

Dynamic Analysis of Dorsal Closure in *Drosophila*: From Genetics to Cell Biology

Review

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Throughout development a series of epithelial bendings, sweepings, and fusions occur that collectively give shape to the embryo. These morphogenetic movements are driven by coordinated assembly and contraction of the actomyosin cytoskeleton in restricted populations of epithelial cells. One well-studied example of such a morphogenetic episode is dorsal closure in *Drosophila* embryogenesis. This process is tractable at a genetic level and has recently become the focus of live cell biology analysis because of the availability of flies expressing GFP-fusion proteins. This marriage of genetics and cell biology is very powerful and is allowing the dissection of fundamental signaling mechanisms that regulate the cytoskeletal reorganizations and contractions underlying coordinated tissue movements in the embryo.

Introduction

Dorsal closure is the last major morphogenetic movement of *Drosophila* embryogenesis and is the process whereby a gaping dorsal epithelial hole—the result of germ band retraction—is sealed closed to cover an otherwise naked extraembryonic amnioserosal sheet (Martinez-Arias, 1993). Lateral epithelium from the two sides of the embryo is drawn up and over the exposed amnioserosa to form a neat, and subsequently invisible, midline seam where the two segmented epithelial edges meet one another. This hole closure process comprises a series of overlapping phases and requires the combined efforts of both epithelium and the amnioserosa. In several regards there are clear parallels between the events of dorsal closure and morphogenetic episodes that occur in higher vertebrates, such as neural tube closure and palatogenesis. In particular, it is likely that there will be significant conservation of mechanism for the events that finally knit together two epithelial faces during any morphogenetic movement.

In this review, we outline the four phases that comprise the dorsal closure process, discuss the genetic clues suggesting which signaling events direct each episode, and, finally, describe recent live studies of dorsal

closure that are unraveling precise roles for each component of the cytoskeletal machinery.

Phases of Dorsal Closure

1. Initiation—Stage 12, Starts ~8 Hours after Egg Laying (AEL) (Figure 1A)

Generally dorsal closure is considered to commence only once the germ band has fully retracted to the posterior end of the developing embryo, leaving the dorsal amnioserosa fully exposed. In reality, the anterior-most epithelial edge begins moving prior to completion of germ band retraction, as this region of amnioserosa and adjacent leading edge has already been revealed by the moving germ band. Subsequent closure is a progressive process spreading from the anterior and posterior ends toward the center of the embryo. It therefore offers good opportunities for observing the full spectrum of temporal stages of epithelial fusion in a single embryo.

The signals that initiate dorsal closure are poorly understood, as these early events have been considerably less well studied than later stages. As a result, there are no genetic clues as to what the primary initiating cues are for dorsal closure. Indeed, the “start” signal may be a complex interplay of cues related to the dorsoventral patterning system and to the mechanical forces generated by germ band retraction. What appears certain is that these initiation cues must somehow trigger the changes in Jun N-terminal kinase (JNK) signaling that are seen in the two main cell types at the commencement of dorsal closure. Coincident with the earliest forward movement, JNK activity is upregulated in the leading edge cells and downregulated in the cells of the amnioserosa (Reed et al., 2001; Stronach and Perrimon, 2001).

At the commencement of dorsal closure, the epithelial cells of the leading edge possess none of the actin-based features seen in later phases and form a somewhat disorganized, scalloped edge. With no actomyosin cable, the cells of the leading edge presumably have very little internal tension and the scalloped appearance of the edge may be caused by these malleable cells being tugged by the amnioserosa cells at restricted points of adhesion (Figure 2A). During this phase, the epithelial edge slowly advances, but there is no apparent movement of epithelia over the amnioserosa, suggesting that hole closure at this time is largely a consequence of amnioserosa cell contraction. Indeed, it is clearly the case that these cells reduce the size of their apical surface substantially during this phase (Figures 2A and 2B; Supplemental Movie S1 [<http://www.developmentalcell.com/cgi/content/full/3/1/9/DC1>]).

2. Epithelial Sweeping—Stage 13, Starts ~9:30 Hours AEL (Figure 1B)

Immediately after the “initiation” phase, leading edge cells begin to polarize as filamentous actin accumulates at their apical edge to form a thick actin cable. The assembly of this cable coincides with, and is probably responsible for, transformation of the leading edge from a scalloped edge into a taut, neat row of cells. Simultaneously, the leading edge cells elongate in a dorsoventral

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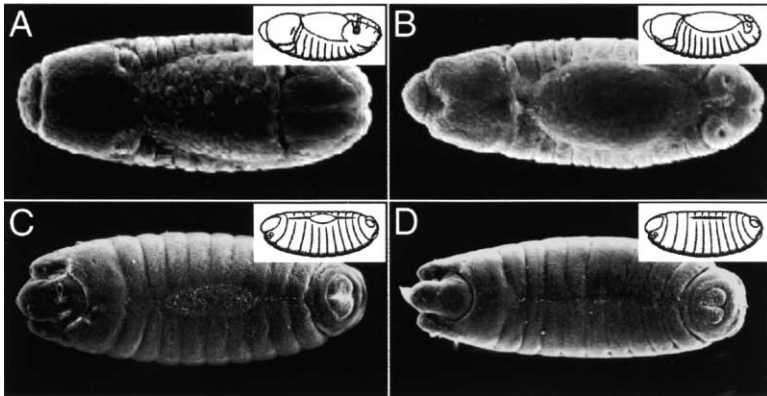


Figure 1. Scanning Electron Micrographs of the Dorsal Surface of *Drosophila* Embryos

Figure illustrates each of the four phases of dorsal closure. The accompanying lateral view icons (modified from (Hartenstein, 1993)) are used in later figures as a staging guide. (A) Initiation; (B) epithelial sweeping; (C) zippering; and (D) termination phases.

direction; it is not clear whether they actively elongate or are tugged forward by the purse-string activity of the contractile actin cable at their “free edge”. Time-lapse movies suggest that at this stage they may sweep forward over the amnioserosal layer (Figures 2B and 2C; Supplemental Movie S1 [<http://www.developmentalcell.com/cgi/content/full/3/1/9/DC1>]), but whether there really is net movement over the amnioserosa or contortion of amnioserosa cells immediately adjacent to the epithelium is still not clear. Previous studies have described front row cell elongation occurring in advance of rows further back (Riesgo-Escovar and Hafen, 1997b), but time-lapse observations suggest that on average these cell shape changes are fairly synchronous for the front few rows of cells (see Supplemental Movie S1). Throughout this stage of dorsal closure the epithelial hole continues to reduce in size, presumably through a combination of amnioserosal cell contraction and contraction of the actin cable operating in a purse-string-like fashion to draw the epithelium forward plus epithelial cell elongation. In addition, it is during this “epithelial sweeping” phase that filopodia and, to a lesser extent, lamellae are first seen protruding from the lead edge cells.

3. Zippering—Stage 14, Starts ~11 Hours AEL (Figure 1C)

The zippering phase of dorsal closure begins once opposing leading edges at the most anterior and posterior ends of the hole are close enough for filopodia to reach

across and touch the opposite epithelial edge (Jacinto et al., 2000). These actin protrusions act to zip the opposing epithelial surfaces together and may also play a key role in ensuring the correct matching of the embryonic segments as the hole closes. However, in addition to filopodia, it is highly likely that both contractility of the actin cable and amnioserosa cell contraction still play roles in bringing the sides together. Certainly, time-lapse studies reveal a continual constriction of amnioserosa cells in the DV axis with occasional cells disappearing from view as their apical face becomes very small and the cell withdraws beneath the surface (Figure 3; see Supplemental Movie S2 [<http://www.developmentalcell.com/cgi/content/full/3/1/9/DC1>]).

4. Termination—Stage 15, Ends ~13 Hours AEL (Figure 1D)

As for the other phases of dorsal closure, the final stage of this process exhibits a temporal progression, spreading like a wave within the seams behind the two advancing zipper fronts. A crucial aspect of termination is that once leading edge cells from opposing sides have made contact they stop moving and form a tight seam along the midline. There must, therefore, be some form of “stop” signal to prevent the cells from overshooting. In one sense this is an example of contact inhibition, and it is of considerable interest that, through dorsal closure, this fundamental cell behavior may now be genetically tractable. A further aspect of termination is that, as well

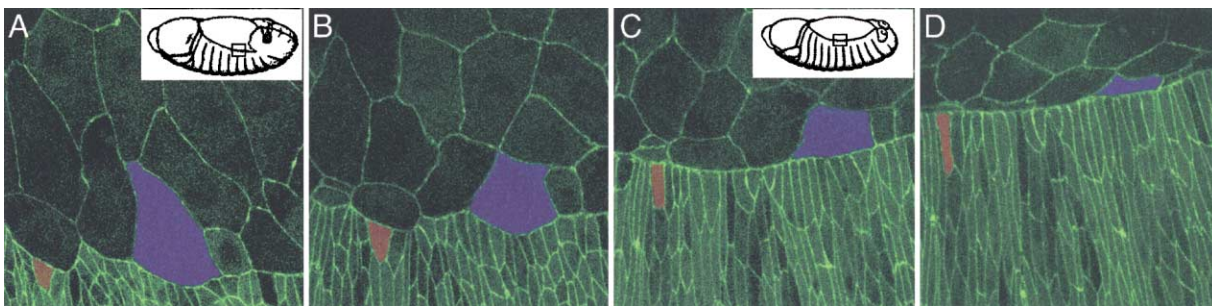


Figure 2. Stills from Movie S1 of an α -Catenin-GFP Embryo

Figure reveals cell:cell junctions and thus cell outlines of both the amnioserosa (larger cells) and the lateral epithelium as it closes over the amnioserosa (i.e., moves upward in these images). The period between (A) and (B) constitutes the “initiation” phase in which the major contributor toward epithelial movement appears to be contraction of amnioserosa cells. By time (C), the leading edge of the epithelium has become taut and the “epithelial sweeping” phase commences, such that the epithelium appears to move forward over the amnioserosal cells (e.g., blue amnioserosal cell between [C] and [D]), while leading edge cells themselves are elongating/stretching (e.g., cell in red).

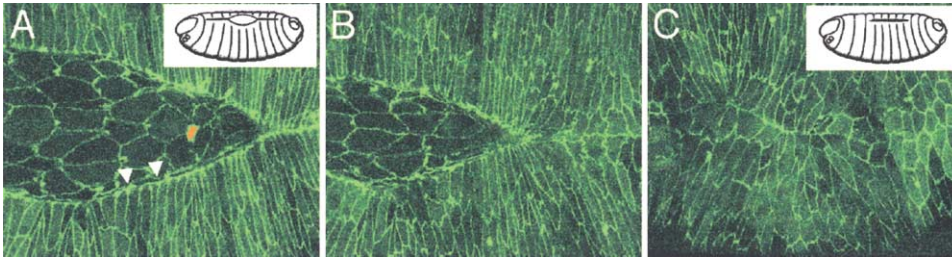


Figure 3. Stills from Movie S2 of a Later Stage α -Catenin-GFP Embryo

Figure illustrates the “zippering” phase between (A) and (B) and finally the “termination” phase, resulting in complete midline fusion, in (C). At the leading edge, bright beads of α -catenin (arrowheads) indicate the adherens junctions that have been proposed to act as linkers for the intracellular segments of actin cable. Highlighted in orange is an example of an amnioserosal cell that disappears beneath the surface.

as stopping, the abutting epithelial cells must also convert the transient adhesions formed between filopodia into tight, permanent, adherens junctions.

Studies of the signaling pathways that regulate dorsal closure have centered on the leading edge epithelial cells that clearly play a critical role throughout this morphogenetic episode. Our focus will be on very recent findings, as there are several good reviews on the signaling aspects of dorsal closure elsewhere. (Noselli, 1998; Noselli and Agnès, 1999; Settleman, 2001; Stronach and Perrimon, 1999).

Defining the Leading Edge Cells

As the germ band retracts to reveal the interface between the leading edge epithelium and the amnioserosa, these two cell populations are clearly morphologically distinguishable (Figure 2A), but they start to be specified at the blastoderm stage. The location and fate of the leading edge cells appears to be broadly specified by the dorsoventral patterning system in which the TGF β -related secreted factor, Decapentaplegic (Dpp), plays a major signaling role. A gradient of Dpp activity controls the expression of the zinc finger transcriptional regulators, *hindsight* and *u-shaped*, which in turn define a competency zone several cells wide that is further restricted to a single row of cells by putative interactions between the presumptive amnioserosa and the dorsal epithelial cells (Stronach and Perrimon, 2001). As part of this process, the JNK pathway is downregulated in the amnioserosa and upregulated in the leading edge cells, activating the localized transcription of *dpp* and a dual specificity phosphatase, *puckered* (*puc*) (Reed et al., 2001; Stronach and Perrimon, 2001). Another likely player in defining the leading edge is the currently less well characterized product of the *raw* gene, which is expressed in the amnioserosa but influences JNK activity in the leading edge epithelium (Byars et al., 1999).

Signaling Dorsal Closure through JNK

One major group of mutants in which dorsal closure is severely disrupted are those that inactivate elements of the JNK signaling pathway (see Figure 4A), suggesting that the JNK cascade must play a central role in directing this movement. Typically, mutations in such genes exhibit a clear dorsal open phenotype and express neither *puc* nor *dpp* in their leading edge cells (Glise et al., 1995;

Glise and Noselli, 1997; Riesgo-Escovar and Hafen, 1997b; Riesgo-Escovar et al., 1996; Sluss et al., 1996).

Hemipterous (Hep) and Basket (Bsk) are the well-established downstream components of the JNK pathway, acting respectively at the level of JNKK and JNK, and leading to activation of Jun, which together with Fos constitutes the AP-1 transcriptional complex and regulates the expression of target genes (Glise et al., 1995; Glise and Noselli, 1997; Hou et al., 1997; Kockel et al., 1997; Riesgo-Escovar and Hafen, 1997b; Riesgo-Escovar et al., 1996; Sluss et al., 1996; Zeitlinger et al., 1997). Connector of Kinase to Ap-1 (Cka), a recently discovered multidomain protein, forms a complex with Hep, Bsk, Jun, and Fos and this complex appears to organize the kinases and facilitate the phosphorylation and activation of AP-1 (Chen et al., 2002).

The final targets of the JNK kinase cascade are Jun and anterior open/yan (Aop), which act as antagonistic transcription factors. Jun, together with Fos, constitute the AP-1 transcriptional complex that activates the expression of target genes—at least *dpp* and *puc*—in the leading edge cells (Glise and Noselli, 1997; Riesgo-Escovar and Hafen, 1997a, 1997b; Riesgo-Escovar et al., 1996; Zeitlinger et al., 1997), while Aop, an ETS domain protein, acts as a transcriptional repressor (Riesgo-Escovar and Hafen, 1997b).

Several more upstream components of the JNK cascade have now also been elucidated. Slipper (Slpr) is a kinase from the mixed lineage kinase (MLK) family that acts at the level of JNKKK and is required for JNK activation during dorsal closure (Stronach and Perrimon, 2002). Misshapen (Msn), a member of the NIK group of Ste20-related kinases, probably acts upstream of Slpr at the level of JNKKKK (Su et al., 1998, 2000), and the *Drosophila* TNF-receptor-associated factor (DTRAF1) has been shown to bind and act upstream of Msn in cell culture transfection experiments (Liu et al., 1999).

A number of Src-like nonreceptor tyrosine kinases may also act upstream of JNK signaling during dorsal closure. Mutations in Shark, a kinase containing SH2 domains and ankyrin repeats, have phenotypes similar to other JNK pathway loss-of-function alleles (Fernandez et al., 2000). Two further src kinases, Src42A and Tec29, successfully complete dorsal closure as single mutants, but in combination show dorsal closure defects and lack expression of *puc* and *dpp* in leading edge cells (Tateno et al., 2000). Given the number of

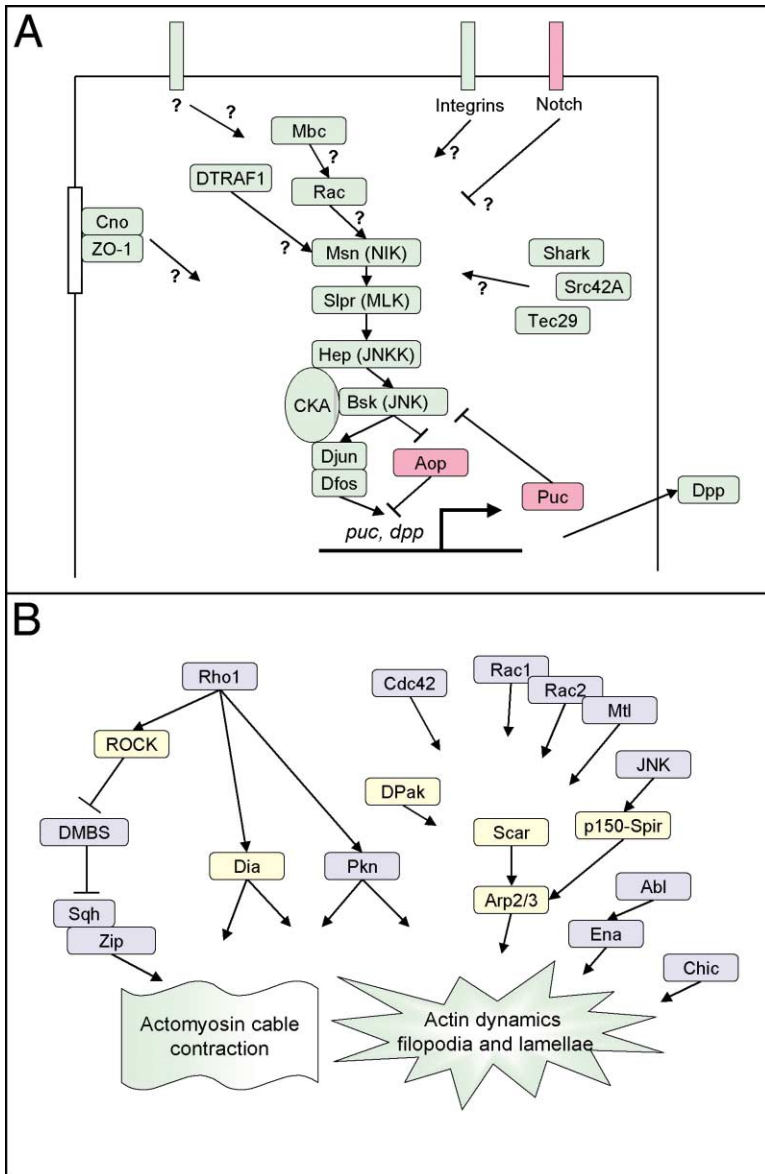


Figure 4. Dorsal Closure Signaling Pathways
(A) Schematic illustration of the JNK signaling events occurring in leading edge epithelial cells from the speculative initial receptor activation events, through to transcriptional activation of target genes. Positive and negative regulators of the pathway are shown in green and red, respectively. This diagram does not include all the proteins mentioned in the text because in several cases it is not clear where those proteins would fit.
(B) In the same leading edge cells, concurrent signaling events are directing actin regulatory events and the assembly, maintenance and contraction of an actin cable and assembly and regulation of dynamic filopodial and lamellar protrusions. The branches that are not supported yet by genetic data are shown in yellow.

players that appear to feed into the JNK cascade, the challenge is to find out how they talk to each other, what the key activating cues—chemical or mechanical—might be, and what receptors receive and transduce these cues.

Morphogenetic Consequences of the JNK Signal

One of the JNK targets, Dpp, is expressed and then secreted by leading edge cells, and appears to be required for elongation of those lateral epithelial cells in rows behind the leading edge; in *thickveins* (*Tkv*—receptor of Dpp) mutants, the leading edge cells initially elongate normally but the more lateral epithelial cells do not (Glise and Noselli, 1997; Riesgo-Escovar and Hafen, 1997a). The simplest explanation of these data is that Dpp acts in a paracrine fashion on the more lateral epithelial cells, but it could also target the leading edge cells themselves or even influence the contractility of the adjacent amnioserosa. What is clear is that overexpression of Dpp, or

expression of an activated form of *Thv*, is largely sufficient to rescue the defects of JNK pathway mutants, suggesting that JNK signaling is operating through Dpp (Chen et al., 2002; Hou et al., 1997; Riesgo-Escovar and Hafen, 1997b; Sluss and Davis, 1997). Cells receiving this signal translocate Mothers against Dpp (*Mad*) along with another Smad, *Medea* (*Med*), to the nucleus to mediate transcriptional effects of Dpp signaling (Affolter et al., 2001).

The other clear transcriptional target of JNK activity, *Puc*, is a dual specificity phosphatase of the VH-1 family. It targets *Bsk* and thus provides negative feedback on the JNK signaling pathway itself. It seems to operate as a brake either during the closure process or at termination, because *puc* mutants exhibit a puckered, overcontraction-type phenotype (Martín-Blanco et al., 1998; Ring and Martinez Arias, 1993). Notch signaling may also have a braking role on JNK signaling during this process, through an activity that is independent of its nuclear interactions (Zecchini et al., 1999).

JNK may be a more universal signal that operates during morphogenetic episodes in vertebrates also. In mouse embryos, closure of the neural tube requires the lips of the folding neural plate to be drawn toward one another and then to fuse together at several nucleation sites, reminiscent of the zippering fronts during fly dorsal closure (Colas and Schoenwolf, 2001; Jacinto et al., 2001). However, in embryos lacking both of the c-Jun terminal kinases (JNK 1 and 2), this process partially fails, leaving embryos with open neural tubes at the level of the hindbrain (Sabapathy et al., 1999).

Small GTPase Signaling within Leading Edge Cells

The Rho family of small GTPases participate in various aspects of dorsal closure, specifically in regulation of the actin cytoskeleton, but several studies using dominant-negative and constitutively active transgenes also implicate Rac and Cdc42 as operating upstream of JNK signaling (Glise and Noselli, 1997; Harden et al., 1995, 1996, 1999). However, expression of *dpp* is not significantly altered in *cdc42* mutant embryos, and the phenotypic consequences of expressing dominant-negative *cdc42* resemble the phenotypes of Dpp pathway mutants more than JNK pathway ones (Genova et al., 2000; Ricos et al., 1999), suggesting that Cdc42 acts downstream of Dpp and may not play a role in early firing of the JNK pathway. Rac remains the best candidate for a small GTPase activator of JNK (Ricos et al., 1999). Indeed, mammalian tissue culture studies reveal that homologs of Msn, Slpr, and Rac1 all participate in a complex that stimulates downstream JNK pathway activity (Dan et al., 2001; Su et al., 1998). Flies have three *rac* genes, *rac1*, *rac2*, and *mtl*, and recent reports describe triple mutants failing to complete dorsal closure (Hakeda-Suzuki et al., 2002). The upstream regulators of Rac activity are currently rather poorly understood. Mutants in *myoblast city* (*mbc*), which encodes a fly homolog of DOCK180/CED-5, exhibit a dorsal open phenotype, and Mbc interacts genetically and physically with Rac1, so it is a possible candidate (Erickson et al., 1997; Nolan et al., 1998). However, *mbc* mutants do not show a reduction in Dpp expression, suggesting that Mbc modulates aspects of Rac signaling other than dorsal closure, or that its function is redundant.

Regulation of the Actomyosin Cable and Cell:Cell Junctions

The most prominent actin machinery at the advancing epithelial front during dorsal closure is a thick, cable-like accumulation of actin and myosin (Young et al., 1993). Zipper (Zip), the nonmuscle myosin heavy chain, is a key component of this actomyosin cable. Assembly and maintenance of the cable in leading edge epithelial cells is dependent on the small GTPase Rho1, a homolog of RhoA in vertebrates, which regulates assembly and contractility of actin stress fibers in 3T3 fibroblasts (Ridley and Hall, 1992). Mutants in *rho1* and *zip* have been shown to interact genetically in *Drosophila* (Halsell et al., 2000), and vertebrate cell culture studies suggest that this functional link acts via Rho-associated kinase (ROCK). ROCK regulates myosin function by repression of myosin light chain phosphatase and direct activation of the myosin light chain (Amano et al., 1996; Kimura et al., 1996), which in flies is encoded by the gene *spaghetti*

squash (*sqh*) (Karess et al., 1991). In mutants of the myosin binding subunit of myosin phosphatase (DMBS), there is excessive accumulation of phosphorylated myosin regulatory light chain (MRLC) and aberrant cable assembly, leading to a failure of dorsal closure. The same is true for embryos overexpressing ROCK, confirming that Rho primarily regulates cable contractility via myosin activity (Mizuno et al., 2002). Further evidence that regulation of myosin is conserved between mammals and flies comes from the observations that DMBS can be phosphorylated by *Drosophila* ROCK, and that DMBS mutations suppress phenotypes caused by mutations in *zip* or *rho1* (Mizuno et al., 2002). However, Rho1 can also regulate the cytoskeleton via different effectors. The kinase Pkn has also been shown to function downstream of Rho1 during *Drosophila* dorsal closure (Lu and Settleman, 1999), and mDia, the murine homolog of *Drosophila* Diaphanous, binds active Rho1 and contributes to the formation of stress fibers in mammalian cells by promoting actin polymerization (Watanabe et al., 1999). Several earlier studies suggest that small GTPases may also have further subtle effects on actin cable assembly and maintenance that reflect differences at the level of each segment. Expression of dominant-negative Rac and Cdc42 throughout the epithelium appears to cause variable disassembly of cable within each segment, suggesting that regulation may be different at segment borders than within the body of each segment. There is evidence that DPak, a downstream effector of Rac1 that is enriched at the leading edge, may be implicated in the regulation of such differences (Harden et al., 1995, 1996, 1999; Ricos et al., 1999).

Interestingly, Magie et al. (2002) have recently shown that Rho1 interacts with p120^{ctn} and regulates cadherin-based adherens junctions in the *Drosophila* embryo, suggesting that the *rho1* mutant phenotype may not only be a consequence of actin cable misregulation but also a result of defective adherens junctions. These cell junctions are probably the anchoring sites for intracellular segments of the actomyosin cable and, just as in the case of “purse-string” driven wound closure (Danjo and Gipson, 1998; Young et al., 1993), their integrity must be crucial for the mechanics of cable contraction. They may also play a role in transmission of extracellular cues into changes in the actin cytoskeleton. Abelson (Abl), a nonreceptor tyrosine kinase, and its target, Ena, a modulator of actin dynamics that localizes to adherens junctions, are both involved in the regulation of this link between junctions and the cytoskeleton (Grevengoed et al., 2001). *abl* mutants interact genetically with *armadillo* and *shotgun* (*Drosophila* β -catenin and *E-cadherin*, respectively), and both fail to undergo coordinated changes in cell shape during dorsal closure. Ena is mislocalized in *abl* mutants and this may be the fundamental defect, since Ena has also been shown to mediate correct junction assembly and actin polymerization at those adherens junctions between follicular epithelial cells (Baum and Perrimon, 2001). It seems likely that adherens junctions are also special sites for the assembly of signaling complexes that direct JNK activity. Indeed, the PDZ domain proteins, Canoe and ZO1, are localized in the adherens junctions and might provide an appropriate scaffold, since *canoe* mutants show a reduction in *pu*

and *dpp* expression in leading edge cells and interact genetically with *hep* and *bsk* (Takahashi et al., 1998).

Function of the Cable

The actomyosin cable clearly has the potential to operate like a purse-string to draw the epithelial hole closed, as appears to happen during repair of an embryonic epithelial or tissue culture wound (Bement et al., 1993; Martin and Lewis, 1992). However, Rho1 mutant embryos, in which actin cable assembly is somewhat disrupted, are still able to close the hole (Magie et al., 1999), and laser cutting experiments that fail to halt the forward movement of epithelium also hint that the cable is not necessary for advancement of the epithelial front (Kiehart et al., 2000). So, does the cable have a major function during dorsal closure? Recent dynamic studies suggest that the cable is indeed contractile and, as such, does operate as a purse-string and maintains tension in the leading edge, at least during the “epithelial sweeping phase” of dorsal closure (Bloor and Kiehart, 2002; Jacinto et al., 2002). Indeed, coincident with the time when an actin cable first assembles, front row cells clearly constrict and the leading edge makes its transition from scallop-edged to straight and taut, suggesting that this movement is driven by cable contraction (Figures 2A and 2B). However, the cable appears to have a second role, whereby it maintains a uniform epithelial advance by preventing individual cells from migrating forward on their own. Experiments in which dominant-negative Rho is expressed in engrailed stripes within the epithelium reveal how cells in the leading edge that fail to maintain a cable spill out and gain a migration advantage over their wild-type neighbors (Bloor and Kiehart, 2002; Jacinto et al., 2002). These results suggest that the cable acts to restrain epithelial cells at the free edge, thus maintaining a taut epithelial edge, which may be necessary for efficient zippering.

It is much more difficult to dissect out the function of small GTPase signaling and indeed of actin contractility machineries during vertebrate morphogenetic episodes, but one study of Rho kinase (ROCK) function in early mouse embryos shows how blocking this Rho effector severely disrupts several morphogenetic movements including fusion of the heart tubes and also neural tube closure (Wei et al., 2001). Moreover, classic transmission electron microscopy studies and cytochalasin D experiments, in which new actin polymerization is blocked during neurulation, suggest that assembly and contraction of apically located actomyosin bundles in neural plate cells may partially underlie mammalian neural tube folding, at least at the head end of the embryo (Morriss-Kay and Tuckett, 1985). Tantalizing new genetic data are beginning to identify genes, such as *shroom*, that may have key roles in regulating the actin reorganizations that drive complex vertebrate morphogenetic epithelial movements (Hildebrand and Soriano, 1999).

Zippering the Epithelial Faces Together

Live imaging of *Drosophila* embryos expressing GFP-actin reveals not only the presence of an actin cable but

also filopodial protrusions (Figure 5A) extending from leading edge epithelial cells as they sweep forward toward the midline (Jacinto et al., 2000). These actin based extensions are first seen during the “epithelial sweeping” stage, coincident with assembly of the actin cable, but, until the zippering phase of dorsal closure, their role, if any, in epithelial movement is unclear. However, from the zippering stage of dorsal closure onward, these actin-rich protrusions appear to be pivotal in drawing together the two epithelial edges. At the zipper front, filopodia and subsequently lamellipodia on opposing epithelial faces contact and engage, and the cells then tug toward one another (Jacinto et al., 2000). Until the “termination” phase of dorsal closure, these filopodial engagements occur only at the anterior and posterior zipper fronts, but in the “termination” phase the gap between opposing epithelial faces is minimal and interactions occur randomly along its length (Figure 5B; Supplemental Movie S3 [<http://www.developmentalcell.com/cgi/content/full/3/1/9/DC1>]). For several minutes after the epithelial faces have met and fully covered the amnioserosa, a final few filopodia are seen to emerge from the seam line, but then “contact inhibition” quells this activity and dorsal closure is complete. Just as for the cell:cell adhesion events occurring between primary mouse keratinocytes stimulated by exposure to Ca^{2+} , and also the seaming together of epithelial sheets during ventral enclosure in the *C. elegans* embryo (Raich et al., 1999; Vasioukhin et al., 2000), it appears that *Drosophila* dorsal closure zippering is “primed” by interdigitation and adhesion between opposing filopodia from the two fusing epithelial fronts (Figures 5C–5G). If the extension of filopodia is blocked by expression of dominant-negative Cdc42 constructs, adhesion of the epithelial front fails, suggesting that filopodia are indeed critical for this fusion event (Jacinto et al., 2000).

The filopodial extensions from leading edge cells may also serve one further key function in the moments preceding final epithelial fusion. We observed filopodia apparently “scanning” epithelial cells on the opposite epithelial sheet (Jacinto et al., 2000), much like the filopodia of a growth cone sensing guidance cues as an axon makes its final turning decisions before locking onto its target in the developing nervous system. There is some evidence to support this postulated “sensing” role for the filopodia of zippering epithelial cells, in that segmental expression of dominant-negative Cdc42 (to block filopodial extension) frequently leads to mismatching of engrailed-GFP-expressing stripes across the midline seam of the embryo (Jacinto et al., 2000). The molecular nature of the matching machinery carried by filopodia, which allows them to marry cells up across the closure seam, is entirely unknown, but is likely to be similar to the machinery that maintains segment identity in the fly embryo. There may be graded expression of a cell:cell adhesion molecule or an “on-off” membrane bound molecule expressed, for example, by the filopodia of anterior compartment cells but not by cells in the adjacent posterior compartment of each segment. In *C. elegans*, where filopodia appear also to form nascent adhesions that prime the assembly of mature adherens junctions across an analogous midline fusion seam (Raich et al., 1999), there is some evidence that *semaphorins* might

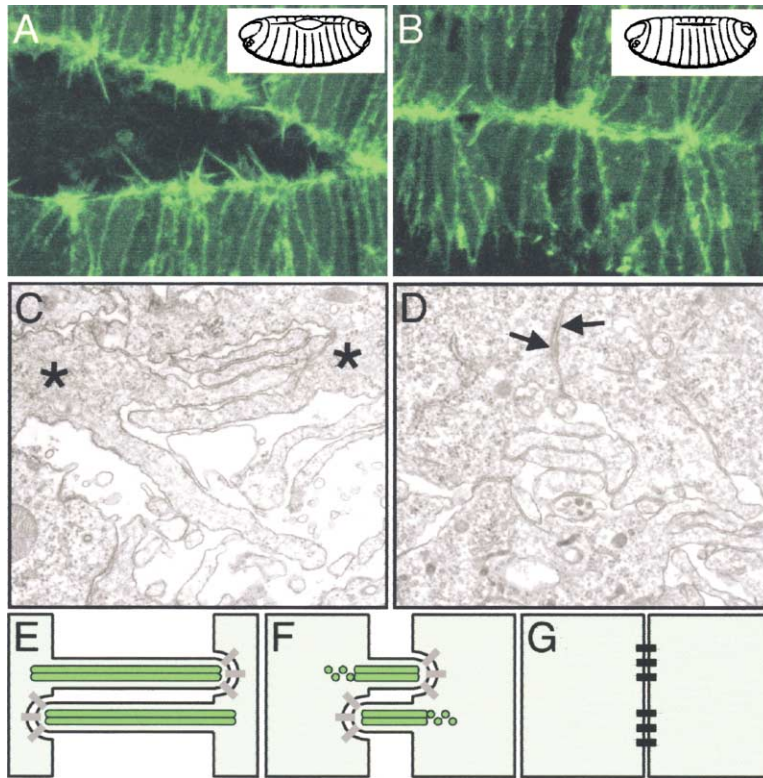


Figure 5. Stills from Movie S3

Stills from Movie 3 during “zippering” (A) and at the “termination” (B) phase in an actin-GFP embryo. Leading edge epithelial cells express both an actin cable and extensive filopodial protrusions which interact between the two opposing epithelial sheets as the zipper front progresses forward (from right to left in these images). Transmission electron micrograph (TEM) views of the interdigitations observed between the filopodia of opposing epithelial cells (C); asterisks indicate the cell bodies of the two interacting epithelial cells. (D) is a TEM section cut further back in the maturing seam and shows how filopodial interdigitations resolve to form junctions (arrows) between the opposing cells. (E)–(G) are schematic views of the filopodial priming events that lead to epithelial adhesion. Green filaments represent polymerized actin and gray and black blocks are transient and mature junctions, respectively.

form part of this cell:cell matching machinery, as mutants in *semaphorin-2a* exhibit incorrect cell contacts between and across cells of the midline seam (Roy et al., 2000).

It seems that filopodia may be key players during fusion of epithelial processes during morphogenetic episodes in vertebrates as well. The best evidence for this comes from the study of palate fusion in mouse embryos whereby the two palatal shelves fuse to form the roof of the mouth. The palatal filopodia, which are expressed only on the medial, fusing, epithelial face, may be dependent on TGF β signals, because TGF β 3 null palatal shelves lack protrusions and these mice are born with cleft palates (Taya et al., 1999).

Future Directions and Speculations

Analyzing the Function of Established Players

The genetics of dorsal closure has already been well explored. More than 30 mutant fly embryos fail in some way in dorsal closure, leading to various shapes and sizes of hole in cuticle preparations of the resulting larvae. Generally, they have names that evoke the defect graphically, such as *kayak*, *basket*, *canoe*, and *coracle* (Borchiellini et al., 1996; Fehon et al., 1994; Glise et al., 1995; Riesgo-Escovar et al., 1996; Sluss et al., 1996; Takahashi et al., 1998; Zeitlinger et al., 1997). These data have helped unravel which signaling cascades are playing a role in driving dorsal closure and which structural and motor proteins are required for the process. But although such studies demonstrate a requirement for each of these components, they generally do not reveal the stage of action, or the precise cell biological involvement of the gene product. Almost certainly it will

be productive to revisit each of these mutants and image them live with GFP-fusion proteins to determine where and how failure occurs in the closure process. These dynamic mutant data, together with live GFP-localization data for all likely component proteins, should reveal which elements are required at each phase of dorsal closure—whether, for example, a particular small GTPase regulator of the actin cytoskeleton is required for early contraction of leading edge cells, or for them to assemble filopodia, or for the elongation of lateral cells, or a combination of these three.

Discovering New Players

Clearly the large-scale mutant screens have not revealed all the key players in dorsal closure. Among the missing components could be, for example, those with a large maternal contribution. Several recent studies have highlighted potential new players using alternative search strategies. Downstream of the JNK cascade activated in leading edge cells there will be regulated expression of more than just the two or three effectors revealed by mutant analysis. SAGE analysis of embryos expressing dominant-negative Bsk compared to embryos expressing constitutively active Hep has provided us with a number of other candidate players, such as the actin regulatory protein Profilin (Chickadee), which may play component roles in directing filopodial protrusion and other aspects of epithelial and amnioserosa cell behavior (Jasper et al., 2001). Other potentially rich sources of new dorsal closure players include “protein trap” screens such as that conducted by the Chia lab, where as yet unidentified proteins clearly localize to potentially interesting intracellular domains within the leading edge epithelium during the dorsal closure process (Morin et al., 2001).

Coordinating Epithelial and Amnioserosa Contributions

Until recently, the role of the amnioserosa during dorsal closure was somewhat underplayed; it was simply considered a passive substratum during the whole process. Analogies with wound healing, which is clearly driven partially by reepithelialization and partially by wound bed connective tissue contraction (Martin, 1997), might have suggested that the amnioserosa played a more active role, and indeed recent studies have now shown that it almost certainly does.

As described earlier, video analysis of the amnioserosa during the dorsal closure period reveals how individual cells within this sheet shrink their apical surface with a contraction bias in the dorsoventral axis (see Supplemental Movie S2 [<http://www.developmentalcell.com/cgi/content/full/3/1/9/DC1>]), and transmission electron microscopy studies confirm this (Rugendorff et al., 1994). However, these descriptive data do not formally rule out that the amnioserosa is being passively squeezed by the advancing epithelial sheets. The best evidence that the amnioserosa is exerting some tension that might aid in drawing the adjacent epithelial sheets together are experiments in which small holes are created in either the amnioserosa or the adjacent lateral epithelium by laser ablation (Kiehart et al., 2000). These holes locally release any potential tension within those tissues. A hole in the amnioserosa leads to gaping of the adjacent epithelium, which is very suggestive that the amnioserosa is actively drawing the epithelium forward. Experiments in which amnioserosa-specific expression of dominant-negative Rac or of constitutively active Rac lead to failure of dorsal closure or over contraction of the amnioserosa and puckering of the leading epithelial edge, respectively (Harden et al., 2002) also provide strong support for amnioserosa contractility as a major factor in dorsal closure and argue that Rac, and not other Rho-family small GTPases, mediate the signals leading to this amnioserosal cell behavior.

It now seems crucial to figure out how the epithelium and amnioserosa coordinate their efforts with one another. If the epithelium is indeed advancing forward over a contracting substrate, then how are the two tissues physically linked, and what are the signals passing between the tissues as they ratchet forward? Adhesions between epithelium and amnioserosa may be at the tips of filopodia and lamellipodial protrusions from the leading edge epithelial cells, but as these protrusions appear to touch down only transiently on the amnioserosa ahead of them, it seems more likely that the firmer links which exist further back between the body of the front row cells and the underlying amnioserosal cells play a central role in this process. Integrins may have a role here since mutations in α and β integrin subunits, *scab* and *mysospheroid*, both result in dorsal closure defects (Brown, 1994; Brown et al., 2000; Stark et al., 1997).

Assembly and Disassembly of Cytoskeletal Machineries

As discussed above, there are no clear indications of how the small GTPases that regulate actin cable and filopodial assembly are themselves activated. Presumably various guanine nucleotide exchange factors (GEFs)

are involved, but as RhoGEF2 is essential for gastrulation (Barrett et al., 1997), there is as yet no direct demonstration of its role in cable assembly. The known GEF for the *Drosophila* Rac, Trio, does not show a dorsal open phenotype. There will likely be significant redundancy at the level of small GTPase regulation, as there are upwards of 20 Rho family GEFs apparent from the *Drosophila* genome (Adams et al., 2000). However, there are good clues from vertebrate tissue culture studies as to signaling steps downstream of the Rhos. As described earlier, Rho itself appears to signal via ROCK and myosin light chain activation. Filopodial and lamellar protrusion via Cdc42, and possibly Rac, appear to be mediated, at least in tissue culture cells, by activation of WASP and Scar proteins and the consequent downstream activation of the ARP2/3 complex which nucleates new actin filament assembly (reviewed in Machesky and Insall, 1999). In *Drosophila*, Scar seems to be the primary regulator of Arp2/3-dependent morphological events and mutations in the homolog of WASP do not cause a dorsal open phenotype (Ben-Yaacov et al., 2001; Zallen et al., 2002). There is also now evidence for involvement of a novel vertebrate myosin, Myosin X, in filopodial protrusion and dynamics (Berg and Cheney, 2002), and while there is no direct *Drosophila* homolog of Myosin X, it is likely that some other fly myosin may too operate in filopodial regulation.

There is currently no genetic evidence supporting a role for the other key cytoskeletal machinery, the microtubule network, in dorsal closure, and yet we know that cell elongations preceding several vertebrate morphogenetic movements, including neurulation, are microtubule dependent (Schoenwolf and Powers, 1987). There are now good clues from tissue culture models that Cdc42-directed microtubule reorganizations play a key role in cell polarity prior to cell migrations (Etienne-Manneville and Hall, 2001), and since leading edge epithelial cells are exquisitely polarized during dorsal closure, it is likely that the microtubule network will turn out to be a key player here also. However, human genetics may offer a clue where the fly has currently failed. A human disorder, Opitz syndrome, in which there are failures of several midline fusion events, has been shown to be due to a defect in the Mid-1 gene which encodes a regulator of the microtubule cytoskeleton (Liu et al., 2001).

During the termination phase of dorsal closure there will be "stop" signals that direct disassembly of the actin motility machineries. These will presumably involve the switching off of the same small GTPases that were activated, and that triggered assembly of the actin motility machinery, in response to the initial "start" signals, and these signals may be partially overlapping with those signals that direct cell:cell adhesions across the midline seam. Clearly screens can be designed to identify mutants that overrun in this process. Such screens will likely uncover signaling events that are fundamental in our understanding of universal "contact inhibition" events.

Dorsal Closure as a Paradigm for Other Epithelial Closure/Zippering Events?

Now we are gaining such a full understanding of the genetics and cell biology of dorsal closure, how can this

help us grasp mechanisms underlying other developmental processes outside of *Drosophila* embryogenesis? As discussed above, there are several obvious parallels with ventral enclosure in *C. elegans* and neural tube closure and palatogenesis in vertebrates. It seems likely that for these movements, and for others not yet as well characterized, there will be a great deal of conservation in the means by which epithelial fronts adhere and fuse together. However, we must be wary of stretching the parallels too far, particularly with regard to the strategies used to bring opposing epithelial faces into contact. It seems unlikely, for example, that identical mechanisms could possibly drive advancement of the leading epithelial edge during dorsal closure and the grossly unrelated movement of two palatal shelves up and over the developing tongue and into contact with one another. One tissue movement that we think might turn out to share rather broader parallels with morphogenetic hole closure events such as dorsal closure is wound reepithelialization. Indeed, there is good evidence for significant parallels between both vertebrate and invertebrate morphogenetic mechanisms and wound repair processes (reviewed in Jacinto et al., 2001). In chick and mouse embryos, an actin cable mediated by activation of the Rho small GTPase draws wounded epithelial holes closed (Brock et al., 1996; Martin and Lewis, 1992), and a recent study in adult flies showed that precisely the same JNK cascades activated during dorsal closure are reactivated at a wound site leading to Puc expression in the repairing epidermis (Ramet et al., 2002). Wound repair studies in mutant flies null for the various genes critical for dorsal closure will reveal whether the tissue movements of wound healing really do recapitulate those of morphogenesis.

Conclusion

Dorsal closure in *Drosophila* embryos is turning out to be a near-perfect model for a paradigm morphogenetic movement that is both genetically tractable and amenable to live cell biology analysis. We now have more than a basic understanding of all phases of dorsal closure, and, while there are still many details to uncover, it promises many more clues about the mechanisms underlying other morphogenetic fusion events in the embryo and may even help us understand how tissues are able to repair at sites of damage.

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References

Adams, M.D., Celniker, S.E., Holt, R.A., Evans, C.A., Gocayne, J.D., Amanatides, P.G., Scherer, S.E., Li, P.W., Hoskins, R.A., Galle, R.F., et al. (2000). The genome sequence of *Drosophila melanogaster*. *Science* 287, 2185–2195.

Affolter, M., Marty, T., Vigano, M.A., and Jazwinska, A. (2001). Nuclear interpretation of Dpp signaling in *Drosophila*. *EMBO J.* 20, 3298–3305.

Amano, M., Ito, M., Kimura, K., Fukata, Y., Chihara, K., Nakano, T., Matsuura, Y., and Kaibuchi, K. (1996). Phosphorylation and activation of myosin by Rho-associated kinase (Rho-kinase). *J. Biol. Chem.* 271, 20246–20249.

Barrett, K., Leptin, M., and Settleman, J. (1997). The Rho GTPase and a putative RhoGEF mediate a signaling pathway for the cell shape changes in *Drosophila* gastrulation. *Cell* 91, 905–915.

Baum, B., and Perrimon, N. (2001). Spatial control of the actin cytoskeleton in *Drosophila* epithelial cells. *Nat. Cell Biol.* 3, 883–890.

Bement, W.M., Forscher, P., and Mooseker, M.S. (1993). A novel cytoskeletal structure involved in purse string wound closure and cell polarity maintenance. *J. Cell Biol.* 121, 565–578.

Ben-Yaacov, S., Le Borgne, R., Abramson, I., Schweisguth, F., and Schejter, E.D. (2001). Wasp, the *Drosophila* Wiskott-Aldrich syndrome gene homologue, is required for cell fate decisions mediated by Notch signaling. *J. Cell Biol.* 152, 1–13.

Berg, J.S., and Cheney, R.E. (2002). Myosin-X is an unconventional myosin that undergoes intrafilopodial motility. *Nat. Cell Biol.* 4, 246–250.

Bloor, J.W., and Kiehart, D.P. (2002). *Drosophila* RhoA regulates the cytoskeleton and cell-cell adhesion. *Development* 129, 3173–3183.

Borchiellini, C., Coulon, J., and Le Parco, Y. (1996). The function of type IV collagen during *Drosophila* muscle development. *Mech. Dev.* 58, 179–191.

Brock, J., Midwinter, K., Lewis, J., and Martin, P. (1996). Healing of incisional wounds in the embryonic chick wing bud: characterization of the actin purse-string and demonstration of a requirement for Rho activation. *J. Cell Biol.* 135, 1097–1107.

Brown, N.H. (1994). Null mutations in the alpha PS2 and beta PS integrin subunit genes have distinct phenotypes. *Development* 120, 1221–1231.

Brown, N.H., Gregory, S.L., and Martin-Bermudo, M.D. (2000). Integrins as mediators of morphogenesis in *Drosophila*. *Dev. Biol.* 223, 1–16.

Byars, C.L., Bates, K.L., and Letsou, A. (1999). The dorsal-open group gene raw is required for restricted DJNK signaling during closure. *Development* 126, 4913–4923.

Chen, H.W., Marinissen, M.J., Oh, S.W., Chen, X., Melnick, M., Perrimon, N., Gutkind, J.S., and Hou, S.X. (2002). CKA, a novel multidomain protein, regulates the JUN N-terminal kinase signal transduction pathway in *Drosophila*. *Mol. Cell Biol.* 22, 1792–1803.

Colas, J.F., and Schoenwolf, G.C. (2001). Towards a cellular and molecular understanding of neurulation. *Dev. Dyn.* 221, 117–145.

Dan, I., Watanabe, N.M., and Kusumi, A. (2001). The Ste20 group kinases as regulators of MAP kinase cascades. *Trends Cell Biol.* 11, 220–230.

Danjo, Y., and Gipson, I.K. (1998). Actin “purse string” filaments are anchored by E-cadherin-mediated adherens junctions at the leading edge of the epithelial wound, providing coordinated cell movement. *J. Cell Sci.* 111, 3323–3332.

Erickson, M.R., Galletta, B.J., and Abmayr, S.M. (1997). *Drosophila* myoblast city encodes a conserved protein that is essential for myoblast fusion, dorsal closure, and cytoskeletal organization. *J. Cell Biol.* 138, 589–603.

Etienne-Manneville, S., and Hall, A. (2001). Integrin-mediated activation of Cdc42 controls cell polarity in migrating astrocytes through PKCzeta. *Cell* 106, 489–498.

Fehon, R.G., Dawson, I.A., and Artavanis-Tsakonas, S. (1994). A *Drosophila* homologue of membrane-skeleton protein 4.1 is associated with septate junctions and is encoded by the coracle gene. *Development* 120, 545–557.

Fernandez, R., Takahashi, F., Liu, Z., Steward, R., Stein, D., and Stanley, E.R. (2000). The *Drosophila* shark tyrosine kinase is required for embryonic dorsal closure. *Genes Dev.* 14, 604–614.

- Genova, J.L., Jong, S., Camp, J.T., and Fehon, R.G. (2000). Functional analysis of Cdc42 in actin filament assembly, epithelial morphogenesis, and cell signaling during *Drosophila* development. *Dev. Biol.* **221**, 181–194.
- 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.
- Grevengoed, E.E., Loureiro, J.J., Jesse, T.L., and Peifer, M. (2001). Abelson kinase regulates epithelial morphogenesis in *Drosophila*. *J. Cell Biol.* **155**, 1185–1198.
- Hakeda-Suzuki, S., Ng, J., Tzu, J., Dietzl, G., and Sun, Y. Harms, M., Nardine, T., Luo, L., and Dickson, B.J. (2002). Genetic analysis of Rac function and regulation during *Drosophila* development. *Nature* **416**, 438–442.
- Halsell, S.R., Chu, B.I., and Kiehart, D.P. (2000). Genetic analysis demonstrates a direct link between rho signaling and nonmuscle myosin function during *Drosophila* morphogenesis. *Genetics* **155**, 1253–1265.
- 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**, 1896–1908.
- Harden, N., Loh, H.Y., 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.
- Harden, N., Ricos, M., Yee, K., Sanny, J., Langmann, C., Yu, H., Chia, W., and Lim, L. (2002). Drac1 and Crumbs participate in amnioserosa morphogenesis during dorsal closure in *Drosophila*. *J. Cell Sci.* **115**, 2119–2129.
- Hartenstein, V. (1993). *Atlas of Drosophila development* (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press).
- Hildebrand, J.D., and Soriano, P. (1999). Shroom, a PDZ domain-containing actin-binding protein, is required for neural tube morphogenesis in mice. *Cell* **99**, 485–497.
- 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.
- Jacinto, A., Martinez-Arias, A., and Martin, P. (2001). Mechanisms of epithelial fusion and repair. *Nat. Cell Biol.* **3**, E117–123.
- Jacinto, A., Wood, W., Balayo, T., Turmaine, M., Martinez-Arias, A., and Martin, P. (2000). Dynamic actin-based epithelial adhesion and cell matching during *Drosophila* dorsal closure. *Curr. Biol.* **10**, 1420–1426.
- Jacinto, A., Wood, W., Woolner, S., Hiley, C., Turner, L., Wilson, C., Martinez Arias, A., and Martin, P. (2002). Dynamic analysis of actin cable function during *Drosophila* dorsal closure. *Curr. Biol.*, in press.
- Jasper, H., Benes, V., Schwager, C., Sauer, S., Clauder-Munster, S., Ansoerge, W., and Bohmann, D. (2001). The genomic response of the *Drosophila* embryo to JNK signaling. *Dev. Cell* **1**, 579–586.
- Karess, R.E., Chang, X.J., Edwards, K.A., Kulkarni, S., Aguilera, I., and Kiehart, D.P. (1991). The regulatory light chain of nonmuscle myosin is encoded by spaghetti-squash, a gene required for cytokinesis in *Drosophila*. *Cell* **65**, 1177–1189.
- Kiehart, D.P., Galbraith, C.G., Edwards, K.A., Rickoll, W.L., and Montague, R.A. (2000). Multiple forces contribute to cell sheet morphogenesis for dorsal closure in *Drosophila*. *J. Cell Biol.* **149**, 471–490.
- Kimura, K., Ito, M., Amano, M., Chihara, K., Fukata, Y., Nakafuku, M., Yamamori, B., Feng, J., Nakano, T., Okawa, K., et al. (1996). Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase). *Science* **273**, 245–248.
- 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.
- 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.
- Liu, J., Prickett, T.D., Elliott, E., Meroni, G., and Brautigan, D.L. (2001). Phosphorylation and microtubule association of the Opitz syndrome protein mid-1 is regulated by protein phosphatase 2A via binding to the regulatory subunit alpha 4. *Proc. Natl. Acad. Sci. USA* **98**, 6650–6655.
- 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.
- Machesky, L.M., and Insall, R.H. (1999). Signaling to actin dynamics. *J. Cell Biol.* **146**, 267–272.
- Magie, C.R., Meyer, M.R., Gorsuch, M.S., and Parkhurst, S.M. (1999). Mutations in the Rho1 small GTPase disrupt morphogenesis and segmentation during early *Drosophila* development. *Development* **126**, 5353–5364.
- Magie, C.R., Pinto-Santini, D., and Parkhurst, S.M. (2002). Rho1 interacts with p120^{cas} and α -catenin, and regulates cadherin-based adherens junction formation in *Drosophila*. *Development*, in press.
- Martin, P. (1997). Wound healing—aiming for perfect skin regeneration. *Science* **276**, 75–81.
- Martin, P., and Lewis, J. (1992). Actin cables and epidermal movement in embryonic wound healing. *Nature* **360**, 179–183.
- 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*, A.M.-A. and M. Bate, eds. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press), pp. 517–607.
- Mizuno, T., Tsutsui, K., and Nishida, Y. (2002). *Drosophila* myosin phosphatase and its role in dorsal closure. *Development* **129**, 1215–1223.
- Morin, X., Daneman, R., Zavortink, M., and Chia, W. (2001). A protein trap strategy to detect GFP-tagged proteins expressed from their endogenous loci in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **98**, 15050–15055.
- Morriss-Kay, G., and Tuckett, F. (1985). The role of microfilaments in cranial neurulation in rat embryos: effects of short-term exposure to cytochalasin D. *J. Embryol. Exp. Morphol.* **88**, 333–348.
- Nolan, K.M., Barrett, K., Lu, Y., Hu, K.Q., Vincent, S., and Settleman, J. (1998). Myoblast city, the *Drosophila* homolog of DOCK180/CED-5, is required in a Rac signaling pathway utilized for multiple developmental processes. *Genes Dev.* **12**, 3337–3342.
- 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.
- Raich, W.B., Agbunag, C., and Hardin, J. (1999). Rapid epithelial-sheet sealing in the *Caenorhabditis elegans* embryo requires cadherin-dependent filopodial priming. *Curr. Biol.* **9**, 1139–1146.
- Ramet, M., Lanot, R., Zachary, D., and Manfrulli, P. (2002). JNK signaling pathway is required for efficient wound healing in *Drosophila*. *Dev. Biol.* **241**, 145–156.
- Reed, B.H., Wilk, R., and Lipshitz, H.D. (2001). Downregulation of Jun kinase signaling in the amnioserosa is essential for dorsal closure of the *Drosophila* embryo. *Curr. Biol.* **11**, 1098–1108.
- Ricos, M.G., Harden, N., Sem, K.P., Lim, L., and Chia, W. (1999). Dcdc42 acts in TGF-beta signaling during *Drosophila* morphogenesis: distinct roles for the Drac1/JNK and Dcdc42/TGF-beta cascades in cytoskeletal regulation. *J. Cell Sci.* **112**, 1225–1235.

- Ridley, A.J., and Hall, A. (1992). The small GTP-binding protein rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors. *Cell* **70**, 389–399.
- 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. *Dev Suppl*, 251–259.
- Roy, P.J., Zheng, H., Warren, C.E., and Culotti, J.G. (2000). mab-20 encodes Semaphorin-2a and is required to prevent ectopic cell contacts during epidermal morphogenesis in *Caenorhabditis elegans*. *Development* **127**, 755–767.
- Rugendorff, A., Younossi-Hartenstein, A., and Hartenstein, V. (1994). Embryonic origin and differentiation of the *Drosophila* heart. *Roux's Arch. Dev. Biol.* **203**, 266–280.
- Sabapathy, K., Jochum, W., Hochedlinger, K., Chang, L., Karin, M., and Wagner, E.F. (1999). Defective neural tube morphogenesis and altered apoptosis in the absence of both JNK1 and JNK2. *Mech. Dev.* **89**, 115–124.
- Schoenwolf, G.C., and Powers, M.L. (1987). Shaping of the chick neuroepithelium during primary and secondary neurulation: role of cell elongation. *Anat. Rec.* **218**, 182–195.
- Settleman, J. (2001). Rac 'n Rho: the music that shapes a developing embryo. *Dev. Cell* **1**, 321–331.
- 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., Goberdhan, D.C.I., Wilson, C., 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.
- Stark, K.A., Yee, G.H., Roote, C.E., Williams, E.L., Zusman, S., and Hynes, R.O. (1997). A novel alpha integrin subunit associates with betaPS and functions in tissue morphogenesis and movement during *Drosophila* development. *Development* **124**, 4583–4594.
- Stronach, B., and Perrimon, N. (2002). Activation of the JNK pathway during dorsal closure in *Drosophila* requires the mixed lineage kinase, slipper. *Genes Dev.* **16**, 377–387.
- Stronach, B.E., and Perrimon, N. (1999). Stress signaling in *Drosophila*. *Oncogene* **18**, 6172–6182.
- Stronach, B.E., and Perrimon, N. (2001). Investigation of leading edge formation at the interface of amnioserosa and dorsal ectoderm in the *Drosophila* embryo. *Development* **128**, 2905–2913.
- Su, Y.C., Maurel-Zaffran, C., Treisman, J.E., and Skolnik, E.Y. (2000). The Ste20 kinase misshapen regulates both photoreceptor axon targeting and dorsal closure, acting downstream of distinct signals. *Mol. Cell. Biol.* **20**, 4736–4744.
- 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.
- 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.
- Tateno, M., Nishida, Y., and Adachi-Yamada, T. (2000). Regulation of JNK by Src during *Drosophila* development. *Science* **287**, 324–327.
- Taya, Y., O'Kane, S., and Ferguson, M.W. (1999). Pathogenesis of cleft palate in TGF-beta3 knockout mice. *Development* **126**, 3869–3879.
- Vasioukhin, V., Bauer, C., Yin, M., and Fuchs, E. (2000). Directed actin polymerization is the driving force for epithelial cell-cell adhesion. *Cell* **100**, 209–219.
- Watanabe, N., Kato, T., Fujita, A., Ishizaki, T., and Narumiya, S. (1999). Cooperation between mDia1 and ROCK in Rho-induced actin reorganization. *Nat. Cell Biol.* **1**, 136–143.
- Wei, L., Roberts, W., Wang, L., Yamada, M., Zhang, S., Zhao, Z., Rivkees, S.A., Schwartz, R.J., and Imanaka-Yoshida, K. (2001). Rho kinases play an obligatory role in vertebrate embryonic organogenesis. *Development* **128**, 2953–2962.
- 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.
- Zallen, J.A., Cohen, Y., Hudson, A.M., Cooley, L., Wieschaus, E., and Schejter, E.D. (2002). SCAR is a primary regulator of Arp2/3-dependent morphological events in *Drosophila*. *J. Cell Biol.* **156**, 689–701.
- Zecchini, V., Brennan, K., and Martinez-Arias, A. (1999). An activity of Notch regulates JNK signalling and affects dorsal closure in *Drosophila*. *Curr. Biol.* **9**, 460–469.
- 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.