groucho and hedgehog regulate engrailed expression in the anterior compartment of the Drosophila wing

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SUMMARY

Drosophila imaginal discs are divided into units called compartments. Cells belonging to the same compartment are related by lineage and express a characteristic set of ‘selector genes’. The borders between compartments act as organizing centres that influence cell growth within compartments. Thus, in the cells immediately anterior to the anterior-posterior compartment boundary the presence of the hedgehog product causes expression of decapentaplegic, which, in turn, influences the growth and patterning of the wing disc. The normal growth of the disc requires that posterior-specific genes, such as hedgehog and engrailed are not expressed in cells of the anterior compartment. Here we show that hedgehog can activate engrailed in the anterior compartment and that both hedgehog and engrailed are specifically repressed in anterior cells by the activity of the neurogenic gene groucho. In groucho mutant discs, hedgehog and engrailed are expressed at the dorsoventral boundary of the anterior compartment, leading to the ectopic activation of decapentaplegic and patched and to a localised increase in cell growth associated with pattern duplications. The presence of engrailed in the anterior compartment causes the transformation of anterior into posterior structures.

Key words: gene regulation, wing development, engrailed, groucho, Drosophila

INTRODUCTION

The adult cuticular structures of Drosophila melanogaster arise from groups of ectodermal cells called imaginal discs. These groups of cells are specified during embryonic development (Bate and Martinez-Arias, 1991; Cohen, 1993) and proliferate extensively during larval life. The development of imaginal discs involves their subdivision into lineage units called compartments (Garcia-Bellido et al., 1973). Anterior and posterior cells are distinguished by the activity of engrailed (en), which is expressed and required throughout development in all posterior cells (Lawrence and Struhl, 1982; Brower, 1986). Dorsal and ventral compartments are specified by the activity of apterous (ap) which is expressed and required in all dorsal cells (Diaz-Benjumea and Cohen, 1993; Blair et al., 1994).

Genetic and developmental experiments involving the confrontation of cells belonging to different compartments (Karlsson, 1981; Diaz-Benjumea and Cohen, 1993) indicate that the borders between compartments influence the ordered growth of the disc. Thus, confrontation between anterior and posterior cells allows the expression of dpp in anterior cells adjacent to the A/P boundary (Tabata and Kornberg, 1994; Basler and Struhl, 1994; Sanicola et al., 1995), where it is required for the development of the disc (Posakony et al., 1991). The role of compartment borders in the control of growth indicates that some mechanism must exist during normal development to prevent the expression of en and ap in the ‘wrong’ compartment. This repression requires the activity of Polycomb (Pc) (Busturia and Morata, 1988; Tong and Russell, 1990).

In this paper we show that the repression of en in the anterior compartment requires the function of the neurogenic gene groucho (gro), and that gro is involved in the control of growth and pattern formation in imaginal discs. gro encodes a nuclear protein expressed ubiquitously in both embryos and imaginal discs (Tata and Hartley, 1993) which is able to interact with the helix-loop-helix (HLH) proteins encoded by both the Enhancer of split complex and hairy. Thus, gro may be a component of protein complexes that can bind to DNA and regulate transcription (Paroush et al., 1994). The requirements for gro have been extensively characterised during the formation of the embryonic nervous system (Schrons et al., 1992; Hartley et al., 1988), where it is required downstream (Lieber et al., 1993) of the membrane receptor Notch (N) during the singling out of neuroblasts (Artavanis-Tsakonas et al., 1991).

In gro mutant wing discs en is ectopically expressed in regions of the dorsoventral boundary (DV) in the anterior compartment. The presence of en-expressing cells in this region triggers a cascade of gene interactions similar to those normally occurring at the anterior-posterior boundary, leading to the expression of dpp in novel positions and to overgrowth and pattern duplications. We also find that the presence of Hedgehog protein (Hb) in the anterior compartment leads to the activation of en, in addition to the ectopic expression of
dpp and ptc (Capdevila and Guerrero, 1994; Basler and Struhl, 1994; Tabata and Kornberg, 1994). In the anterior wing margin, the presence of en causes the transformation of anterior bristles into posterior hairs, confirming the role of en in the distinction of anterior and posterior elements in the wing margin (Garcia-Bellido and Santamaria, 1972; Gubb, 1985; Hidalgo, 1994). These observations suggest that en regulation in the imaginal disc is dependent on a variety of mechanisms, operating both in the anterior and posterior compartments. Some aspects of en regulation in the anterior compartment rely on cell-to-cell interactions mediated by Hh and Gro proteins.

**MATERIAL AND METHODS**

**Drosophila strains**

We used the viable allele gro\(^{1}\), the lethal alleles gro\(^{E73}\) and gro\(^{E48}\) (Preiss et al., 1988), and the gro deletions Df(3R)E(spl)RA7.1, Df(3R)E(spl)RX22 and Df(3R)E(spl)RX1 that include gro and adjacent genes of the Enhancer of split complex (Schrons et al., 1992). Viable phenotypes were scored in all combinations between gro\(^{1}\) and both lethal alleles and the deficiencies. The cell marker mutations used were bald (bld\(^{1}\)) and forked (f\(^{66}\)). The shaggy allele used was sgg\(^{m11}\) (Siegfried et al., 1992). hh\(^{Mrt}\) homozygous flies were generated after removal of associated lethals in the original hh\(^{Mrt}\) strain (Tabata and Kornberg, 1994) by meiotic recombination.

**Generation of mitotic recombination clones**

**gro clones**

Mitotic recombination was induced by X-rays (dose 1000R; 300 R/min, 100 Kv, 15 mA, 2 mm Al filter). Irradiated larvae were timed in hours after egg laying (AEL). Clones were scored in males of genotypes f\(^{66}\); mwh bld f\(^{+}\)/gro (twin clones) and f\(^{66}\); mwh M(3)w f\(^{+}\)/gro (M + clones). The different gro alleles (gro) used were gro\(^{E73}\), gro\(^{E48}\), Df(3R)E(spl)RX22 (Preiss et al., 1988), and Df(3R)E(spl)RA7.1 (Schrons et al., 1992). The cytological position of the f\(^{+}\) insertion is 87F. Mitotic recombination proximal to the f\(^{+}\) insertion produces homozygous gro cells labeled with the marker forked (f).

In the twin experiments, the mean number of cells in clones induced at 48-72 hours AEL were: 150 f gro\(^{E73}\) vs. 180 bld gro\(^{+}\) (39 twin clones), and 76 f Df(3R)E(spl)RA7.1 vs. 136 bld E(spl)\(^{+}\) (24 twin clones). The mean number of cells in twin clones induced at the same age in the control experiment f\(^{66}\); mwh bld f\(^{+}\)/+ were 134 f vs. 114 bld (20 twin clones). In M + experiments, the mean number of cells were: f M(3)w\(^{+}\)gro\(^{E73}\) 972 cells (39 clones) and f M(3)w\(^{+}\)E(spl)RA7.1

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**Fig. 1.** gro viable phenotypes. (A) Wild-type and (B-F) gro/Df(3R)E(spl)RA7.1 wings. gro mutant wings show varying degrees of overgrowth and pattern duplications in the anterior compartment. Weak phenotypes correspond to distal duplications of the LIII vein (B,E), and thickening of the proximal anterior wing margin (F). Strong phenotypes correspond to disappearance of the LII vein and formation of ectopic and thicker LIII vein (C), and overgrowth of the proximal wing margin with duplications of the LIII vein (D). The regions of the wing margin between the duplicated LIII vein bears sensory elements typical of distal regions of the wing (D,E). In the legs, the tarsal segments are duplicated (G,H), with a low frequency, and the femur and tibia may fuse lengthways (I). (J) Higher magnification of the tibia-femur junction in.
260 cells (26 clones). The mean number of cells in control $M^+$ experiment in $f$; $M(3)w^f/+$ was 1727 (32 clones).

**shaggy; gro clones**

$sgg^{m11.1};Df(3R)E(spl)BX22$ mutant clones were obtained by X-ray mitotic recombination in $sgg^{m11.1};Dp(1;3)w^67k$. $sgg^+/Df(3R)E(spl)BX22$ male larvae. Mitotic recombination proximal to the $sgg^+$ duplication generates $sgg^{m11.1};Df(3R)E(spl)BX22$ homozygous cells. Control $sgg$ clones were obtained in $sgg^{m11.1}/+$; TM3/+ sisters, after X-ray induced mitotic recombination on the X chromosome. In all cases, $sgg^{m11.1}$ clones were identified by the differentiation of ectopic sensory elements in the wing blade (Simpson et al., 1988; Blair, 1992b).

**Other methods**

Whole-mount in situ hybridisation with digoxigenin-labeled probes and immunocytochemistry were performed as described by Cubas et al. (1991) and Ruiz-Gomez and Ghysen (1993).

Wings were mounted for microscopic examination in lactic acid:ethanol (1:1).

**RESULTS**

**gro viable phenotypes in imaginal development**

Genetic combinations between the viable allele $gro^I$ and either lethal alleles or deficiencies (see Material and methods) cause a variety of phenotypes. These phenotypes have greater penetrance and expressivity in combinations of $gro^I$ with deficiencies that remove $gro$ and adjacent genes of the $E(spl)$ complex, than in combinations of $gro^I$ with $gro$ lethal point alleles. Here we will focus on the effects on wing development. The phenotype of $gro$ in the wing consists of overgrowth and pattern duplications in regions close to the D/V compartment boundary in the anterior compartment. The phenotype is variable, both in the region of the wing affected and in the degree of overgrowth. Weak phenotypes consist of distal bifurcation of vein LIII and thickening of the proximal costa (Fig. 1F). Extreme phenotypes consist of overgrowth and pattern duplications in proximal (Fig. 1D) or distal regions of the anterior wing margin, including duplication and triplication of the LIII vein and disappearance of the LII vein (Fig. 1B,C,E). In these cases, the sensory elements that appear in the affected regions are typical of distal regions of the wing (Fig. 1E), irrespective of the position of the overgrowth. This range of phenotypes has not been observed in the case of mutations in other neurogenic genes, such as Delta and Notch, suggesting that they identify a novel function of $gro$ that may be independent of the neurogenic gene pathway.

The same genetic combinations of $gro$ alleles cause the appearance of clusters of macro- and microchaetae in particular regions of the head, thorax and tergites (de Celis et al.,

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**Fig. 2.** Mosaic analysis of $gro$ lethal alleles and $gro$ deficiencies. (A,B) Dorsoventral $gro^{E73}$ clone causes the differentiation of a thicker LIII vein and a distal duplication of the LIII vein. Mutant cells do not differentiate sensory elements in the wing margin, instead they cause a weak notching of the wing. (C) Dorsoventral $E(spl)^{RA7.1}$ clone causing a mirror-image duplication of regions around the proximal part of the anterior wing margin. (D) Small $gro^{E73}$ clone in the anterior wing margin causing the absence of sensory organs and the displacement of the LII vein. (E) Ventral $E(spl)^{RA7.1}$ clone producing the differentiation of a thicker LII vein, and distal overgrowth. (F) Dorsal $E(spl)^{RA7.1}$ clone causing the thickening of the LII vein and the absence of sensory elements in the anterior wing margin.
**Fig. 3. engrailed expression in gro mutant wing discs.** (A) *en* is expressed in the posterior compartment in wild-type discs. (B,C) In gro mutant discs (gro/E(spl)^M), clusters of anterior cells also express *en*. These clusters appear in distal (B) or in proximal (C) regions of the presumptive wing margin. (D,E) Double staining of third instar wing discs with anti-cut (brown) and anti-en (black) antibodies in gro mutant discs reveals that *en*-expressing cells appear in both dorsal and ventral regions abutting the presumptive wing margin.

**Fig. 4. wg and en expression in gro mutant discs and the phenotype of sgg; gro double mutant clones.** (A) Double *wg-en* staining in gro mutant disc shows that *en*-expressing cells (black) are mainly included in the *wg* realm of expression (brown). (B) Control anterior and posterior sgg clones. (C,D) Double mutant sgg; gro clones in the anterior compartment differentiate sensory elements similar to those of the anterior wing margin, and in the posterior compartment differentiate long hairs (E) typical of the posterior wing margin. Note that the number of bristles that appear in sgg; gro double-mutant clones is reduced compared to sgg single-mutant clones. This may be due to a transformation of the external part of the sensory organ to neurons, caused by the neurogenic effect of gro.
These phenotypes are similar to those produced by mutations in other neurogenic genes (de Celis et al., 1991). The phenotype in the legs consists of duplications of tarsal segments and tibia (Fig. 1G,H) and in proximodistal fusion of the tibia with the femur (Fig. 1I,J). These phenotypes appear with very low frequency, and were not analysed further.

Overgrowth phenotypes associated with pattern duplications only occur when gro mutant cells are at the dorsoventral boundary of the anterior compartment

To analyse the effect of the complete absence of gro function, and to define where the gro gene is required, we studied several
Away from the wing margin gro is not required to repress en expression

We have analysed whether the absence of gro function can lead to ectopic en expression away from the D/V boundary region in the anterior compartment, by studying the expression of en in clones of the gro deficiency Df(3R)E(spl)P22. The frequency of E(spl)P22 clones induced at 48-72 hours AEL in the anterior compartment of the wing is 5%. However, we only found ectopic expression of en in 3 out of 170 wing discs after irradiation at 48-72 hours AEL. In all three cases ectopic en expression was found in regions close to the presumptive D/V boundary. The low frequency of ectopic en expression and its consistent location suggests that the requirement of gro to repress en is restricted to the D/V boundary.

To analyse further if en is derepressed in regions of the wing away from the D/V boundary, which we designate as ‘internal’, we examined clones simultaneously mutant for the genes shaggy (sgg) and gro (see Materials and methods). sgg mutant cells in the wing blade differentiate bristles or hairs typical of the wing margin, and the type of structure formed depends on the position in the wing of the sgg cells (Simpson et al., 1988). The type of sensory elements differentiated by sgg:gro mutant clones in the anterior compartment is indicative of the state of en activity, as en function determines the differences between anterior bristles and posterior hairs (García-Bellido and Santamaria, 1972). We found 8 sgg:gro double-mutant clones in the anterior compartment which differentiated anterior bristles (Fig. 4B,C), as did single-mutant sgg clones (Simpson et al., 1988; Blair, 1992b) (Fig. 4A). However, sgg:gro clones in the posterior compartment differentiate hairs typical of the posterior wing margin (Fig. 4E). These results confirm that anteriorly en is not being expressed in internal regions of the wing in gro mutant clones, and suggest that the properties of the D/V boundary that define the gro requirement to repress en are independent of sgg inactivation.

hedgehog, patched and decapentaplegic are also ectopically expressed at the dorsoventral boundary of the anterior compartment in gro mutant discs

The ectopic expression of en in gro mutant discs, and the pattern alterations in gro wings, suggests that other posterior genes, such as hh, and anterior genes normally expressed at the A/P boundary, such as decapentaplegic (dpp) and patched (ptc), are also expressed incorrectly in gro mutant wing discs. We find that hh, dpp and ptc are all ectopically expressed in the anterior compartment of gro mutant discs, in clusters of cells positioned along the D/V boundary (Fig. 5A,B,C). The ectopic clusters of dpp- and ptc-expressing cells are similar in size to each other and include more cells than do the hh and en ones (compare Fig. 3B with 5A, and 5B with 5C). Double staining using en and dpp probes in gro mutant discs shows that the realm of expression of dpp includes the en-expressing cells, and also adjacent dorsal and ventral cells (Fig. 5D,E). The coexpression of en and dpp in anterior cells in gro discs is surprising, as it appears that en can repress dpp expression in the posterior compartment (Sanicola et al., 1995). However, we notice that the ectopic expression of en is consistently lower than the normal expression of en in the posterior compartment. It is therefore possible that the levels of en expression in the anterior compartment in gro mutant discs are insufficient to

gro null alleles in genetic mosaics. Mosaics of the lethal alleles groE72 and groE48 and the gro deficiencies Df(3R)E(spl)P22 and Df(3R)E(spl)P22 (see Material and methods) were induced between 48 and 72 hours after egg laying. The phenotypes of gro lethal alleles and of gro deficiencies are very similar to each other, and will be described together as the gro phenotype. The phenotype of gro mutant cells depends on the region of the wing they occupy. In the anterior compartment, all gro clones abutting the wing margin cause local overgrowth phenotypes and pattern duplications (Fig. 2A,C,E). gro clones induced after the separation of the dorsal and ventral compartments respect the D/V boundary. Interestingly, gro clones abutting the wing margin and restricted to either the dorsal (17 clones) or ventral (14 clones) wing surfaces cause the overgrowth of both dorsal and ventral cells (Fig. 2E,F), indicating a non-autonomous effect of mutant cells on wild-type cells of the opposite wing surface. A similar, non-autonomous effect on growth across the D/V border occurs with clones of en mutant alleles in the posterior wing (Lawrence and Struhl, 1982).

In addition to overgrowth phenotypes most gro clones abutting the wing margin cause the absence of sensory organs and weak scalloping of the margin (Fig. 2A,B,F). gro clones do not cause any apparent phenotype in inter-vein regions, but, when the clones appear in vein regions, extra-vein tissue is differentiated (Fig. 2A). These latter effects on wing margin formation and vein differentiation are similar to those caused by N mutant clones (de Celis and García-Bellido, 1994) and suggest that N and gro functions are related in these processes.

The position of the overgrowth in gro mutant wings is correlated with the presence of clusters of cells that ectopically express en

The phenotypes of gro viable allelic combinations and of gro lethal alleles in clones are similar to those described in the hh gain of function allele Moonrat (hhMrt) (Tabata and Kornberg, 1994; Felsenfeld and Kennison, 1995) suggesting that lack of gro could be causing incorrect expression of genes such as en and hh in the anterior compartment. Indeed, we find that this is the case; in gro mutant wing discs en is ectopically expressed in the anterior compartment (Fig. 3B,C). The frequency of gro mutant discs with ectopic expression of en is similar to the frequency of gro mutant wings with overgrowth phenotypes (10%), suggesting a causal relationship between them. In addition there is a good correlation between the positions of ectopic en clusters and the regions in which overgrowth and pattern duplication occur in gro mutant wings.

The position of the ectopic en-expressing cells was mapped with respect to the expression of cut (ct) and wingless (wg). In mature wing discs ct and wg are expressed by both dorsal and ventral cells along the D/V boundary, the domain of wg being broader than that of ct (Blochinger et al., 1993; Blair, 1993). Ectopic en-expressing cells can appear in either dorsal or ventral compartment, or simultaneously in both (Fig. 3D,E), but always along the D/V boundary. These ectopic en-expressing cells occur mainly within the wg realm of expression along the D/V boundary (Fig. 4A). The en-expressing clusters consist of few cells, suggesting that they are generated late in development. That small clusters of anterior en-expressing cells include dorsal and ventral cells implies that cells belonging to different lineages were recruited to express en.
repress \textit{dpp}. The ectopic and overlapping expression patterns of \textit{en}, \textit{hh}, \textit{ptc} and \textit{dpp} in clusters of anterior cells are similar to those that occur in anterior cells along the normal \textit{A/P} boundary (Raftery et al., 1991; Blair, 1992a).

\textbf{gro is also required in the setting up of \textit{en} expression during embryonic development}

To investigate whether \textit{gro} may also be important in regulating \textit{en} at other stages in development, we studied the expression of \textit{en} in \textit{gro} mutant embryos. It is known that there is a strong maternal component of \textit{gro} (Schröns et al., 1992), and that in \textit{gro} embryos derived from germ line clones, the expression of several segmentation genes, such as \textit{hairy} and \textit{en} is altered (Paroush et al., 1994). In embryos lacking only zygotic \textit{gro} function we find that at stage 11 \textit{en} stripes are broader in \textit{gro} mutants than in wild type (Fig. 6A,B). Furthermore, cells expressing \textit{en} ectopically appear in the anterior compartment (Fig. 6), although the number and position of these cells is variable (Fig. 6D,E,F). Similarly, in \textit{gro} embryos, cells expressing \textit{hh} ectopically can be identified (Fig. 6C,G). These observations suggest that zygotic \textit{gro} function is also required to establish the normal pattern of \textit{en} and \textit{hh} expression during embryogenesis.

\textbf{\textit{hh} can activate the expression of \textit{en} in the anterior compartment}

Since it has been shown that \textit{hh} is activated by \textit{en} (Tabata et al., 1992; Sanicola et al., 1995), it seems likely that the ectopic expression of \textit{hh} observed in \textit{gro} mutants is a consequence of the de-repression of \textit{en}. We have also investigated the possibility that \textit{hh} activates \textit{en}. To do this, we studied the expression of \textit{en} in discs with altered expression of \textit{hh}. \textit{hh}\textsuperscript{Mrt} is a gain-of-function allele that results in expression of \textit{hh} in the anterior compartment (Tabata and Kornberg, 1994; Felsenfeld and Kennison, 1995). We generated \textit{hh}\textsuperscript{Mrt} homozygous flies (see Material and methods) which allowed us to study the effects of high levels of \textit{hh} in the anterior compartment. Homozygous \textit{hh}\textsuperscript{Mrt} flies show a constant phenotype characterised by the thickening and duplication of the \textit{LII} vein, the absence of the \textit{LII} vein, and the differentiation of posterior hairs and distal bristles along the anterior wing margin (Fig. 7). These phenotypes would be expected if \textit{dpp} were expressed throughout the anterior wing blade and \textit{en} were expressed at the presumptive anterior wing margin. We therefore analysed \textit{en} expression in \textit{hh}\textsuperscript{Mrt} homozygous discs, and found that \textit{en} is expressed in the presumptive anterior wing margin (Fig. 7F), in a very similar pattern to that of \textit{hh} expression (Fig. 7E). The coincidence of \textit{hh} and \textit{en} expression patterns in the anterior compartment in \textit{hh}\textsuperscript{Mrt} wing discs indicates that \textit{Hh} protein is capable of activating \textit{en} transcription. The failure to detect ectopic \textit{en} expression in heterozygous \textit{hh}\textsuperscript{Mrt} discs (Felsenfeld and Kennison, 1995) suggests that \textit{en} activation by \textit{hh} is sensitive to levels of \textit{hh} expression.

In \textit{hh}\textsuperscript{Mrt} homozygous wing discs, \textit{dpp} is also expressed ectopically, and is detected in a much broader domain than those of \textit{en} and \textit{hh} (Fig. 7H). The comparison of \textit{en} and \textit{dpp} expression in \textit{hh}\textsuperscript{Mrt} discs (Fig 7G,H) shows that regions with higher levels of \textit{en} (the presumptive wing margin) have lower or no \textit{dpp} expression, and that only in regions where ectopic expression of \textit{en} is lower is \textit{dpp} coexpressed with \textit{en}. The implication is that high levels of \textit{en} expression are required to repress \textit{dpp}. We observe a similar relationship between \textit{cubitus interruptus dominant} (\textit{ci}\textsuperscript{D}) and \textit{en} expression. In wild-type discs \textit{ci}\textsuperscript{D} expression occurs in the anterior compartment (Fig. 7I), and \textit{ci}\textsuperscript{D} is repressed by \textit{en} during embryogenesis (Eaton and Kornberg, 1990). In \textit{hh}\textsuperscript{Mrt} homozygous discs, the expression of \textit{ci}\textsuperscript{D} is reduced or absent only in the region of maximal ectopic expression of \textit{en}. These results are in agreement with the coexpression of \textit{en} and \textit{ci}\textsuperscript{D} late in wing disc development (Blair, 1992a).

\section*{DISCUSSION}

We have identified two novel mechanisms operating in the regulation of \textit{en} expression in the wing imaginal disc. First, \textit{en} is specifically repressed at the D/V boundary of the anterior compartment by the activity of \textit{gro}. Second, \textit{en} can be activated in the anterior compartment by the secretable protein (Lee et al., 1992). Ectopic \textit{en} expression in the anterior compartment causes a transformation of anterior into posterior structures in the wing margin. The presence of ectopic \textit{en} in the anterior compartment leads to an ectopic activation of \textit{dpp} in novel positions, and to overgrowth and pattern duplications (Fig. 8). We find that in \textit{gro} and \textit{hh}\textsuperscript{Mrt} mutants, \textit{en} and \textit{dpp} can be coexpressed in the anterior compartment, a situation that seems to be incompatible with the repression of \textit{dpp} mediated by \textit{en}. It is possible that the level of \textit{en} expression in these anterior cells is lower than necessary to repress \textit{dpp}, as additional mechanisms of \textit{en} repression operate in the anterior compartment (Busturia and Morata, 1988).

\textbf{Repression of \textit{en} and \textit{hh} by \textit{gro} in the anterior compartment}

We find that in \textit{gro} mutant discs \textit{en} and \textit{hh} are expressed at the D/V boundary of the anterior compartment in clusters of cells whose position is correlated with the appearance of overgrowth and pattern duplications in \textit{gro} mutant wings. The Gro protein is localised in the nucleus and may function as a transcriptional corepressor that interacts with basic HLH transcription factors such as its neighbours in the \textit{E(spl)} complex (Tata and Hartley, 1993; Paroush et al., 1994). \textit{gro} is required for neuroblast segregation where it is functionally related to \textit{Notch}. This functional relationship appears to be conserved in other processes later in development (e.g. vein and chaetae differentiation). However, the repression of posterior genes by \textit{gro} does not appear to involve the action of \textit{N}, as in later development \textit{N} loss-of-function mutations cause the absence of the wing margin, but no pattern duplications (de Celis and Garcia-Bellido, 1994). It is still possible that \textit{N} is involved in the localisation of \textit{gro} corepressors at the D/V boundary, and that \textit{N} may be indirectly related to \textit{en} repression in this region. We suggest that \textit{N} and \textit{gro} are related functionally only when the expression of \textit{gro} corepressors is dependent on \textit{N} signalling, as in the case of \textit{E(spl)} during neurogenesis (Jennings et al., 1994).

Strikingly, the repression of posterior genes by \textit{gro} is only operative in the vicinity of the D/V boundary. It is therefore possible that the particular cell proliferation properties and gene expression patterns at the D/V boundary (Schubiger and...
Palka, 1987; Williams et al., 1993) require an additional level of en repression to that exerted by Pc. Consistent with the localised requirement for gro, some putative gro partners, the HLH proteins of the E(spl) complex, are expressed at higher levels along the D/V boundary (J. de Celis and S. Bray, in preparation). It is possible, therefore, that the restricted expression of the E(spl) complex limits the region in which Gro is capable of repressing en in the anterior wing.

Fig. 7. Developmental characterisation of the hh gain of function allele hh\textsuperscript{Mrt}. Homozygous hh\textsuperscript{Mrt} flies survive to pharate adults that die inside the puparium. hh\textsuperscript{Mrt}/hh\textsuperscript{Mrt} wings (A) show transformation of the anterior wing margin into posterior, and differentiate a thicker and duplicated LIII vein (D). B and C show higher magnifications of the anterior (B) and posterior (C) wing margin in hh\textsuperscript{Mrt}/hh\textsuperscript{Mrt} wings. The anterior wing margin is formed by both long hairs typical of the posterior margin and bristles, typical of distal regions of the wing (arrows in B). Interestingly, despite the transformation of the sensory organs, their spacing is typical of the anterior compartment. Alteration in the expression patterns of hh (E), en (F,G), dpp (H) and ci\textsuperscript{D} in hh\textsuperscript{Mrt}/hh\textsuperscript{Mrt} wing discs. E,F,I and J are RNA expression patterns, and G is a higher magnification of the presumptive wing blade of an hh\textsuperscript{Mrt}/hh\textsuperscript{Mrt} disc stained with anti-en-inv antibody. Note the similarity in the ectopic expression of en and hh along the presumptive wing margin (E, F), the broader expression of dpp in which lower dpp levels (H) coincides with maximal expression of en (G), and the reduction in ci\textsuperscript{D} expression in the same region (J). I shows the normal expression of ci\textsuperscript{D}. In I and J anterior is to the right.
Trans-activation of en mediated by hh

It is surprising to find that hh can activate en in the anterior compartment. This result implies that the normal repression of en in this compartment depends on an additional pathway, besides that mediated by Pc. This second pathway of en-repression could be mediated by ptc since it is known that hh antagonises the repression by ptc of downstream genes, such as dpp, during imaginal development and wg during embryonic development (Capdevila et al., 1994; Ingham and Hidalgo, 1993). Interestingly, it has already been suggested that ptc might mediate en repression in the anterior compartment on the basis of the phenotypes of ptc clones. The ptc phenotype is corrected in such clones when they are simultaneously mutant for en (Hidalgo, 1994). The activation of en by hh provides a simple mechanism to explain en expression in the cells of the anterior lineage that lie immediately adjacent to the A/P compartment border of the mature wing disc (Blair, 1992a). Whether hh activation of en operates to maintain en expression in the posterior lineage during normal development is not known.

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