaptic density protein (PSD-95), *discs large* tumor suppressor protein (DLG), nitric oxide synthetase from rat brain (NOS), human tight junction protein ZO-1HS, erythrocyte membrane protein p55, an intracellular protein tyrosine phosphatase (PTP-meg), and the putative Friedriech ataxia gene, x11 (references in text). PSD-95 and DLG each have three copies of this motif (e.g., PSD1 etc.), while DSH and the other proteins contain a single copy. Amino acid identities or conservative changes with respect to DSH are shown in **CAPITAL BOLD** letters while amino acids with no matches to *dsh* are shown in *lowercase italics*. Conservative groupings include (A,V,L,I); (K,R); (D,E); (S,T).

dsh or *armadillo* activity and the indistinguishable phenotype of *dsh*, *wg* and *arm* mutant embryos (Perrimon and Mahowald, 1987; Peifer et al., 1991) strongly suggest that *dsh, wg, arm* and perhaps other segment polarity genes act together during pattern formation in both embryos and discs.

```
GAATTCCGAA GAAGTAAAAG AAACGCGTGC GAGAGCGAGA GGGCTAGAGC GACCAGGGGG AGAAAAGCAA      70
AATCGAGTGA ATTTTGTTTG TTGTATCGGC TTTTCCCTGG CTTAATCCCG TTTTCCCTGT GATTTTTCCC     140
CACCAACTTC CCACGCCACC TCCCCACCGC ATCCATCGCA TTTCGTTTTG ATCGCGTTTT ATCGCGTCGA     210
GGAGTTTTCC CGTGGATTTC CGCAGTCGGC GCAGCTAAA ATG GAC GCG GAC AGG GGC GGC GGG CAG    276
                                          M  D  A  D  R  G  G  G  Q>
GAG ACG AAG GTG ATA TAC CAC ATC GAC GAT GAG ACG ACG CCG TAT CTG GTG AAG ATC CCC    336
 E  T  K  V  I  Y  H  I  D  D  E  T  T  P  Y  L  V  K  I  P>
ATT CCA TCC GCC CAG GTG ACG CTG CGC GAT TTC AAG CTG GTG CTG AAC AAG CAG AAC AAC    396
 I  P  S  A  Q  V  T  L  R  D  F  K  L  V  L  N  K  Q  N  N>
AAC TAC AAG TAC TTC TTC AAG TCA ATG GAC GCC GAT TTC GGT GTG GTC AAA GAG GAA ATA    456
 N  Y  K  Y  F  F  K  S  M  D  A  D  F  G  V  V  K  E  E  I>
GCC GAC GAC TCC ACC ATA CTG CCC TGC TTC AAT GGG CGA GTG GTG TCC TGG CTG GTC TCC    516
 A  D  D  S  T  I  L  P  C  F  N  G  R  V  V  S  W  L  V  S>
GCC GAT GGT ACC AAT CAG TCA GAT AAC TGC TCC GAG CTG CCA ACC AGT GAG TGT GAA TTG    576
 A  D  G  T  N  Q  S  D  N  C  S  E  L  P  T  S  E  C  E  L>
GGC ATG GGT CTG ACC AAC AGG AAG CTA CAG CAG CAA CAG CAG CAG CAT CAG CAG CAG CAA    636
 G  M  G  L  T  N  R  K  L  Q  Q  Q  Q  Q  Q  H  Q  Q  Q  Q>
CAA CAA CAG CAG CAG CAG CAC CAG CAA CAG CAA CAA CAA CAG CAG CAG CAG GTG CAG CCT    696
 Q  Q  Q  Q  Q  Q  H  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  V  Q  P>
GTC CAG CTG GCG CAG CAG CAA CAG CAG CAG GTG CTC CAT CAC CAG AAG ATG ATG GGC AAT    756
 V  Q  L  A  Q  Q  Q  Q  Q  Q  Q  V  L  H  H  Q  K  M  M  G  N>
CCG CTG CTG CAG CCG CCA CCG CTC ACA TAT CAA TCG GCA TCC GTG CTC TCC AGC GAT CTG    816
 P  L  L  Q  P  P  P  L  T  Y  Q  S  A  S  V  L  S  S  D  L>
GAC TCG ACC AGT CTC TTT GGC ACC GAA TCC GAA TTG ACG CTG GAC CGC GAC ATG ACC GAC    876
 D  S  T  S  L  F  G  T  E  S  E  L  T  L  D  R  D  M  T  D>
TAT AGC AGC GTT CAG CGA TTG CAG GTG CGC AAG AAG CCG CAG AGG CGC AAA AAA CGG GCG    936
 Y  S  S  V  Q  R  L  Q  V  R  K  K  P  Q  R  R  K  K  R  A>
CCC AGC ATG TCG CGC ACC TCC TCG TAC AGC TCC ATA ACC GAC TCG ACC ATG TCC CTA AAT    996
 P  S  M  S  R  T  S  S  Y  S  S  I  T  D  S  T  M  S  L  N>
ATC ATT ACC GTC TCC ATC AAC ATG GAG GCG GTC AAC TTT CTG GGC ATA TCC ATT GTC GGG   1056
 I  I  T  V  S  I  N  M  E  A  V  N  F  L  G  I  S  I  V  G>
CAA TCG AAT CGC GGC GGC AAC GGC GGC ATC TAC GTG GGC AGC ATC ATG AAA GGC GGC GCA   1116
 Q  S  N  R  G  G  N  G  G  I  Y  V  G  S  I  M  K  G  G  A>
GTG GCA CTC GAT GGA CGC ATC GAA CCG GGC GAC ATG ATC CTC CAA GTG AAC GAT GTC AAC   1176
 V  A  L  D  G  R  I  E  P  G  D  M  I  L  Q  V  N  D  V  N>
TTC GAG AAC ATG ACC AAC GAC GAG GCG GTA CGT GTG CTG AGA GAA GTC GTT CAG AAG CCG   1236
 F  E  N  M  T  N  D  E  A  V  R  V  L  R  E  V  V  Q  K  P>
GGA CCC ATC AAG CTG GTG GTG GCC AAG TGC TGG GAC CCA AAT CCC AAG GGC TAC TTC ACC   1296
 G  P  I  K  L  V  V  A  K  C  W  D  P  N  P  K  G  Y  F  T>
ATT CCG CGC ACG GAG CCG GTG CGA CCC ATC GAT CCC GGT GCT TGG GTG GCG CAC ACT CAG   1356
 I  P  R  T  E  P  V  R  P  I  D  P  G  A  W  V  A  H  T  Q>
GCC CTC ACA TCG CAC GAC AGT ATT ATT GCC GAC ATT GCG GAG CCG ATT AAG GAG CGA CTG   1416
 A  L  T  S  H  D  S  I  I  A  D  I  A  E  P  I  K  E  R  L>
GAC CAG AAT AAC CTC GAG GAG ATC GTT AAG GCG ATG ACG AAG CCG GAC AGC GGC CTG GAG   1476
 D  Q  N  N  L  E  E  I  V  K  A  M  T  K  P  D  S  G  L  E>
ATC AGG GAT CGC ATG TGG CTA AAG ATA ACG ATT CCC AAT GCA TTT ATC GGC GCC GAT GCG   1536
 I  R  D  R  M  W  L  K  I  T  I  P  N  A  F  I  G  A  D  A>
GTC AAT TGG GTG CTG GAG AAT GTG GAG GAT GTT CAG GAT AGG CGG GAG GCG CGA AGG ATC   1596
 V  N  W  V  L  E  N  V  E  D  V  Q  D  R  R  E  A  R  R  I>
GTC TCG GCG ATG CTG CGC AGC AAT TAC ATC AAG CAT ACG GTC AAT AAG TTG ACC TTC TCG   1656
 V  S  A  M  L  R  S  N  Y  I  K  H  T  V  N  K  L  T  F  S>
GAG CAG TGC TAC TAC GTG GTG AAC GAG GAG CGC AAT CCC AAT CTG TTG GGC CGA GGA CAT   1716
 E  Q  C  Y  Y  V  V  N  E  E  R  N  P  N  L  L  G  R  G  H>
CTG CAT CCG CAC CAG CTG CCG CAC GGG CAT GGC GGC CAT GCG CTT AGC CAT GCG GAC ACC   1776
 L  H  P  H  Q  L  P  H  G  H  G  G  H  A  L  S  H  A  D  T>
GAG AGC ATC ACC AGC GAC ATC GGG CCG CTA CCG AAC CCG CCC ATT TAT ATG CCA TAT TCG   1836
 E  S  I  T  S  D  I  G  P  L  P  N  P  P  I  Y  M  P  Y  S>
GCC ACG TAC AAT CCA AGT CAC GGC TAT CAG CCG ATC CAG TAC GGC ATC GCC GAG CGA CAC   1896
 A  T  Y  N  P  S  H  G  Y  Q  P  I  Q  Y  G  I  A  E  R  H>
ATC TCG TCG GGA TCG AGT AGC AGC GAT GTG CTG ACC AGC AAG GAT ATC TCG GCG TCG CAG   1956
 I  S  S  G  S  S  S  S  D  V  L  T  S  K  D  I  S  A  S  Q>
AGC GAC ATC ACC TCG GTG ATC CAT CAG GCC AAC CAG CTG ACC ATC GCC GCT CAT GGC TCT   2016
 S  D  I  T  S  V  I  H  Q  A  N  Q  L  T  I  A  A  H  G  S>
AAC AAA TCA TCC GGC TCC TCC AAT CGG GGC GGC GGC GGA GGC GGT GGC GGT GGC GGC AAC   2076
 N  K  S  S  G  S  S  N  R  G  G  G  G  G  G  G  G  G  G  N>
AAT ACC AAC GAT CAG GAC GTA TCC GTA TTT AAT TAC GTA TTG TAG AAACCTTTC TTTTTGCAAT  2140
 N  T  N  D  Q  D  V  S  V  F  N  Y  V  L  *>
CAAATTGTGT TTTTAATTAA TTAAATTAAA TATGTACGAT TATTCTTTTT TATATACGAG TATACAAAGC     2210
AAGCAAACAG CAAACCACAA TATAATAATT ATAATTATAA TAATAATAAT AATAATAATT ATAATAATAA     2280
TAATAATACT TATGTATATG TATGGTATGT GTAAGTCATA GAAAAGAACG CTAAACACCC AGCCGCAACG     2350
AGTCAAGGAT CAGCGAGGGC TCGTGAGGCA GCAGGAGGAT CAGCACAGGG ATATCCGTGT CCGGCAGCAA     2420
TCGGTTGTGG ACCCGATCAG ATCAGATCAA CTGAGATATA TGATATATGA TATATATATA TTATATTATA     2490
TATTATTATA TATATATATA TATATATGTG TTTGTGTGCA GGAACGGATG ATGCGGAATG GAGCGGAAGA     2560
GCAGGAACGG CAGCATGACG GGCGAGAATG ATTTTGAATA AAGTTCTAAG AATTTAGTAA TCACAAAGTA     2630
AATAAATACA AATAAAATG(A)42
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Fig. 8. Sequence of the *dsh* gene. The DNA sequence of the *dsh* gene was determined by sequencing the *dsh* cDNAs. A single long open reading frame is observed. Three motifs are underlined in bold: a long string of 34 Q residues beginning at base 597, the region corresponding to the DHR/GLGF motif beginning at bp 1026 and a string of 10 G residues beginning at bp 2043. A poly(A) addition signal is underlined in bold at bp 2631 just 14 bp from the 42 residue poly(A) tail. This sequence has been submitted to GenBank (Accession no. U02491).

dsh and *wg* together specify circumferential positional value in leg discs

The polar coordinate model of pattern formation proposed a set of rules for cell interactions that have proven remarkably robust in predicting tissue behavior (French et al., 1976; Bryant et al., 1981). However, a molecular basis for this model has not been described (Bryant, 1993). A specific prediction of the model is that genes that specify circumferential positional values should be required in cells all along the proximal distal axis of the leg but their activity should be restricted to a particular sector around the circumference of the leg. Previously, it has not been possible to test this prediction by clonal analysis since *wg* is non autonomous (therefore clones are rescued) and *arm* is cell lethal hence precluding locating the site of origin of clonal tissue (Peifer et al., 1991). Our finding that *dsh* clones lead to pattern regulation in a restricted portion of the leg disc circumference provides a unique test of the circumferential signal hypothesis.

While a complete discussion of the implications of the polar coordinate model is beyond the scope of this paper, a brief discussion of a few examples will be illustrative (Girton, 1982; Wilkins and Gubb, 1991; Held, 1993 provide additional discussion of this topic). Pattern regulation will occur when cells are confronted with neighbors of incorrect positional values. This can occur either by loss (e.g. due to cell death) or when values are mis-specified (e.g. as in a ventral clone adopting a more dorsal value; Bryant, 1975; Haynie and Bryant, 1976; Adler, 1981). The predicted outcomes are different depending upon whether the disparity at the confrontation is greater or less than half of the positional values (shortest intercalation rule). Thus, it is significant that fate map studies show that the anterior half of the leg disc contains more positional values than the posterior region (Bryant, 1980), an observation that is consistent with the effects of loss of *wg* signal in leg discs.

Two predictions of the polar coordinate model are a circumferentially restricted requirement and regulative growth accompanying pattern regulation. Clones of *dsh* that occur in the posterior compartment or the dorsal-most region of the anterior compartment of the leg disc cause no defects. Clones that occur in the anterior/ventral one third of the leg disc cause bifurcations of the leg. The location of clones that cause patterning defects overlaps the territory in which *wg* function is required and spans more than half of the positional values on the leg disc fate map (Fig. 5). Clones associated with pattern regulation expand distally while clones in the posterior compartment do not lead to pattern regulation and remain long and thin thus satisfying the prediction that regulative growth accompanies pattern regulation. The defects caused by *dsh* clones fall into two categories, diverging duplications and converging triplications (e.g., Fig. 4). Like *wg* defects, they can be explained by loss or mis-specification of ventral values followed by regulative growth. The two clones shown in Fig. 4G,H occurred in the anterior/ventral region of the disc and produce pattern abnormalities similar to those seen in *wg* mutants with only slight differences due to their clonal nature. The pattern defect associated with the *dsh* clone in Fig. 4G is a converging triplication which is similar to the converging duplication seen in *wg* mutants (Fig. 4C). A polar coordinate explanation for these legs is loss of ventral values followed by

regulative growth. The reason for duplication versus triplication is that the clone affects only a patch of tissue thus leaving a point of symmetry on either side while *wg* mutants affect the whole disc including the perimeter of the pattern. Thus, the effects of *dsh* clones satisfy the two predictions of the polar coordinate model.

The question of autonomy deserves special mention. Mosaic analysis can be used to distinguish a gene involved in the sending of a signal from one involved in the reception or response to a signal. Genes involved in the sending of a signal tend to give no phenotype in clones because neighboring normal cells can provide the diffusible function. For example, clones of *wg* give no patterning phenotype indicating that normal cells can rescue or compensate for the mutant cells (Baker, 1988a). In contrast, clones of *dsh* are not rescued by adjacent normal cells suggesting that *dsh* functions on the receiving side of a signal. The fact that both mutant and normal tissue participate in the ectopic structures formed is expected from the regulative growth that follows from *dsh* cells being unable to receive the correct positional value signal. The mixed nature of the ectopic outgrowths does not imply non autonomy of *dishevelled*'s cellular function.

Death versus mis-specification

Positional confrontations that lead to pattern regulation can occur either by loss of cells or by mis-specification of positional values. Positional values can be lost through surgical removal (French et al., 1976) or genetically induced cell death (Girton, 1982). Loss of *armadillo* activity is cell lethal and *arm* clones produce pattern abnormalities similar to those produced by cell death (Peifer et al., 1991). Positional confrontation can also occur due to mis-specification of positional values. For example, ectopic expression of *wingless* under heat-shock control leads to mis-specification and pattern regulation (Struhl and Basler, 1993). *dsh* clones could induce pattern abnormalities either because *dsh* cells die or because *dsh* cells are unable to respond to the *wingless* signal and thus adopt a default positional value. Several observations are relevant here. Firstly, *dsh* cells survive in clones demonstrating that loss of *dsh* function is not generally cell lethal. This does not rule out the possibility that a subset of *dsh* cells die as a consequence of not having dsh^+ function. Secondly, duplications show symmetry of ventral structures when the regenerated tissue is wild type but when ventral regions are mutant, those cells produce other structures suggesting that they adopt a default value (e.g., Fig. 4G). Thirdly, *wg* embryos secrete an excess of a single type of denticle in a repeating pattern and this pattern duplication is not accompanied by cell death or by ectopic cell proliferation (Bejsovec and Martinez Arias, 1991), suggesting that cells have adopted a default value. In contrast, many *dsh* clones are associated with ectopic cuticle elements inside the leg and/or dark fragments of tissue which might be melanized (e.g., Fig. 4G). This tissue could represent dying cells although dead tissue was never observed when genetically induced death was known to occur (Girton, 1982). Nevertheless, it is conceivable that *dsh* clones cause continued low levels of cell death and that late dying cells do not have time to be cleared thus accounting for why a pulse of cell death never left traces of dying cells while *dsh* clones may leave such traces. Alternatively, excess tissue might be formed if *dsh* cells are unable to participate in the pattern and are mechanically displaced during metamorphosis. Additional studies will be required to distinguish between death versus mis-specification as a mechanism.

dsh encodes an intracellular protein

The sequence of *dsh* suggests an intracellular protein that shares an amino acid motif with seven proteins that are localized to cell junctions (DLG; PSD-95; ZO-1) or to junctional-like complexes (p55) raising the possibility that DSH may localize to junctional complexes. DSH also has a string of 34 glutamine residues near the amino terminus. Such glutamine-rich strings are thought to be involved in protein-protein interactions (Pascal and Tjian, 1991; Su et al., 1991) which would be consistent with a protein interacting in a complex.

Recent studies indicate that cell signaling events often occur in apical regions of epithelial cells and that proteins mediating these interactions are localized in apical junctions (reviewed in Woods and Bryant, 1993, 1992; Bryant et al., 1993). The transfer of WG protein during signaling can occur in the apical part of the cell immediately basal to the adherens junctions (Gonzalez et al., 1991). The ARM protein, a β-catenin homologue, is part of a membrane-associated complex that includes a large cadherin-like glycoprotein and is localized at the adherens junction (Peifer et al., 1993). In embryonic cells that receive the WG signal, ARM protein is released from the membrane and accumulates in the cytoplasm (Peifer, personal communication). Mutations of *dsh*, *porc* and *wg* block the relocalization of ARM and thus function upstream of ARM (Peifer, personal communication). The intracellular nature of the *dsh* protein and the autonomous requirement for *dsh* function for patterning demonstrate that DSH acts downstream of WG. Coupled with the ARM studies, this puts DSH function between reception of the WG signal and redistribution of ARM in response to that signal in embryos. β-catenin molecules such as ARM serve as a link between the adhesive junctions of epithelial cells and the cytoskeleton (Peifer, 1993). Thus transduction of the WG signal may involve a DSH-mediated modulation of ARM and ultimately a change in the organization of the cytoskeleton. We cannot determine whether DSH interacts with the same gene products during both cell fate choice and cell polarity from this study. Our results do show that DSH is an intracellular protein that is required for the response to a *wingless* signal. The territories that require this signal and the consequences of interfering with it indicate that a *dsh*-requiring *wg* signal specifies circumferential positional value in leg discs and thus provides a molecular basis for a theoretical function predicted by the polar coordinate model (Bryant, 1993).

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