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The *Drosophila* tumor suppressor gene *lethal(2)giant larvae* is required for the emission of the Decapentaplegic signal

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SUMMARY

The *Drosophila* tumor suppressor gene *lethal(2) giant larvae* (*lgl*) encodes a cytoskeletal protein required for the change in shape and polarity acquisition of epithelial cells, and also for asymmetric division of neuroblasts. We show here that *lgl* participates in the emission of Decapentaplegic (Dpp), a member of the transforming growth factor β (TGFβ) family, in various developmental processes.

During embryogenesis, *lgl* is required for the *dpp*-dependent transcriptional activation of *zipper* (*zip*), which encodes the non-muscle myosin heavy chain (NMHC), in the dorsalmost ectodermal cells – the leading edge cells. The embryonic expression of known targets of the *dpp* signaling pathway, such as *labial* or *tinman* was abolished or strongly reduced in *lgl* mutants. *lgl* mutant cuticles exhibited phenotypes resembling those observed in mutated partners of the *dpp* signaling pathway. In addition, *lgl* was required downstream of *dpp* and upstream of its receptor Thickveins (Tkv) for the dorsoventral patterning of the ectoderm. During larval development, the expression of *spalt*, a *dpp* target, was abolished in mutant wing discs, while it was restored by a constitutively activated form of Tkv (Tkv^{Q253D}). Taking into account that the activation of *dpp* expression was unaffected in the mutant, this suggests that *lgl* function is not required downstream of the Dpp receptor. Finally, the function of *lgl* responsible for the activation of Spalt expression appeared to be required only in the cells that produce Dpp, and *lgl* mutant somatic clones behaved non autonomously. We therefore position the activity of *lgl* in the cells that produce Dpp, and not in those that respond to the Dpp signal. These results are consistent with a same role for *lgl* in exocytosis and secretion as that proposed for its yeast ortholog *sro7/77* and *lgl* might function in parallel or independently of its well-documented role in the control of epithelial cell polarity.

Key words: *Drosophila*, tumor suppressor gene, *decapentaplegic*, signaling pathway

INTRODUCTION

Inactivation of the *Drosophila* tumor suppressor gene *lethal(2)giant larvae* (*lgl*; *l(2)gl* – FlyBase), leads to uncontrolled proliferation of the neuroblasts of the optic lobes and of the epithelial cells of the larval imaginal discs. The *lgl* mutant larvae never pupariate, and during the delayed larval stage disc cells become non-adhesive and undifferentiated, with no apparent polarity. When transplanted in a wild-type adult host, mutant tissues grow in a dispersed manner, completely invading the tissues of the host to finally kill it (Gateff, 1982; Woodhouse et al., 1998). Re-introduction of one copy of the wild-type gene into a mutant animal is sufficient to rescue the tumoral phenotype (Jacob et al., 1987), conforming to the standard definition of a tumor suppressor gene (Gateff and Schneiderman, 1969; Harris et al., 1969; Knudson, 1971).

Lgl belongs to a novel family of WD-40-containing proteins with homologs in many species, including humans (Strand et al., 1995; Koyama et al., 1996), mouse (Tomotsune et al., 1993), yeast (Kagami et al., 1998; Larsson et al., 1998), *C. elegans* (U51993) and the more divergent Tomosyn in rat (Fujita et al., 1998) and *Drosophila* (C617762).

The *lgl* gene encodes a protein (P127 or Lgl) of the cytoskeleton mainly localized to the internal face of the plasma membrane (Strand et al., 1994a). Lgl is present in the cytoplasm as a multiprotein complex containing at least ten components, including the product of the *zipper* gene, which encodes the non-muscle myosin heavy chain (NMHC) (Young et al., 1993; Strand et al., 1994b), a serine-threonine kinase capable of phosphorylating Lgl (Kalmes et al., 1996) and the *Drosophila* homolog of the yeast nucleosome-associated protein 1 (NAP1) (Ishimi and Kikuchi, 1991; Li et al., 1999). In addition, Lgl is able to form homopolymers (Strand et al., 1994b). These different observations implicate Lgl in a cytoskeletal network (Strand et al., 1994b) whose disruption might cause the overgrowth phenotype.

These biochemical features are consistent with the recently reported function for *lgl* in regulation of cell-shape change and epithelial cell polarity (Manfruelli et al., 1996; Bilder et al., 2000). Lgl appears to act in this latter process in cooperation with the two other neoplastic tumor suppressor genes characterized in *Drosophila*, *discs-large* (*dlg*; *dlg1* – FlyBase) and *scribble* (*scrib*; *dlg2* – FlyBase) (Bilder et al., 2000). Moreover, Lehman et al. (Lehman et al., 1999) have clearly demonstrated that the two yeast homologs of *lgl*, *sro7* and *sro77*, are involved in exocytosis by interacting directly with Sec9, a component of the t-SNARE complex. *sro7/77* can be functionally replaced by the human *lgl* homolog to rescue their mutant phenotypes (Kagami et al., 1998). A similar function has been proposed for rat Tomosyn, in that it facilitates secretory vesicles fusion to specific membrane domain in neurons (Fujita et al., 1998).

Lgl function is also essential for asymmetric cortical localization of all known basal determinants in mitotic neuroblasts and is therefore necessary for asymmetric division and creation of intrinsic differences between daughter cells (Peng et al., 2000; Ohshiro et al., 2000). Similar to its function in epithelial cell polarization, Lgl seems to cooperate with Dlg in asymmetric division. All these observations point towards a function of the Lgl family proteins in some specific aspects of intracellular proteins sorting and proteins targeting to specific membrane domains.

The use of *lgl* temperature-sensitive alleles had led us to implicate *lgl* function in shape change of various epithelial cells in the embryo, including the dorsalmost ectodermal cells, the leading-edge (LE) cells (Manfruelli et al., 1996), and such a function might be related to that described above. At dorsal closure, the LE cells have to change their shape and to undergo a remodeling of their polarity. During germband shortening, all the epithelial ectodermal cells of the trunk display a characteristic apical-basal polarity – their apical membrane facing the outside of the embryo. At the end of germband shortening, when dorsal closure begins, the LE cells are the first ectodermal cells to change their shape (Young et al., 1993; Ring and Martinez Arias, 1993). They elongate in the dorsoventral axis and, concomitantly, they acquire a planar polarity, such that the membrane domain facing the amnioserosa has now changed from its initial basolateral character to a typical apical polarity (Martin-Blanco, 1998). For example, it no longer expresses Fasciclin III (FasIII), a basolateral marker (Young et al., 1993; Ring and Martinez Arias, 1993).

The shape change initiated in the LE cells propagates from cell to cell along the dorsoventral axis (Young et al., 1993; Ring and Martinez Arias, 1993). The onset of dorsal closure is associated with an accumulation of actin and NMHC in these LE cells. It has been suggested that the nonmuscle myosin could, as a mechanically contractile element, drive the epidermal sheet movement (Young et al., 1993). Mutations in the *zipper* gene (Nusslein-Volhard et al., 1984; Young et al., 1993) result in a prominent dorsal opening that resembles the dorsal holes observed in mutants of the DJNK (*Drosophila* Jun N-terminal Kinase) pathway (Martin-Blanco, 1997; Noselli, 1998). The amounts of actin and NMHC in the LE cells are greatly reduced in all those mutants (see, for example, Hou et al., 1997). Cell differentiation and morphogenesis during dorsal closure are mediated by the activation of the DJNK signaling pathway, which in turn allows, from the middle of germband shortening, the expression of *dpp* (*decapentaplegic*), the *Drosophila* homolog of transforming growth factor β (TGFβ), in the LE cells (Martin-Blanco, 1997; Noselli, 1998).

As *lgl* seems to be involved in dorsal closure, our aim was to position its function in a differentiation process in which cell-shape change and epithelial polarity remodeling are triggered by a well-characterized signaling pathway. The results show that, during dorsal closure, *lgl* functions in the transmission of the Dpp signaling and this observation has prompted us to investigate the function of *lgl* in other *dpp*-regulated developmental processes. The analysis has revealed a new aspect of a general function for *lgl* in polarized membrane proteins targeting, and suggests its participation in exocytosis and secretion of the Dpp morphogen.

MATERIALS AND METHODS

Drosophila strains

Wild-type embryos were from the Oregon R strain. The lgl^{ts3} hypomorphic temperature-sensitive allele has previously been described (Manfruelli et al., 1996; DeLorenzo et al., 1999). Three amorphic mutations were used: a deficiency, *Dfnet62/CyO*, which totally uncovered the *lgl* locus (Korochkina and Golubovsky, 1978), lgl^{4}*/CyO* (Mechler et al., 1985) and lgl^{4W3}*/CyO* (Bilder et al., 2000).

UAS-tkv^{Q253D}, *en*-GAL4 and *paired*-GAL4 were obtained from M. Frasch, A. Gallet and L. Fasano, respectively. The UAS-*dpp*, *dpp{blk1}40C.6*-GAL4 lines were provided by the Bloomington *Drosophila* Stock Center. The different chromosomes were recombined either with the lgl^{4} or with the lgl^{ts3} mutant chromosomes. The UAS-*lgl* line has already been described (Manfruelli et al., 1996). The UAS-GFP line was as described (Mollereau et al., 2000).

The *yw*,P(ry^{+},hs-FLP); *arm-lacZ,M(2)z*,P(ry^{+},neo-FRT)4OA/*CyO* and the ywf^{36a}, P(ry^{+},hs-FLP) ck^{CH52},P(f^{+}),P(ry^{+},neo-FRT)4OA/*CyO* stocks were kindly provided by H. Stocker. The other stocks used to generate somatic clones were obtained from the Bloomington *Drosophila* Stock Center.

In situ hybridization on whole-mount embryos and discs

Digoxigenin (DIG)-labeled RNA probes were generated from a *dpp* DNA fragment (generous gift from S. Chauvet) and a *wg* DNA fragment (generous gift from T. Sagnier) with the T3-T7 polymerase riboprobe kit (Promega) and DIG-UTP (Boehringer). They were used for whole-mount in situ hybridization of fixed staged embryos as described (Francois et al., 1994). Embryos were treated for hybridization as described (Tautz and Pfeifle, 1989). DNA probes were DIG-labeled according to the DIG-DNA Labeling Mixture protocol (Boehringer). The *zip* probe was obtained by labeling a PCR-amplified genomic fragment corresponding to the ninth exon of the *zipper* locus. The *lab* probe is described in Diederich et al. (Diederich et al., 1989). The DIG-labeled RNA and DNA probes were detected with the aid of a preadsorbed anti-DIG antibody coupled to alkaline phosphatase (Boehringer) and NBT/BCIP as substrate. The embryos were mounted in Geltol medium (Immunotech, France) for further observation under a Zeiss Axiophot microscope.

For fluorescent in-situ hybridization on imaginal discs, the *dpp*-DIG-UTP probe was revealed as described previously (Zaffran and Frasch, 2000).

Immunohistochemistry

Embryos and discs were fixed and stained with antibodies according to the protocol described previously (Ashburner, 1989). The following primary antibodies were used: anti-NMHC (1/500

dilution; Young et al., 1993); anti-Fasciclin III (1/2 dilution; Developmental Studies Hybridoma Bank); anti-Phosphotyrosine (1/200 dilution; anti-PY20 from Transduction Laboratories); anti-Spalt (1/500 dilution; de Celis and Barrio, 2000); anti-En (1/10 dilution; Developmental Studies Hybridoma Bank); anti-Eve and anti-Tinman (1/5000 and 1/800 dilution, respectively; Azpiazu and Frasch, 1993); anti-Dlg (1/200; Woods and Bryant, 1993); anti-Patched (1/200; Maschat et al., 1998); and mouse anti-βGal (1/1000, Promega). Affinity-purified secondary antibodies (Jackson Immuno Research Laboratories) were either coupled to alkaline phosphatase and used at a 1/1000 dilution or conjugated to TRITC, FITC or Cy5 and used at a 1/100 dilution. The stained embryos were either mounted in Geltol medium (Immunotech, France) for further observation under a Zeiss Axiophot microscope or, when fluorescent, in Vectashield (Vector Laboratories) for observation under a Zeiss LSM 410 confocal microscope.

Cuticle preparation

Crosses between homozygous lgl^{ts3}males and females were carried out at 29°C. After 48 hours spent by the flies at 29°C, eggs were collected for 4 hour periods and allowed to develop for 24 hours at 29°C. The embryos thus obtained were dechorionated, devitellinized in a heptane/methanol solution (v/v) and mounted in Hoyer's medium (van der Meer, 1977). Cuticles phenotypes were observed under phase-contrast microscopy.

Rescue experiments

For rescue in embryos, lgl^{ts3}/lgl^{ts3}*; prd*-GAL4 males, lgl^{ts3}/lgl^{ts3}*; en*-GAL4 males and lgl^{ts3}/lgl^{ts3}*; arm*-GAL4 males were crossed either with $lgl^{ts3}/lgl^{ts3;}$ UAS-*dpp/MKRS* females or with $lgl^{ts3}/lgl^{ts3;}$ UAS-tkv^{Q253D}*/MKRS* females at 29°C. Embryos were collected 48 hours after the temperature shift to warrant the penetrance of the mutant phenotype. Cuticles were then prepared as described above.

For rescue in larvae, *yw;* $lgl^4/CyOy^+$*;* UAS-*lgl* females were crossed with *yw;* $lgl^4/CyOy^+$*; dpp*-GAL4 males and *yw;* $lgl^4/CyOy^+$*; en*-GAL4 males. *yw;* $lgl^4/CyOy^+$*;* UAS-*dpp/MKRS* females or *yw;* $lgl^4/CyOy^+$*;* UAS- tkv^{Q253D}*/MKRS* females were crossed with *yw;* $lgl^4/CyOy^+$; *en*-GAL4 males. These crosses were incubated at 22°C for 24 hours. Vials were then shifted to 29°C until the third instar larval stage. Homozygous lgl^4 larvae were recognized with the aid of the *y* marker.

Generation of mutant *lgl* somatic clones

Homozygous mutant *lgl* clones were generated by using the autosomal FLP recombinase technique (Xu and Rubin, 1993). The chromosome bearing the lgl^4 mutation was recombined with a P(ry^+,neo-FRT)40A chromosome to obtain lgl^4, P(ry^+,neo-FRT)40A chromosomes, which were selected both for G418 resistance and for the presence of the *lgl* mutation. The selected chromosomes were balanced over CyO.

Somatic clones in the wing imaginal discs were produced from the offspring of a cross between *yw,* P(ry^+, hs-FLP); *arm-lacZ, M(2)z,* P(ry^+, neo-FRT)4OA/CyO (or the same strain without the *M(2)z* mutation) males and *w;* lgl^4, P(ry^+, neo-FRT)40A/ CyO females. 48- to 72-hour-old larvae were heat shocked at 37°C for 2 hours. Homozygous mutant clones were visualized by the loss of the ubiquitous marker *lacZ*. Third instar larvae were dissected and imaginal discs were stained with anti-βgal, anti-Spalt and anti-Patched antibodies. Affinity-purified secondary antibodies (Jackson Immuno Research Laboratories) were conjugated to FITC, TRITC or Cy5 and used as described above.

Somatic clones in adults were produced from a cross between *ywf* ^{36a},P(ry^+,hs-FLP); ck^{CH52},P(f^+),P(ry^+, neo-FRT)40A/CyO males and *w;* lgl^4, P(ry^+, neo-FRT)40A/ CyO females. Larvae aged for at least 72 hours after hatching were heat-shocked at 37°C for 2 hours. They were allowed to develop until eclosion at 25°C. Wings were dissected and mounted in DPX medium before observation.

RESULTS

lgl is required for the accumulation of the *zipper* gene product in the LE cells

In lgl^{ts3} embryos, in which both maternal and zygotic functions of *lgl* were reduced, the dorsalmost cells of the epidermis (LE cells) do not elongate and, consequently, hamper the dorsal closure (Manfruelli et al., 1996). In a wild-type embryo, NMHC begins to specifically accumulate in LE cells at the onset of dorsal closure, before the cells have initiated their elongation (Young et al., 1993; Fig. 1A-C). By contrast, in mutant embryos issued from homozygous lgl^{ts3} parents, the concentration of NMHC in the LE cells was greatly reduced (Fig. 1D). Conversely, neither the localization nor the amount of Lgl was impaired in *zipper* mutant embryos (not shown). In a wild-type embryo, Lgl is ubiquitously expressed and is mainly located to the basolateral membrane of the epithelial cells (Strand et al., 1994a), whereas, in lgl^{ts3} mutant embryos, the Lgl mutant protein is no longer associated to the plasma membrane and is found in the cytoplasm (Manfruelli et al., 1996, DeLorenzo et al., 1999). The replacement of Ser311 by a Phe residue in the Lgl^{ts3} protein (DeLorenzo et al., 1999) appears therefore to be crucial to the membrane localization of Lgl, probably by perturbing its binding to the cytoskeleton, and Lgl is required for the specific accumulation and localization of NMHC in LE cells.

In addition, the mutant LE cells did not acquire their novel polarity as shown by Phosphotyrosine (PY), Discs-large (Dlg) (Woods and Bryant, 1993) or FasIII (Martinez-Arias, 1993) stainings (Fig. 1E-J). Interestingly, in the mutant, the epithelial polarity was affected only in those LE cells but was preserved in all other ectodermal cells of the future epidermis (Fig. 1). In particular, cell junctions were correctly assembled and positioned, as probed with anti-Dlg and anti-PY (Muller and Wieschaus, 1996, Woods et al., 1997).

zip is a transcriptional target of the *dpp* signaling pathway in LE cells and its activation requires *lgl* function

The accumulation of the NMHC protein in LE cells at the onset of dorsal closure was accompanied by an activation of *zip* transcription in these cells (Fig. 2A). This high level of mRNA was maintained until completion of dorsal closure, when epidermal cells had acquired their final shape (not shown). This observation strongly suggests that *zip* transcription is activated in the LE cells in response to the activation of the DJNK-Dpp signaling pathway and is responsible, at least in part, for the observed accumulation of NMHC.

To investigate the involvement of Dpp in *zip* transcriptional activation, we examined *zip* mRNA accumulation in LE cells in mutant embryos for *thickveins* (*tkv*), which encodes a Type I receptor for Dpp. The localized accumulation of *zip* mRNA did not prevail any longer in this mutant (Fig. 2B), thus confirming that *zip* transcription requires the Dpp pathway. The occurrence in the *zip* promoter region of a GC-rich sequence (Mansfield et al., 1996), which well matches the consensus sequence reported for *Drosophila* Mad/Medea (Smad family proteins)-binding sites (Xu et al., 1998), further strengthens the possibility that *zip* could be a direct transcriptional target of the *dpp* signaling pathway.

Mutations in other components of the JNK pathway (*bsk* and

jun) gave rise to the same downregulation of *zip* in LE cells, which correlates with the lack of accumulation of NMHC protein already observed in mutants of all characterized genes of the DJNK-Dpp signaling pathway (not shown). By contrast, in *puc* mutants, *zip* expression was, overall, similar to that in a wild-type embryo, even though some perturbations were noticed (not shown; Martin-Blanco et al., 1998).

Upregulation of *zip* transcription was also abolished in lgl^{ts3} mutant embryos at restrictive temperature (Fig. 2C). Therefore, at least part of the *lgl* function in the dorsal closure process might be mediated by its effect on the transcriptional activation of *zip*. In contrast, the expression of *dpp* (Fig. 2), and particularly its onset, as well as the timing of its decrease in the margin cells (not shown), were not noticeably affected in lgl^{ts3} mutant embryos. Thus, this positions *lgl* function downstream of *dpp* transcriptional activation in LE cells.

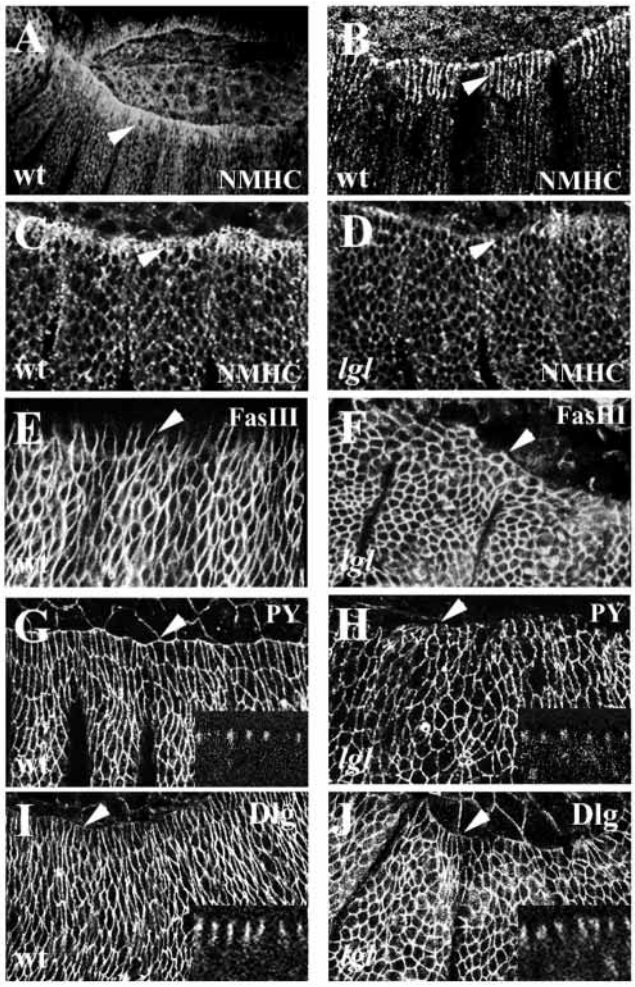

Fig. 1. Distribution of NMHC and membrane markers in lgl^{ts3} mutant embryos at dorsal closure. Whole-mount embryos were stained at the beginning of dorsal closure as follows and observed under a confocal microscope: with anti-NMHC (A-D), with anti-Fasciclin III (FasIII), which labels the lateral membrane domain of epithelial cells (E,F), with anti-Phosphotyrosine (anti-PY), a marker of the most apical region of the lateral membrane (G,H), and with anti-Discs-large (Dlg), a marker of septate junctions (I, J). (A,B,C,E,G,I) Wild-type embryos; (D,F,H,J) lgl^{ts3} mutant embryos at 29°C. Inserts (G-J) show *z*-views of dorsal epidermis cells. (A-C) NMHC is strongly expressed in the LE cells, where it accumulates at the onset of dorsal closure. (D) In mutant embryos, LE cells do not change their shape and fail to accumulate NMHC (compare with C, a wild-type embryo (slightly younger than those in A,B) that has not yet changed its LE cells shape). (E) Fas III and (I) Dlg are absent from the membranes facing the amnioserosa in the LE cells during dorsal closure, while in lgl^{ts3} embryos, FasIII (F) and Dlg (J) are evenly distributed on the whole surface of the cell membranes. (G) In LE cells, PY is detected in the membrane facing the amnioserosa, as well as in the basolateral membranes in contrast to the situation prevailing in mutant embryos (H). In other ectodermal cells, epithelial cell polarity is maintained in lgl^{ts3} mutant embryos and cell junctions are correctly positioned (inserts in G-I). Arrowheads indicate the LE cells.

lgl function is necessary in various embryonic developmental processes that are dependent on *dpp* signaling

The results described above have prompted us to investigate the effect of *lgl* mutations on the expression of some known *dpp* signaling targets during embryogenesis.

The mature pattern of *lab* (*labial)* expression in the midgut depends on an inductive information issued from the adhesive visceral mesoderm (Bienz, 1994) that is mediated by the secreted product of *dpp* expressed in parasegment 7 of this tissue. In lgl^{ts3} embryos, the midgut expression of *lab* was strongly reduced or even undetectable, whereas its expression in the intercalary segment, which is not dependent on *dpp* (Immergluck et al., 1990), remained unaltered (Fig. 2F,G). This observation is consistent with a function for *lgl* in midgut morphogenesis (Manfruelli et al., 1996).

As a result of the expression of *dpp* in the dorsal ectoderm, *tinman* expression is activated in the underlying dorsal mesodermal cells, allowing their specification and their subsequent differentiation. The *even skipped* (*eve*) gene, which is expressed in a subset of pericardial cells and in precursor cells of a dorsal muscle, is also a *dpp* target in the dorsal mesoderm (Bodmer and Frasch, 1998). As shown in Fig. 2H-K, the expression of these two genes was downregulated in several regions of the heart in lgl^{ts3} mutants, and this was accompanied by an abnormal morphogenesis of the dorsal vessel (not shown).

The function of *dpp* is crucial to the specification and the differentiation of the dorsal epidermis in the embryo. Cuticle preparations of lgl^{ts3} homozygous embryos, reared at restrictive temperature (29°C), displayed a large variety of phenotypes illustrated in Fig. 3. The various phenotypes, probably due to the fact that lgl^{ts3} is a hypomorphic allele, were all related to the establishment of the dorsoventral axis. The most frequent ones consisted in a lack of internal head structures and head involution (86% of the total lgl^{ts3}cuticles), as well as in a lack of externalization of the Filzkörpers (90%) (Fig. 3C). The cephalopharyngeal skeleton and the Filzkörpers are derived respectively from the anterior dorsal region and from the posterior dorsolateral region (Jürgens et al., 1986). A large proportion of cuticles (45%) appeared twisted (Fig. 3B) and they were similar to cuticles prepared from *screw, shrew, tolloïd* or *twisted-gastrulation* mutants. As already mentioned, dorsal closure is blocked and 58% of the cuticles therefore displayed holes in their dorsal epidermis (Fig. 3A). Finally, lateral extension of the denticle belts was compared with that in a *scab* mutant, in which the differentiation of the dorsal epidermis was affected although no appearance of

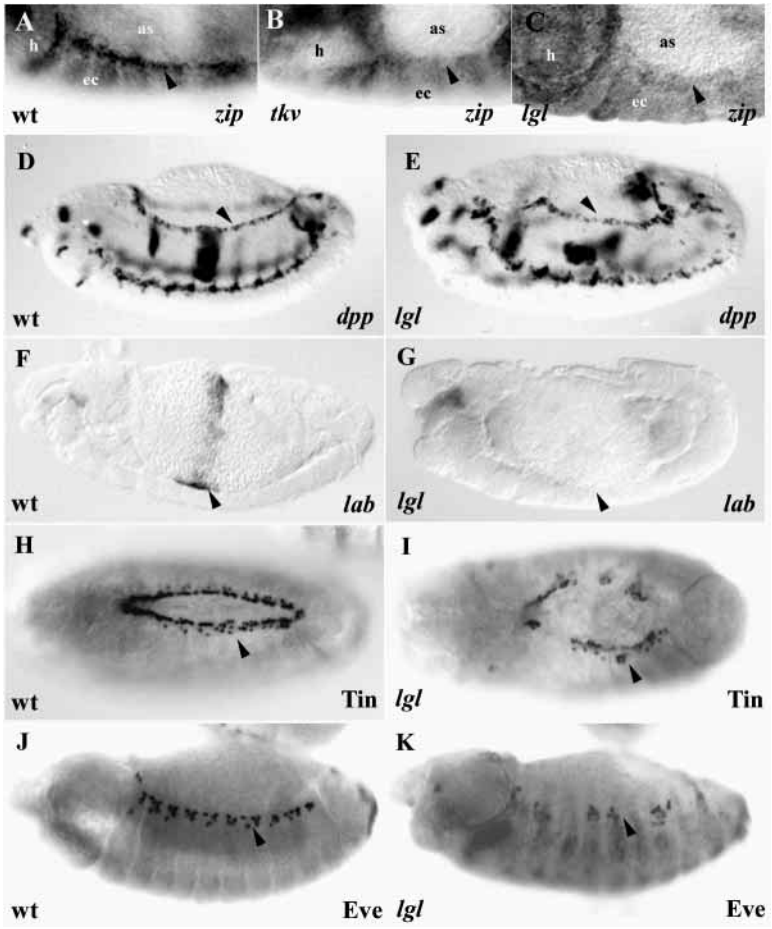

Fig. 2. Downregulation of the expression of dpp targets in lgl^{ts3} mutant embryos. (A,D,F,H,J) Wild-type embryos; (C,E,G,I,K) lgl^{ts3} homozygous embryos reared at 29°C; (B) tkv^{7} homozygous embryo. (A-C) In situ hybridizations of whole-mount embryos probed with a DIG-labeled *zipper* cDNA. The views are focused on one of the two LE cells rows (arrowheads) where *zip* transcript accumulates in a wild-type embryo at the onset of dorsal closure (A) but fails to accumulate either in a tkv^{7} homozygous embryo (B) or in a lgl^{ts3} homozygous embryo at 29°C (C). as, amnioserosa; ec, lateral ectoderm; h, head. (D,E) In situ hybridizations of whole-mount embryos probed with a DIG-labeled *dpp* cDNA. (D) In a stage 13 wild-type embryo, *dpp* is expressed in the LE cells (arrowhead), as well as in subsets of other cells such as those in the visceral mesoderm. (E) A lgl^{ts3} mutant embryo at the same stage expresses *dpp* in the LE cells (arrowhead), as does the wild-type embryo. Note the lower expression of *dpp* in the PS7 visceral mesoderm. (F,G) In situ hybridizations of whole-mount embryos probed with a DIG-labeled *lab* cDNA. *lab* expression was no longer detected in a lgl^{ts3} mutant embryo (arrowhead). The most extreme phenotype, with almost complete absence of *lab* expression, is shown here. Persistence of a labeling in the intercalary segment should be noted in mutant embryos. (H,I) Tin is expressed in the cardial cells in wild-type embryos and absent from most of the cardial cells in mutant embryos (arrowheads). (J,K) The same situation prevails in the case of Eve expression. The anti-Eve antibody labels a subset of pericardial cells and of dorsal muscles precursors. For all embryos shown, anterior is leftwards and the dorsal side is in focus. The genotypes are mentioned in the lower left and the probe or the antibody used in the lower right of each panel.

ventralization could be detected (Nusslein-Volhard et al., 1984). On the average, an increase of 15% in the length of ventral denticle belts in lgl^{ts3} mutant cuticles could be measured, reaching up to 60% of lateral extension in some mutant individuals (not shown).

In conclusion, these phenotypes, which were typical of a disruption in the dorsoventral patterning, were reminiscent of the defects encountered when the *dpp* signaling pathway is disrupted. In contrast, cuticles never displayed a ventralization with lack of amnioserosa and dorsal epidermis differentiation, which is however the phenotype the most sensitive to a lowering of the *dpp* gene dose. As a matter of fact, complete ventralization was not observed either in mutants in *dpp* signaling pathway genes which have a maternal expression (such as *punt*, for example, Letsou et al., 1995). A small number (less than 8%) of cuticles from lgl^{ts3} embryos displayed segmental abnormalities such as fusion of denticle belts or extended regions of naked cuticles (Fig. 3D), suggesting that *lgl* is not crucial to the function of other secreted morphogens such as Wingless or Hedgehog.

However, gain-of-function experiments clearly demonstrate that *lgl* function is required downstream of *dpp* for the specification of the dorsal epidermis. Ubiquitous ectopic expression of *dpp* in the embryo causes a dorsalization of cuticles (Staehling-Hampton et al., 1994). A similar dorsalization prevailed when *dpp* or Tkv^{Q253D}, a constitutively activated form of the Dpp receptor, was expressed in a wild-type embryo in the *paired*-expressing cells (Fig. 3E,G). Dorsalization was significantly reduced when *dpp* was ectopically expressed in lgl^{ts3} mutant embryos (compare Fig. 3E with 3F), suggesting that the *dpp* function requires *lgl* activity for the establishment of the dorsoventral polarity in embryos. By contrast, an absence of ventral denticles persisted in a *lgl* mutant background when Tkv^{Q253D} was ectopically expressed (Fig. 3H). Similar results were obtained with other GAL4 drivers, *en*-GAL4 (Fig. 3I,J) or *arm*-GAL4 (not shown), even though in this latter case the dorsalization observed with UAS-*dpp* in lgl^{ts3} was clearly higher than with *prd*-GAL4, probably because *arm*-GAL4 is expressed in all ectodermal cells and (or) at a higher level. Therefore, *lgl* appears to fulfill its function downstream of the *dpp* activation step, but not downstream of Tkv, after the receptor has been activated.

The function of *lgl* is required for *dpp*-dependent activation of *spalt* in wing imaginal discs

Growth and patterning of the wing imaginal disc rely on the localized expression of *dpp*, which in turn induces the transcriptional activation of its targets, including *spalt* (Nellen et al., 1996). Wing imaginal discs from homozygous null *lgl* alleles display a disorganized growth as early as the beginning of third instar larval stage and they do not form regular epithelial cells sheets, contrasting with what is encountered in a wild-type larva. However clear neoplasia in mutant wing discs become detectable only during the delayed larval life. In order to allow a clearer recognition of their shape, most of the analyses in mutants were conducted on wing discs from early

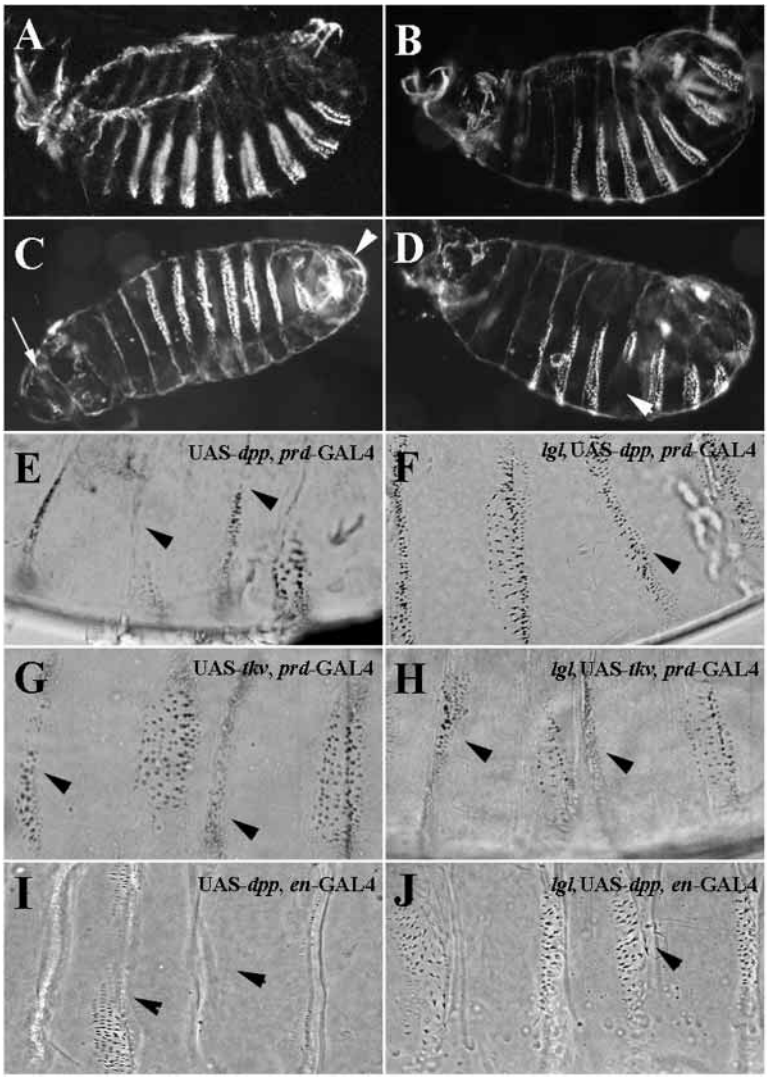

Fig. 3. The dorsoventral patterning of the embryonic ectoderm mediated by the *dpp* signaling is affected in lgl^{ts3} mutant embryos. (A-D) Cuticles of lgl^{ts3} homozygous embryos reared at 29°C. (A) Absence of dorsal closure producing a large hole in the dorsal ectoderm is visible in 58% of lgl^{ts3} mutant embryos. (B) Shows the twisted phenotype and a lack of involution of the head. (C) The absence of cephalopharyngeal skeleton (arrow) and the lack of externalization (or the absence) of the Filzkörpers (arrowhead) were the most frequently encountered phenotypes. (D) Mutant cuticle showing a rare phenotype consisting in a partial lack of ventral cuticle (arrowhead). Cuticle preparations of wild-type (E,G,I) or lgl^{ts3} mutant (F,H,J) embryos in which have been expressed Dpp (E,F,I,J) or constitutively activated Tkv (G,H) under the control of *prd* (E-H) or *en* (I,J) regulatory elements. The extent of dorsalization was assessed by the reduction of the number of ventral cuticles (arrowheads). Dorsalization by ectopic expression of Dpp in wild-type embryos (E,I) is more pronounced than that induced by Tkv^{Q253D} in wild-type embryos (G; data not shown) probably because Dpp can diffuse some distance away from its source of production. As *prd* is a pair-ruled gene, dorsalization is observed in only one of two segments in (E,H). Cuticle in F is very similar to that in wild-type, indicating that *lgl* is required for *dpp* mediated dorsalization (compare E with F). More than 80% of mutant cuticles were phenotypically wild-type whereas in control embryos 100% of them were dorsalized. By contrast, the extent of dorsalization is roughly identical in G,H, indicating that Tkv^{Q253D} can rescue *lgl* function in this process. (I,J) When *dpp* is induced in *en*-expressing cells, the ventral denticles of all the segments can potentially be affected owing to the expression of *en* in the epidermal cells that gives rise to the anteriormost row of denticle belts. Dorsalization was also more effective in wild-type (I) than in lgl^{ts3} embryos (J). In all panels, cuticles are oriented with the anterior towards the left. Arrowheads point to wild-type (F,J) or phenotypically mutant (E,G-I) denticle belts.

third instar larvae, whose morphology still roughly resembled that of wild-type larvae. At this stage, the expression domain of *dpp* in the wing disc was larger than in late third instar larvae and the Spalt expression domain narrower.

The *dpp*-dependent expression of Spalt was abolished or strongly reduced in the presumptive wing blade of *lgl* mutant disc (Fig. 4A,B), while *dpp* expression itself was normally initiated and not noticeably affected (Fig. 4C,D). The polarity of the epithelial cells in mutant discs, as probed with Dlg or Armadillo (Arm) was still preserved (Fig. 4F), in contrast to what is observed in discs from delayed lgl^4 homozygous third instar larvae (Woods et al., 1997; Bilder et al., 2000). Furthermore, En (Fig. 4G,J,P) and Ptc (not shown) were normally expressed in mutant wing discs, strongly suggesting that the *lgl* function is involved in the *dpp* signaling pathway itself.

spalt expression in lgl^4 mutant wing imaginal discs was restored by expressing Tkv^{Q253D}, but not Dpp, in the posterior compartment with the aid of an *en*-GAL4 driver (Fig. 4G-L). In this experiment, Spalt expression was restored only in the posterior compartment. In order to be able to recognize the imaginal disc cells that require the *lgl* function in *dpp* signaling (i.e. the cells that emit the Dpp signal or the cells that receive it), *lgl* has been specifically expressed in the *dpp* territory within a lgl^4 mutant background. Such an ectopic expression led to a rescue of Spalt expression in the presumptive wing blade (Fig. 4M-O). By contrast, no rescue of Spalt expression (Fig. 4P-R) could be obtained by restoring *lgl* function in the posterior compartment. These results indicate that *lgl* function is required in the cells that produce Dpp to activate Spalt expression. Moreover, when *lgl* expression was restored in *dpp*-expressing cells, Spalt expression in the wing blade was detected in the cells that did not express *dpp* (arrowhead in Fig. 4N-O). This observation of a Spalt expression domain extending out of the dpp expression domain suggests that the function of *lgl* is not required for the reception of the Dpp signal.

Likewise, in eye imaginal discs, *lgl* seems to play a role related to *dpp* signaling pathway. *Dpp* function in this tissue is rather involved in the onset of the morphogenetic furrow movement than in ommatidia differentiation (see, for example, Burke and Basler, 1996b). In a wild-type eye disc, Spalt was detected in all the differentiated ommatidia, while in lgl^4 mutants, its expression was restricted to only a few disorganized ommatidia (Fig. 5A,B). Furthermore, the advance

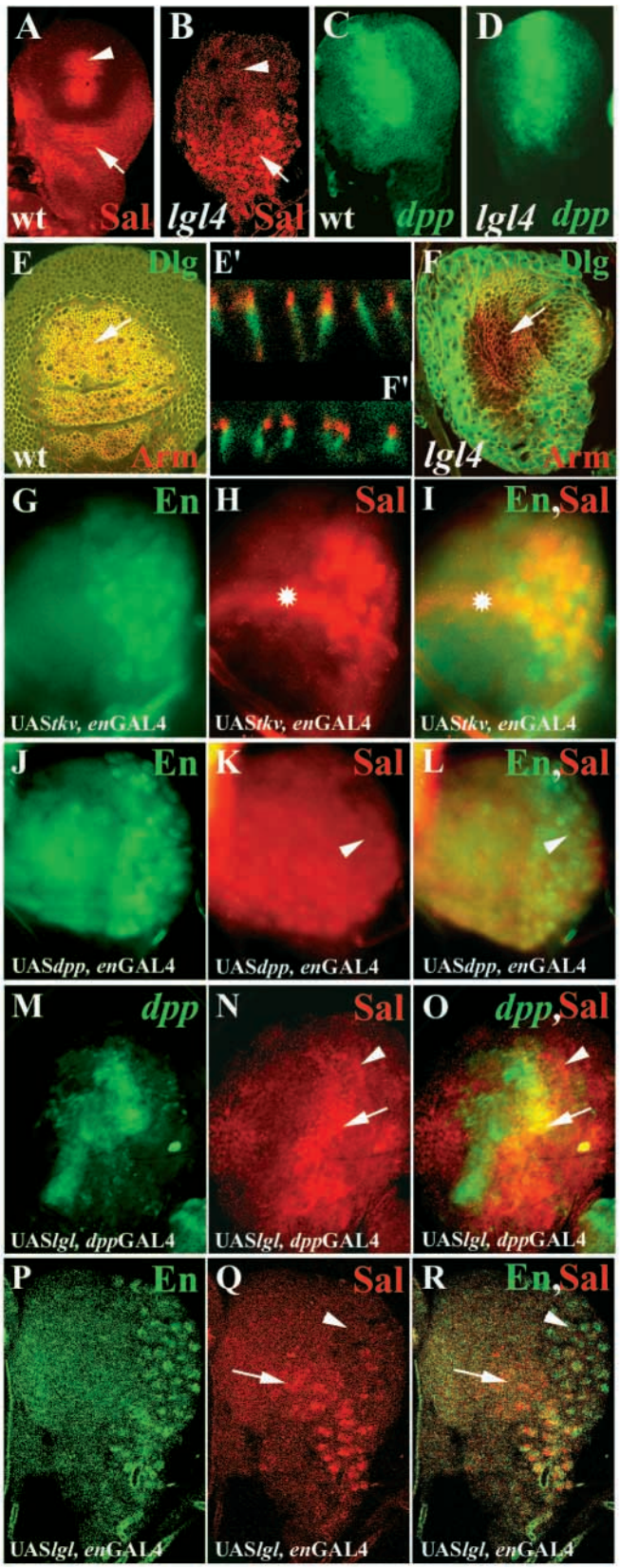

Fig. 4. Expression of Spalt, a *dpp* target, in lgl^4 imaginal wing discs. In all panels (except E,F), Spalt (Sal) expression was revealed using an anti-Spalt antibody (in red). (A,B) The expression of Sal in the center of the presumptive wing blade (arrowhead) is abolished in early third instar mutant larva. The *dpp*-independent expression of Sal in the notum (arrow) is not affected by *lgl* loss of function. (C,D) *dpp* expression, revealed by in situ hybridization with a DIG-labeled *dpp* probe (in green) is normally initiated in a lgl^4 mutant disc from early third instar larva. (E,F) En face optical sections of imaginal discs. Epithelial cell polarity probed with anti-Dlg (green) and anti-Arm (red) antibodies. In spite of a disorganization of the epithelial folds in lgl^4 mutant discs (F), Dlg and Arm are normally located at the plasma membranes. (E′,F′) *z*-views of the epithelial layers at positions marked by an arrow in (E,F). As in the wild type, Arm is located in the mutant to the apicalmost region of the lateral membrane, above the septate junctions labeled with Dlg. (G-R) All discs are lgl^4 mutants. (I,L,O,R) are merged views of the two separate views on their left. (G-I) *en*-Gal4 driven expression of a constitutively activated form of Tkv in the posterior compartment of a lgl^4 mutant disc induces the expression of Sal in the posterior compartment, marked with anti-En antibody. The star indicates a trachea. (J-L) Under the same conditions as in (G-I), expression of *dpp* in *en*-expressing cells is unable to induce Sal expression in the posterior compartment (arrowhead). (M-O) Sal expression is restored in the presumptive wing blade (arrowhead) of an lgl^4 mutant wing disc by expression of *lgl* under the control of *dpp*-Gal4. The *dpp* domain of expression was assessed by UAS-GFP (green). (P-R) expression of *lgl* in the posterior domain of a lgl^4 mutant disc cannot rescue Sal expression in the presumptive wing blade (arrowhead). The arrows in M-R point to *dpp*-independent Sal expression.

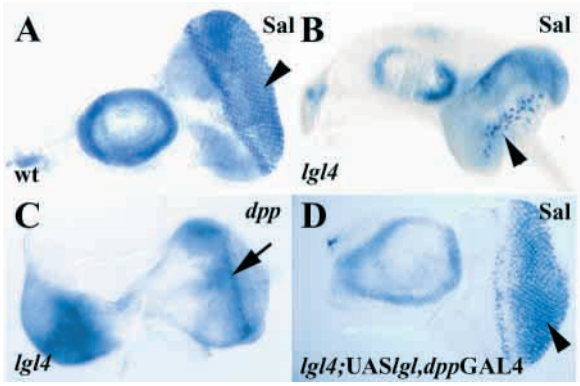

Fig. 5. *lgl* function in the eye imaginal disc. (A,B,D) Anti-Sal staining, which labels a small number of photoreceptors in every differentiated ommatidia. (C) In situ hybridization with a DIG-labeled *dpp* probe. At late third instar larval stage, *lgl* mutant eye discs are smaller than in the wild type (compare the respective sizes of the eye disc with the antenna disc in A,B, for example). (D) The differentiation of ommatidia is almost completely restored when *lgl* is expressed in *lgl* mutant discs under the control of *dpp*-Gal4.

of the morphogenetic furrow was slowed down. This phenotype is reminiscent of that prevailing in the class of 'furrow-stop mutants', including *dpp* (Heberlein et al., 1993).

In the mutant discs, the expression of *dpp* was normal in the delayed morphogenetic furrow (Fig. 5C). Restoration of *lgl* function in the *dpp* expression domain was sufficient to almost completely rescue the mutant phenotype (compare Fig. 5A with 5D). It is not known whether Spalt expression in the eye disc is dependent on *dpp* signaling, but this latter result indicates that *lgl* function in the eye disc is required only in the cells that express or have expressed *dpp*, confirming the results obtained in the wing discs.

Loss-of-function *lgl* somatic clones behave non autonomously with respect to *dpp* signaling

Cell autonomy of the Dpp signal has been analyzed in *lgl* loss-of-function somatic clones in the wing imaginal disc. Two different null alleles were used (lgl^4 and lgl^{4W3}) that led to similar results. Spalt expression was taken as an indicator of the activity of the Dpp signaling cascade and Patched as a marker of the A/P compartment boundary (Maschat et al., 1998).

All the mutant clones scored (about 200 clones scored) that encompassed the Spalt expression domain exhibited a wild-type pattern of Spalt expression (Fig. 6). This observation, which holds even in the case of large clones produced in *Minute* background, indicates that *lgl* function, at least with respect to *spalt* activation, is not required in the cells that receive Dpp and respond to it. Because the expression of Spalt was abolished in mutant discs that were entirely lgl^4, this behavior was interpreted as the result of a non autonomous function of *lgl* with respect to *dpp*-dependent Spalt expression: the mutant cells might be rescued by the surrounding wild-type cells that normally expressed and secreted Dpp.

However, we have never detected mutant cells within the anterior compartment in close apposition to the A/P compartment boundary, where Dpp is produced. In contrast, numerous mutant cells in large clones (25 mutant clones scored) were able to reach the A/P compartment boundary when they were located in the posterior compartment. It appears therefore that mutant cells appeared to be excluded from the region in which Dpp was produced at third instar larval stage. A striking example is shown in Fig. 6A in which a twin clone of wild-type cells reaches the A/P frontier and is much larger than the mutant clone that is composed of only a few cells expressing spalt, and that is located farther away from the A/P compartment boundary. *lgl* mutant cells seem to be eliminated from the Dpp domain; they probably cannot survive and the surrounding wild-type cells might not be able to rescue them. This clonal analysis, which has to be strengthened by other experiments, strongly suggests that the function of *lgl* is required only in the *dpp*-expressing cells of the imaginal wing disc. As a matter of fact, a clonal analysis by Posakony et al. had demonstrated a localized requirement for wild-type *dpp* expression along the A/P compartment boundary of the developing wing (Posakony et al., 1990). They never recovered *dpp* mutant clones located in this domain in adult wings.

Analysis of mitotic clones in the adult wing was consistent with these observations. Dpp plays a dual function in wing morphogenesis: it regulates growth and patterning of the wing disc during larval development and promotes the differentiation of veins during pupal development (de Celis, 1997). Under the conditions of induction that we have used, three types of situations have been encountered (around 200 clones were analyzed). Phenotypes of loss of veins were observed only when mutant clones were present on both sides of the wing blades (~2%, Fig. 7A,B), as already shown in the case of *dpp* mutant mosaics (de Celis, 1997). By contrast, numerous genetically mutant clones (~20%), which occupied only one surface of the wing blade and encompassing veins, did not affect vein differentiation (Fig. 7C). These results are consistent with the hypothesis that *dpp* expression in one surface is sufficient to provide *dpp* function to the other surface (de Celis, 1997). Finally, the large majority of mutant clones (~80%), which were found in intervein regions, did not display any mutant phenotypes and were as large as the wild-type twin clones.

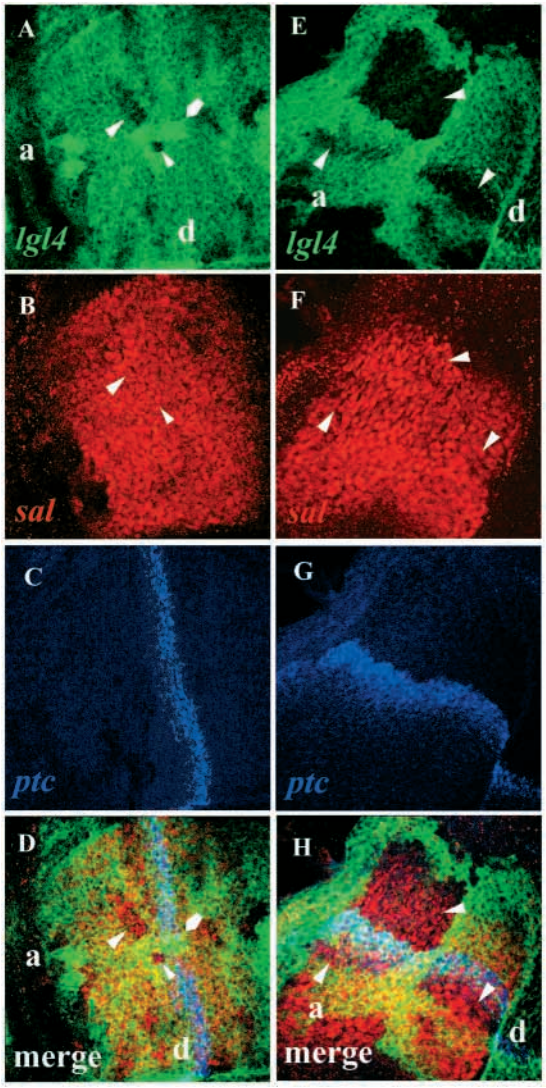

Fig. 6. Non-autonomous behavior of lgl^4 mutant clones with respect to *dpp* signaling in wing imaginal discs. Homozygous clones for lgl^4 have been produced by the FLP-FRT system in a wild-type (A-D) or in a *Minute* background (E-H). Mitotic recombinations have been induced during the second larval stage. Mutant clones were visualized by the absence of the *arm-lacZ* marker (green in A,E). The activity of the *dpp* signaling pathway was assessed by the expression of Spalt (red in B,F). The position of the A/P boundary was assigned by the expression of Patched (blue in C,G). (D,H) Merged images. Filled arrowheads point to mutant clones. The large arrows in A,D show an example of a twin clone lying just anteriorly to the A/P boundary. a, anterior; d, dorsal. The general structure of the wing discs containing numerous large mutant clones was repeatedly deeply affected.

Taken together, all these observations indicate a non autonomous function of *lgl* in the *dpp* signal transduction pathway during wing morphogenesis.

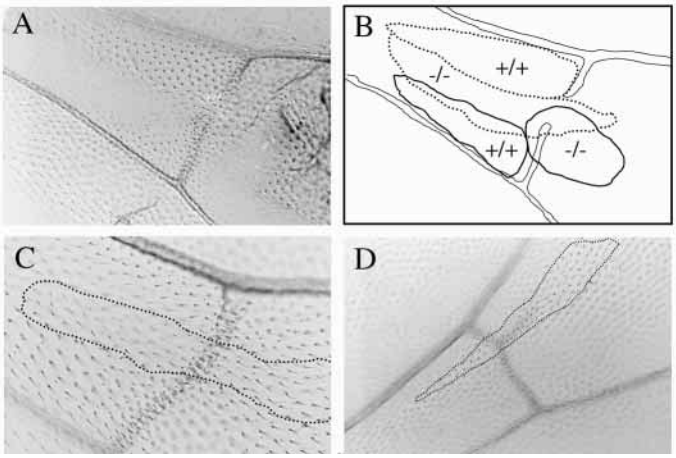

Fig. 7. Mosaic analysis of lgl^4 wings. (A) Example of lgl^4 clones occupying dorsal and ventral wing blade surfaces and causing loss of veins. (B) Schematic representation of ventral (unbroken lines) and dorsal (broken lines) clones in (A) of homozygous lgl^4 (–/–) and twin wild-type (+/+) clones. (C) Example of a lgl^4 clone (broken line) encompassing the posterior cross-vein that differentiates despite the presence of a large clone in the ventral surface. (D) Example of ectopic vein material within a lgl^4 mutant clone.

DISCUSSION

A function for *lgl* in Dpp secretion?

The results reported herein demonstrate that at least part of the *lgl* function is involved in the *dpp* signaling pathway. All the known *dpp* targets considered in this study were underexpressed or even not expressed at all in *lgl* mutants. In addition, whenever technically possible, we have shown that the *dpp* signaling pathway mutant phenotype was rescued by the expression of a constitutively active form of Tkv, thus assessing the specificity of *lgl* action in the process. This observation implies that the function of *lgl* resides in a participation either in the secretion and (or) maturation of Dpp, or in its long range diffusion or, eventually, in the correct activation of its receptor.

Several lines of evidence presented in this article suggest that the *lgl* function is required in cells that express Dpp rather than in cells that receive the signal. Spalt expression in mutant wing discs is recovered by restoring *lgl* activity in the Dpp domain of expression. In this situation, Spalt expression can be detected not only in the cells that produce *dpp*, but also in groups of mutant cells which do not express *dpp* (Fig. 4). Imaginal discs Dpp-dependent expression of *spalt* can be rescued in *lgl* mutant clone cells by surrounding wild-type cells. This observation supports the idea that the Dpp signal can diffuse and interact correctly with its receptor in the wing blade in *lgl* mutant cells, thus pointing towards a function for *lgl* in the production or the secretion of Dpp by the cells in which it is transcribed.

So far, any straightforward approach to the analysis of the processing or the secretion of Dpp in mutant cells has been hampered mainly because of the lack of efficient antibodies. We have nevertheless been able to detect a precursor form of Dpp that is synthesized in lgl^4 mutant disc cells and present at a level similar to that observed in wild-type cells (N. A., unpublished) by using an antibody directed against the N-terminal part of the protein (Groppe et al., 1998). This antibody however does not recognize the C-terminal moiety of the Dpp protein which constitutes its processed and secreted form.

It has been clearly demonstrated that the yeast homologs of Lgl, Sro7p and Sro77p, participate in polarized exocytosis and are associated to Sec9, a plasma membrane SNARE (Lehman et al., 1999). This protein family seems to be functionally conserved, as *lgl* or its human homolog (*HUGL*), are able to rescue the *sro7/77* mutant phenotypes in yeast (Kagami et al., 1998). Moreover, a Sec9/Snap25 homolog is also present in the *Drosophila* genome (Lloyd et al., 2000). By taking into account these functional homologies, a function for *lgl* can be postulated in exocytosis.

lgl in the control of epithelial cell polarity

Secretion relies on intracellular vesicular trafficking and on the polarized exocytosis machinery (Mellman and Warren, 2000). Recent studies have demonstrated that Lgl function is essential for the establishment of the polarities of epithelial cells (Manfruelli et al., 1996; Bilder et al., 2000) and of dividing neuroblasts (Peng et al., 2000; Ohshiro et al., 2000). An important issue is therefore to understand whether the role of Lgl in Dpp secretion is direct or simply a consequence of the loss of epithelial cell polarity. Analysis of the temporal requirement for Lgl function argues in favor of Lgl being necessary for the establishment of cell polarity, rather than for its maintenance. Moreover, alteration in Dpp signaling can be observed in *lgl* mutants in epithelial cells that are correctly polarized and this supports a direct function for Lgl in Dpp secretion.

The epidermis is not affected in homozygous lgl^4-null mutant larvae that no longer contain the maternal Lgl protein responsible for a normal embryonic development (Gateff and Schneiderman, 1969; Manfruelli et al., 1996). lgl^4 larvae develop a cuticle that possesses the hallmarks of a wild-type cuticle by all the criterions used, thus indicating that the apical secretion of cuticle components has not been altered. Markers for epithelial cell polarity are localized in the correct position (Peng et al., 2000) in stage 16 embryos when Lgl is no longer detected (Strand et al., 1994a). Likewise, lgl^{ts3} embryos in which the Lgl protein has lost its cortical location have maintained their typical epithelial cell polarity (Fig. 1) and their capacity to secrete normal cuticle components (Fig. 3; see also Manfruelli et al., 1996; Ohshiro et al., 2000). In neuroblasts, Lgl seems to exert its action early during mitosis to recruit basal determinants to the cortex but it does not contribute to their maintenance in this latter location (Ohshiro et al., 2000). The polarity of epithelial wing disc cells is preserved until the middle of the third instar larval stage, long after the maternal Lgl contribution has ceased (Fig. 4; Gateff and Schneiderman, 1969).

In contrast, the need for Lgl function appears crucial whenever cell polarity has to be established. In neuroblasts, its function during mitosis is to assist Miranda and Prospero localization (Ohshiro et al., 2000), when a new asymmetric division requires basal localization of cortical determinants. Asymmetric division is equally impaired in late lgl^4 embryos in which the maternal contribution of Lgl might become too low to ensure the efficiency of the process (Peng et al., 2000). In embryos issued from germline clones, lacking both maternal

and zygotic *lgl* activities, the cells of the ectoderm form but they do not acquire their epithelial cell polarity (Bilder et al., 2000).

While embryogenesis proceeds, some epithelial cells have to change or remodel their polarity. This is the case, for example, for the LE cells during dorsal closure and our data show that Lgl is also involved in this process. During larval stages, imaginal disc cells actively proliferate and have to continuously reconstruct their membrane junctions and their polarity in different steps that are dependent on the exocytosis machinery. Loss of activity of *lgl* might be responsible for the progressive alteration in cell polarity leading to neoplasms in *lgl* mutant animals.

In conclusion, *lgl* activity appears to be required whenever targeting to the membrane of new components is crucial to the acquisition of cell polarity.

Alteration of Dpp signaling precedes epithelial cell polarity disruption in *lgl* mutants

Our data support the hypothesis that the effect of *lgl* on Dpp signaling does not result, as an indirect consequence, from altered cell polarity and, accordingly, we have provided some examples in which the two processes could be uncoupled. Epidermal cells in lgl^{ts3} embryos are normally polarized and secrete cuticle but they are unable to promote cuticle dorsalization in response to an ectopic expression of Dpp while they induce this same dorsalization in response to Tkv^{Q253D}.

The cell polarity in lgl^{ts3} embryos is not altered when the targets of the Dpp signaling have to be activated. When *tin* and *eve* expressions have to be induced within the mesoderm, the dorsal ectoderm, which produces Dpp, shows a wild-type epithelial cell polarity and can secrete dorsal cuticle. The mesodermal and endodermal midgut cells display a normal cell polarity when *labial* expression has to be induced by Dpp (Manfruelli et al., 1996). Similarly, *zip* accumulates in LE cells before they undertake their shape and polarity changes (Fig. 1). Dpp is actually expressed well ahead of the initiation of dorsal closure and its expression in LE cells rapidly decreases at the onset of this process. Finally, Spalt is not expressed in *lgl* mutant wing discs that express Dpp, even though their epithelial polarity is not yet disturbed (Fig. 4). The rescue in lgl^{4} mutants of Spalt expression by a constitutively activated form of the Dpp receptor, Tkv, strongly suggests that its localization to a specific domain of the cell membrane or (and) its integration within the membrane has not been impaired, although they are both likely to be dependent on cell polarity.

It seems reasonable to assume that there is a unique exocytosis pathway mediated by *lgl* to ensure both cell polarity control and secretion. *Dlg* and *scrib* might participate in this same pathway, as, indeed, they strongly interact genetically with *lgl* and share with this gene a large panel of identical mutant phenotypes (Bilder et al., 2000). Lgl, however, does not strictly colocalize with Dlg and Scrib in either epithelial cells (Bilder et al., 2000) or in neuroblasts (Peng et al., 2000). In addition, the Dlg cortical localization does not require *lgl* function (Peng et al., 2000; Ohshiro et al., 2000). One could therefore anticipate an *lgl* action, within a separate and distinct pathway, in parallel to that of *dlg* and *scrib*, and further experiments are needed to address this issue.

Whatever the situation, Lgl probably mediates a specific secretion pathway for Dpp that other secreted morphogens such as Wingless or Hedgehog or cuticle proteins do not share. This is consistent with the function of *lgl* in targeting specific cortical proteins to particular membrane domains in epithelial cells or neuroblasts.

lgl and the control of the actomyosin cytoskeleton

In yeast, *sro7/77*-mediated polarized exocytosis relies on a complex regulation and interaction with the actomyosin cytoskeleton. *Sro7/77* display a strong genetic interaction with *myo1* (encoding a Type II myosin homolog of NMHC) and with *myo2* (encoding an unconventional Type V myosin) (Kagami et al., 1998). In addition, Myo1P can physically interact with Sro7P (Kagami et al., 1998), in a manner resembling that prevailing between Lgl and NMHC (Strand et al., 1994b). These observations are in favor of Lgl serving as a functional link between the actomyosin cytoskeleton polarity and a specific polarized exocytosis pathway, although the precise function exerted by Lgl in such a process has yet to be deciphered. In yeast, as in flies, *myo1 (zipper)* and *sro7/77 (lgl)* display a negative genetic interaction (Kagami et al., 1998; Peng et al., 2000; Ohshiro et al., 2000), as already mentioned during the fly dorsal closure (P. Manfruelli, PhD thesis, Marseille, 1996). Loss-of-function alleles of *lgl* suppress the dorsal closure phenotype in homozygous *zip* mutants. Conversely, overexpression of *lgl* enhances the dorsal closure phenotype.

The data reported in this article rather state a role for Lgl in the transcriptional activation of *zip* in LE cells, raising a puzzling question about the in vivo functional significance directly associated with the interaction demonstrated in vitro between Lgl and NMHC. As a matter of fact, even in LE cells in which there is an important accumulation of NMHC, there is no particular colocalization of the two proteins. The same situation is encountered in other cells in which NMHC also accumulates (Young et al., 1991; Edwards and Kiehart, 1996; C. De Lorenzo, PhD thesis, Marseille, 1998). Similarly, in yeast, an abnormal localization of actin and myosin becomes visible only long after the initial defect of exocytosis encountered in *sro7/77* mutants (Lehman et al., 1999). These observations might indicate that the Lgl-NMHC physical interaction observed in vitro is not relevant in vivo and might not be functional in the LE cells. One could rather anticipate a general ability of Lgl to bind myosins. Accordingly, it is noteworthy that, in yeast, another myosin-encoding gene, *myo2*, interacts positively with *sro7/77* (Kagami et al., 1998), and that, in *Drosophila* neuroblasts, two functionally different cytoskeletal actomyosin networks, one being repressive and the other positive, seem to control the cortical localization of basal determinants (Peng et al., 2000).

Tumor suppression and TGFβ signaling

It is particularly intriguing that *lgl*, a *Drosophila* tumor suppressor gene, could play a role in a signaling pathway such as the *dpp* pathway. Dpp is a member of the superfamily of TGFβs, which have been described as important key regulators in carcinogenesis (Padgett et al., 1998). TGFβ receptors and components of the TGFβ signal transduction pathway are mutated in a number of human tumors (for review see Riggins et al., 1997). Homozygous mutant animals for Smad3 have recently been obtained that are viable but that spontaneously display colorectal adenocarcinomas (Zhu et al., 1998).

However, in the wing discs, which are tissues that develop tumors in the absence of *lgl* function, *dpp* stimulates growth and proliferation (Burke and Basler, 1996a). This leads to a paradox, implying that a tumor suppressor gene, which is likely to encode a negative regulator of cell proliferation, could be primarily involved in a cell signaling pathway that activates proliferation.

While it is tempting to speculate that tumors arise in *lgl*-deficient animals as a direct consequence of the perturbation of *dpp* signaling, this has not been demonstrated in our study, and tumors could well be due to another *dpp*-independent function of *lgl*. In particular, the reported role of *lgl* in establishing epithelial cells polarity might be implicated in tumorigenesis (Bilder et al., 2000). Current work is in progress to address this question specifically.

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