Control of cell fate and polarity in the adult abdominal segments of *Drosophila* by *optomotor-blind*

Artyom Kopp and Ian Duncan*

Department of Biology, Washington University, 1 Brookings Drive, St Louis, MO 63130, USA

*Author for correspondence (e-mail: duncan@biodec.wustl.edu)

**SUMMARY**

In an accompanying report (Kopp, A., Muskavitch, M. A. T. and Duncan, I. (1997) *Development* 124, 3703-3714), we show that Hh protein secreted by posterior compartment cells patterns the posterior portion of the anterior compartment in adult abdominal segments. Here we show that this function of Hh is mediated by *optomotor-blind* (*omb*). *omb* mutants mimic the effects of loss-of-function alleles of *hh*: structures from the posterior of the anterior compartment are lost, and often this region develops as a mirror image of the anterior portion. Structures from the anterior part of the posterior compartment are also lost.

In the pupa, *omb* expression in abdominal histoblasts is highest at or near the compartment boundary, and decreases in a shallow gradient toward the anterior. This gradient is due to activation of *omb* by Hh secreted by posterior compartment cells. In contrast to imaginal discs, this Hh signaling is not mediated by *dpp* or *wg*. We describe several gain-of-function alleles that cause ectopic expression of *omb* in the anterior of the segment. Most of these cause the anterior region to develop with posterior characteristics without affecting polarity. However, an allele that drives high level ubiquitous expression of *omb* (*Qd*F0) causes the anterior tergite to develop as a mirror-image duplication of the posterior tergite, a pattern opposite to that seen in *omb* mutants. Ubiquitous expression of *hh* causes similar double-posterior patterning. We find that *omb* alleles suppress this effect of ectopic *hh* expression and that posterior patterning becomes independent of *hh* in the *Qd*F0 mutant. These observations indicate that *omb* is the primary target of Hh signaling in the adult abdomen. However, it is clear that other targets exist. One of these is likely *Scuffy*, a novel gene that we describe, which acts in parallel to *omb*.

To explain the effects of *omb* alleles, we propose that both anterior and posterior compartments in the abdomen are polarized by underlying symmetric gradients of unknown origin. We suggest that *omb* has two functions. First, it specifies the development of appropriate structures both anterior and posterior to the compartment boundary. Second, it causes cells to reverse their interpretation of polarity specified by the underlying symmetric gradients.

Key words: *optomotor-blind*, *hedgehog*, pattern formation, segment polarity, *Drosophila*

**INTRODUCTION**

The epidermis of each segment in *Drosophila* is subdivided into anterior and posterior lineage compartments defined by expression of the *engrailed* (*en*) gene (Lawrence and Struhl, 1996, and references therein). The anterior-posterior compartment boundary has organizing properties that are essential for pattern formation (Lawrence and Struhl, 1996; Tabata et al., 1995). Posterior cells secrete a short-range inducer encoded by *hedgehog* (*hh*) (Lee et al., 1992; Mohler and Vani, 1992; Tabata et al., 1992), which causes anterior cells at the compartment boundary to express the long-range morphogens *decapentaplegic* (*dpp*) or *wingless* (*wg*) (Alexandre et al., 1996; Dominguez et al., 1996; Hepker et al., 1997; Ingham, 1993; Zecca et al., 1995). In the thoracic imaginal discs, these morphogens are responsible for most or all patterning in both compartments (Brook and Cohen, 1996; Jiang and Struhl, 1996; Lecuit et al., 1996; Nellen et al., 1996; Penton and Hoffmann, 1996; Zecca et al., 1995). In the embryonic epidermis, Wg secreted by anterior cells at the compartment boundary patterns only the posterior portion of the anterior compartment (Bokor and DiNardo, 1996; Lawrence et al., 1996), and the anterior portion of the posterior compartment (Dougan and DiNardo, 1992). The remainder of the anterior compartment is patterned by Hh secreted by posterior cells from the preceding segment (Bokor and DiNardo, 1996; Heemskerk and DiNardo, 1994). Such intersegmental signaling is precluded in the thoracic imaginal discs, as these are physically isolated from cells of other segments. Since the thoracic discs are derived from only a portion of the segment straddling the compartment boundary (Simcox et al., 1991), they may lack pattern elements equivalent to those specified by *hh* in the embryonic epidermis.

In this report, we focus on patterning in the epidermis of the adult abdomen. The abdominal cuticle is not produced by imaginal discs, but develops from histoblast nests that are continuous with the larval epidermis. There are three major histo-
blast nests in each hemisegment: an anterior dorsal, posterior dorsal and ventral nest (Madhavan and Madhavan, 1980). Histoblasts do not divide during the larval stages, but undergo rapid division during the pupal stage, when their progeny spread over the surface of the segment and replace the degenerating larval epidermal cells.

The abdominal epidermis appears to be patterned by two interacting systems: a hh-dependent system that directs patterning near the compartment boundary, and a more global mirror-symmetric system that is responsible for patterning throughout the entire segment (Kopp et al., 1997). In the absence of hh signaling, this global patterning system generates a mirror-symmetric, double-anterior pattern. The function of hh in the abdominal segments appears to be twofold. First, Hh, or an unknown morphogen induced in anterior cells by hh, functions to specify a range of cell fates in the posterior of the anterior compartment and may also be involved in determining cell fates in the posterior compartment. Second, hh signaling causes cells in the posterior of the anterior compartment to reverse their interpretation of the underlying symmetric polarity. This function of hh appears to require a certain threshold level of hh activity (Kopp et al., 1997).

In this paper, we show that both functions of hh are mediated by the gene optomotor-blind (omb). omb is a homolog of the vertebrate Brachyury gene, and encodes a T-box DNA-binding protein thought to function as a transcription factor (Pflugfelder et al., 1992a,b). omb is required for normal development in the CNS (Brunner et al., 1992; Pflugfelder et al., 1992b; Poeck et al., 1993b), leg and wing (Grimm and Pflugfelder, 1996; Lecuit et al., 1996b; Nellen et al., 1996), and adult abdomen (this report). The locus is complex, and alleles affecting only the CNS (e.g. ombH32), wing (bsfd (bs) and lacquered (lq)) or abdomen (Quadroon (Qd) alleles) are known (Bangia et al., 1986; Pflugfelder et al., 1990; this report).

In the wing imaginal disc, omb is a target of dpp, which in turn is induced anterior to the compartment boundary by hh (Lecuit et al., 1996b; Nellen et al., 1996). omb is expressed symmetrically about the compartment boundary and is required for distal outgrowth of the wing blade (Grimm and Pflugfelder, 1996). Here we demonstrate that omb also functions downstream of hh in the adult abdomen, although here its regulation is independent of dpp. We show that omb mediates both the morphogenetic and polarity-reversal functions of hh, and is probably the most important target of hh signaling in the adult abdomen. In addition, we describe a novel gene, Scruffy (Scf), that acts in parallel to omb in the abdomen, and probably also the wing.

**MATERIALS AND METHODS**

The mutations QdF2, QdF3 and Scf were recovered incidentally in different X-ray mutagenesis experiments. The alleles QdF2E1, QdF2E7 were selected after irradiation of the QdF allele. QdF was isolated and very generously provided to us by Dr Barry Ganetzky. omb null mutants and the UAS-omb line were kindly provided by Dr Gert Pflugfelder and Dr Stephan Grimm. All alleles used are described elsewhere (Kopp et al., 1997). The FRT19A and hs-FLP38 lines are described by Xu and Rubin (1993) and Chou and Perrimon (1992), respectively. The en-lacZ reporter used was the XboZ construct of Hama et al. (1990). The hs-GAL4 driver is described by Brand and Perrimon (1993).

To generate somatic omb− clones, flies of the genotype y59b omb− f56a FRT19A ly+ omb− FRT19A; hs-FLP38/+ were heat shocked for 2 hours during embryonic or larval stages. Alternatively, y59b omb− f56a ly+ omb− f− animals were irradiated with 1000 rad of X-rays. The omb− alleles used were l(1)omb92, l(1)omb988 and l(1)bi241 (Pflugfelder et al., 1990; Poeck et al., 1993a).

Heat-shock treatments, cuticle mounts, pupal abdominal epidermis whole mounts and all staining procedures were as described (Kopp et al., 1997). The omb RNA probe was prepared as described by Poeck et al. (1993b). The omb cDNA was kindly provided by Dr Gert Pflugfelder.

In situ hybridization of phage clones to polytene chromosomes was as described (Cai et al. 1994). The omb genomic walk (Pflugfelder et al., 1990) was a gift from Dr Gert Pflugfelder and Dr Helmut Roth.

**RESULTS**

**Dominant alleles of omb cause anterior to posterior transformations within abdominal tergites**

Several gain-of-function alleles of omb cause transformations of anterior tergite toward the posterior. The first such allele identified, Quadroon (QdF), causes the posterior pigment band in each abdominal tergite to be expanded to the anterior (Thompson, 1959) (Fig. 1B). Allelism of QdF with omb was established by Pflugfelder et al. (1990). We have isolated two other dominant alleles, Qd and Qd2, that have phenotypes similar to QdF. Both are associated with chromosomal breaks at the locus of omb (4C). A fourth dominant allele, QdF, was discovered by Dr Barry Ganetzky and very kindly provided for analysis. To isolate additional gain-of-function alleles, we irradiated the QdF allele and screened for enhancement of the abdominal phenotype. Of the seven enhancers isolated, three (QdF1E1, QdF1E3 and QdF1E4) have chromosomal breaks at 4C, suggesting they result from additional mutations at omb. We have not been able to separate the remaining enhancers from QdF by recombination, suggesting they are also allelic to omb. The effects of the Qd mutations appear to be specific to the adult abdomen; we see no abnormalities in embryos or in adult structures derived from imaginal discs. The Qd mutations are described in Table 1.

Allelism of QdF with omb was established by the finding that two X-ray-induced reversions cause an omb− phenotype when hemizygous. Allelism of QdF with omb was demonstrated by in situ hybridization of phage clones from the omb genomic walk to mutant polytene chromosomes. The breakpoints in QdF and QdF fall within the clones XIII-35 and XI-40, respectively (Fig. 4 in Pflugfelder et al., 1990), placing them in the 5′ region of omb. Since deficiencies and loss-of-function alleles of omb have no dominant phenotype, the Qd mutations are clearly gain-of-function alleles.

**The phenotypes of QdF mutants**

With the exception of QdF, the Qd mutations can be arranged in a phenotypic series according to their strengths (Fig. 1). Qd+/− heterozygotes show a relatively weak expansion of the posterior pigment band, whereas QdF and QdF+ heterozygotes are stronger and similar in phenotype to QdF homozygotes (Fig. 1B,C). The enhanced QdF alleles QdF1E1 and QdF1E4 cause an extreme expansion of the posterior pigment band, resulting in tergites that are totally black. The remaining enhanced QdF alleles are similar to QdF and QdF when het-
erozygous. Several heterozygous combinations of Qd alleles show, in addition to very strong pigment band expansion, patches of unpigmented posterior hairy zone (PHZ)-like cuticle within the tergite (Fig. 1D). engrailed does not become activated in these PHZ patches (not shown), indicating that they retain anterior compartment identity. None of the mutations causes any alteration in polarity or bristle morphology in the tergite, and none affects the sternites or the pleura (not shown).

In contrast, the QdFub allele has a dramatic effect on both polarity and bristle patterning. In QdFub hemizygotes and heterozygotes, the anterior tergite and intersegmental membrane (ISM) are deleted and replaced with a mirror-image duplication of the posterior tergite and PHZ (Fig. 2A). Ectopic macrochaetes are often, but not always, present at the anterior edge of the duplicated tergite structures, and sometimes also in the central tergite (Figs 2A, 3C, 6D). The lines of polarity reversal are not fixed precisely with respect to cuticular pattern. In the most extreme phenotype, polarity is reversed exactly in the middle of the tergite and in the middle of the PHZ (Figs 2A, 3C). More frequently, the line of polarity reversal is shifted anteriorly in the tergite and posteriorly in the PHZ (Fig. 6D). The phenotype is stronger in hemizygous males than heterozygous females, and is stronger in more posterior segments. The intertergal region is often compressed, and the dorsal longitudinal muscles underlying the tergites show irregular spacing and attachment sites. The effects of QdFub are much weaker ventrally; trichome polarity at the extreme anterior of the sternite and in the neighboring pleura is disturbed, but sternal bristles are unaffected (not shown).

Surprisingly, polarity reversal in QdFub occurs within the en-expressing domain, rather than at the presumptive segment boundary (Fig. 3C). Examination of mid-stage pupal epidermis shows that en-expressing histoblasts do not migrate beyond the segment boundary (which is marked by specialized larval border cells – Kopp et al., 1997). This suggests that QdFub causes the transformation of most or all of the ISM region of the posterior compartment into ectopic PHZ with reversed polarity.

Ubiquitous omb expression from a UAS-omb transgene (Grimm and Pflugfelder, 1996) causes a phenotype very similar to that of QdFub. We subjected hs-GAL4/UAS-omb pupae to a single 2-hour heat shock between 12 and 30 hours after puparium formation (APF). These animals died prior to tergite pigmentation. However, the effects on polarity were dramatic: in pharate adults rescued from pupal cases, ISM and anterior tergite were completely replaced by a mirror-image duplication of posterior tergite and PHZ (Fig. 2B). No ectopic macrochaetes were observed, since ectopic omb expression

### Table 1. Quadroon alleles of omb

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<th>Allele</th>
<th>Cytology</th>
<th>Viability in hemizygote</th>
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<tr>
<td>QdFub</td>
<td>In(1)4C;20 +</td>
<td>viable, sterile</td>
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<tr>
<td>QdFub</td>
<td>Tp 4C;4D,het.</td>
<td>viable</td>
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<td>QdFub</td>
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Fig. 1. Phenotypes of Qd mutations. (A) Wild-type pattern of the dorsal cuticle of the third abdominal segment. Each tergite can be divided into three regions: an acrotergite (ac) that contains undecorated sclerotized cuticle, a central region containing an array of microchaetes (mi), and a posterior region that contains a dark pigment band (pb) and a row of large macrochaetes (ma) at its posterior edge. All of the tergite, except the acrotergite, is covered with trichomes. For convenience, we define the posterior boundary of the tergite to be the posterior edge of the pigment band. The intertergal cuticle is unpigmented and composed of an anterior trichome-bearing region (the posterior hairy zone or PHZ) and a posterior region of naked cuticle (the intersegmental membrane or ISM). All trichomes and bristles in the abdomen are oriented from the anterior to the posterior. The tergite and anterior portion of the PHZ develop from the anterior dorsal histoblast nest; the rest of the PHZ and the ISM develop from the posterior dorsal nest. (B-D) Phenotypes of QdFub/B (B), QdFub/c (C) and QdFub/QdFub (D). Note the expansion of the pigment band in B-D, and the patches of unpigmented PHZ-like cuticle in D.
was induced after the sensitive period for bristle development (Kopp et al., 1997). Weaker disturbances in polarity could be induced as late as 35-36 hours APF, i.e. until the replacement of most larval epidermal cells by histoblasts.

**omb** alleles cause defects that are reciprocal to those of the **Qd** alleles

Hemizygotes for the **omb** loss-of-function alleles **l(1)omb^282**, **l(1)omb^2498** and **l(1)hbd** (Pflugfelder et al., 1990; Poeck et al., 1993a) mostly die as late larvae or early pupae; only a small percentage survive to the late pharate adult stage. Among the latter, we observe the loss of structures that lie within the posterior region of the anterior compartment and the anterior region of the posterior compartment. In many hemisegments, especially those more anterior in the animal, posterior tergite and PHZ are deleted and replaced with a mirror-image duplication of the anterior tergite (Fig. 4A). This phenotype is exactly reciprocal to the phenotype of **Qd^Fab**. The deleted posterior region is more extensive in some segments than in others. Usually, the three anterior rows of microchaetes are unaffected (Fig. 4A). Less frequently, only one or two anterior rows are left and the tergite is greatly reduced in size. A small number of hemitergites are missing altogether. The expression pattern of **en** indicates that duplicated anterior tergite structures develop exclusively from the anterior compartment, and that the portion of the PHZ from the posterior compartment is transformed into ISM and lost (Fig. 3B). In favor of the latter possibility, **en** stripes are significantly narrowed in hemisegments that have undergone double anterior patterning.

Other hemisegments display weaker phenotypes, including deletion of the PHZ, absence of the pigment band, abnormal trichome polarity in the PHZ and posterior tergite, and transformation of macrochaetes into microchaetes in **omb** animals. Trichome polarity in the posterior sternite is reversed and, occasionally, a few sternal bristles and neighboring pleural trichomes are affected (Fig. 8C). Like the **Qd** alleles, **omb** loss-of-function alleles have no effect on embryonic segmentation.

**Clonal analysis**

We used both the FLP-FRT method (Golic, 1991; Xu and Rubin, 1993) and X-irradiation to generate **omb** clones in the abdomen. Analysis of these clones indicates that **omb** function is required in the histoblasts, and that this requirement is limited to the posterior tergite and PHZ. Even large clones containing up to 19 marked bristles have no effect on polarity or cell fates when located in the anterior tergite (Fig. 5A). However, clones in the posterior tergite and PHZ cause an **omb**-phenotype: trichome polarity is reversed and the pigment band is missing (Fig. 5B,C). Polarity of the marked bristles is also often reversed, whereas neighboring wild-type bristles remain unaffected. Even when induced at the early embryonic stage, clones in the posterior tergite remain small and contain no more than 2-3 bristles, suggesting that **omb** expression may be important for cell survival or proliferation in this region. Although our results establish that **omb** is required within the histoblasts of the posterior tergite and PHZ, we have not been able to establish strict autonomy of **omb** function because the cell markers used in our experiments (y and f) can be scored only in bristles in the abdomen.

**The expression pattern of omb**

In the abdominal epidermis of wild-type pupae, **omb** is expressed in a stripe in the middle of each segment (Fig. 6A). This stripe is 2-3 cells wide in the larval epidermal cells (LEC), and is continuous with expression in the central part of the ventral histoblast nest and the posterior of the anterior dorsal nest. After fusion of the anterior and posterior dorsal histoblast nests, **omb** expression becomes sharply asymmetric (Fig. 6B). The level of **omb** transcript accumulation is high posteriorly and trails off in a shallow gradient toward the anterior. In some preparations, a steep declining gradient toward the posterior can also be seen. **omb** transcripts can be detected until shortly after the replacement of all LEC by histoblasts at approximately 40 hours APF.

In the dorsal abdomen, the compartment boundary is created by fusion of the anterior and posterior dorsal histoblast nests. This suggests that the high point of **omb** expression likely coincides with the compartment boundary. To test this, we double labelled for **omb** (by in situ hybridization) and **en** (by X-gal staining of an **en-lacZ** enhancer trap). No gap was seen between the **omb** and **en** staining, although the extent of overlap...
was difficult to determine. We also double labelled for en protein and the omb enhancer trap omb-GAL4 (Calleja et al., 1996; Lecuit et al., 1996). The latter does not reproduce the normal omb expression in the abdomen; expression begins very late (approx. 30 hours APF) and is limited to a spotty stripe 2-3 cells wide that shows no evidence of an anterior gradient. Nevertheless, this partial stripe of omb-GAL4-expressing cells abuts the en stripe from the anterior and does not overlap it. These observations suggest that peak levels of omb expression lie immediately anterior to the compartment boundary and that the steeply declining levels to the posterior probably lie within the posterior compartment.

In both the LEC and the histoblasts, omb transcripts are tightly localized to one small, intense spot per cell. In the large, polyploid LEC, this spot is seen to be at the periphery of the nucleus (Fig. 6A). Two spots of omb transcript per nucleus are often observed in females heterozygous for Qd\textsuperscript{Fab}, a complex rearrangement that would be expected to disrupt somatic pairing of omb, suggesting that the omb transcript accumulates at or near the omb locus.

In the Qd mutants, omb is expressed ectopically in both histoblasts and LEC. Expression is most dramatically altered in Qd\textsuperscript{Fab} (Fig. 6D), which causes high level expression in all histoblasts and in all LEC except the ‘border cells’, a specialized row located at the segment boundary (Kopp et al., 1997). Ubiquitous omb expression in the histoblasts is consistent with the phenotype of Qd\textsuperscript{Fab}, in which all cells assume the identities normally specified by omb. Moreover, the level of ectopic omb expression in Qd\textsuperscript{Fab} is correlated with the strength of the cuticular phenotype: expression is stronger in more posterior segments, and is stronger in hemizygous males than in heterozygous females. Ectopic omb expression is also observed ventrally. We find that the expression of hh is unaffected in Qd\textsuperscript{Fab} (not shown), ruling out the possibility that double-posterior patterning in Qd\textsuperscript{Fab} results from ectopic expression of hh at the anterior of the segment (Kopp et al., 1997).

The other Qd mutants cause ectopic activation of omb at a lower level than Qd\textsuperscript{Fab} (Fig. 6C). This suggests that low level expression of omb in anterior histoblasts causes only transformation to the posterior, whereas high level ectopic expression can induce polarity reversal. The same appears to be true for hh (Kopp et al., 1997).

\textit{omb} is the primary target of \textit{hh} in the anterior compartment

In the adult abdomen, Hh protein secreted by posterior compartment cells controls pattern in the posterior portion of the anterior compartment (Kopp et al., 1997). The requirement for omb in this same region, and the close similarity between the phenotypes of hh and omb mutants, suggest that omb may mediate the function of hh in the anterior compartment. This is strongly supported by our finding that omb mutations are epistatic to hh alleles.

A gain-of-function allele of hh, hh\textsuperscript{Mir}, causes replacement of
the anterior tergite by a mirror-image duplication of the ISM, PHZ and posterior tergite (Kopp et al., 1997; Fig. 7A). The basic defect in this mutant is that it drives an ectopic stripe of hh expression at the anterior edge of the anterior compartment. The effects of hhMir in the tergite and PHZ are suppressed by l(1)omb282, a loss-of-function allele of omb. In l(1)omb282 ; hhMir/+ double mutants, both the normal and mirror-image duplicated PHZ and posterior tergite structures are missing, and replaced with a mirror-image double anterior pattern very similar to that seen in omb− single mutants (Fig. 7B). However, all segments display double anterior patterning in the double mutant, whereas mirror-image duplications are observed in only a fraction of the segments in omb− single mutants. This indicates that hh has additional targets in the abdomen. l(1)biD4; hhMir/+ double mutants have a phenotype very similar to that of hhMirRevBx1, a partial reversal of hhMir (Kopp et al., 1997), suggesting that l(1)biD4 is a hypomorphic allele. The omb mutants do not suppress the formation of ectopic ISM by hhMir, consistent with the observation that omb is not required in the ISM region of the posterior compartment.

Shifting hh1ls2 mutants to the restrictive temperature after pupariation results in defects similar to those caused by omb null alleles. In particular, mirror-image duplication of the anterior tergite is observed in some segments (Kopp et al., 1997; Fig. 7C). In QdFab, hh1ls2 double mutants raised at the restrictive temperature after pupariation, the phenotype is indistinguishable from that of QdFab single mutants (Fig. 7D). Thus, in QdFab mutants, patterning of the posterior tergite is independent of hh. This, and the finding that an omb null allele is epistatic to hhMir, suggest that omb is the most important mediator of hh function in the anterior compartment of the dorsal abdomen.

Consistent with this, we find that omb expression in the pupal abdomen is dependent on hh. omb expression is greatly reduced both in histoblasts and in LEC in hh1ls2 pupae shifted to the restrictive temperature (not shown). Conversely, omb is expressed in all but the most anterior histoblasts of the anterior compartment in hhMir (Fig. 6E). Ubiquitous or near-ubiquitous expression of omb in the anterior compartment is also seen in pupae in which hh expression is driven by a hs-hh transgene (Tabata et al., 1992) (not shown).

In addition to patterning the posterior tergite, hh functions late in pupal development to specify the acrotergite (Struhl et al., 1997a,b; Kopp et al., 1997). This function of hh is not mediated by omb, since the acrotergite develops normally in omb null mutants and omb is not expressed in the acrotergite primordium.

**Identification of a gene that functions in parallel to omb**

The variable phenotype of omb null alleles suggests that additional genes may contribute to posterior patterning in the tergite. Redundant gene functions have also been invoked to explain the wing phenotype of omb (Grimm and Pfugfelder, 1996). Here we report a novel gene, Scruffy (Scf), that appears to act in parallel with omb. The Scf gene was identified by an X-ray-induced dominant allele that causes weak polarity reversal in the anterior tergite. This allele, Scf1, is associated with an inversion, In(3L)65A:66F3. A stronger dominant allele, Scf2, was isolated after further irradiation of Scf1. The phenotype of Scf2 is very similar to that of QdFab, with the exception that no macrochaetes are present in the anterior of the tergite (Fig. 8A).

Seven X-ray-induced reversions of Scf1 were recovered (Table 2). The chromosomal breaks associated with these indicate that Scf is located at one of the Scf inversion breakpoints, probably in 66F1-3. The deficiency Df(3L)Scfr11 complements all available deficiencies and mutations in the 66F region.

Scf appears to be dispensable for normal development, as all three cytologically normal reversions of Scf1 (Scfr5, Scfr8 and Scfr10) are viable and phenotypically normal when homozygous. However, when these revertants are combined with omb null alleles, strong enhancement of the omb− phenotype is seen in both the abdomen and wing (Fig. 8). The frequency of complete anterior duplications in the abdominal tergites of l(1)omb282, Scfr10/Scfr8 double mutants is strongly increased in comparison with the l(1)omb282; Scfr8/+ animals (Table 3). In sternites, the enhancement is dramatic: the polarity of all sternal bristles is completely reversed in the double mutant, whereas few, if any, bristles are affected in omb− single mutants (Fig. 8C,D). The wing phenotype of omb is also strongly enhanced in the double mutant (Fig. 8E,F).

To position Scf function with respect to omb in the developmental pathway(s) that pattern the posterior tergite, we examined the l(1)omb282; Scf+/+ double mutant. The phenotype appears identical to that of the Scf1 single mutant (Fig. 8A,B), indicating that Scf functions either downstream of, or in parallel to, omb. Consistent with this, we find that omb

![Fig. 4. Phenotypes of l(1)omb282. (A) Posterior tergite and PHZ are deleted and replaced with a mirror-image duplication of anterior tergite (at); ISM is unaffected. Arrowhead points to the line of polarity reversal. (B) The pigment band is absent, macrochaetes are transformed to microchaetes (mi) and most of the PHZ is deleted.](image-url)
expression is not affected in Scf² mutants (not shown). If Scf were to act downstream of omb, the omb⁻ Scf⁻ double mutant would be expected to be indistinguishable from the omb⁻ single mutant. Therefore, the strong enhancement seen in the double mutant indicates that Scf⁺ functions in parallel to omb. The ability of the Scf² allele to direct essentially normal posterior tergite and PHZ patterning in the absence of omb suggests that Scf and omb encode related proteins.

**DISCUSSION**

Previously (Kopp et al., 1997), we presented evidence that the posterior tergite is patterned by Hh protein secreted by posterior compartment cells. We inferred that hh has two roles in the abdomen. First, it induces anterior compartment cells to produce posterior tergite structures, including macrochaetes and posterior pigment band. Second, hh controls polarity in the posterior tergite, primarily by causing cells to reverse their interpretation of an underlying mirror-symmetric polarization. In this report, we present evidence that both functions of hh are mediated by omb.

**omb controls cell fates in both anterior and posterior compartments**

The effects of both loss- and gain-of-function alleles indicate that omb is the major determinant of posterior cell fate within the abdominal tergite. Although the effects of omb null alleles are variable, the most common phenotypes seen include disappearance of the posterior pigment band, transformation of macrochaetes into microchaetes and deletion of the PHZ. In extreme cases, the entire posterior tergite and PHZ are missing, and replaced with a mirror-image duplication of the anterior tergite. Similar effects are seen in omb⁻ somatic clones, indicating that omb function is required within the histoblasts of the posterior tergite and PHZ.

Consistent with its requirement in the posterior tergite, we find that omb is expressed in the posterior portion of the anterior compartment in a gradient whose high point is at or near the compartment boundary. Ectopic omb expression in the anterior of the segment in the gain-of-function Qd alleles is correlated with expansion of the posterior pigment band toward the anterior. Surprisingly, most Qd alleles do not cause transformation of microchaetes to macrochaetes. These mutants may cause ectopic expression of omb too late to influence bristle type. The effects of ectopic hh expression indicate that bristle patterning occurs much earlier than pigment patterning in tergites (Kopp et al., 1997).

In contrast to other Qd mutants, Qd Fab causes the entire anterior tergite to develop as a mirror-image duplication of the posterior tergite. Similar effects are seen when ubiquitous omb expression is driven by hs-GAL4. Why ectopic omb expression has such different effects in different mutants is not clear. However, it seems likely that the level of ectopic omb expression

**Table 2. Scruffy alleles**

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<thead>
<tr>
<th>Allele</th>
<th>Cytology</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scf⁻</td>
<td>In(3L) 65A12-15; 66F3</td>
<td>Dominant, weak, homoz. viable</td>
</tr>
<tr>
<td>Scf²</td>
<td>In(3L) 64E; 66F*</td>
<td>Dominant, strong, homoz. lethal</td>
</tr>
<tr>
<td>Scf⁻R5</td>
<td>Normal*</td>
<td>Homozygous viable, normal</td>
</tr>
<tr>
<td>Scf⁻R8</td>
<td>Normal*</td>
<td>Homozygous lethal</td>
</tr>
<tr>
<td>Scf⁻R9</td>
<td>In(3L) 66F; 70F*</td>
<td>Homozygous lethal</td>
</tr>
<tr>
<td>Scf⁻R10</td>
<td>Normal*</td>
<td>Homozygous viable, normal</td>
</tr>
<tr>
<td>Scf⁻R11</td>
<td>Dr(3L) 66E3-4; 66F1-2*</td>
<td>Homozygous lethal</td>
</tr>
<tr>
<td>Scf⁻R13</td>
<td>T(2;3) 65A; 66E*</td>
<td>Homozygous lethal</td>
</tr>
<tr>
<td>Scf⁻R14</td>
<td>Complex; no breaks in 66*</td>
<td>Homozygous lethal</td>
</tr>
</tbody>
</table>

*All mutations were induced in Scf¹ background and carry In(3L) 65A12-15; 66F3.

**Table 3. Frequency of hemitergites with different cuticular phenotypes (A2 through A5 scored)**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>omb; Scf⁻R¹⁰/TM3</th>
<th>omb; Scf⁻R¹⁰/Scf⁻R¹⁰</th>
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<tbody>
<tr>
<td>Missing</td>
<td>2.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Complete polarity reversal</td>
<td>18.1</td>
<td>25.0</td>
</tr>
<tr>
<td>Partial polarity reversal</td>
<td>22.8</td>
<td>11.0</td>
</tr>
<tr>
<td>Abnormal PHZ</td>
<td>2.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Ma transformed into Mi</td>
<td>2.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Abnormal PHZ &amp; Ma tr. into Mi</td>
<td>53.7</td>
<td>17.0</td>
</tr>
<tr>
<td>Total scored</td>
<td>99.9</td>
<td>100.0</td>
</tr>
</tbody>
</table>

PHZ, posterior hairy zone; Ma, macrochaetes; Mi, microchaetes
determines whether expansion of posterior patterning to the anterior or mirror-symmetric double posterior patterning will occur. The level of ectopic expression in \( Qd^{lab} \) is strikingly higher than in the other mutants. Moreover, the extent of polarity reversals in \( Qd^{lab} \) is correlated with the level of ectopic \( omb \) expression; both are greater in hemizygous males than in heterozygous females, and are also greater in more posterior segments of both sexes. A similar dose dependence is observed for \( hh \); low levels of ectopic \( hh \) expression promote posterior cell fates, whereas higher levels cause polarity reversals (Kopp et al., 1997).

\( omb \) also appears to function in the posterior compartment, where it is required for PHZ development. In wild type, the posterior compartment is composed of PHZ anteriorly and ISM posteriorly. In \( omb^- \) animals, the entire posterior compartment develops as ISM, whereas in \( Qd^{lab} \) and \( hs-GAL4/UAS-omb \) animals, the entire compartment produces PHZ. It seems likely that the weak posterior expression of \( omb \) that we see in wild type is responsible for PHZ development in the posterior compartment.

Evidence as to whether the level of \( omb \) expression is instructive in determining cell fate is contradictory. In some strong heterozygous combinations of \( Qd \) alleles, patches of cells in the middle and posterior tergite secrete unpigmented, PHZ-like cuticle. This suggests that higher levels of \( omb \) may specify more posterior cell fates. However, in \( Qd^{lab} \) and \( hs-GAL4/UAS-omb \) animals, the entire range of posterior tergite and PHZ structures is present and patterned apparently normally in both the anterior and posterior of the segment. This indicates that cells must receive patterning instructions from a source other than the \( omb \) gradient. As discussed below, it appears that polarity in abdominal segments is determined by an underlying system that has mirror symmetry. It seems likely that this same system can also specify the diversity and arrangement of cuticular structures, and predominates when \( omb \) expression is absent or reaches a threshold level.

**\( omb \) reverses underlying polarity in the posterior of the segment**

We show that, in the absence of \( omb \) function, the tergite can undergo double-anterior patterning, whereas ubiquitous expression induces reciprocal, double-posterior patterning. In a previous report, we demonstrated that similar phenotypes are caused by loss of function and ectopic expression of \( hh \); ectopic \( en \) expression also results in a mirror-symmetric pattern. We concluded that adult abdominal segments are polarized symmetrically by a system that appears to be independent of \( hh \) and that \( hh \) signaling causes cells in the posterior tergite to

**Fig. 6.** Expression pattern of \( omb \) in the dorsal pupal epidermis. Arrowheads in A-E point to the specialized larval border cells that mark segment boundaries (Kopp et al., 1997). (A) Wild-type expression at 18 hours APF. adn, anterior dorsal histoblast nest; pdn, posterior dorsal nest. Histoblasts are distinguished from the polyploid larval cells by their smaller size. Note the tight perinuclear localization of the \( omb \) transcript in larval cells. (B) Wild-type expression at 35 hours APF. The sharp posterior limit of expression likely corresponds to the compartment boundary. (C) \( Qd^{2+}/+ \) female. Low level expression is observed in all histoblasts, and in some LEC at ectopic locations (arrows). (D) \( Qd^{lab}/+ \) female. \( omb \) is expressed at a high level in all histoblasts and all LEC except the specialized border cells; expression is highest near the compartment boundary. (E) \( hh^{Mtr} \). \( omb \) expression is greatly increased in the anterior compartment. However, little or no expression is observed in the posterior compartment and at the anterior edge of the anterior compartment, the regions that will presumably form ISM cuticle (arrows).
reverse their interpretation of this underlying polarity (Kopp et al., 1997). Results presented here suggest that this polarity-reversal function of hh is mediated by omb.

Ectopic expression of en reveals a cryptic line of polarity reversal within the anterior compartment (Kopp et al., 1997). This line is located within the pigment band of the normal tergite and roughly corresponds to the position of polarity reversal in the double anterior tergites produced by hh^ts2 animals. Surprisingly, polarity reversal in omb^- animals occurs anterior to this line. This difference may have a trivial explanation; tergite width is reduced in omb^- relative to hh^ts2 animals due to the presence of ISM in omb^- but not hh^ts2 mutants. As a consequence, fewer rows of bristles are present in omb^- than in hh^ts2, giving the appearance of a more anterior

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Fig. 7.  omb mediates the function of hh. Arrowheads point to the lines of polarity reversal. (A) hh^Mr. Anterior tergite is deleted and replaced with a mirror-image duplication of posterior tergite (pt), PHZ and ISM. (B) l(l)omb^ts2; hh^Mr. Posterior tergite and PHZ structures are absent and replaced with a mirror-image duplication of the anterior tergite (at); note the similarity to l(l)omb^ts2 single mutants (Fig. 4A). However, the duplicated ISM persists. (C) Anterior duplication in hh^ts2. (D) Posterior duplication in Qd^Hub; hh^ts2.

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Fig. 8.  Scf acts in parallel with omb. (A) Dorsal cuticle of Scf^+/-. Note the similarity to Qd^Hub (Fig. 2A). Arrowheads point to the lines of polarity reversal. (B) l(l)omb^ts2; Scf^+/-. (C) Sternite of l(l)omb^ts2 hemizygote. Sensillae (arrowheads in C, D) mark the anterior of the sternite. Only the trichomes in the posterior of the sternite are affected (arrow). (D) Sternite of l(l)omb^ts2; Scf^R11/Scf^R11. Note reversal of bristle polarity. (E) Wing of l(l)omb^ts2. (F) Wing of l(l)omb^ts2; Scf^R11/Scf^R11, shown at the same magnification as E.
polarity reversal. Consistent with this, extreme compression of the tergite in omb<sup>−/−</sup>; hh<sup>Mir</sup> animals (Fig. 7B) is associated with a further reduction in the number of bristles. Differential tergite compression may also explain why polarity reversal appears to occur further anterior in Qd<sup>Fab</sup> than in hh<sup>Mir</sup>.

In this report, we identify a line of polarity reversal within the posterior compartment. This line is revealed in Qd<sup>Fab</sup> and hs-GAL4/UAS-omb animals, in which the entire posterior compartment develops as PHZ. In these animals, PHZ trichomes are directed posteriorly in the anterior portion of the posterior compartment and anteriorly in the posterior portion (Fig. 3C). Like the line of polarity reversal in the anterior compartment (Kopp et al., 1997), the line separating trichomes of opposite polarity in the PHZ does not appear rigidly fixed, as its position within the en stripe is variable. It is not clear whether polarity reversal occurs in the posterior compartment in wild type, since the polarity of ISM cuticle cannot be determined.

As summarized in Figure 9, we suggest that underlying polarity in abdominal segments is specified by a mirror-symmetric gradient that has inflection points at the lines of polarity reversal within the anterior and posterior compartments (Fig. 9A). The nature of this gradient is unknown, but it appears to be independent of hh (Kopp et al., 1997). We suggest that omb serves to establish normal polarity by causing cells to reverse their interpretation of this underlying symmetric polarity. In omb<sup>−/−</sup> animals, this reversal frequently fails, causing the tergite to revert to the default double anterior pattern (Fig. 9B), whereas ubiquitous omb expression causes all cells to reverse polarity, generating double posterior tergite patterns (Fig. 9C). An extreme omb overexpression phenotype is seen in Qd<sup>Fab</sup>; hh<sup>Mir</sup> double mutants; these show very strikingly the alternation of polarities present in our model (Fig. 9D).

**omb is the main target of hh in the anterior compartment**

Our epistasis tests provide compelling evidence that omb is a major target of hh signaling in the abdomen. Consistent with these tests, we find that omb expression in histoblasts is dependent on hh. Although omb appears to be the primary target of hh in the abdomen, we find that polarity reversal occurs in all segments in omb<sup>−/−</sup>; hh<sup>Mir</sup> double mutants, but only in some segments in omb<sup>−/−</sup> single mutants. This, and the lack of effect of omb mutants in the acrotergite, suggest that hh has additional targets in the abdomen.

It is unclear whether hh activates omb directly or through a secondary morphogen induced at the compartment boundary. In the wing disc, omb is activated on both sides of the compartment boundary by dpp (Lecuit et al., 1996; Nellen et al., 1996). However, in the abdomen, dpp is not expressed in a pattern that could account for omb activation and functional tests indicate that it plays no role in anteroposterior patterning

Fig. 9. Model for how omb specifies pattern and polarity in the abdominal segment. (A) Underlying polarity is determined by a mirror-symmetric gradient that has inflection points within the anterior (a) and posterior (p) compartments (see text). In the absence of omb, this gradient specifies mirror-symmetric double anterior tergite (at) pattern in the anterior compartment (solid black arrows). The posterior compartment produces only intersegmental membrane (ism). We suggest this also has mirror-symmetric polarity (dashed grey arrows), although this is not known. (B) In wild type, omb (grey lines) specifies posterior tergite (pt) structures in the anterior compartment, and posterior hairy zone (phz) in the posterior compartment. The extent of omb expression in the posterior compartment is unclear (dashed grey line). omb also causes cells in both compartments to reverse their interpretation of underlying polarity. (C) In Qd<sup>Fab</sup>, ubiquitous expression of omb induces pt development throughout the anterior compartment and phz throughout the posterior compartment, and causes all cells in both compartments to reverse polarity. (D) Abdominal pattern in Qd<sup>Fab</sup>; hh<sup>Mir</sup> animal. Note correspondence of this phenotype to that shown schematically in C.
(Kopp et al., 1997). Similar evidence rules out wg as mediating omb activation by hh (Shirras and Couso, 1996; Kopp et al., 1997). omb may be activated in the abdomen by a long-range morphogen that has not yet been identified. Based on the expression pattern of omb, one would expect this hypothetical morphogen to diffuse more freely in the anterior than the posterior compartment. Such asymmetric diffusion is shown by Wg protein in the embryonic epidermis (Gonzalez et al., 1991). Alternatively, the asymmetric expression pattern of omb could be explained by direct activation by hh and repression by en.

**omb function is partially redundant**

Grimm and Pflugfelder (1996) suggested that some functions of omb may be shared by other genes. Here we report such a gene, Scruffy (Scf). Scf was identified by a gain-of-function allele that causes a phenotype very similar to that of QdΔab. Scf+ appears to be dispensable, since several Scf revertants are homozygous viable and wild type. Epistasis tests indicate that Scf acts in parallel to omb and may be an additional target of hh.

Our discovery of Scf underscores the importance of analyzing gain-of-function mutations as a means of identifying new genes. Developmental redundancy appears to be widespread (Tautz, 1992), and poses a serious problem for genetic analysis; it has been estimated that about 2/3 of all genes in *Drosophila* have no readily identifiable loss-of-function phenotype (Miklos and Rubin, 1996). Although most workers have avoided gain-of-function alleles, our observations, and those of others (e.g. Simmonds et al., 1995), indicate that such mutations provide an effective way to identify and determine the function of redundant genes.

This work would not have been possible without the generous help of Dr Barry Ganetzky, who provided the QdΔab mutant, and Dr Gert Pflugfelder, who provided the omb− and UAS-omb stocks, the omb cDNA, and the omb genomic walk. We would like to acknowledge the contributions of Dr Stephan Grimm, who constructed the UAS-omb stock, and Dr Helmut Roth, who made most of the omb genomic walk. We are grateful to Dr Tom Kornberg for the hs-hh and en-lacZ stocks, and to Kathy Matthews at the Bloomington Stock Center and Linda Treeger at the Mid-America Stock Center for various other stocks. We also thank Dianne Duncan for useful advice, Paula Kiefel for technical help, and Richard Emmons and Mark Kankel for support and discussions. This work was supported by NIH grant GM32318 to I.D.

**REFERENCES**


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