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The *Drosophila* JNK pathway controls the morphogenesis of imaginal discs during metamorphosis

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SUMMARY

In *Drosophila*, the Jun-N-terminal Kinase-(JNK) signaling pathway is required for epithelial cell shape changes during dorsal closure of the embryo. In the absence of JNK pathway activity, as in the *DJNKK/hemipterous* (*hep*) mutant, the dorsolateral ectodermal cells fail both to elongate and move toward the dorsal midline, leading to dorsally open embryos. We show here that *hep* and the JNK pathway are required later in development, for correct morphogenesis of other epithelia, the imaginal discs. During metamorphosis, the imaginal discs undergo profound morphological changes, giving rise to the adult head and thoracic structures, including the cuticle and appendages. *hep* mutant pupae and pharate adults show severe defects in discs morphogenesis, especially in the fusion of the two lateral wing discs. We show that these defects are accompanied by a loss of expression of *puckered* (*puc*), a JNK phosphatase-encoding gene, in a subset of peripodial cells that ultimately delineates the margins of fusing discs. In further support of a role of *puc* in discs morphogenesis, pupal and adult *hep* phenotypes are suppressed by reducing *puc* function, indicative of a negative role of *puc* in disc morphogenesis. Furthermore, we show that the small GTPase Dcdc42, but not Drac1, is an activator of *puc* expression in a *hep*-dependent manner in imaginal discs. Altogether, these results demonstrate a new role for the JNK pathway in epithelial morphogenesis, and provide genetic evidence for a role of the peripodial membrane in disc morphogenesis. We discuss a general model whereby the JNK pathway regulates morphogenesis of epithelia with differentiated edges.

Key words: JNK, *hep*, *puc*, *DCdc42*, Imaginal disc, Morphogenesis, Peripodial membrane, Metamorphosis

INTRODUCTION

Epithelial morphogenesis is a fundamental and widespread process controlling cell, tissue and organ development, hence the ultimate shape and organization of metazoans. The ability of fields of cells to translate spatial and temporal information into appropriate morphogenetic activity is crucial for the making of a functional organism. In *Drosophila*, several genes have been identified that are important for correct morphogenesis, including both structural and regulatory (signaling) activities. For example, during mid-embryogenesis, the dorsal part of the embryo is progressively covered by the lateral ectodermal cells undergoing dorsal closure (DC): these epithelial cells elongate dorsally and move in concert toward the dorsal midline where they fuse at the end of the process (Martinez Arias, 1993; Noselli, 1998). It was recently shown that the Jun-N-terminal Kinase (JNK) pathway plays an essential role in this process, by controlling specific gene activity in a single row of cells leading the migrating ectoderm (Glise et al., 1995; Glise and Noselli, 1997; Harden et al., 1996, 1995, 1999; Hou et al., 1997; Kockel et al., 1997; Liu et al., 1999; Lu and Settleman, 1999; Martin-Blanco et al., 1998; Ricos et al., 1999; Riesgo-Escovar and Hafen, 1997a,b, 1996; Sluss and Davis, 1997; Sluss et al., 1996; Su et al., 1998; Zeitlinger et al., 1997). In JNK pathway mutant embryos, these leading edge (LE) cells lose both their identity and ability to move dorsally. Thus, a regional defect can affect the ability of a larger field of ectodermal cells to undergo proper morphogenesis. A current model proposes that the LE cells may play an organizer role for morphogenesis of the whole ectoderm through the coupling of JNK and *dpp* signaling pathways (for review, see Goberdhan and Wilson, 1998; Ip and Davis, 1998; Noselli, 1998; Noselli and Agnes, 1999).

We are interested in the signaling mechanisms governing the morphogenesis of epithelia, and the way these are modulated in order to generate morphogenetic diversity. One way to study this is to compare related processes that are controlled by the same pathway in a single organism. The identification of the putative flexible parts would permit an understanding of how a single pathway may control apparently different morphogenetic processes and thus contribute to diversity. In this context, we investigated the role of *hep* and the JNK pathway in metamorphosis.

Drosophila is an holometabolous insect, i.e., the adult form (imago) derives from complete metamorphosis during the pupal stage. The transition from larval to adult body shape is

mediated by specialized epithelial cell sacs called imaginal discs, which develop from precursors that are specified during embryonic development (Cohen, 1993; Fristrom and Fristrom, 1993). The larvae can thus be seen as a two-faced animal, one (the larva) hosting the other (the future adult). By the end of third instar larval stage, sharp variations in the titer of the steroid hormone 20-hydroxyecdysone induces the entry of the larvae into pre-pupal development and discs begin their dramatic morphogenesis (Fristrom and Fristrom, 1993; Riddiford, 1993). Within 12 hours, the monolayered imaginal discs transform into recognizable adult structures (wings, thorax, legs, head) by a series of morphogenetic changes. These include elongation/shaping and eversion of individual discs (the so-called evagination), followed by spreading and fusion of neighboring discs. During this metamorphic process, adult tissues will replace larval ones, thus forming a continuum of adult, neo-formed epidermis (Fristrom and Fristrom, 1993).

Whereas loss of both maternal and zygotic *hep* function induces deficient dorsal closure leading to embryonic lethality (Glise et al., 1995; Glise and Noselli, 1997), loss of zygotic *hep* function induces defects in disc morphogenesis causing pupal lethality.

Here, we show that *hep* and, by extension, the JNK pathway are required for the correct morphogenesis of the imaginal discs. One major defect observed in *hep* mutants is the absence of wing disc fusion. This aberrant morphogenetic behavior is accompanied by a loss of *puc* gene expression in cells of the peripodial membrane, a specialized stretched epithelium that leads the spreading discs and is ultimately found at the suture sites joining adjacent discs. As in the process of dorsal closure, *hep* controls the differentiation of leading margins in imaginal discs, suggesting a general model for JNK pathway function in the morphogenesis of epithelia with differentiated or 'free' margins (Trinkaus, 1969).

MATERIALS AND METHODS

Genetics

A description of genetic markers and chromosome balancers used in this study can be found in Lindsley and Zimm (1992). The hep^{1} allele is an insertion of a P element in the 5′ untranslated region of the *hep* gene, and was used to generate lethal alleles by imprecise excision (hep^{r39} and hep^{r75}; Glise et al., 1995). The puc^{E69} allele is a P *lacZ* enhancer-trap insertion in the *puc* gene (Ring and Martinez Arias, 1993). The *dpp-lacZ* line was kindly provided by K. Basler (Zecca et al., 1995).

GAL4 targeted expression

Targeted expression of UAS-driven transgenes (Brand and Perrimon, 1993) was induced using the following GAL4 lines: MS1096-GAL4 (wing disc: wing pouch, notum and peripodial membrane; Capdevila and Guerrero, 1994); *dpp*-GAL4 (all discs); GMR-GAL4 (eye-antennae specific). The UAS lines used in this study are: UASDrac1V12 and UAS-Dcdc42V12 (Luo et al., 1994). Recombinant chromosomes with these lines and puc^{E69} are described in Glise and Noselli (1997).

Targeted expression in a *hep* mutant background was done using the following strains: $ywhep^{r39}$/*FM7*; *UAS-Dcdc42V12*,puc^{E69}/*TM6B* and $ywhep^{r75}$/*FM7*; *UAS-Drac1V12*,puc^{E69}/*TM6B*.

To test *puc* expression in a *hep* mutant background, the following genetic crosses were performed: $ywhep^{r}$/*FM7;UAS-X*, puc^{E69}/*TM6B* females were crossed by *+/Y; Z-GAL4* males. X represents any of the *UAS*-driven reporter genes whereas Z represents any *GAL4* line described above.

Staging of larvae and pupae

Larvae were grown on yeasted polenta medium containing 0.05% of bromophenol blue, and collected at the wandering stage. For staging of late L3 larvae, animals were selected according to the intensity of gut staining (Andres et al., 1993). The selection of *hep* mutant male larvae (*hep/Y*) was done using the *yellow* (*y*) marker (mouthparts have a clear brown color compared to the wild type). Pupae were collected as white puparia (Bainbridge and Bownes, 1981) and kept in Petri dishes between 0 and 8 hours after puparium formation (APF). Prepupal stages were identified as described in Bainbridge and Bownes (1981).

Overnight egg collections made on yeasted agar plates were counted and transferred into bottles for growth. The entire progeny was scored for the number of eclosed or dead pupae. Alternatively, individual cohorts of late L3 larvae from bottle cultures were transferred to Petri dishes and scored for pupal lethality.

X-gal and antibody staining

Dissected larvae and prepupa were fixed for 20 minutes at room temperature in 4% paraformaldehyde and washed in 1× PBS. Staining for β-galactosidase activity was performed according to standard protocols (Ashburner, 1989). Antibody staining was performed using the following antibodies: commercial rabbit anti-β-gal (Cappel), mouse anti-BR-C (kindly provided by G. Guild, University of Pennsylvania, USA) anti-mouse biotinylated antibody (Vectastain, Vector Inc, CA, USA) and anti-rabbit FITC (Fluorescein)-tagged secondary antibody (Immunotech/Beckman/Coulter, Inc.). For double immunostaining, samples were incubated overnight at room temperature (RT) with both primary antibodies in 1× PBT (1× PBS, 0.03% Triton X-100). After several washings, samples were incubated for a couple of hours with respective secondary antibodies in 1× PBT at RT. After washing, a streptavidin/biotin amplification step was performed for 30 minutes to enhance BR-C signal which was revealed by streptavidin-TRITC (Rhodamine).

puc expression was monitored using the *puc-lacZ* enhancer trap line puc^{E69} (Ring and Martinez Arias, 1993). *dpp* expression was monitored using the dpp^{P10638}-*lacZ* line (Zecca et al., 1995). Photographs were taken with a CCD camera (DAGE-MTI. INC., Michigan, USA) mounted on a Zeiss Axiophot microscope. Fluorescence microscopy was carried out using a LSM10 Zeiss confocal microscope. Pictures were captured using Matrox Intellicam 2.0 software and assembled with Adobe Photoshop 3.0 software.

RESULTS

hemipterous pupal and adult phenotypes

Loss of both maternal and zygotic *hep* function leads to embryonic dorsal closure (DC) defects (Glise et al., 1995; Glise and Noselli, 1997), a process of epithelial morphogenesis by which lateral ectodermal cells elongate dorsalward and ultimately fuse at the dorsal midline (Martinez Arias, 1993; Noselli, 1998; Young et al., 1993). By contrast, zygotically deficient *hep* animals die later during the pupal stage, thus indicating *hep* requirement in at least two different processes and stages. The anterior (head and thorax) and abdominal regions of the adult body derive from different structures, the imaginal discs and the histoblasts, respectively (Fristrom and Fristrom, 1993). Dissected null *hep* mutant pupae (>10 hours after puparium formation; h APF) show incomplete metamorphosis of the anterior body region, whereas abdominal structures seem to form properly (see below and data not

shown). These observations suggest that *hep* is involved in imaginal disc morphogenesis specifically. Interestingly, a viable P insertion allele of *hep* (*hep*^{1}) causes poorly penetrant (approx. 5%) adult head and thoracic abnormalities (Fig. 1; Glise et al., 1995). The phenotypes range from slight to strong unilateral deletions of imaginal disc-derived adult structures like the wings (hence the name of the gene; Fig. 1B,C), legs and eyes (data not shown). The most frequent and typical phenotype is a cleft of variable width and depth at the dorsal midline of the thorax (Fig. 1A). In strongly affected adults, a large cleft can separate two deformed dorsal hemithoraces, and, more rarely, all wing disc derivatives (wing and dorsal mesothorax) can be missing, and a dramatically bent fly results (Fig. 1D). In this extreme case, the wing disc is improperly located in the abdominal cavity (Fig. 1E), with well differentiated adult wing tissues (data not shown). These observations suggest that *hep* controls the correct morphogenesis and/or positioning of imaginal discs. We took advantage of the unique and late lethality associated with strong, lethal *hep* alleles (*hep*^{r75} and *hep*^{r39}; Glise et al., 1995) to study the origin of these defects during pupal development.

hep mutations induce disc morphogenetic defects

Dissected discs from third instar (L3) *hep*^{r} larvae show various phenotypes. First, there is a delay in *hep*^{r} larval development. Second, there is a variable reduction of the size of mutant discs as compared to wild type (approximately 30% reduction in strong cases; Figs 2, 3). This phenotype is observed in staged larvae and white pupae (data not shown), indicating that the delayed development may not be the cause of this defect. The origin of this growth phenotype will be addressed elsewhere. Third, and though the overall disc morphology is correct, malformed and misfolded discs can also be observed, with a higher frequency in smaller discs, suggesting a growth origin of these defects (data not shown). Because the major events in disc morphogenesis take place during pre-pupal (extending from 3 hours before to 12 hours APF; Fristrom and Fristrom, 1993) and early pupal development, we analyzed the development of *hep*^{r} mutant discs undergoing morphogenesis in staged prepupae.

Overall, metamorphosis is strongly affected in *hep*^{r} mutants, as evidenced by an important disorganization of pupal tissues. We found that the pattern of defects varied from individual to individual. Therefore, we will only describe those defects that are the most frequently observed and which best reflect the *hep* pupal phenotype, i.e., the morphogenesis of the wing imaginal discs. To better visualize morphogenetic defects and have a view of the overall organization in each larva, we used different markers to label specific disc domains (*puc-lacZ*; *dpp-lacZ*; see Materials and Methods).

The analysis of dissected *hep* mutant pupae revealed one major defect: the absence or aberrant spreading and fusion of the two lateral wing discs. Wing discs contribute to the wings and almost all the dorsal thorax (Cohen, 1993; Fristrom and Fristrom, 1993). By 8 hours APF, the wing discs meet and fuse at the dorsal midline (Fig. 2A), a region that is frequently affected in *hep* adult mutants, as described above (Fig. 1). In strong *hep* alleles (*hep*^{r75} or *hep*^{r39}, data not shown), the two wing discs remain in their initial position in the pre-pupae, and do not spread and meet at the dorsal midline (Fig. 2C). In these mutants, the morphology of the discs is strongly affected, suggesting that folding and/or eversion are not completely normal. In some cases, we observed that eversion does take place, indicating that *hep* is not absolutely required for this step. However, and as mentioned above, even in these cases spreading and fusion did not take place, leading to the open thorax phenotype. If *hep* function is only partially absent, as in the viable *hep*^{1}/*hep*^{r75} allelic combination, wing disc morphogenesis can proceed almost normally. In these conditions, discs can spread and fuse, though in some cases one disc does not reach the midline (Fig. 2B), a behavior that may lead to the unilateral defects shown in Fig. 1D,E.

Because the thorax does not close in *hep*^{r75} pupae, the internal tissues, including the gut and larval tissues become extruded, thus presenting a similar phenotype to *hep* embryos which do not close dorsally. Although eye-antenna discs can fuse in *hep*^{r75}, the head does not form (data not shown). It is not clear whether this is an indirect consequence of aberrant thorax formation or if *hep* eye-antenna discs are not able to complete morphogenesis. On the ventral side, leg discs also appear to evert and fuse, but their shape is frequently abnormal (data not shown).

These results show that *hep* is required for the formation of the head and thoracic structures during metamorphosis, with a strong effect on the most distant imaginal tissues, the wing discs. This suggests that the initial position of discs (the distance separating contralateral discs is different from disc to disc) contributes to their *hep*-dependent fusion pattern (see Discussion).

puckered is expressed in the peripodial membrane of imaginal discs

The *puckered* (*puc*) gene encodes a JNK MAPK phosphatase that negatively regulates the activity of the JNK pathway during dorsal closure (Glise and Noselli, 1997; Martin-Blanco et al., 1998; Ring et al., 1993). It is specifically expressed in a population of lateral cells (the leading edge) that delineates the boundary between the ectoderm and the amnioserosa (Ring and Martinez Arias, 1993). Importantly, *puc* expression is activated by *hep* in the leading edge (Glise et al., 1995), to initiate a negative feedback loop (Martin-Blanco et al., 1998). Therefore, *puc* is a marker of both JNK activity and the margins of moving epithelia during dorsal closure. It was therefore of particular interest to analyze the expression pattern of *puc* during larval and pupal development, using the *puc*^{E69} allele, a P *lacZ* enhancer-trap line inserted in the *puc* gene (Ring and Martinez Arias, 1993).

In the wild type, expression of *puc* becomes detectable in the third instar larva (epidermis, spiracles; data not shown) and slightly increases throughout this stage. It then becomes stronger during prepupariation and decreases by the end of this stage (data not shown). Interestingly, *puc* is specifically expressed in particular cell populations in all thoracic discs. In the proximal part of the wing, haltere and leg discs, *puc* is strongly expressed in the stalk region (Fig. 3), where imaginal discs connect to the larval epidermis. Further, *puc* is also expressed in rows of cells in wing, eye-antenna, haltere and leg discs, in a pattern that is reminiscent of the margin expression observed in the ectoderm during dorsal closure (Fig. 3; Ring and Martinez Arias, 1993). These cells are on the dorsal side of imaginal discs, and are part of a particular structure of the discs, the peripodial epithelium or peripodial membrane (pm).

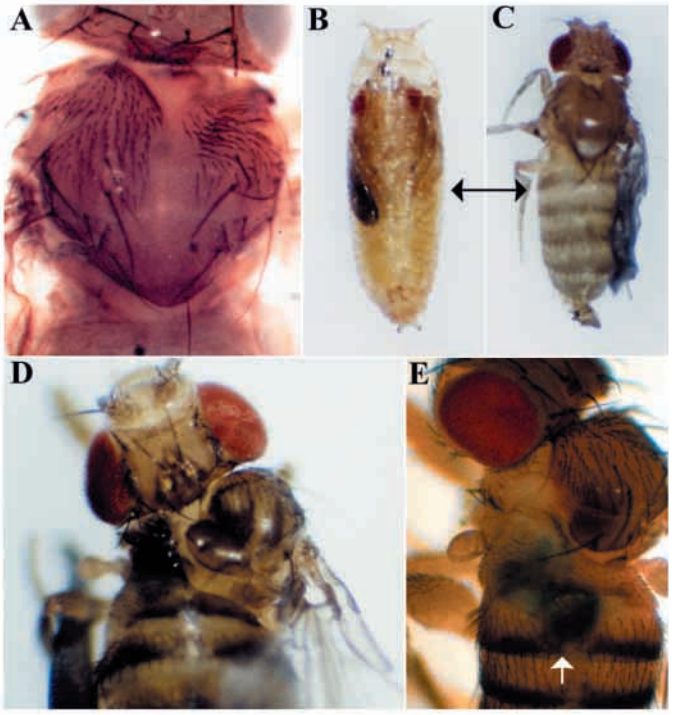

Fig. 1. *hemipterous* pupal and adult thoracic phenotypes. *hep*^{1} hemi- or homozygous flies show a series of head (not shown) and thoracic phenotypes, ranging from an abnormal spacing of rows of bristles at the dorsal midline (A), to a partial (B,C) or complete absence of external structures derived from the head, leg (not shown) and wing imaginal discs (D,E). In B and C, the wing part of the wing disc is missing (double arrow), leading to 'hemipterous' pupae and adult. In E, the wing disc is mislocated in the abdomen (white arrow).

The peripodial membrane is an epithelium made of squamous cells easily distinguishable from those of the disc proper due to their bigger and more spaced nuclei. During metamorphosis, cells of the peripodial membrane play an active role through the dramatic change of their shape, either by intensive stretching or contraction (Fristrom and Fristrom, 1993; Milner et al., 1984, 1983). In addition to this role, the peripodial membrane also contributes to some parts of the adult integument, especially in regions where adjacent discs will suture. To confirm that *puc* is expressed in the peripodial epithelium, double immunostaining was performed using an antibody directed against β-galactosidase (reflecting *puc* expression) and another against the conserved N terminus of the Broad-complex (Br-C) protein isoforms (DiBello et al., 1991). Br-C is ubiquitously expressed in the nuclei of disc cells, and serves here as a marker of peripodial membrane vs columnar epithelial cells. The results show that *puc* is expressed in the peripodial membrane of all thoracic discs, at

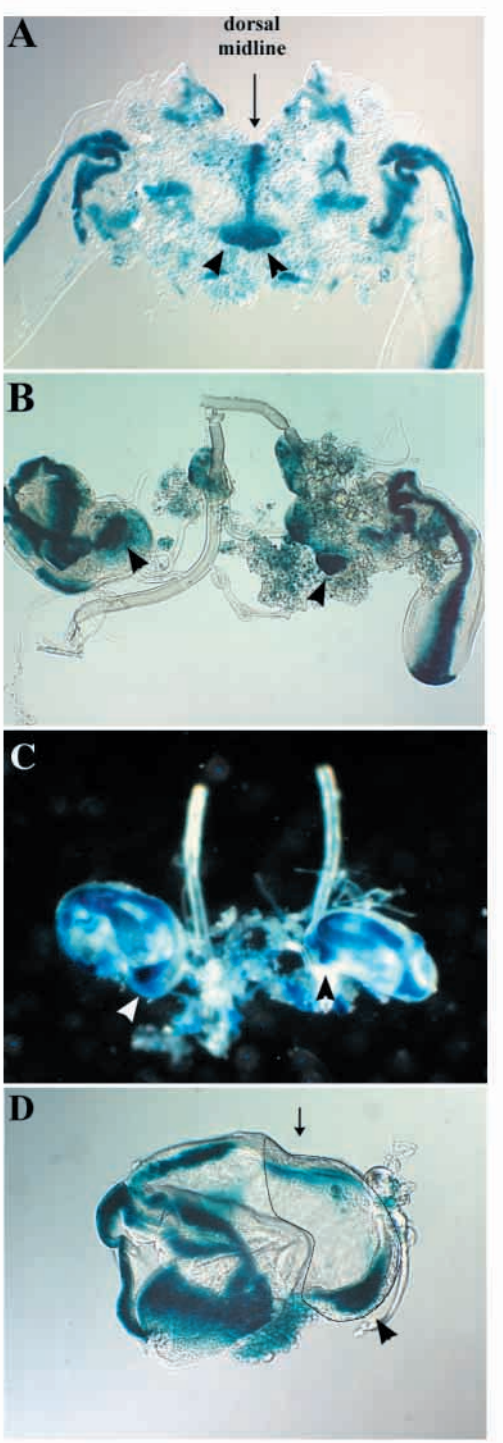

Fig. 2. Aberrant spreading and fusion of wing discs in *hemipterous* pupae. Wild-type (A) and mutant (B-D) pupae were dissected at 8 hours after puparium formation, when the two contralateral heminota have met and fused in the wild type. The *dpp-lacZ* reporter gene is expressed in several places in the disc, in particular at the dorsal midline in two patches where discs fuse (A). B is an example of a partial defect, where only one of the two wing discs underwent normal morphogenesis. The other disc (left) did not spread, and fusion of the discs does not occur. This defect may well result in the adult phenotype shown in Fig. 1D and E. In *hep*^{r75}/Y larvae (C), none of the wing discs have spread, leading to unfused discs and an open thorax. The absence of dorsal thorax closure will result in the externalization of more internal organs (not shown). D, is an enlargement of a *hep*^{r75}/Y wing disc showing that *dpp* expression in the thorax part (outlined) is normal at this late stage. Arrowheads mark the domain of *lacZ* expression in the notum. In D, the arrow marks the anterior domain of *dpp* expression in the thoracic part of the disc (compare to A). (A) *dpp*-lacZ/+; (B) *hep*^{r75}/*hep*^{1}; *dpp*-lacZ/+; (C,D) *hep*^{r75}/Y; *dpp*-lacZ/+.

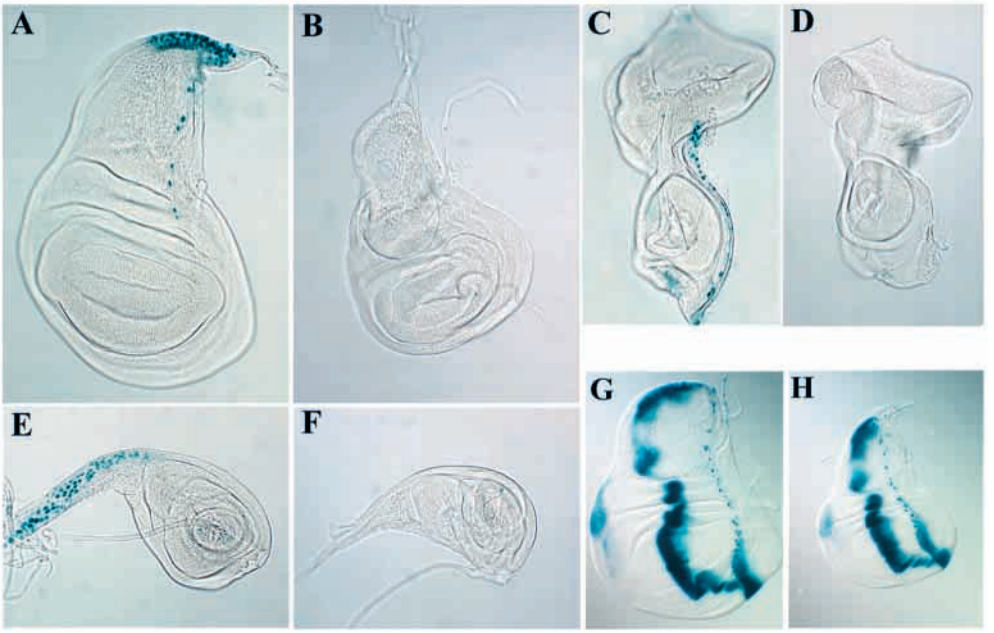

Fig. 3. *hemipterous* controls *puckered*, but not *decapentaplegic* expression in imaginal discs. *lacZ* expression of the enhancer-trap lines puc^{E69} and dpp^{P10638} in wild-type (A,C,E,G) and hep^{r75} (B,D,F,H) imaginal discs. *puc* and *dpp* expression overlap in the stalks and a subset of cells of the peripodial membrane. *puc* expression is under the control of *hep* function in all discs (A-F), whereas *dpp* expression is not (G,H). Note the reduced size of *hep* discs.

the boundary between peripodial and columnar epithelia (Fig. 4). Due to a more sensitive detection using antibody staining, we found that *puc* is expressed in a larger subset of peripodial membrane cells than that revealed using X-gal stainings (compare with Fig. 3). These experiments thus confirm the restriction of *puc* expression in the peripodial membrane.

Later during prepupariation, *puc* expression is maintained in the peripodial membrane and marks the presumptive suture sites of imaginal discs with their neighbors (data not shown). By the end of this stage, *puc* staining is found at the frontier between sutured discs (Fig. 6). These data suggest that *puc*, and the JNK pathway, are required in a specific subset of peripodial cells for morphogenesis of imaginal discs.

hep controls *puc* expression in imaginal discs

In embryos, *hep* activates *puc* and *dpp* expression in ectodermal margins. *puc* activation serves to initiate a negative feedback loop that controls the levels of JNK activity during dorsal closure (Glise et al., 1995; Glise and Noselli, 1997; Martin-Blanco et al., 1998).

To test whether these important regulatory links also exist during disc morphogenesis in larvae, we analyzed the expression pattern of *puc* and *dpp* in L3 larvae using strong *hep* alleles (see Materials and Method; Glise et al., 1995). Both in hep^{r39} (data not shown) and hep^{r75} male larvae, *puc* expression in imaginal tissues is dramatically reduced (Fig. 3). Since the loss of *puc* staining may reflect a delay in the onset of its expression, white-pupae were dissected and stained to reveal *puc-lacZ* expression. As in L3 larval stage, *puc* expression is absent in *hep* white-pupa discs whereas wild-type *puc* expression slightly increases at this developmental stage (data not shown). In contrast, *puc* expression is still detectable in the epidermis, mouthparts, and spiracles both in the wild type and in *hep* mutants, indicating an *hep*-independent expression of *puc* in these tissues as well as providing an internal control (data not shown).

In contrast, we found that the *dpp-lacZ* line dpp^{P10638}, which is expressed in a pattern very similar to that of *puc* in the peripodial membrane (Zecca et al., 1995), is not controlled by *hep* function in L3 and 5 h APF discs (Fig. 3G,H and data not shown). Similarly, in later stage discs (L3 to 8 hours APF), *dpp* expression was not changed in regions close to the dorsal midline, like the notum (Fig. 2D, and data not shown).

Together, these results show that *hep* is required for the normal expression of *puc* in the peripodial membrane of thoracic discs, suggesting that the subset of *puc*-expressing

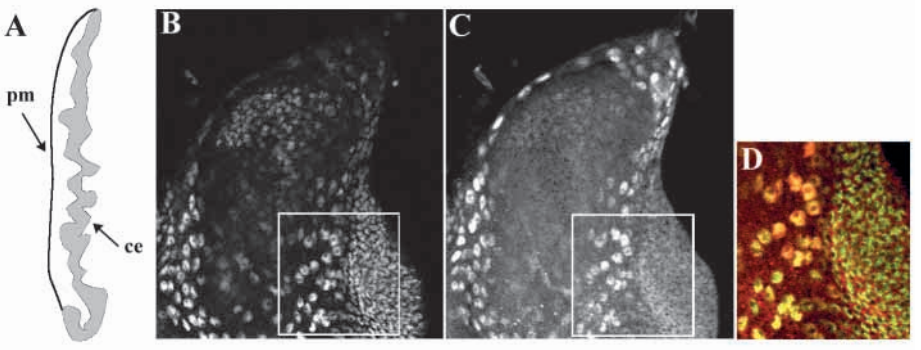

Fig. 4. *puckered* is highly expressed in the marginal cells of the peripodial membrane. The imaginal discs are two-sided, sac-like structures. A represents a schematic longitudinal section of a wing disc showing the two sides of the disc, the squamous peripodial membrane (pm) and the columnar epithelia (ce). Peripodial cells are recognizable by their larger and more spaced nuclei. *puc* expression is restricted to cells of the peripodial membrane. Anti-Broad antibodies mark all nuclei (B, rhodamine) in both epithelia, whereas anti-β-galactosidase antibodies (C, fluorescein) only mark cells of the peripodial membrane that border the columnar epithelium (D, overlay).

Table 1. Suppression of *hep^{r75}* lethal phase by *puc*

	ywhep^{r75}FRT/Y			*ywhep^{r75}FRT/Y; puc^{E69}/+*		
	Pupae	Pharate	Adult	Pupae	Pharate	Adult
ywhep^{r75}FRT/FM6; +/+ × +/Y; puc^{E69}/TM3, Sb	48 (84%)	10 (16%)	0 (0%)	0 (0%)	35 (100%)	0 (0%)

cells in the peripodial membrane is the primary site of JNK activity during discs morphogenesis. They also highlight an important difference with the situation found during dorsal closure where the JNK and *dpp* pathways are coupled (see Discussion).

puc supresses *hep* phenotypes

During the process of dorsal closure, *hep* and *puc* have opposing effects on their common target, basket/DJNK (Riesgo-Escovar et al., 1996; Sluss et al., 1996). To begin to analyse the role of *puc* during disc morphogenesis, we looked at genetic interactions between *hep* and *puc*. In addition to dominantly suppressing the embryonic lethality associated with the *hep^{1}* allele (Martin-Blanco et al., 1998; data not shown), we found that *puc* mutations also strongly suppress the adult phenotypes associated with this allele. The extent of suppression allowed a normally embryonic lethal stock (*hep^{1}/hep^{1}*) to be kept (*hep^{1}/hep^{1}; puc^{E69}*/TM3, Sb). Moreover, reduction by half of *puc* activity also suppresses *hep^{r}* early pupal development arrest, allowing a large proportion of hemizygous males to proceed to late pupal stage (Table 1). In support of the overall rescuing activity of *puc*, the thoracic discs of *hep^{r}/Y*; *puc^{E69}*/+ males, although smaller than in wild type, are homogeneous in size and bigger than those of *hep^{r}/Y*;+/+ (data not shown). The suppressing activity of *puc* was also observed in trans-heterozygous *hep^{r}/hep^{1}* adult females. In this particular viable allelic combination, the thoraces show frequent defects, including clefts and bristle duplications (data not shown). In the presence of only one copy of *puc*, these defects are strongly reduced (Table 2). These results indicate that *puc* has a negative regulatory function in discs, suggesting that the regulatory link established between *hep* and *puc* during DC is well conserved in imaginal discs during metamorphosis.

Dcdc42, but not *DRac1*, can activate *puc* expression in discs in a *hep*-dependent manner

It was previously shown that the small GTPases of the Rho family, *DRac1* and *Dcdc42*, can positively regulate the *Drosophila* JNK pathway in embryos (Glise et al., 1995; Harden et al., 1995). To further investigate the conservation of JNK pathway function in disc morphogenesis, activated forms of *DRac1* (UAS-*Drac1V12*) and *DCdc42* (UAS-*DCdc42V12*; Luo et al., 1994) were expressed in different subsets of imaginal disc cells using the UAS-GAL4 system (Brand and Perrimon, 1993). Because GAL4 lines driving specific expression in the peripodial membrane do not exist, we used GAL4 lines that are expressed in different domains of the columnar epithelium (*dpp*-GAL4; GMR-GAL4; MS1096, data not shown) to test for a potential activating role of *Drac1* and *Dcdc42* in discs; among those, some are also expressed in the peripodial epithelium (*dpp*-GAL4 and MS1096). Targetted expression of either *Drac1* or *Dcdc42* results in a strong ectopic expression of *puc* in patterns specific of each GAL4 driver used (Figure 5; data not shown). Figure 5 shows the results obtained using a *dpp*-GAL4 line which directs expression in all imaginal discs (see Fig. 3 for *dpp* expression pattern in the wing disc). In the wing, expression is prominent along the anteroposterior boundary. The effects of *Drac1* and *Dcdc42* on *puc* are similar, although not exactly the same in terms of the activation levels and pattern of activated cells (data not shown). In a *hep^{r}* mutant background, only *Dcdc42*-mediated *puc* ectopic expression is strongly suppressed (Fig. 5), indicating that *hep* is required downstream of *Dcdc42* to activate *puc*. However, suppression is not complete, since *puc* expression remains in some cells of the discs, indicating an *hep*-independent activation of *puc*. Similar results were also observed in embryos (Glise and Noselli, 1997).

In addition to its potent activity on *puc* expression, Dcdc42 also induces dramatic defects in the overall disc morphology, including a reduction of the size and an aberrant shape (Fig. 5). These defects are also strongly suppressed by *hep*, reinforcing the notion that a Dcdc42, *hep, puc* cascade plays an important morphogenetic activity in imaginal discs. Interestingly, we found that in *dpp*-GAL4/+; UAS-*Dcdc42V12*/+ animals, aberrant and premature fusion of the mesothoracic leg discs (T2) is frequent in L3 larvae (Fig. 5E), a phenotype that may result from an excess of fusion activity due to an hyperactivity of the JNK pathway.

These data suggest that *Dcdc42* may play a role in disc morphogenesis in combination with *hep* and *puc*. In addition, they provide evidence that cells of the columnar epithelium are competent for JNK activity, and that a limiting factor for JNK activation lies upstream of the small GTPases. In this respect, the disc epithelium is very similar to the lateral ectodermal cells during dorsal closure.

Table 2. Suppression of the thorax phenotypes of *hep^{r75}/hep^{1}* females by *puc*

	ywhep^{r75}/hep^{1} (%)	*ywhep^{r75}/hep^{1}; puc^{E69}/+* (%)
Strong cleft	10	0
Weak cleft	13	0
Extra-scutellar bristles	54	<1

DISCUSSION

The complex morphogenetic movements taking place during metamorphosis are precisely coordinated to promote the transformation of the maggot into a perfect adult. Here, we show that the functions of the *hep* and *puc* genes, both members of the JNK pathway, are crucial for several aspects of imaginal discs morphogenesis. Most particularly, the localized expression and activity of *puc* and *hep* provide the first genetic evidence for a role of the peripodial membrane in metamorphosis, as well as suggesting a common basis for the morphogenesis of all imaginal discs.

Roles of the JNK pathway in imaginal disc morphogenesis

Imaginal discs undergo a series of dramatic changes in shape during prepupal development, and though each disc has a specific and stereotyped morphogenesis, several common features can be distinguished (for review, see Fristrom and Fristrom, 1993). Early in puparium development, the columnar epithelium elongates in the sac, a process required to shape the future appendages (legs and wings) as well as preparing for eversion. Then, the dramatic process of disc eversion leads the internal parts to fold out through the open stalks, leading to a reversal of the inner/outer pattern of the sac. After eversion, the adjacent discs spread to meet their neighbors along suture margins and thus promote complete adult thoracic epidermis closure. In contrast to the process of evagination which has been studied in cultured discs (Fristrom et al., 1977; Milner, 1977), the spreading and suture of adjacent discs is poorly described. It is worth mentioning that the extent of spreading and timing of fusion vary from disc to disc, suggesting different genetic requirements for their respective morphogenesis. Among all discs, the wing imaginal discs have to spread the most extensively.

The study of imaginal disc metamorphosis in *hep* mutant larvae revealed an important role of the JNK pathway in the process of spreading and fusion of the wing discs. In strong *hep* alleles, wing discs remain in their initial position in the pupae, and frequently show abnormal shapes. This phenotype is accompanied by a loss of *puc* expression in the peripodial membrane from L3 larval stage onward. Interestingly, in larvae where *hep* function is only partially reduced (hep^{1}/hep^{r75}), wing discs undergo morphogenesis to varying extent, leading to thorax phenotypes in the adults (Figs 1 and 2). *hep* is required in the peripodial membrane to control *puc* expression in a subset of cells ultimately adopting a margin position during spreading of the discs and their suture with their neighbors. We propose that this marginal activation of *hep* and the JNK pathway controls the spreading of the wing discs during metamorphosis. This role of *hep* is further supported by the premature fusion of leg discs expressing activated *Dcdc42*, a phenotype that can be interpreted as a gain of spreading/fusion activity due to the hyperactivation of the *hep* pathway early in disc morphogenesis.

puc is highly expressed in rows of cells delineating margins between the two epithelia making each imaginal disc, the peripodial membrane and the columnar epithelium. These cells represent a sub-population of peripodial cells with intermediate characteristics: they are thicker than those that are more distally and centrally located, but less than the cells of the columnar epithelium proper (Milner et al., 1984, 1983). The *hep*-dependent expression of *puc* in these cells suggest that they have an important role in metamorphosis.

During dorsal closure, JNK activity in the LE controls *dpp* expression, which is proposed to control the movement of the whole ectoderm through long-range signaling (Goberdhan and Wilson, 1998; Noselli, 1998; Noselli and Agnes, 1999). As occurs during dorsal closure, *dpp* is expressed in domains overlapping that of *puc* in discs. However, in striking contrast to dorsal closure, *dpp* expression appears not to be under the control of *hep* (Figs 2 and 3). Though a direct regulatory link between *hep* and *dpp* is not conserved, the expression of *dpp* in the peripodial membrane, together with the adult phenotypes of some *dpp* and other *dpp* pathway heteroallelic combinations strongly suggest that the *dpp* pathway has a role in disc metamorphosis (Chen et al., 1998; Hudson et al., 1998; Morimura et al., 1996; Simin et al., 1998). The co-expression of *dpp* and *puc* in the peripodial membrane suggests that the JNK and *dpp* pathways may cooperate in disc morphogenesis, as they do during dorsal closure. A cooperation between these 2 pathways may well represent a common basis for morphogenesis of epithelia with differentiated margins.

The peripodial epithelium and disc morphogenesis

The fact that *hep* regulates *puc* expression in cells of the peripodial membrane indicates a requirement of the JNK pathway in these cells, and reveals at least one site of JNK activity during metamorphosis. The role of this structure, which covers one side of the larval imaginal discs, has been controversial and unclear. It is proposed to play an active role during metamorphosis, by undergoing successive stretching and contraction (Fristrom and Fristrom, 1993; Milner et al., 1984, 1983). Our results provide a genetic confirmation of a role of the peripodial membrane, by showing a clear link between abnormal disc morphogenesis and spreading, and cell fate determination in the peripodial membrane mediated by the JNK pathway.

One important question is whether the defects observed in *hep* mutants are only the result of a lack of peripodial membrane function. This question could be addressed directly by generating mutant *hep* clones in the peripodial membrane specifically, using directed mosaics (Duffy et al., 1998). If *hep* function is indeed restricted to the peripodial membrane, then it would strongly argue in favor of a model in which the peripodial membrane would orchestrate the process. Such a role was proposed in dorsal closure of the leading edge. Interestingly, an hedgehog-mediated organizing role of the peripodial membrane during regeneration of the T2 leg disc was recently reported (Gibson and Schubiger, 1999), as well as a novel structural basis for the long-range activity of signaling molecules in the wing disc (Ramirez-Weber and Kornberg, 1999). Whether *hep* and the JNK pathway contribute to metamorphosis through related mechanisms will be further investigated.

By several criteria, the so-called leading edge of the embryonic ectoderm is very similar to the peripodial membrane cells expressing *puc*: it expresses *puc* in a *hep*-dependent manner, and marks the future sites of epithelial suture. In addition, it is found at the boundary between a columnar and a stretched/squamous epithelium. These similarities suggest that the same signal(s) may activate *hep* and the JNK pathway in both developmental processes. Consistent with this view, we found that other members of the JNK pathway, including *puc* and probably *Dcdc42*, play a role in metamorphosis. A role for *Dcdc42* during pupal development has been previously reported to control epithelial cell shape (Eaton et al., 1995). Finally, the cleft thorax phenotypes displayed by other genes involved in dorsal closure, like Dfos and ZO-1 (Riesgo-Escovar and Hafen, 1997a; Takahashi et al., 1998; Zeitlinger et al., 1997), reinforces the notion that most of the members of the JNK pathway have a role in metamorphosis.

The observation that *Drac1V12* can activate *puc* expression independently of *hep* suggest that another JNK-related activity

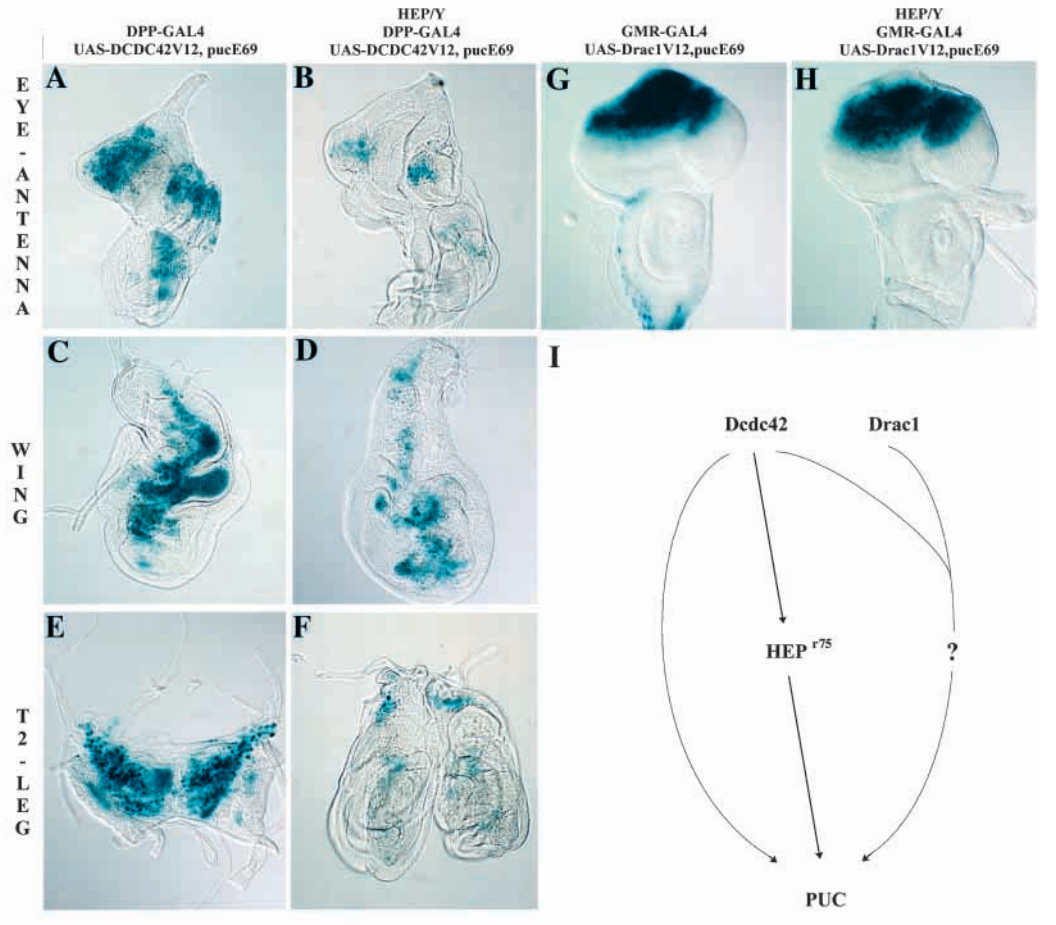

Fig. 5. *Dcdc42* activates *puckered* in a *hemipterous*-dependent manner in the columnar epithelium. An activated form of *Dcdc42* (*Dcdc42V12*) can activate *puc*^{E69} ectopically in imaginal discs (A,C,E; compare with the normal expression pattern of *puc* in wild-type discs in Fig. 3A,C,E), leading to a premature fusion of T2 leg discs (E). The *hep*^{r75} mutation strongly suppresses *Dcdc42* gain-of-function phenotypes and premature fusion of leg discs (B,D,F), indicating an *hep* requirement for *Dcdc42V12* activity in the imaginal discs. In contrast, *Drac1V12* activation of *puc* is not suppressed by *hep* (G,H). The incomplete suppression of these phenotypes by *hep* suggests that not only *Dcdc42*, but also *Drac1*, can use different routes to activate *puc* expression (I).

is present in discs. In support of this view, a novel MAPKK of the JNKK family has been isolated, DMKK4, that may have a partially redundant function with *hep* (Han et al., 1998b). Further, *Drosophila* homologs of another stress-activated MAPK pathway, the p38 pathway, have been identified recently in flies that may also play a role in pupal development (Han et al., 1998a,b; Suzanne et al., 1999), a question that can now be addressed using the *Drosophila* p38 MAPKK *licorne* mutants (Suzanne et al., 1999).

The study of dorsal closure and metamorphosis provides two

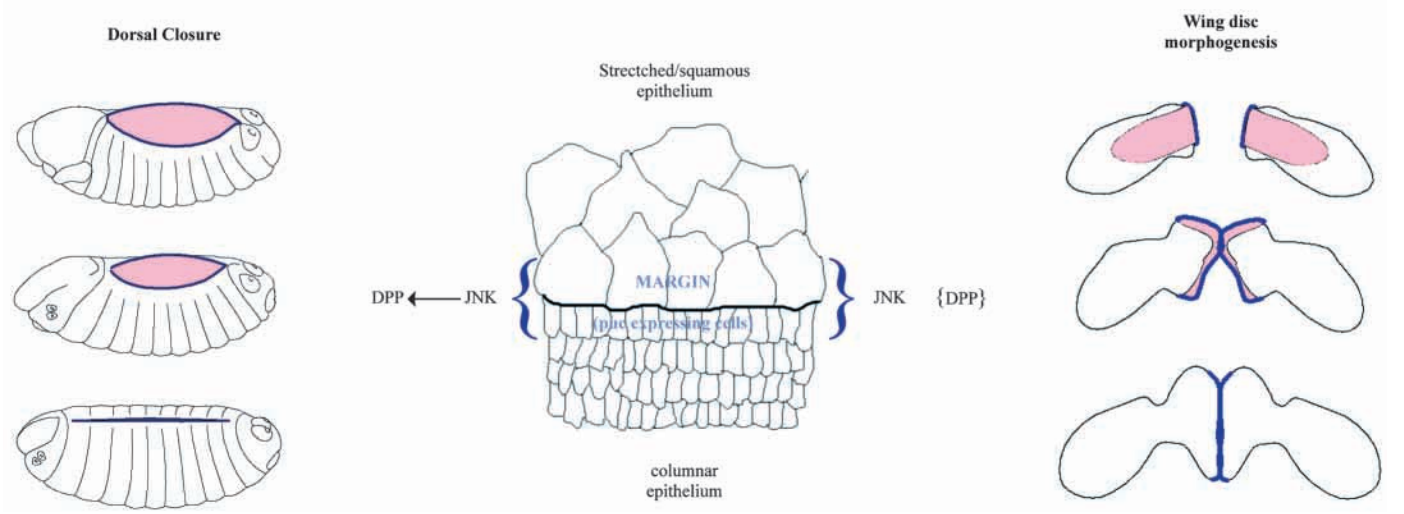

Fig. 6. Comparison of dorsal closure and wing imaginal disc morphogenesis. The two processes (dorsal closure, embryonic stages 11-13; wing disc metamorphosis, 0-7 h APF) are summarized. The squamous tissues (amnioserosa and peripodial membrane) are in pink and the *puc*-expressing cells, which delineate the boundary between the squamous and columnar epithelia in each process, are shown in blue. See text for details.

complementary examples of epithelial morphogenesis, where dorsal closure can be viewed more like a two-dimensional process, whereas disc morphogenesis represents a more complex process involving movements in a third dimension. Despite their apparent differences, these two processes share several features, including gene-specific expression of *puc* and *dpp*, and their genetic requirement for members of the JNK pathway. In both processes, margins are established that develop at the boundary between two epithelia, one stretched (peripodial membrane and amnioserosa), and the other columnar in shape (lateral ectoderm and columnar imaginal tissue). Based on these similarities, we propose that the JNK pathway regulates the determination of the leading cells in moving epithelia (Fig. 6). As evidenced by the mutant phenotypes of *hep* at embryonic and pupal stages, the making of margins is crucial for the morphogenesis of entire epithelial structures (dorsal ectoderm and imaginal discs), especially during the spreading and fusion of sheets of cells that ultimately make a continuous tissue.

This general view may be extended to related processes in other multicellular organisms. In the worm *C. elegans* for example, the morphogenetic process known as ventral enclosure displays several common features with dorsal closure. The leading edges of the ectoderm accumulate F-actin to form a purse-string (Williams-Masson et al., 1997; Young et al., 1993), move toward each other and suture at the ventral midline. As in dorsal closure, ventral enclosure relies on cell elongation along the DV axis, suggesting that the underlying mechanisms may be conserved (Williams-Masson et al., 1997). In vertebrates, several observations suggest that the process of wound-healing is controlled by a network of molecules involved in the JNK and TGF-β pathways (Agnes and Noselli, 1999; Martin, 1997), leading to the proposal that both dorsal closure, and imaginal disc closure, may represent nice genetic models of wound-healing (Agnes and Noselli, 1999).

In conclusion, dorsal closure, metamorphosis and related processes in other organisms will provide complementary models to unravel the intimate mechanisms whereby modulation of a single conserved pathway may create the fascinating diversity of epithelium movement accompanying metazoan development.

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REFERENCES

Agnes, F. and Noselli, S. (1999). Dorsal closure in Drosophila. A genetic model for wound healing?. *C. R. Acad. Sci. III* **322**, 5-13.

Andres, A., Fletcher, J. C., Karim, F. D. and Thummel, C. S. (1993). Molecular analysis of the initiation of insect metamorphosis: a comparative study of Drosophila ecdysteroid-regulated transcritption. *Dev. Biol.* **160**, 388-404.

Ashburner, M. (1989). Drosophila: A laboratory manual. New York: Cold Spring Harbor Laboratory Press.

Bainbridge, S. P. and Bownes, M. (1981). Staging the metamorphosis of Drosophila melanogaster. *J. Embryol. Exp. Morphol.* **66**, 57-80.

Brand, A. H. and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118**, 401-415.

Capdevila, J. and Guerrero, I. (1994). Targeted expression of the signaling molecule decapentaplegic induces pattern duplications and growth alterations in Drosophila wings. *EMBO J.* **13**, 4459-4468.

Chen, Y., Riese, M. J., Killinger, M. A. and Hoffmann, F. M. (1998). A genetic screen for modifiers of Drosophila decapentaplegic signaling identifies mutations in punt, Mothers against dpp and the BMP-7 homologue, 60A. *Development* **125**, 1759-1768.

Cohen, S. M. (1993). Imaginal disc development. In *The Development of Drosophila melanogaster*, vol. 2 (ed. M. Bate and A. Martinez Arias), pp. 747-841. New York: Cold Spring Harbor Laboratory Press.

DiBello, P. R., Withers, D. A., Bayer, C. A., Fristrom, J. W. and Guild, G. M. (1991). The Drosophila Broad-Complex encodes a family of related proteins containing zinc fingers. *Genetics* **129**, 385-397.

Duffy, J. B., Harrison, D. A. and Perrimon, N. (1998). Identifying loci required for follicular patterning using directed mosaics. *Development* **125**, 2263-2271.

Eaton, S., Auvinen, P., Luo, L., Jan, Y. N. and Simons, K. (1995). CDC42 and Rac1 control different actin-dependent processes in the Drosophila wing disc epithelium. *J. Cell Biol.* **131**, 151-164.

Fristrom, D. and Fristrom, J. W. (1993). The metamorphic development of the adult epidermis. In *The Development of Drosophila melanogaster*, vol. 2 (ed. M. Bate and A. Martinez Arias), pp. 843-897. New York: Cold Spring Harbor Laboratory Press.

Fristrom, J., Fristrom, D., Kekete, E. and Kuniyuki, A. (1977). The mechanisms of evagination of imaginal discs of Drosophila melanogaster. *Am. Zool.* **17**, 671-684.

Gibson, M. C. and Schubiger, G. (1999). Hedgehog is required for activation of engrailed during regeneration of fragmented drosophila imaginal discs. *Development* **126**, 1591-1599.

Glise, B., Bourbon, H. and Noselli, S. (1995). hemipterous encodes a novel Drosophila Map kinase kinase, required for epithelial cell sheet movement. *Cell* **83**, 451-461.

Glise, B. and Noselli, S. (1997). Coupling of Jun amino-terminal kinase and Decapentaplegic signaling pathways in Drosophila morphogenesis. *Genes Dev.* **11**, 1738-1747.

Goberdhan, D. C. and Wilson, C. (1998). JNK, cytoskeletal regulator and stress response kinase? A Drosophila perspective. *BioEssays* **20**, 1009-1019.

Han, S. J., Choi, K. Y., Brey, P. T. and Lee, W. J. (1998a). Molecular cloning and characterization of a Drosophila p38 mitogen- activated protein kinase. *J. Biol. Chem.* **273**, 369-374.

Han, Z. S., Enslen, H., Hu, X., Meng, X., Wu, I. H., Barrett, T., Davis, R. J. and Ip, Y. T. (1998b). A conserved p38 mitogen-activated protein kinase pathway regulates Drosophila immunity gene expression. *Mol. Cell. Biol.* **18**, 3527-3539.

Harden, N., Lee, J., Loh, H. Y., Ong, Y. M., Tan, I., Leung, T., Manser, E. and Lim, L. (1996). A Drosophila homolog of the Rac- and Cdc42-activated serine/threonine kinase PAK is a potential focal adhesion and focal complex protein that colocalizes with dynamic actin structures. *Mol. Cell Biol.* **16**, 1896-908.

Harden, N., Loh, H., Chia, W. and Lim, L. (1995). A dominant inhibitory version of the small GTP-binding protein Rac disrupts cytoskeletal structures and inhibits developmental cell shape changes in *Drosophila*. *Development* **121**, 903-914.

Harden, N., Ricos, M., Ong, Y. M., Chia, W. and Lim, L. (1999). Participation of small GTPases in dorsal closure of the Drosophila embryo: distinct roles for Rho subfamily proteins in epithelial morphogenesis. *J Cell Sci* **112**, 273-284.

Hou, X. S., Goldstein, E. S. and Perrimon, N. (1997). Drosophila Jun relays the Jun amino-terminal kinase signal transduction pathway to the Decapentaplegic signal transduction pathway in regulating epithelial cell sheet movement. *Genes Dev.* **11**, 1728-1737.

Hudson, J. B., Podos, S. D., Keith, K., Simpson, S. L. and Ferguson, E. L. (1998). The Drosophila Medea gene is required downstream of dpp and encodes a functional homolog of human Smad4. *Development* **125**, 1407-1420.

Ip, Y. T. and Davis, R. J. (1998). Signal transduction by the c-Jun N-terminal kinase (JNK) – from inflammation to development. *Curr. Opin. Cell Biol.* **10**, 205-219.

Kockel, L., Zeitlinger, J., Staszewski, L. M., Mlodzik, M. and Bohmann,

D. (1997). Jun in Drosophila development: redundant and nonredundant functions and regulation by two MAPK signal transduction pathways. *Genes Dev.* **11**, 1748-1758.

Lindsley, D. and Zimm, G. (1992). *The Genome of Drosophila melanogaster*. San Diego: Academic press.

Liu, H., Su, Y. C., Becker, E., Treisman, J. and Skolnik, E. Y. (1999). A Drosophila TNF-receptor-associated factor (TRAF) binds the ste20 kinase Misshapen and activates Jun kinase. *Curr. Biol.* **9**, 101-104.

Lu, Y. and Settleman, J. (1999). The Drosophila pkn protein kinase is a Rho/Rac effector target required for dorsal closure during embryogenesis. *Genes Dev.* **13**, 1168-1180.

Luo, L., Liao, Y. J., Jan, L. Y. and Jan, Y. N. (1994). Distinct morphogenetic functions of similar small GTPases: Drosophila Drac1 is involved in axonal outgrowth and myoblast fusion. *Genes Dev.* **8**, 1787-1802.

Martin, P. (1997). Wound healing – aiming for perfect skin regeneration. *Science* **276**, 75-81.

Martin-Blanco, E., Gampel, A., Ring, J., Virdee, K., Kirov, N., Tolkovsky, A. M. and Martinez-Arias, A. (1998). puckered encodes a phosphatase that mediates a feedback loop regulating JNK activity during dorsal closure in Drosophila. *Genes Dev.* **12**, 557-570.

Martinez Arias, A. (1993). Development and patterning of the larval epidermis of Drosophila. In *The Development of Drosophila melanogaster*, vol. 1 (ed. M. Bate and A. Martinez Arias), pp. 517-608. New York: Cold Spring Harbor Laboratory Press.

Milner, M. J. (1977). The eversion and differentiation of *Drosophila melanogaster* leg and wing imaginal discs cultured in vitro with an optimal concentration of beta-ecdysone. *J. Embryol. Exp. Morphol.* **37**, 105-117.

Milner, M. J., Bleasby, A. J. and Kelly, S. L. (1984). The role of the peripodial membrane of leg and wing imaginal discs of Drosophila melanogaster during evagination and differentiation in vitro. *Whilelm Roux's Archiv. Dev. Biol.* **193**, 180-186.

Milner, M. J., Bleasby, A. J. and Pyott, A. (1983). The role of the peripodial membrane in the morphogenesis of the eye-antennal disc of *Drosophila melanogaster*. *Whilelm Roux's Archiv. Dev. Biol.* **192**, 164-170.

Morimura, S., Maves, L., Chen, Y. and Hoffmann, F. M. (1996). decapentaplegic overexpression affects Drosophila wing and leg imaginal disc development and wingless expression. *Dev. Biol.* **177**, 136-151.

Noselli, S. (1998). JNK signaling and morphogenesis in Drosophila. *Trends Genet.* **14**, 33-38.

Noselli, S. and Agnès, F. (1999). Roles of the JNK signaling pathway in Drosophila morphogenesis. *Curr. Opin. Genet. Dev.* **9,** 466-472.

Ramirez-Weber, F. A. and Kornberg, T. B. (1999). Cytonemes: cellular processes that project to the principal signaling center in Drosophila imaginal discs. *Cell* **97**, 599-607.

Ricos, M. G., Harden, N., Sem, K. P., Lim, L. and Chia, W. (1999). Dcdc42 acts in TGF-β signaling during Drosophila morphogenesis: distinct roles for the Drac1/JNK and Dcdc42/TGF-β cascades in cytoskeletal regulation. *J. Cell Sci.* **112**, 1225-1235.

Riddiford, L. M. (1993). Hormones and *Drosophila* development. In *The Development of Drosophila melanogaster*, vol. 2 (ed. M. Bate and A. Martinez Arias), pp. 899-939. New York: Cold Spring Harbor Laboratory Press.

Riesgo-Escovar, J. R. and Hafen, E. (1997a). Common and distinct roles of DFos and DJun during Drosophila development. *Science* **278**, 669-672.

Riesgo-Escovar, J. R. and Hafen, E. (1997b). Drosophila Jun kinase regulates expression of decapentaplegic via the ETS-domain protein Aop and the AP-1 transcription factor DJun during dorsal closure. *Genes Dev.* **11**, 1717-1727.

Riesgo-Escovar, J. R., Jenni, M., Fritz, A. and Hafen, E. (1996). The Drosophila Jun-N-terminal kinase is required for cell morphogenesis but not for DJun-dependent cell fate specification in the eye. *Genes Dev.* **10**, 2759-2768.

Ring, J. M. and Martinez Arias, A. (1993). puckered, a gene involved in position-specific cell differentiation in the dorsal epidermis of the Drosophila larva. *Development* **Supplement** 251-259.

Simin, K., Bates, E. A., Horner, M. A. and Letsou, A. (1998). Genetic analysis of punt, a type II Dpp receptor that functions throughout the Drosophila melanogaster life cycle. *Genetics* **148**, 801-813.

Sluss, H. K. and Davis, R. J. (1997). Embryonic morphogenesis signaling pathway mediated by JNK targets the transcription factor JUN and the TGF-beta homologue decapentaplegic. *J. Cell Biochem.* **67**, 1-12.

Sluss, H. K., Han, Z., Barrett, T., Davis, R. J. and Ip, Y. T. (1996). A JNK signal transduction pathway that mediates morphogenesis and an immune response in Drosophila. *Genes Dev.* **10**, 2745-2758.

Su, Y. C., Treisman, J. E. and Skolnik, E. Y. (1998). The Drosophila Ste20-related kinase misshapen is required for embryonic dorsal closure and acts through a JNK MAPK module on an evolutionarily conserved signaling pathway. *Genes Dev.* **12**, 2371-2380.

Suzanne, M., Irie, K., Glise, B., Agnes, F., Mori, E., Matsumoto, K. and Noselli, S. (1999). The drosophila p38 MAPK pathway is required during oogenesis for egg asymmetric development. *Genes Dev.* **13**, 1464-1474.

Takahashi, K., Matsuo, T., Katsube, T., Ueda, R. and Yamamoto, D. (1998). Direct binding between two PDZ domain proteins Canoe and ZO-1 and their roles in regulation of the jun N-terminal kinase pathway in Drosophila morphogenesis. *Mech. Dev.* **78**, 97-111.

Trinkaus, J. (1969). *Cells into Organs - The Forces that Shape the Embryo.* Englewood Cliffs, New Jersey: Prentice-Hall, Inc.

Williams-Masson, E. M., Malik, A. N. and Hardin, J. (1997). An actin-mediated two-step mechanism is required for ventral enclosure of the C. elegans hypodermis. *Development* **124**, 2889-2901.

Young, P. E., Richman, A. M., Ketchum, A. S. and Kiehart, D. P. (1993). Morphogenesis in Drosophila requires nonmuscle myosin heavy chain function. *Genes Dev.* **7**, 29-41.

Zecca, M., Basler, K. and Struhl, G. (1995). Sequential organizing activities of engrailed, hedgehog and decapentaplegic in the Drosophila wing. *Development* **121**, 2265-2278.

Zeitlinger, J., Kockel, L., Peverali, F. A., Jackson, D. B., Mlodzik, M. and Bohmann, D. (1997). Defective dorsal closure and loss of epidermal decapentaplegic expression in Drosophila fos mutants. *EMBO J.* **16**, 7393-7401.