Coupling of Jun amino-terminal kinase and Decapentaplegic signaling pathways in Drosophila morphogenesis

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Dorsal closure in Drosophila embryos involves the migration of two lateral epidermis toward the dorsal midline to establish the dorsal ectoderm. Previous work showed that this morphogenetic movement depends on the activities of a Jun amino (N)- terminal kinase kinase (JNKK) encoded by the hemipterous (hep) gene, and of a JNK encoded by basket. Hep is required for cell determination in the leading edge of migrating epithelia, by controlling specific expression of the puckered (puc) gene in these cells. During dorsal closure, decapentaplegic (dpp), a member of the transforming growth factor-β (TGF-β) superfamily, is expressed in the row of cells making up the leading edge of the epithelia. Here, we show that the small GTPases Dcdc42, Drac1, and the Hep JNKK control dpp expression in this migratory process. Appropriate dpp and puc expression in the leading edge also depends on the inhibitory function of the puc gene. Further, our data suggest that the leading edge is the source of a JNK autocrine signal, and exclude a role of Dpp as such a ligand. Dorsal closure couples JNK and dpp signaling pathways, a situation that may be conserved in vertebrate development.

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Movement and folding of epithelia are basic processes in metazoan development that are required to establish germ layers and differentiated structures in the whole body. An important issue is to understand the regulatory pathways linking pattern formation and morphogenetic activity in these crucial events (Leptin 1995).

During Drosophila embryogenesis, two symmetrical cell sheets undergo coordinate migration from lateral to dorsal positions and fuse along the dorsal midline. This process, dorsal closure (DC), allows the embryo to be covered by epidermis on the dorsal side (Fig. 1A). In the context of a probable dorsoventral inversion in animal evolution, it has been proposed that DC is equivalent to yolk incorporation in vertebrates (for review, see Nübler-Jung and Arendt 1994). DC in Drosophila provides a good model system to study cell sheet movement. First, it is a simple and well-described process in which movement of epithelia depends strictly on cell elongation, requiring neither cell division nor cell recruitment (Young et al. 1993; Martinez Arias 1993). Additionally, extensive genetic screens have revealed several different loci in the genome that affect this process (Jürgens et al. 1984; Nüsslein-Volhard et al. 1984; Wieschaus et al. 1984), therefore offering a means to identify the entire cellular pathway.

In these DC mutants, the ectoderm does not migrate to cover the dorsal region and the resulting embryos die with a characteristic "dorsal open" phenotype. Based on molecular data, the DC genes can be divided into two main classes. In the first group, as expected of a process involving cell movement, are genes with important roles in cell adhesion [1(1) myospheroid; MacKrell et al. 1988], the cytoskeleton [zipper; Young et al. 1993] and cell junctions [coracle, canoe; Febon et al. 1994; Miyamoto et al. 1995]. These functions are crucial for establishing and/or maintaining cell shape and the integrity of the whole cell sheet. The second class comprises genes with nonstructural, signal-dependent regulatory functions. One of these regulatory genes is hemipterous [hep], which encodes a mitogen-activated protein kinase kinase (MAPKK) most related to vertebrate Jun amino (N)-terminal kinase kinase (JNKK; Glise et al. 1995). In the absence of hep function, specific expression of the puckered (puc) gene in cells of the leading edge is abolished, and movement does not occur. These data suggested that DC relies on JNK activation. In support of this view, recent work identified basket (bsk) as a Drosophila JNK gene (Riesgo-Escovar et al. 1996; Sluss et al. 1996). Further, Bsk is activated by Hep in vitro, and bsk and hep mutations provoke similar dorsal open phenotypes. An
important question concerns the nature of the JNK activating signals employed in DC.

In addition to components of the Drosophila JNK pathway, other signaling molecules have a role in DC. These are members of the decapentaplegic (dpp) signal transduction pathway, including schnurri, a nuclear zinc finger protein [Arora et al. 1995; Grieder et al. 1995; Staehling-Hampton et al. 1995], and the type I and type II dpp receptor genes thick veins (tkv; Affolter et al. 1994; Brummel et al. 1994; Nellen et al. 1994; Penton et al. 1994), and punt [Letsou et al. 1995; Ruberte et al. 1995], respectively. dpp, a member of the transforming growth factor-β (TGF-β) superfamily [Padgett et al. 1987; for review, see Kingsley 1994], is expressed in a complex pattern that reflects multiple signaling roles during Drosophila development. In early embryos, dpp is established in a gradient to specify dorsal fates [Ferguson and Anderson 1992]. Later, a dpp signal crosses embryonic cell layers to control specific gene expression in the gut [Panganiban et al. 1990; for review, see Bienz 1994] and visceral and cardiac mesoderm [Frasch 1996]. During larval development, a direct and long range Dpp morphogen activity participates in imaginal disc patterning and growth [Nellen et al. 1996], in combination with two other secreted molecules, hedgehog and wingless [for review, see Ingham 1995]. Although dpp mutations specifically affecting DC are unknown, genes encoding Dpp signal transducers like tkv and punt have a clear zygotic dorsal open phenotype [Jürgens et al. 1984; Nüsslein-Volhard et al. 1984; Affolter et al. 1994; Letsou et al. 1995]. The similar DC phenotype shown by hep and dpp signal transducers therefore suggests a possible link between dpp and JNK signaling in controlling dorsal closure.

In this paper, we have identified this link by showing that hep controls dpp expression in the leading edge of dorsal epithelia. This identifies dpp as a novel target of JNK activity and suggests a coupling of both pathways in DC. We also demonstrate that hep-mediated regulation of the two downstream targets dpp and puc depends on the upstream activities of the Drosophila Drac1 and Ddec42 genes, which encode small GTP-binding proteins with important functions in controlling cell shape and signaling [for review, see Hall 1994; Voitek and Cooper 1995; Kyriakis and Arruck 1996; Symons 1996]. We show that constitutively activated forms of Drac1 and Ddec42 can mediate hep-dependent ectopic dpp and puc expression in the ectoderm. These results represent the first genetic demonstration of a link between these small GTP-binding proteins and JNK signaling in a multicellular organism, and suggest a strong evolutionary conservation of the JNK pathway in vertebrates and Drosophila. Further, we show that hep and puc act antagonistically to ensure appropriate dpp expression in the leading edge. Finally, experiments in which we modulate JNK activity in the leading edge suggest the existence of an autocrine JNK signal in these cells, but exclude Dpp...
as such a ligand. We discuss the role of JNK-mediated patterning in epithelial morphogenetic activity.

**Results**

**hep controls dpp expression in the leading edge**

The normal embryonic pattern of dpp expression is complex and dynamic (St. Johnston and Gelbart 1987), and we focus here on those dpp transcripts expressed in the dorsal ectoderm. In this region, the pattern of dpp expression can be divided into two discrete phases. First, during early development (stages 5-10; for a detailed description of embryogenesis, see Campos-Ortega and Hartenstein 1985), dpp shows a broad expression in the dorsal region (data not shown; St. Johnston and Gelbart 1987), which is refined secondarily (stages 11-15), into two discrete stripes bordering this domain dorsally and ventrally (Fig. 1B-D). When dorsal closure begins (stage 13), the dorsal-most stripe is one-cell-wide and corresponds to the leading edge of the migrating ectoderm (Fig. 1D). Instead of a continuous line of cells, only about one cell in two discrete stripes bordering this domain dorsally and ventrally (Fig. 1B-D). When dorsal closure begins (stage 13), the dorsal-most stripe is one-cell-wide and corresponds to the leading edge of the migrating ectoderm (Fig. 1D). dpp expression remains in these cells throughout DC, although expression levels are diminished later.

In hep mutant embryos, expression of dpp is normal until germ band extension. At this stage, the onset of dpp expression in the leading edge is affected dramatically. Instead of a continuous line of cells, only about one cell per segment expresses dpp detectably (Fig. 1E). In wild-type embryos, a dotted expression is observed, but only very transiently (data not shown), indicating that the re-initiation of dpp expression in the ectoderm margins is not completely synchronous. The speckled pattern of dpp expression persists in hep embryos, but the signal fades during germ-band retraction (Fig. 1F). When DC begins (stage 13), dpp transcripts are no longer detected in the leading edge, in contrast to wild type (Fig. 1D,G). Targeted expression of a hep cDNA in the ectoderm completely restores dpp transcripts in the leading edge (data not shown). It is noteworthy that defects in dpp expression are restricted to the dorsal-most ectodermal stripe, as no change was detected in the neighboring ventral-most dpp ectodermal stripe (Fig. 1G). Similar results were obtained using either of two hep alleles, hep1 or hep75 (Glise et al. 1995). These observations strongly support the specific role in DC assigned to hep (Glise et al. 1995).

Therefore, **hep** is required for normal dpp expression in the leading edge during dorsal closure. This result suggests a role for dpp in this process and identifies dpp as a transcriptional target of the Hep/JNKK pathway.

**puc is a repressor of dpp expression in the ectoderm**

Mutations in the puc gene affect the final step of DC, which corresponds to the suture of the two lateral leading edges at the dorsal midline (Ring and Martinez Arias 1993). Expression of the puc gene, as revealed by a puc-lacZ enhancer-trap line (pucE69), is restricted to the leading edge (Fig. 2D; Ring and Martinez Arias 1993; Glise et al. 1995). As for dpp, puc expression in these cells requires hep function (Glise et al. 1995). puc encodes a protein related to vertebrate dual-specificity MAPK phosphatases of the CL100 family (E. Martin-Blanco, A. Gampel, and A. Martinez Arias, pers. comm.). To test for a regulatory function of puc in the leading edge, the two target genes, puc and dpp, were used as in vivo markers of JNK pathway activity.

In puc mutant embryos, the levels both of dpp transcripts (Fig. 2C) and of puc-lacZ activity (Fig. 2E) are increased in the leading edge compared with a wild-type situation. Intriguingly, ectopic expression of puc and dpp is also induced in the ectoderm, that is, outside the normal domain of puc expression (Fig. 2D,E). In puc mutant embryos, dpp and puc appear overexpressed in the margins (C,E), and ectoderm cells express both genes ectopically (B,E), as compared to wild type (A,D). puc-lacZ staining is also observed in the amnioserosa (E). Note that ectopic expression of puc and dpp takes place outside the normal domain of puc expression (D,E). B is a stage 14 embryo, in which ectopic expression of dpp is readily observed in the thoracic and head regions. C is a stage-13 embryo in which dpp ectopic expression is not yet fully established, but it shows an increase of dpp transcripts in the leading edge. Anterior is left. D and E are dorsal views and A-C are lateral views. In A-C, dorsal is up. Genotypes: [A] w embryo; [B,C] pucE69/pucE69; [D] pucE69/TM3, Sb; [E] pucE69/puc810.

![Figure 2. puc is a repressor of the JNK target genes puc and dpp. Expression of puc and dpp in wild-type (A,D) and puc mutant embryos (B,C,E), as revealed by whole-mount dpp in situ hybridization (A-C) or puc-lacZ X-gal stainings (D,E). In puc mutant embryos, dpp and puc appear overexpressed in the margins (C,E), and ectoderm cells express both genes ectopically (B,E), as compared to wild type (A,D). puc-lacZ staining is also observed in the amnioserosa (E). Note that ectopic expression of puc and dpp takes place outside the normal domain of puc expression (D,E). B is a stage 14 embryo, in which ectopic expression of dpp is readily observed in the thoracic and head regions. C is a stage-13 embryo in which dpp ectopic expression is not yet fully established, but it shows an increase of dpp transcripts in the leading edge. Anterior is left. D and E are dorsal views and A-C are lateral views. In A-C, dorsal is up. Genotypes: [A] w embryo; [B,C] pucE69/pucE69; (D) pucE69/TM3, Sb; (E) pucE69/puc810.](https://genesdev.cshlp.org/content/1740/GENES%26DEVELOPMENT.png)
In summary, puc is both a repressor and a target of hep function in the leading edge. This conclusion is consistent with the molecular data indicating that puc encodes a MAPK phosphatase (E. Martin-Blanco, A. Gampel, and A. Martinez Arias, pers. comm.). The combined and antagonistic functions of hep and puc maintain appropriate levels of puc and dpp activities in migrating epithelia during DC. These results indicate that a leading edge cell identity, and DC, depend on a balance between JNK activation and puc repression.

**Drac1 and Dcdc42 are components of the Drosophila JNK pathway**

To elucidate in part how hep drives specific gene expression in dorsal epithelia, we set out to identify the small GTPases involved in DC. In mammalian cells, distinct small GTPases are involved in different MAPK pathways. In particular, the vertebrate JNK and p38 pathways are regulated by the Rac and Cdc42 small GTPases (Bagrodia et al. 1995; Coso et al. 1995; Minden et al. 1995). To overcome the absence of identified mutants in the corresponding Drosophila Drac1 and Dcdc42 genes (Luo et al. 1994; Harden et al. 1995), we used the GAL4 system (Brand and Perrimon 1993) to target expression of modified Drac1 and Dcdc42 forms to specific tissues or cell types.

Activated Drac1 (Drac1V12) and Dcdc42 (Dcdc42V12; Luo et al. 1994) were expressed in populations of ectodermal cells, using GAL4 constructs expressed in the whole ectoderm (69B), in segmental stripes (en–GAL4 or wg–GAL4), or in the leading edge (LE–GAL4). In these experiments, both target genes puc and dpp are induced ectopically in ectodermal cells in a pattern specific to GAL4 drivers (Fig. 3B–D; data not shown). Interestingly, we also observe an expression in amnioserosa cells, where Drac1V12 and Dcdc42V12 are not expressed [Fig. 3B; see below]. Not all of the induced ectodermal cells show ectopic expression, indicating that some cells are refractory to activated small GTPases. Additionally, it appears that Drac1V12 and Dcdc42V12 induce distinct, though partly overlapping, responses [Fig. 3, cf. B and C]. This suggests a specific effect of each, as observed in other studies (Luo et al. 1994; Eaton et al. 1995; Murphy and Montell 1996). Dcdc42V12 appears to be a good inducer of ectodermal cells located more ventrally, whereas Drac1V12 seems more active in cells nearest to the leading edge (Fig. 3B,C). The specificity of the dominant Drac1V12 and Dcdc42V12 constructs as inducers of JNK activity was tested further in experiments using activated Dras2 (Dras2V14; Brand and Perrimon 1993) or Drafl, which are likely to function in two separate Ras pathways. Neither Dras2V14 nor Drafl constructs were able to activate the JNK target genes in the ectoderm [data not shown], suggesting that Dras2 and Drafl do not participate in the DC pathway.

Expression of activated Drac1V12 and Dcdc42V12 in the ectoderm can induce a strong activation of the two JNK target genes puc and dpp. These findings support the notion that the small GTPases Drac1 and Dcdc42 par-
these embryos. Taken together, these results lead us to place Drac1 and Dcdc42 upstream of hep in the DC pathway.

Previous reports showed that dominant-negative Drac1 affects the accumulation and rearrangement of cytoskeletal proteins in the leading edge (Harden et al. 1995) and that both dominant-negative Drac1 and Dcdc42 affect DC (Harden et al. 1995; Riesgo-Escovar et al. 1996; data not shown). Here, we have demonstrated that both Dcdc42 and Drac1 participate in nuclear responses as well, through activation of the Hep/JNK pathway and its downstream targets, dpp and puc. These results represent the first genetic demonstration that the small GTPases Drac1 and Dcdc42 are upstream activators of JNK in a multicellular organism, suggesting a strong conservation of the JNK pathway in metazoan (see Discussion).

The leading edge is the source of a JNK-activating signal

As shown above, puc mutations have a cell nonautonomous effect, as revealed by the ectopic expression of dpp and puc (Fig. 2B, E). The response is slightly different in the dorsal and ventral neighbors of the leading edge cells, because amnioserosa cells express puc only, whereas ectodermal cells express both puc and dpp. In puc embryos, expression in the amnioserosa precedes expression in ectodermal cells, suggesting that the amnioserosa is more sensitive to ectopic activity. Formally, these observations indicate that the leading edge cells have the potential to activate neighboring cells, and that restriction of activation to the leading edge is via puc itself. Closer observation of puc embryos indicates that ectopic expression of puc in the amnioserosa occurs preferentially in cells bordering the leading edge (Fig. 2E). Taken together, these observations suggest that the leading edge is the source of a JNK-regulated diffusible signal, whose range of activation is increased in puc mutants. To better understand how this putative signal might be controlled in the leading edge, we perturbed normal JNK activity in these cells and analyzed cell fate changes in amnioserosa and ectodermal cells, using puc as a marker. When activated Drac1V12 is expressed in the ectoderm using an en–GAL4 line, puc expression is induced in the ectoderm, as shown above (Fig. 3). Interestingly, puc expression is also detected in the amnioserosa (Fig. 3B), suggesting an inducer activity of ectodermal cells in these embryos. To test whether the leading edge, the probable site of JNK activity, is sufficient to induce puc nonautonomously in neighboring cells, activated Drac1 was expressed using a LE–GAL4 line, which targets expression specifically in the leading edge (Fig. 4A, D). Expression of Drac1V12 in the leading edge specifically induces ectopic puc–lacZ in neighboring amnioserosa and ectodermal cells (Fig. 4C, F). Interestingly, this ectopic puc expression is observed predominantly in cells close to the leading edge, as noted previously in puc embryos. Using a UAScdc42V12 construct driven by an LE–GAL4 line, ectopic induction was observed rarely, reinforcing the notion that Drac1V12 and Dcdc42V12 have different effects on JNK activity. The weaker effect observed than for puc embryos (Figs. 2 and 4, cf. E and C, respectively) presumably reflects the presence of one wild-type copy of the puc gene in these embryos. Therefore, augmenting JNK activity in the leading edge, either by removing repressor functions (as in puc mutants) or by overactivation (as in activated Drac1 embryos), results in a failure of the leading edge to restrict JNK signaling.

These data provide evidence for the existence and nature of a JNK-activating signal for DC. This signal, originating from the leading edge, is diffusible and able to activate the JNK pathway in cells neighboring its source. Additionally, its range of activity depends on levels of JNK signaling, suggesting that the JNK pathway is activated, at least in part, by autocrine signaling.

Dpp is not a JNK-activating signal

The conclusion that the leading edge is a local source of a JNK-activating signal is interesting, as it might reflect a general mechanism for epithelial movement (see Discussion).
We have shown that dpp, which encodes a secreted molecule with signaling properties, is expressed in the leading edge in a hep-dependent manner. An attractive model is that Dpp itself could be the autocrine signal described above. To test this idea, ectopic expression of dpp or of an activated tkv receptor [UAS-tkv(253D)], Nellen et al. 1996] were induced using different GAL4 lines [en-GAL4, 69B, or LE-GAL4], and resulting puc and endogenous dpp expression patterns were analyzed. Ectopic dpp or tkv(253D) have no effect on puc and endogenous dpp, in either ectoderm or amnioserosa cells (data not shown). These results therefore rule out the possibility that Dpp is sufficient to activate the JNK pathway via an autoregulatory loop. However, these observations do not exclude a paracrine signaling role for Dpp during DC, directed toward amnioserosa and/or ectodermal cells.

Discussion

Conservation of the JNK pathway in invertebrates

In recent years, the study of different MAPK pathways in eukaryotes has provided a complex view of how these kinase cascades work and what they control. A current, simplified view, is that at least three related, functionally separate MAPK pathways exist in vertebrate cells: the ERK, JNK, and p38 pathways, respectively, so named for the three different MAPks involved [Derijard et al. 1995; for review, see Cooper 1994; Davis 1994; Cano and Mahadevan 1995; Waskiewicz and Cooper 1995]. In Drosophila, components of the ERK [for review, see Dickson and Hafen 1994; Wassarman et al. 1995; Duffy and Perrimon 1996] and JNK pathways [Glise et al. 1995; Riesgo-Escovar et al. 1996; Sluss et al. 1996] have been described, and we recently isolated a Drosophila p38 kinase homolog [M. Suzanne, B. Glise, and S. Noselli, unpubl.]. From these findings, we conclude that Drosophila has conserved a set of three MAPK pathways, as in vertebrates. RAS/ERK signaling in Drosophila is well conserved [Wassarman et al. 1995], and the same may be true for the Drosophila JNK pathway [Fig. 5]. First, both Drosophila and vertebrate pathways are involved in the regulation of the actin cytoskeleton, cell shape (Ridley and Hall 1992; Ridley et al. 1992; Young et al. 1993; Glise et al. 1995; Harden et al. 1995; Nobes and Hall 1995), and stress responses [Coso et al. 1995; Minden et al. 1995; Bagrodia et al. 1996; Riesgo-Escovar et al. 1996; Sluss et al. 1996; for review, see Vojtek and Cooper 1995]. In addition, the molecular cascade leading to JNK activation involves homologous molecules in both systems. Previous studies have demonstrated the role of the Drosophila JNKK (Hep), JNK (Bsk) and dual-specificity MAPK phosphatase (puc) in DC [Ring and Martinez Arias 1993; Glise et al. 1995; Riesgo-Escovar et al. 1996; Sluss et al. 1996; E. Martin-Blanco, A. Gampel, and A. Martinez Arias, pers. comm.] Here, we demonstrate that the small GTPases Drac1 and Dcdc42 act upstream of the Hep JNKK to control gene expression, a situation well established in vertebrate cells [Coso et al. 1995; Minden et al. 1995; Bagrodia et al. 1996]. In addition, dominant-negative forms of these small GTPases induce dorsal open phenotypes when expressed generally in the ectoderm [Harden et al. 1995; Riesgo-Escovar et al. 1996]. Interestingly, the recent analysis of a mouse hep homolog, Mkk7, indicates that it is also a target of rac in cell cultures [P.M. Holland, M. Suzanne, J.S. Campbell, S. Noselli, and J.A. Cooper, unpubl.]. Whether Dcdc42 and Drac1 induce cytoskeletal and nuclear responses independently, as shown recently for vertebrate Rac and Cdc42 [Lamarche et al. 1996], must be characterized fur-
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ther in Drosophila. A novel mediator of rac and cdc42 signaling, p65\textsuperscript{PAK}, was identified in vertebrate brain cells (Manser et al. 1994). Activated rac and cdc42 bind to the PAK serine/threonine kinase, which is required to mediate JNK activation and nuclear responses. Recently, a Drosophila PAK homolog was isolated, and shown to be accumulated in cells of the leading edge during DC (Harden et al. 1996). These data are consistent with a role for PAK as a component of the Drosophila JNK pathway. Taken together, all present data indicate a high degree of conservation of JNK pathways in vertebrate and insect cells.

Coupling of JNK and Dpp pathways in Drosophila development

During Drosophila development, a complex genetic control sets the \textit{dpp} expression pattern. The importance of such control is illustrated by the dramatic effects on development of perturbing \textit{dpp} expression, either spatially (e.g., Capdevilla and Guerrero 1994; Staehling-Hampton and Hoffmann 1994; Zecca et al. 1995) or quantitatively (Morimura et al. 1996). In early embryos, \textit{dpp} expression along the dorsal-ventral axis is controlled by a dorsal protein gradient of maternal origin (for review, see Chasan and Anderson 1993). Later in embryogenesis, \textit{dpp} expression in the visceral mesoderm is under the direct control of the homeotic protein Ultrabithorax (Capovilla et al. 1994). During wing imaginal disc development, expression of \textit{dpp} along the anteroposterior boundary depends on a cascade involving the segment polarity genes \textit{engrailed} and \textit{hedgehog} (Zecca et al. 1995; for review, see Ingham 1995). All these data indicate that the \textit{dpp} gene can respond to several different developmental pathways in multiple cell types. In this paper, we identify the JNK signaling pathway as a novel key regulator of \textit{dpp} expression in a migratory process.

During DC, \textit{dpp} expression is affected dramatically in the leading edge of dorsal ectoderm, in \textit{hep} embryos. \textit{dpp} expression in the leading edge is blocked at its onset when a dotted, rather than uniform, expression pattern is detected. This suggests that \textit{hep} affects propagation and/or maintenance of \textit{dpp} in these cells, rather than triggering it. However, in experiments using dominant activated Drac1 and Dcdc42, we show that JNK signaling can trigger new \textit{dpp} expression in the ectoderm as well. These loss- and gain-of-function experiments indicate a close coupling of both pathways in Drosophila. This apparent specificity is reinforced by the inability of either activated Dras2 or Draf to modify \textit{dpp} expression in the ectoderm. Taken together, these findings suggest that \textit{dpp} is a specific JNK target in Drosophila development.

The coupling between JNK and \textit{dpp} in DC depends on an apparently simple link: The JNK pathway activates a secondary signaling cascade, via the direct control of its ligand, Dpp (Fig. 5). No JNK-mediated \textit{dpp} autoregulation seems to occur in this process, as revealed by inability of either \textit{dpp} or \textit{tkv} to activate the JNK target genes. Two situations are encountered in JNK and \textit{dpp} coupling during DC. Initially, when \textit{dpp} is still silent in the leading edge, JNK and \textit{dpp} pathways appear to function sequentially. Once \textit{dpp} expression is established, however, the two pathways work in parallel and cooperate. Such coupling of JNK and \textit{dpp} pathways suggests two nonexclusive possibilities. First, the initial, sequential activation of JNK and \textit{dpp} may indicate important timing requirements, that is, \textit{dpp} should not be activated before JNK for proper DC to occur. This hypothesis could be tested in uncoupling experiments, where \textit{dpp} would be activated before the JNK pathway. In another hypothesis, coupling might serve as a way to control the JNK/Dpp ratio in the leading edge. However, we were unable to detect any effect on DC resulting from the overexpression of \textit{dpp} in the ectoderm or leading edge (data not shown).

Another important question is whether coupling of JNK and \textit{dpp} is conserved in vertebrates. Recent cloning and analysis of a novel mouse MAPKK, TAK1 (Yamaguchi et al. 1995), suggests that it is. TAK1 activity is enhanced in cells treated with TGF-\beta or BMP-4, the closest vertebrate Dpp homolog. In this model, however, coupling seems to be the opposite of what we observe in Drosophila, as JNKK activity depends on TGF-\beta. Although TGF-\beta/TAK1 coupling has yet to be confirmed in the animal, it appears to be specific to the JNK pathway (Yamaguchi et al. 1995), as it is in Drosophila. Future studies will surely allow the cooperation between JNK and TGF-\beta family members during vertebrate development to be characterized further.

JNK patterning and migrating epithelia

Despite its general importance in cell and developmental biology, the way an epithelium embarks on a migratory behavior is poorly understood. The study of DC in Drosophila should provide interesting clues on how pattern formation organizes migrating epithelia. Accumulated data indicate that migrating epithelia are not homogeneous structures, but rather, count at least two different cell identities: [1] a leading edge identity, as revealed by specific gene expression (\textit{puc} and \textit{dpp}; Ring and Martinez Arias 1993; Glise et al. 1995) and specific protein accumulation (Martinez Arias 1993; Young et al. 1993; Harden et al. 1996); [2] a non-leading edge identity. The importance of pattern formation and leading edge determination for epithelial migration can be evaluated by mutant analysis. In \textit{hep} embryos, the absence of a leading edge cell identity, as revealed by the absence of specific gene expression, results in a motionless epithelium, which otherwise shows no additional defects. The determination of the leading edge is therefore crucial for the whole migrating epithelium.

In a previous study, we showed evidence for sustained Hep activity during the spreading phase of DC, which lasts for -2 hr (Glise et al. 1995). Here, our data support the important notion that, during DC, the leading edge identity could be maintained by autocrine JNK-mediated signaling. During normal development, this activity seems to be restricted to the leading edge, as shown by...
the restricted puc and dpp gene activities. Under conditions of high, abnormal JNK activity in the leading edge, like those encountered in puc or activated Drac1 embryos, JNK pathway activity expands from the margins and can be detected in neighboring cells. The inducibility of cells neighboring the leading edge suggest in turn that a limiting factor in JNK pathway activation is the spatial restriction of its signal in the leading edge. Whether normal JNK activity in the leading edge has the potential to signal to other cells remains to be established. However, JNK-dependent production of Dpp, a signaling molecule that can mediate long-range morphogenetic activities (Nellen et al. 1996), suggests that the leading edge could have an organizing role for the entire migrating epithelium. Determining the developmental role of dpp in DC will be a future goal toward an understanding of how TGF-β signaling can organize cell movement in epithelia. Whether the mechanisms employed in DC, that is, patterning, epithelium boundary determination, and cell signaling are conserved features of migrating tissues in other metazoa will be an exciting issue to address.

Materials and methods

Genetics and GAL4 targeted expression

A description of genetic markers and balancer chromosomes can be found in Lindsay and Zimm [1992]. The pucE69 and puc810 alleles were kindly provided by A. Martinez Arias [Ring and Martinez Arias 1993]. Homozygous germ-line clones for the hep75 mutation were induced by mitotic recombination as described (Glise et al. 1995).

Targeted expression of UAS-driven transgenes [Brand and Perrimon 1993] was induced using the following GAL4 lines: 69B (ectodermal expression), Brand and Perrimon (1993); en-GAL4 (engrailed like striped expression in the ectoderm), wg-GAL4 (wingless-like striped expression in the ectoderm); LE-GAL4 (leading edge specific expression), kindly provided by E. Martin Blanco and A. Martinez Arias, Cambridge University, UK). The UAS lines used in this study are: UAS-Drac1V12, UAS-Dedc42V12 [kindly provided by L. Luo and Y.N. Jan, Luo et al. 1994]; UAS-Dras2V14 (Brand and Perrimon 1993); UAS-Dra800 [a gift of N. Perrimon, Harvard University, Cambridge, MA]; UAS-dpp and UAS-tkvQ2053D (Nellen et al. 1996). puc expression was monitored using the puc-lacZ enhancer-trap line pucE69 [pH7/ lacZ<sup>E69</sup>], Ring and Martinez Arias 1993). The puc-lacZ transgene was recombined on to either GAL4 or UAS chromosomes for convenience.

For targeted expression in a hep mutant background, the following strains were constructed and used: y w hep<sup>y</sup>/FM6; en-GAL4/en-GAL4; y w hep<sup>y</sup>/FM6; wg-GAL4/wg-GAL4; y w hep<sup>y</sup>/FM6, UAS-Drac1V12, puc<sup>E69</sup>/TM3, Sb; y w hep<sup>y</sup>/FM6, UAS-Dedc42V12, puc<sup>E69</sup>/TM3, Sb. To test dpp and puc expression in a hep mutant background, the following genetic crosses were made: y w hep<sup>y</sup>/y w hep<sup>y</sup>; X-GAL4 females were crossed to y w hep<sup>y</sup>/y; UAS-Z, puc<sup>E69</sup>/TM3, Sb males. X represents any GAL4 promoter or pattern element, whereas Z represents any of the UAS-driven reporter genes described above. The progeny of these crosses was collected and examined either for dpp mRNA by in situ hybridization or puc<sup>E69</sup> lacZ expression by X-Gal stainings.

Histology

In situ hybridizations were performed according to Tautz and Pfeifle [1989], using a dpp DNA probe encompassing the entire coding region (clone BB1), St. Johnston et al. 1990). For X-Gal stainings, embryos were collected and stained for β-galactosidase activity according to standard protocols [Ashburner 1989]. Photographs were taken under a Zeiss Axiohot microscope, and figures were assembled using Adobe Photoshop 3.0 software.

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