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The *Drosophila* segment polarity gene *patched* is involved in a position-signalling mechanism in imaginal discs

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Summary

We demonstrate the role of the segment polarity gene *patched* (*ptc*) in patterning in the cuticle of the adult fly. Genetic mosaics of a lethal allele of *patched* show that the contribution of *patched* varies in a position-specific manner, defining three regions in the wing where *ptc* clones, respectively, behave as wild-type cells, affect vein formation, or are rarely recovered. Analysis of twin clones demonstrates that the reduced clone frequency results from a proliferation failure or cell loss. In the region where clones upset venation, they autonomously fail to form veins and also non-autonomously induce ectopic veins in adjacent wild-type cells. In heteroallelic combinations with lethal alleles, two viable alleles produce distinct phenotypes: (1) loss of structures and mirror-image duplications in the region where *patched* clones fail to proliferate; (2) vein abnormalities in the anterior compartment. We propose that these differences reflect independently mutable functions within the gene. We show the pattern of *patched* transcription in the developing imaginal wing disc in relation to the expression of certain other reporter genes using a novel double-labelling method combining non-radioactive detection of *in situ* hybridization with β-galactosidase detection. The *patched* transcript is present throughout the anterior compartment, with a stripe of maximal intensity along the A/P compartment border extending into the posterior compartment. We propose that the *patched* product is a component of a cell-to-cell position-signalling mechanism, a proposal consistent with the predicted structure of the *patched* protein.

Key words: imaginal discs, pattern formation, cell communication, segment polarity, *patched*, *Drosophila*.

Introduction

The body of the *Drosophila* adult is derived from a series of embryonic primordia (imaginal discs) which undergo extensive rounds of cell division during the larval stages of development (Auerbach, 1936; Nöthiger, 1972). Although these cells receive instructions about their segmental identity during embryogenesis (Akam, 1987; Peifer *et al.* 1987), clonal analysis has shown that their early lineage, with the exception of the anterior–posterior compartmentalisation event (Garcia-Bellido *et al.* 1973), is largely indeterminate (Garcia-Bellido and Merriam, 1971; Bryant, 1970). The information required for the patterning of cells in different structures is thus an emergent property, which presumably depends upon communication between cells as they proliferate. A large body of data shows that imaginal disc cells respond to changes in the identity of neighbouring cells whether these alterations are made by surgical or genetic changes (reviewed in Whittle, 1990). When normally distant parts of a mature wing disc are juxtaposed and allowed to proliferate, the intervening pattern of cell types is regenerated by a process known as intercalation (Haynie and Bryant, 1976). In some genetic mosaics, wild-type cells respond to neighbours of changed genotype by differentiating cell types in changed patterns (Stern, 1956; Simpson and Schneiderman, 1975; Mohler, 1988; Santamaria *et al.* 1989) or polarities (Gubb and Garcia-Bellido, 1982; Vinson and Adler, 1987). In contrast to the early events of embryonic patterning (Ingham, 1988), virtually nothing is known about the molecular basis of this process.

Amongst the many mutations that disrupt adult morphogenesis are viable alleles of some of the segment polarity genes, a class originally defined by embryonic lethal mutations (Nüsslein-Volhard and Weischaus, 1980; Nüsslein-Volhard *et al.* 1984). An increasing body of evidence has implicated these genes in cell interactions (Martinez-Arias *et al.* 1988; DiNardo *et al.* 1988; Mohler, 1988). Here we show that mutations of the segment polarity gene *patched* (*ptc*) disrupt cell patterning in the cuticle of the adult fly. Using genetic mosaics of *patched* we find a requirement for this gene in cells in specific regions of the wing, which is not rescued by neighbouring cells; moreover in some locations the effects of *patched* mutations extend beyond the limit of the mosaic, suggesting a role for the gene in a signal

relay mechanism. We describe the pattern of *patched* transcription in the developing imaginal discs in relation to the expression of certain other genes and compare this to the requirements revealed by the mosaic analysis and the phenotypes of the viable alleles of *patched*.

Materials and methods

Drosophila *stocks*

14 *patched* alleles were generously supplied by C. Nüsslein-Volhard. ptc^{G12}, ptc^{G13} and ptc^{G20} were recovered in an F_1 screen at the University of Sussex on a *cinnabar, brown, speck* chromosome following 400 Grays irradiation from a ^{60}Co source. Eight alleles were recovered in a similar F_1 screen following a dysgenic cross between the Harwich P-element containing stock and the same M cytotype stock used in the irradiation screen. The mutation *tufted* (Sturtevant, 1948) and *T(2:3)dp*, which behaves as if it carries an allele of *tufted*, were obtained from stock centres. ptc^{S2} was found as an allele of *tufted* during a gamma-irradiation mutagenesis screen in this laboratory (Whittle, 1980). ptc^{RX67} was kindly supplied by P. Simpson (see Table 1 for a description and source of *ptc* alleles), the *engrailed–lacZ* reporter genotype by C. Hama and T. Kornberg and the *neuralized–lacZ* reporter by D. Clements and J. Merriam.

Mitotic recombination

Mosaics were induced by 100 Grays gamma irradiation from a ^{60}Co source in *straw pawn* $ptc^x/M(2)c^{33a}$, *stw pwn* $ptc^x/M(2)S7$ and in *stw pwn* ptc^x/*shavenoid* genotypes, ptc^x representing the *patched* allele under examination. Wings were examined under phase-contrast illumination for the phenotypes of the cell marker mutations *pawn* (*pwn*) and *shavenoid* (*sha*) (Fig. 2).

Non-radioactive detection of in situ *hybridization in whole mounts of imaginal disc*

Larvae and prepupae were collected from the media and walls of a yeasted cornmeal–agar milk bottle, washed and dissected in phosphate-buffered saline (PBS), pH 7.2. The anterior halves of animals were separated, inverted and stored on ice for less than 30 min; fixed for 15 min on ice in 4 % paraformaldehyde in PBS and washed three times for 1 min in diethylpyrocarbonate-treated PBS plus 0.1 % Tween 20 (PBT). The prehybridization treatment and hybridization conditions are based on the non-radioactive whole mount *in situ* hybridization protocol for *Drosophila* embryos of Tautz and Pfeifle (1989). The inverted heads were digested for 3 min at 22 °C in 50 μg ml^{-1} proteinase K in PBT and washed twice for 2 min in 2 mg ml^{-1} glycine in PBT. After two 1 min washes in PBT, the heads were fixed for a further 20 min in 4 % paraformaldehyde in PBS at 22 °C, then washed for 5 min in 2 mg ml^{-1} glycine in PBT, twice for 5 min in PBT, 5 min in PBT:hybridization buffer (1:1), and 3–5 h in hybridization buffer (50 % formamide, 5×SSC, 100 μg ml^{-1} denatured herring sperm DNA and 0.1 % Tween 20) at 45 °C. The heads were hybridized overnight at 45 °C in hybridization buffer containing 1 μg ml^{-1} denatured digoxigenin–dUTP-labelled DNA. Heads were washed for 20 min at 22 °C in the hybridization solution then in a series of hybridization solution: PBT dilutions (4:1, 3:2, 2:3, 1:4) for 20 min each and finally in PBT twice for 20 min. Incubation in polyclonal sheep anti-digoxigenin Fab fragments conjugated to alkaline phosphatase (Boehringer Mannheim) at 0.375 i.u. ml^{-1} in PBT for 1 h at 22 °C was followed by four 20 min washes each in PBT and two 5 min washes in AP buffer (100 mM NaCl, 50 mM $MgCl_2$, 100 mM Tris pH 9.2 and 0.1 % Tween 20). Finally, they were developed in the 'brown' alkaline phosphatase substrate (Kit III, SK-5300, Vector Laboratories) or in AP buffer with nitroblue tetrazolium salt (337.5 μg ml^{-1}) and 5-bromo-4 chloro-3-indolyl phosphate toluidinium salt (175 μg ml^{-1}) for 1 h at 22 °C in the dark. The reaction was stopped by transfer to PBT. Individual discs were dissected in PBS, mounted in Hydromount (National Diagnostics) and photographed using Differential Interference Contrast photomicroscopy. For detection of β-galactosidase activity, the material was incubated for 10 min (*en–lacZ*) or 3 h (*neu–lacZ*) at 37 °C in X-gal staining solution (Simon *et al.* 1985) after the initial fixation and 1 wash in PBT, then the hybridization protocol was continued. Larvae heterozygous for *en–lacZ* or *neu–lacZ* were collected from an outcross to Canton S wild-type flies.

The probe for detection of *patched* expression was prepared by random primer labelling (Feinberg and Vogelstein, 1983) using digoxigenin-modified dUTP and the *patched* cDNA plasmid, pC7. This plasmid was isolated (Phillips, unpublished) from an 8–12 h embryonic cDNA library generously provided by N. Brown using genomic probes from the *patched* region (Nakano *et al.* 1989). The probe was prepared using the Non-radioactive DNA Labelling and Detection Kit (cat. no. 1093 657, Boehringer Mannheim) as described in the instructions, using 100 ng of linear template DNA in a 20 μl reaction.

Results

Allelism between patched *and* tufted *mutations*

A new allele of the adult-viable recessive mutation tufted (Sturtevant, 1948) found in this laboratory (Whittle, 1980) is an embryonic recessive-lethal mutation with a segment-polarity larval cuticular phenotype identical to that of *patched* (Nüsslein-Volhard and Wieschaus, 1980; Nüsslein-Volhard *et al.* 1984). All 14 extant *patched* lethal alleles failed to complement the lethality of this new allele of *tufted*, producing instead embryos arrested with a typical *patched* larval cuticle; in addition they failed to complement the adult-viable phenotype of *tufted* (Fig. 1). This new lethal allele was therefore designated ptc^{S2} and *tufted* redesignated ptc^{tuf}.

Recovery of new ptc *alleles*

We carried out F_1 mutagenesis screens to identify new mutations that failed to complement features of the ptc^{tuf} phenotype. This procedure yielded 10 new lethal alleles of *patched*, and another adult-viable allele (Table 1). Among the 10 new lethal alleles, 8 were induced by P-M-dysgenesis, four of these being associated with P insertions at 44D3,4 (Hidalgo, unpublished). One of these, ptc^{P78} has been used to clone the *patched* gene (Nakano *et al.* 1989).

Patched mutations upset cuticular patterning in the wing

The combined evidence from the heteroallelic combinations of the 26 lethal and 3 viable alleles shows that the patterning of structures formed by all the imaginal discs and histoblast nests is dependent upon *patched* activity. Of these 78 heteroallelic combinations, only 5 have a wild-type phenotype (Roberts and Whittle,

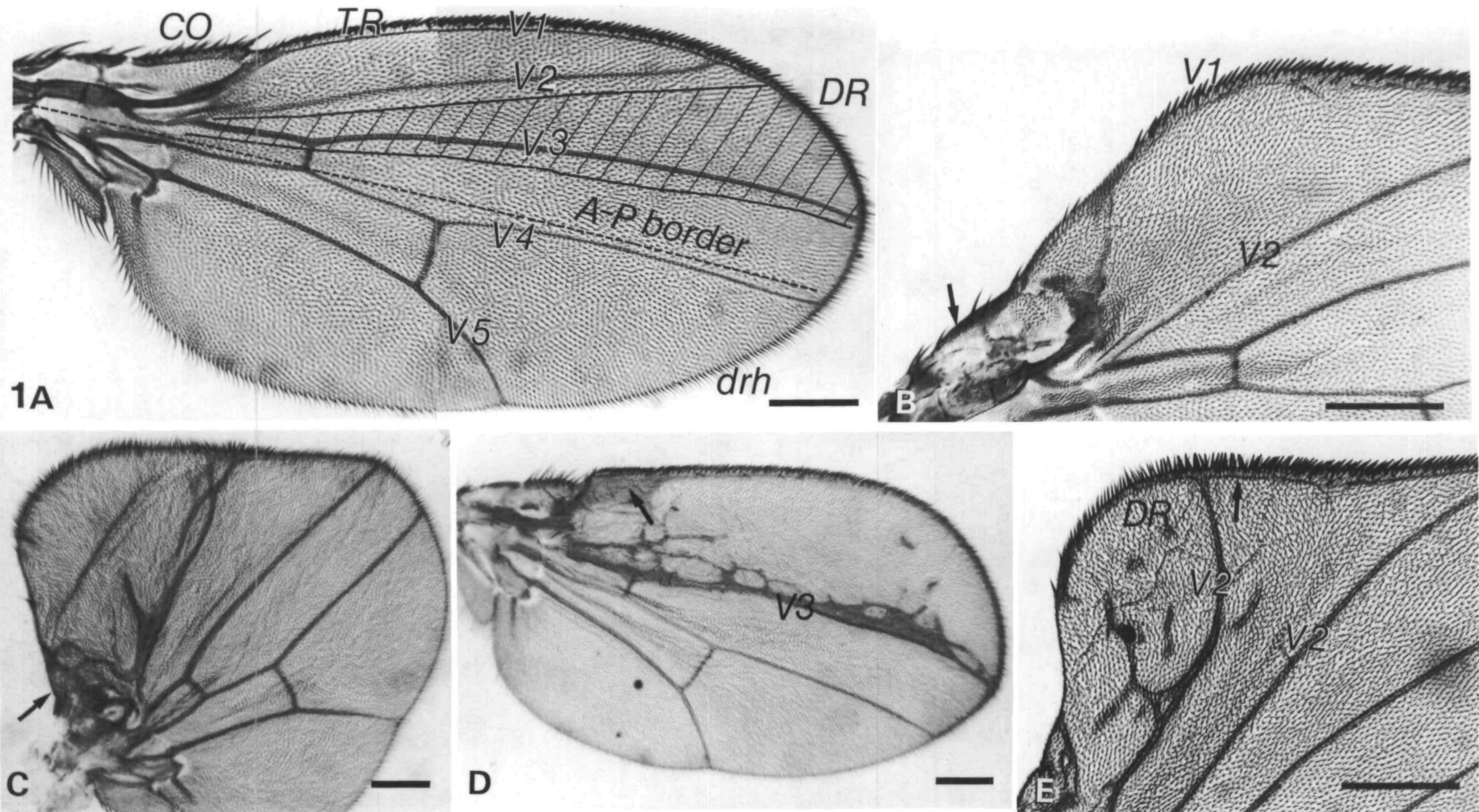


Fig. 1. A range of morphological patterns are produced in wings of *patched* adult-viable genotypes. (A) A wild-type wing showing the almost invariant pattern of veins (V1 to V5), the triple row (TR) and double row (DR) bristles of the anterior margin vein (V1) and the costal area (CO) and double row hairs (drh). The A/P border is shown by the dashed line. The solid line shows the proposed boundaries of regions 1,2 (shaded) and 3 (see Discussion). (B) Anterior edge of a wing from a ptc^{tuf} homozygote. The costal bristles are absent (arrow) and vein 1 is distorted. (C) Wing of genotype ptc^{tuf}/ptc^{S2}. There are gross disturbances in the leading edge of the anterior compartment; vein 2 is partially duplicated, the costal area is absent (arrow) and the wing is foreshortened on its proximo-distal axis. (D) Wing of genotype ptc^{G20}/ptc^{IN}. Vein 1 is broadened at the base (arrow), vein 2 has not formed and vein 3 is broadened and plexate. The shape and area of the wing anterior to vein 3 are both changed. (E) Anterior edge of a ptc^{tuf}/ptc^{S2} wing showing the duplication (V2′) of vein 2, the mirror-imaging of double row bristles (DR) and the change in orientation of the TR bristles at the plane of mirror symmetry (arrow). In all photomicrographs the bar represents 200 μm.

Table 1. *Provenance of mutations shown to define a single complementation group, the* patched *gene*

Allele superscript	Embryonic lethal (L) or adult-viable (V)	Mutagen	Original source
tuf	V	none, spontaneous	Sturtevant (1948)
6C 7M 8H 9B 1F 1N IIB IIC2 IIC8 IIE IIR IIU IIW IIX	L	Ethyl methane sulphonate	Max Planck Institute Tübingen Nüsslein-Volhard *et al.* (1984)
S2	L	gamma irradiation	University of Sussex (Whittle, 1980)
G12 G13	L	gamma irradiation	University of Sussex
RX67	L	ethyl methane sulphonate	P. Simpson, Strasbourg
G20	V	gamma irradiation	University of Sussex
P15 P66 P76 P78 P88 P93 P98 P201	L	P-M dysgenesis	University of Sussex
Associated with the rearrangement *T (2;3) dp*	V	reportedly spontaneous	Lindsley and Grell (1968)

unpublished). Here we report only upon the effects within the wing disc and its derivatives.

There is considerable phenotypic variability in the effects on the wing both within and between different *patched* genotypes. Wing phenotypes of ptc^{tuf} homozygotes range from wild-type to loss of costal bristles and moderate duplication of the base of vein 1 with the adjacent triple row margin. In the most subtle changes seen in the wing blade from this genotype, the base of vein 1 at its junction with the costa may be swollen or bulge out anteriorly. More extensive changes at the anterior margin include the loss of bristles of the medial and distal costa (Fig. 1B), broadening of the region between vein 1 and vein 2 with accompanying change of shape in vein 1 (Fig. 1B), or duplications of the base of vein 1 and associated triple row bristles (not shown). Combinations of ptc^{tuf} with lethal *patched* alleles cause more extensive deletions of the costa and duplications of veins 1 and 2. Vein 2 may be duplicated partially or through its entire length and the triple row bristles often show a change in their orientation (or 'polarity') between the sites at which the duplicate veins touch the margin (Fig. 1C and E). This local duplication of structures forms part of the most extreme change seen in *patched* wings in which the costa is also absent and the wing shape is broader, foreshortened and increased in the area occupied by the duplicated vein 2 (Fig. 1C and E). More or less severe changes of this kind are typical of the majority of adult-viable *patched* genotypes.

A contrasting phenotypic change in vein 2 and upsets involving vein 3 are seen only in genotypes that include the viable allele ptc^{G20}. In ptc^{G20} homozygotes the costal, triple row and double row bristles remain intact and show no polarity reversals but the wing shape and vein pattern are changed. In these wings, vein 2 is partially or completely deleted and veins 1 and 3 are broadened and/or plexate. In combination with lethal *patched* alleles, ptc^{G20} may cause loss of bristles in the costa, but the phenotypes of veins 1, 2 and 3 are not substantially different from those of ptc^{G20} homozygotes (Fig. 1D). In contrast to the changes seen in the anterior compartment, no *patched* genotype has yet been found in which posterior compartment structures are upset.

Although allelism between *patched* and *tufted* mutations was established on the basis of the wing blade disturbance, many combinations of *patched* lethal alleles with ptc^{tuf} have their more obvious effects in the derivatives of the eye antennal disc, or increase the number and upset the pattern of large bristles on the notum, scutellum and abdominal tergites (Roberts and Whittle, unpublished). The range and type of wing phenotypic changes described, illustrated in Fig. 1, make it clear that there is no simple 'phenotypic series' of graded and increasingly severe effects amongst the allelic combinations of *patched* mutations; indeed some combinations have phenotypically wild-type wings but severe disturbances in other disc derivatives.

Mosaics of patched *lethal alleles indicate position-specific* patched *requirements*

To analyse the cellular basis of the *patched* requirement in wings, we generated mosaic animals bearing clones of cells homozygous for *patched* lethal mutations (which we shall call *ptc* clones). The behaviour of *ptc* clones induced during wing disc growth by mitotic recombination could be divided into three categories, showing an important dependence upon the position of the clone. In region 1 of the wing blade (see Figs 1A and 3A for the limits of these regions), the recovery of *ptc* clones was significantly reduced, whilst in region 2 *ptc* clones affected vein pattern both autonomously and in neighbouring wild-type cells, and, in region 3, including the entire posterior compartment, there were no

Table 2. *Number and location of mitotic recombination clones found in the wing*

Genotype irradiated		Genotype of clone	Number of clones in wing between (Anterior) V1–V2	V2–L3	V3–A/P	(Posterior) A/P–V5	V5–margin	Total clones
	S	*sha/sha*	14	22	15	17	14	82
stw pwn ptc^{S2}/ *sha* experimental twin system	T	*stw pwn* ptc^{S2}/ *stw pwb* ptc^{S2} with *sha/sha*	0	12 (11)	8 (3)	10 (0)	5 (0)	35
	S	*stw pwn* ptc^{S2}/ *stw pwn* ptc^{S2}	0	1 (1)	0	2 (0)	1 (0)	4
stw pwn/sha	S	*sha/sha*	0	0	1	3	2	6
control twin system	T	*stw pwn/stw pwn* with *sha/sha*	6 (0)	7 (0)	6 (0)	11 (0)	6 (0)	36
	S	*stw pwn/stw pwn*	0	0	0	0	0	0
stw pwn ptc^{S2}/* experimental not-twin	S	*stw pwn* ptc^{S2}/ *stw pwn* ptc^{S2}	3 (0)	12 (12)	27 (13)	13 (0)	8 (0)	63
	All	*stw pwn* ptc^{S2}/ *stw pwn* ptc^{S2}	3 (0)	24 (23)	35 (16)	23 (0)	13 (0)	98

S, single clone; T, twin clones; *, unmarked chromosome or carrying *M(2)*C^{33a} or *M(2)S7*.
Numbers in parenthesis are clones associated with ectopic venation.
V1, 2, 3 and 5, veins 1, 2, 3 and 5.
A/P, Anterior/Posterior border.

phenotypic consequences of the presence of the *ptc* clone.

There is a significant shortfall in *ptc* clones recovered between the anterior wing margin and vein 2 (Fig. 1A and Table 2). Mitotic recombination in larvae of the genotype *stw pwn* ptc^{S2}/*sha*, proximal to *straw*, should produce twin daughter clones which are either ptc^- or ptc^+ marked by *straw pawn* and *shavenoid*, respectively. The twin/single *shavenoid* clone ratio between the anterior margin and vein 2 is 0/14 compared to 35/68 in the remainder of the wing ($P<0.001$, Fisher's exact 2×2 test). Of 98 marked clones in total, only three lay in this area whereas this fraction for *multiple wing hairs* clones is 35/351 (Whittle, unpublished). Anterior compartment *ptc* clones are occasionally fragmented (5 cases, Fig. 2D) or considerably smaller than their *shavenoid* twin, particularly when the *shavenoid* twin is anterior to the *ptc* clone (6 cases in 20 sets of twins, Fig. 2B). Two lines of evidence suggest that region 1 extends posterior to vein 2. *ptc* clones are infrequent along the posterior edge of vein 2 as well as anterior to this vein and never form triple row bristles. One of the surviving *ptc* clones recovered in region 1 was associated with ectopic venation and we envisage that the essential requirement for *patched* in region 1 is superimposed on a vein patterning role similar to that in region 2 (see below). *In vivo* culture of disc primordia from embryos arrested during segmentation from two different hetero-allelic combinations of *patched* embryonic lethal alleles (ptc^{1N}/ptc^{G12} and ptc^{S2}/ptc^{G12}) (Simcox *et al.* 1989 and Simcox and Whittle, unpublished) provides confirming evidence that *patched* is required for development of region 1. The cells of these disc primordia proliferate in the abdomen of adult flies and, when transplanted to metamorphosing larvae, form extensive areas of double row bristles and posterior hairs characteristic of regions 2 and 3, but never form costal or triple row bristles (8 embryos tested).

Clones that do form in the anterior compartment never form part of any vein and wild-type cells bordering these clones often form ectopic vein not usually found at these locations (Fig. 2). Ectopic venation was found in 23 of 24 clones between veins 2 and 3, and in 16 of 35 clones between vein 3 and the A/P compartment border (Table 2). 24 clones were totally surrounded by vein tissue made of wild-type cells immediately adjacent to the clones (Fig. 2A,B,D), and all ectopic veins bordered *ptc* clones. *ptc* clones associated with extra venation are found in a proximo-distal sector of the anterior compartment on both dorsal and ventral surfaces (Fig. 1A, hatched area), and their borders in this region are smooth and straight (Fig. 2A–E) unlike wild-type clones (Garcia-Bellido and Merriam 1971; Bryant 1970) and *ptc* clones in the posterior compartment (data not shown). We have seen 7 cases of large *ptc* clones with straight borders posterior and adjacent to vein 3 that have no ectopic veination (e.g. Fig. 2F). The posterior boundary of region 2 can be defined by a group of 4 clones, including one shown in Fig. 2E, which are surrounded by ectopic vein except at the posterior edge where the clone extends into region 3. The boundary is posterior to the wild-type vein 3

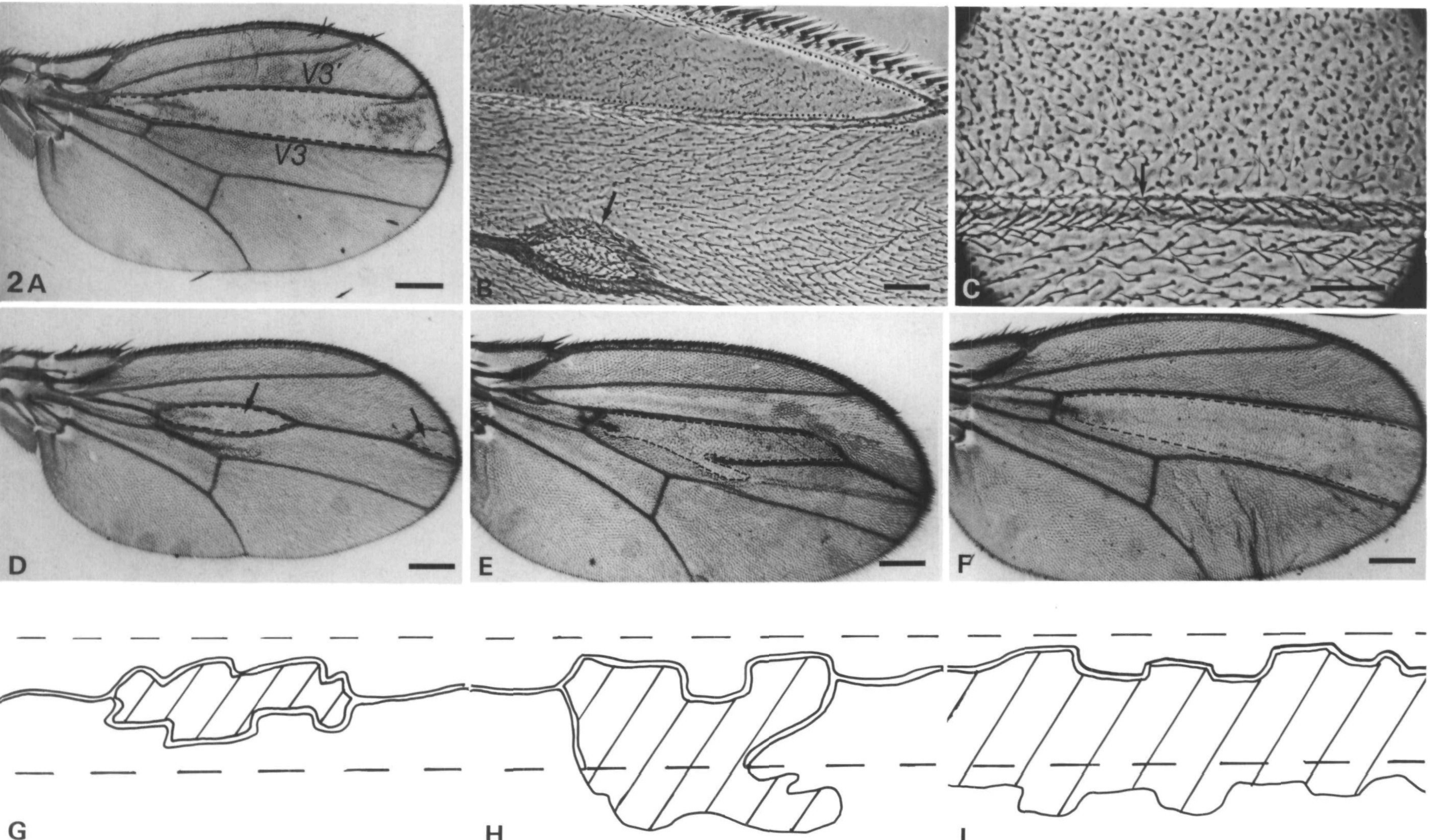


Fig. 2. ptc^{S2} genetic mosaics show position-dependent effects on wing pattern. (A) A large clone (dashed line) immediately bordered by an ectopic vein anteriorly (V3′). (B) A small clone (dashed) bordered by veins (arrow) anterior to which is a large *shavenoid* twin clone (dotted). (C) Higher magnification of veins with wild-type trichomes (arrow) bordering *ptc* clone. (D) Some clones (dashed line) are split (arrows) but still bordered by veins. Some clones are bounded by veins except on their posterior edge (dashed line) (E), whilst (F) other large clones smoothly abut vein 3 and the A/P border (dashed line). This clone crosses the margin, covering both dorsal and ventral surfaces. In G, H and I we have represented diagrammatically, possible earlier stages of clones shown in D, E and F respectively, before 'vein smoothing' (see Discussion). The dotted line indicates the limits of the postulated competence region (region 2). In A,D,E and F the bar represents 200 μm and in B and C 50 μm.

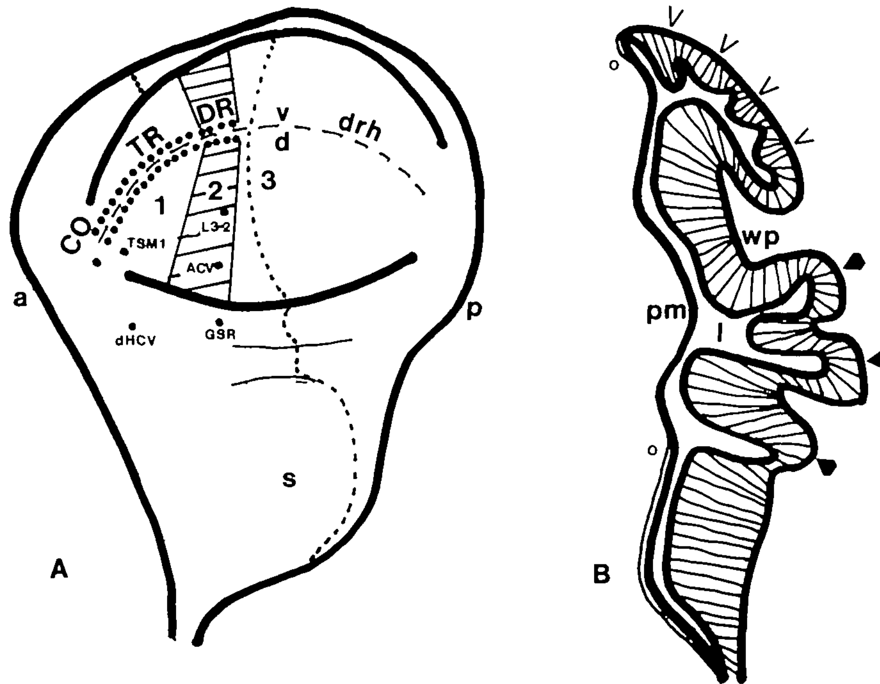


Fig. 3. Diagrammatic representation of morphological features and fate map of the wing disc. (A) 'Flat' aspect of the wing disc as viewed from the peripodial side. The dotted line marks the anteroposterior border (a anterior, p posterior) and the dashed line the dorsoventral border (d dorsal, v ventral). The heavy line marks the fold that surrounds the wing pouch, primordium of the adult wing blade. Light lines mark folds of the middle disc region and stalk (s). Features of the presumptive wing margin, which follows the d–v border, are primordia for the costal (CO), triple row (TR) and double row (DR) bristles and the double row hairs (drh) as in Fig. 1A. Heavy dots mark the site of primordia for individual bristles or sensilla that express *neu–lacZ* in late 3rd instar early prepupal discs. The multiply innervated bristles (recurved) are one type of marginal bristle present in both TR and DR on both sides of the D/V border for which a precursor is identifiable at this stage both immunohistologically (Hartenstein and Posakony, 1989) and by *neu–lacZ* expression (Phillips, unpublished). The early twin sensilla of the margin (TSM1), dorsal humeral cross vein sensilla (dHCV), the giant sensillum of the radius (GSR), the anterior cross vein sensillum (ACV) and the middle sensillum of vein 3 (V3–2) derive from precursors which can be detected at this stage by immunohistology (Murray *et al.* 1984) and by *neu–lacZ* expression (Phillips, unpublished). The shaded area is the approximate location of region 2. (B) Cross-section of a wing disc adapted from Milner *et al.* (1984) indicating the relative depth and folding of the columnar epithelium including the wing pouch (wp) which projects into the disc lumen (l). The pouch faces the peripodial membrane (pm), the centre part of which (between open circles) is squamous epithelium (Milner *et al.* 1984). Three folds (closed arrow heads) are present in the columnar epithelium dorsal to the wing pouch which correspond to folds seen in the middle of the disc in A. Four thickenings of the columnar epithelium (open arrow head) are present ventral to the wing pouch.

position and anterior to the A/P border. All the data in Table 2 relate to clones of ptc^{S2} but similar ectopic venation has been found close to shavenoid clones in the genotype ptc^{P76}/sha, suggesting that this P-element disrupted allele behaves similarly to ptc^{S2}.

Patched expression revealed by in situ *hybridisation to whole-mount discs*

We have used a non-radioactive method (see Materials and methods) to detect *in situ* hybridization of a *patched* cDNA probe to RNA in whole-mount imaginal discs and applied this simultaneously with detection of *β*-galactosidase activity to discs from stocks carrying *lacZ* reporter P-element inserts in either the *engrailed* gene (*en–lacZ*) or the *neuralized* gene (*neu–lacZ*). This novel double-labelling procedure allows us to relate the pattern of *patched* expression to features of the imaginal disc fate map (Fig. 3A).

In imaginal wing discs from third instar larvae and prepupae, cells in a stripe parallel to the A/P border express *patched* intensely (Fig. 4A–I)). The stripe follows the border of the posterior compartment as defined by expression of *en–lacZ* (Fig. 4B–E). No unlabelled cells intervene between *patched* and *en–lacZ* expressing cells in the double-labelled preparations. Some cells of the stripe express only *patched* whilst others express both *patched* and *en–lacZ* (Fig. 4C–E). In the wing pouch, posterior to the *patched* stripe, *patched* expression decreases abruptly. By contrast, anterior to the stripe, expression decreases over several cell diameters to a lower level which is maintained thoughout the anterior compartment. The contrast between anterior and posterior staining is stronger in prepupal discs (Fig. 4H,I) than in larval discs (Fig. 4A,G).

Interpretation of the relationship of the stripe relative to the compartment border is complicated by the complex three-dimensional structure of the disc which

is formed by an invagination of a group of cells that straddle the A/P border in the embryonic epithelium. The invagination flattens and maintains only a narrow attachment to the larval epithelium, called the stalk (Fig. 3A). Therefore the A/P border runs continuously from the stalk, up one side of the flattened disc and down the other side, back to the stalk. One side of the disc is a folded columnar epithelium and on the other the epithelium is stretched to form the squamous cells of the peripodial membrane (Fig. 3B). The wing pouch, the primordium for the adult wing blade, bulges against the peripodial membrane (Fig. 3B). Because the membrane is very thin, the features visible from the peripodial side are primarily those of the columnar wing pouch (Fig. 4A,B,D,F–I). The stripe of *patched*-expressing cells in the pouch is 2–3 cells wide and is interrupted by a gap consisting of 15–20 cells expressing *patched* at a lower level at the intersection of the A/P border with the dorsal–ventral compartment (D/V) border as defined by expression of the *neu–lacZ* insertion (Fig. 4F–H). β-galactosidase activity is detected in the precursors of bristles and sensilla in the discs of larvae carrying this insertion. In the wing disc, these precursors include two rows of multiply innervated bristles (recurved), which lie on either side of the D/V border on the primordium of the anterior wing margin, as well as campaniform sensilla on veins 1, 2 and 3, and macroachaetes on the costa (Fig. 3A). The stripe of *patched* expression is separated from the primordia of vein 3 sensilla by 2–3 cells (Fig. 4F). In the middle of the disc, the A/P border and the *patched* stripe descend into and emerge from three major folds (Fig. 4B,C) and cross the stalk near the posterior edge of the disc. At the top of the pouch, the stripe descends into a fold and ascends laterally. Stripes of *patched*-expressing cells border both sides of the posterior compartment in the stalk (Fig. 4C) as would be expected in a pouch-like invagination. The extension of this border across the upper part of the peripodial side is undetectable probably because these cells are thin. *patched* is also expressed in the other imaginal discs (Phillips, in preparation).

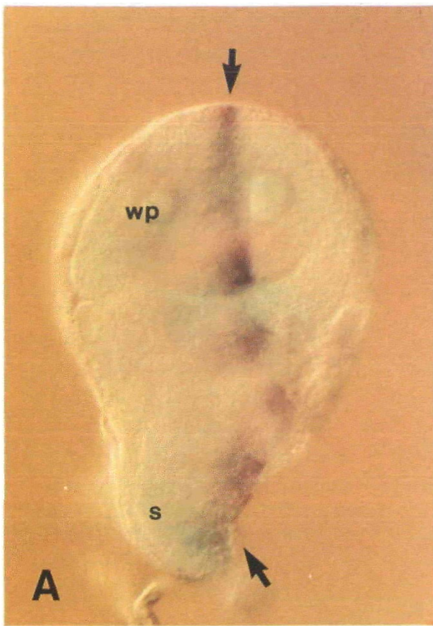
Fig. 4. Patterns of expression of *patched* in whole-mount imaginal wing discs. Hybridization is indicated by brown or purple (A,H) precipitate from a non-radioactive detection technique (Materials and methods). All discs are oriented wing pouch (wp) at top and stalk (s) down; anterior compartment (a) left and posterior compartment (p) right. A stripe of *patched* expression (between arrows) is the primary feature of wild-type III instar larval (A) and brown prepupal (I) wing discs. Discs from *en–lacZ* larvae prepared for simultaneous detection of *patched* and *en–lacZ* expression (blue green) show the relationship between *patched* and the posterior compartment (B,C,D,E). The stripe of *patched* expression is adjacent to the A/P border as viewed from the peripodial surface (B,D) or from the columnar surface (C,E). The stripe descends into and emerges from folds (solid arrowhead) in the middle region of the disc on both surfaces (B,C). The A/P border runs diagonally from the back of the pouch on the columnar side of the disc (C). As a result the posterior compartment underlies the anterior compartment and creates a blue background in this region (*) when viewed from the peripodial side (B). On the columnar side (C) clusters of cells with intense hybridization (open arrowhead) are visible ventral to the back of the pouch. In the stalk, expression flanks both sides of the posterior compartment (arrows). (D,E) Some cells of the stripe express only *patched* (arrowheads) while others express both *patched* and *en–lacZ* (arrow). (F,G,H) Discs from *neu–lacZ* larvae prepared for simultaneous detection of *patched* and *neu–lacZ* expression (blue green) show the position of *patched* expression relative to identified primordia on the wing disc fate map, including the presumptive wing margin (m) and sensilla of vein 3 (V3–2, and ACV, see Fig. 3A). A gap in *patched* stripe on the wing pouch is apparent at the presumptive wing margin in mid-third instar larvae (arrow) in (G) when the precursors of the marginal bristles are first detected and persists through white (F) and brown (H) prepupal stages. Developmental stages were assigned by the criteria of Bainbridge and Bownes (1981).

Discussion

We have presented genetic evidence that the segment-polarity gene *patched* is required in some cells of the wing disc for normal spatial patterning. The expression pattern of *patched* in the wing disc is complex spatially and temporally. We offer an interpretation of the role of *patched* that integrates these observations, suggesting it to be a component in a cell-to-cell position-signalling mechanism which is required in at least two processes in the developing wing: (1) *patched* is required autonomously for cell viability and/or proliferation in the region adjacent and anterior to vein 2, and (2) *patched* is required for vein formation throughout the anterior compartment. The role of *patched* in cell communication is evident in the way that wild-type cells respond to adjacent ptc^{-} cells by forming ectopic vein.

We distinguish three regions in our analysis of *patched* (1, 2 and 3, Figs 1A and 3A), which must represent different cellular ‘environments’ with respect to the contribution of *patched*. In region 1, few *ptc* clones are recovered and those that do survive are small and/or fragmented and do not form veins. Our twin clone analysis shows that *ptc* clones are not lost because of migration away from this region or through the failure of flies carrying such clones to eclose, but because the cells of *ptc* clones fail to proliferate or die before cuticle formation. However, even small clones are infrequent and no disturbance symptomatic of late loss of *ptc* clones has been seen, favouring the interpretation that the absence of *ptc* clones is due to a continuous or intermittent requirement for *patched* throughout disc growth. The finding that costal and triple row bristles are not produced by cultured tissue heteroallelic for *patched* lethal alleles is further evidence for this requirement.

Cells can survive in region 2 without *patched*; nevertheless all cells in this region require *patched* expression for normal development. The consequences of forming *patched* mosaics are two-fold and differ from those seen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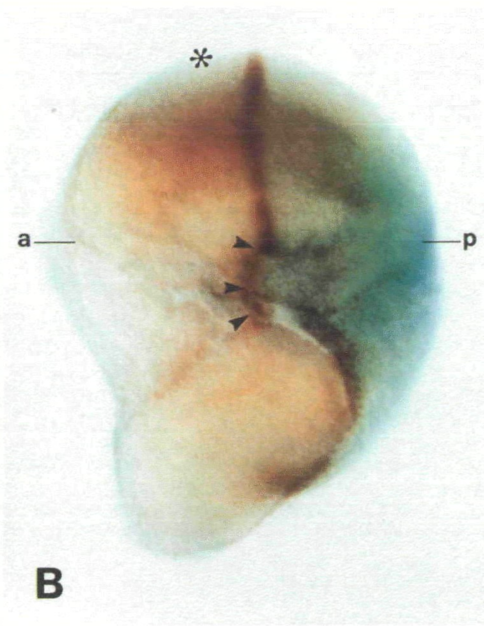
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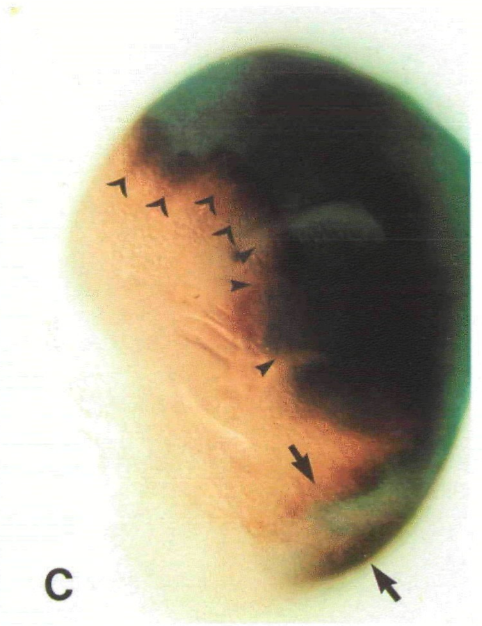
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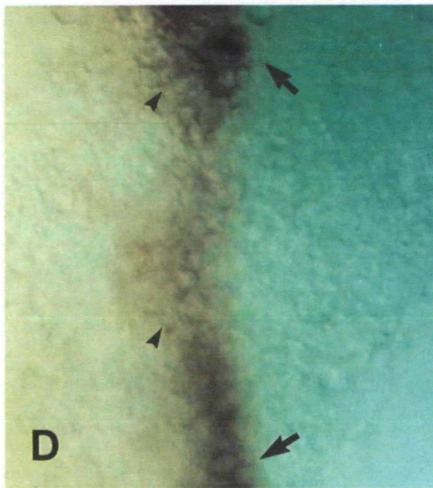
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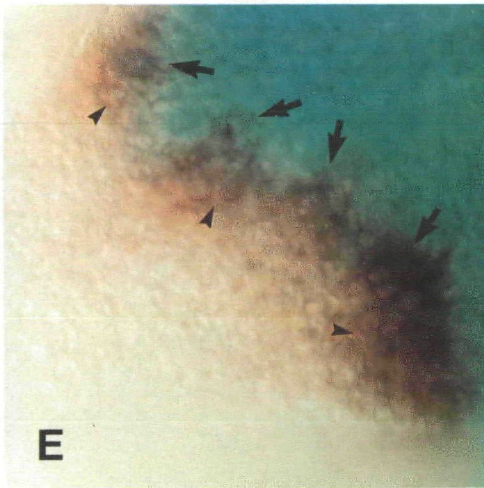
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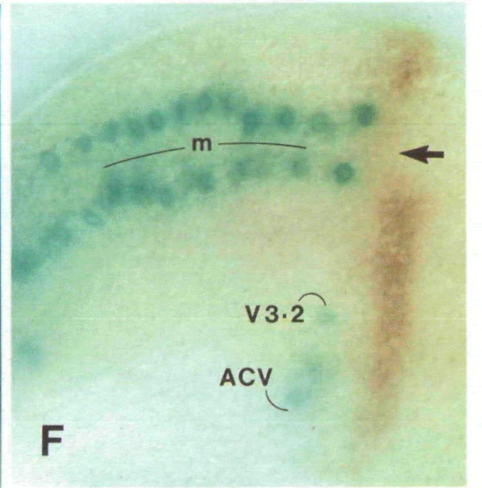
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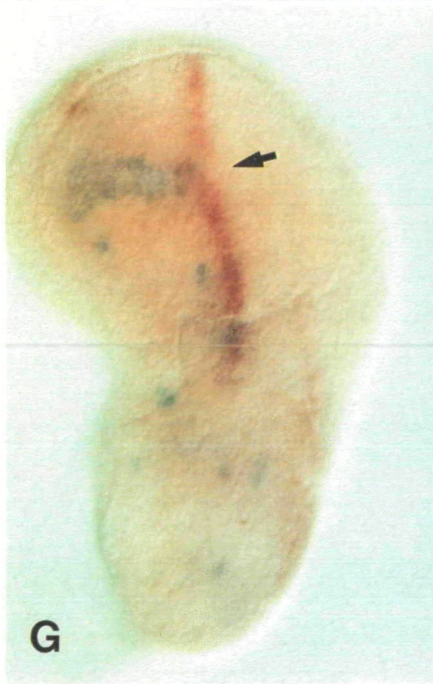
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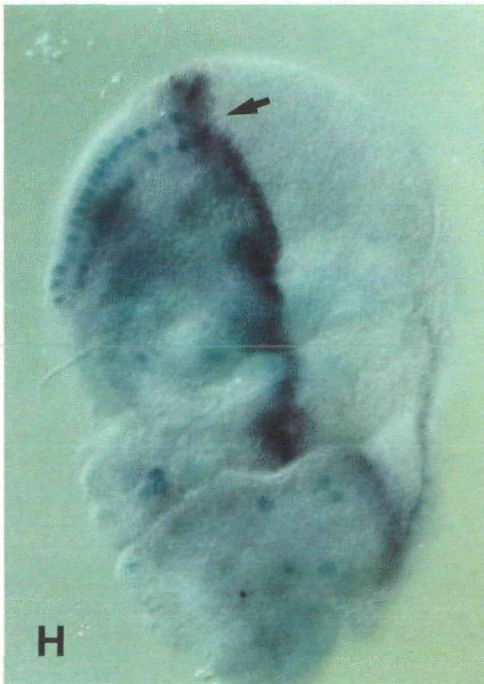
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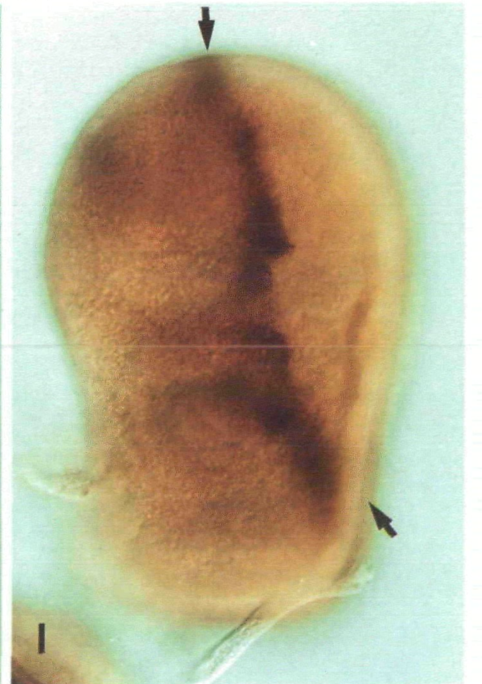
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in region 1. First, cells require *patched* before they can participate in forming veins. Second, the removal of *patched* expression from cells of region 2 causes their immediate neighbours to form veins, so implying a role for *patched* in signalling between cells in this region. We propose that *ptc* clones lead to the formation of veins in adjacent cells either because a positive induction signal is generated by default, or because *patched* is required in a lateral inhibition system for vein spacing in the anterior compartment. *ptc* clones in the centre of the region competent to form vein 3 (region 2) prompt vein formation on all sides (Fig. 2D,G), whereas clones straddling this region are only bounded by a vein within the region (Fig. 2E,H). Clones in which the majority of cells are outside and posterior to region 2 are subtly different; their anterior borders are atypically straight where the clones have entered region 2 and presumably induced vein 3 (Fig. 2F,I). We suggest that, once cells have become committed to differentiating a vein, a straightening or smoothing process occurs. In wild-type wings, this smoothing process would be undetectable, but in our mosaics, because the clone borders are also the vein borders the adjustment of the presumptive vein cells 'smooths' the outline of the marked *ptc* clone. The approximate position of the veins is established by the end of the larval period (Garcia-Bellido, 1977). However, specification of individual cells to form veins probably occurs during metamorphosis. Expression of *patched* increases throughout the anterior compartment at this stage, consistent with a role in this process in both regions 1 and 2.

Within region 3 *ptc* clones have irregular outlines, except where they obey the A/P compartment restriction. Clones in the posterior compartment participate in the formation of veins 4 and 5. Therefore *patched* is not involved in posterior vein patterning.

The boundaries between regions where *ptc* clones behave differently (regions 1, 2 3), appear to correspond to the boundaries of 'zones of positional homology' defined by the position-dependent response of clones homozygous for *shaggy* mutations (Ripoll *et al.* 1988; Simpson *et al.* 1988). Homozygous *shaggy* clones differentiate bristles rather than trichomes but the particular bristle morphology varies with position, resembling that of the adjacent marginal bristles defining 5 zones (A–E) in their Fig. 4 (Simpson *et al.* 1988).

We now consider the phenotypes produced by several adult-viable *patched* genotypes in the light of our distinction between the *patched* requirement in regions 1 and 2. The viable alleles, ptc^{tuf} and ptc^{G20}, confer distinct spectra of abnormalities. Allelic combinations with ptc^{tuf} delete parts of the costa and expand the triple row region. The altered patterns of marginal bristles and venation shown in Fig. 1B,C and E, in particular the mirror imaging in Fig. 1E, would be consistent with the consequences of loss of cells with the positional values corresponding to the costa and triple row bristles on the wing disc fate map (Bryant, 1975). However, adult-viable genotypes that include ptc^{G20} have a very different wing phenotype (Fig. 1D). Polarity reversals and pattern duplications are very rare but veins are abnormal. Veins 1 and 3 are widened or plexate while vein 2 is often interrupted or deleted. This suggests that ptc^{G20} is specifically deficient for the *patched* activity required in vein regulation while ptc^{tuf} is deficient for the cell viability function in region 1. If *patched* is required for both lateral inhibition and specification of vein-forming cells, as the clonal analysis implies, then cells that are hypomorphic for this activity might be competent to form veins but unable to regulate the amount of vein, resulting in broadened veins. Vein 2 might be supressed by cells having either too little or too much activity (see below).

We detect an intense stripe of *patched* expression that straddles the A/P border in imaginal discs. In contrast, during embryogenesis, *patched* expression is restricted to cells that do not produce *engrailed* protein and a regulatory relationship between these genes has been proposed (Martinez-Arias *et al.* 1988; Dinardo *et al.* 1988; Hooper and Scott, 1989; Nakano *et al.* 1989; Hidalgo and Ingham, unpublished). Large *ptc* clones abutting the A/P border for much of its length fail to show any phenotypic changes (Fig. 2F). We suggest three possible interpretations of this result: (1) if the role of *patched* in this stripe is not cell-autonomous, *patched*-expressing cells of the posterior compartment could 'rescue' a large anterior *ptc* clone and *vice versa*. (2) the expression of *patched* in this region may have no function, being a relic of the embryonic expression pattern (Nakano *et al.* 1989; Hooper and Scott, 1989) or (3) the high level of *patched* expression in this area might have an autonomous role in vein suppression thus defining the posterior boundary of region 2. *ptc* clones could not be used to detect this function because they are also incompetent to form veins.

The data presented in this paper mean that *patched* joins the growing collection of genes shown to be important in imaginal disc patterning that are also deployed during embryonic segment patterning, and which are known in that context as segment polarity genes (Nüsslein-Volhard and Wieschaus, 1980; Baker, 1988*a*, 1988*b*, Mohler 1988; Perrimon and Mahowald 1987; Busson *et al.* 1988; Grau and Simpson 1987; Simpson and Grau 1987; Diaz-Benjumea and Garcia-Bellido, 1990). The hydropathy profile of the *patched* coding sequence suggests that it is a multi-transmembrane domain protein (Nakano *et al.* 1989; Hooper and Scott 1989). This and the domineering non-autonomy of *ptc* clones are strongly consistent with our proposed role for *patched* in cell-to-cell signalling during pattern formation.

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