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Cell Sheet Morphogenesis: Dorsal Closure in *Drosophila melanogaster* as a Model System

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Abstract

Dorsal closure is a key process during *Drosophila* morphogenesis that models cell sheet movements in chordates, including neural tube closure, palate formation, and wound healing. Closure occurs midway through embryogenesis and entails circumferential elongation of lateral epidermal cell sheets that close a dorsal hole filled with amnioserosa cells. Signaling pathways regulate the function of cellular structures and processes, including Actomyosin and microtubule cytoskeletons, cell-cell/cell-matrix adhesion complexes, and endocytosis/vesicle trafficking. These orchestrate complex shape changes and movements that entail interactions between five distinct cell types. Genetic and laser perturbation studies establish that closure is robust, resilient, and the consequence of redundancy that contributes to four distinct biophysical processes: contraction of the amnioserosa, contraction of supracellular Actomyosin cables, elongation (stretching?) of the lateral epidermis, and zipping together of two converging cell sheets. What triggers closure and what the emergent properties are that give rise to its extraordinary resilience and fidelity remain key, extant questions.

Keywords

biomechanics; morphogenetics; amnioserosa; ingression; oscillation; Actomyosin

INTRODUCTION

Morphogenesis

Morphogenesis—the development of cell, tissue, organ, and organismal form—can be viewed as one of the final frontiers in developmental biology. Early developmental events, some during oogenesis, pattern the embryo. Such patterning coordinates cell proliferation

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with specification and the establishment of cell fates. Screens for genes expressed maternally, zygotically, or both, coupled with classical embryology and modern molecular genetic and reverse genetic approaches in model systems, have helped establish the molecular mechanisms by which anterior/posterior (A/P), dorsal/ventral (D/V), and left/right axes are specified. Such approaches have also provided key insight into how cascades of gene expression coupled with sophisticated and interacting signaling pathways further specify how cells are determined and differentiate. Ultimately, the transcriptional identity of cells lays the groundwork for morphogenesis by ensuring that cells are equipped to change shape and move in the choreographed ways that result in development through intermediate stages to adult form. The molecular and cellular mechanisms that drive such changes in form constitute morphogenesis.

Which Morphogenetic Movements Does Dorsal Closure Model?

The dorsal closure stage of embryogenesis in *Drosophila melanogaster* serves as a key model system for cell sheet morphogenesis in chordates. Perhaps the morphogenetic movement most akin to closure is found in *Ciona intestinalis*, an ascidian in which neural tube closure involves the Cadherin-dependent fusion of neural tube folds in a zipping process driven by apical, Actomyosin-mediated contractions (Hashimoto et al. 2015). Movements comparable to dorsal closure in vertebrates include neural tube closure, palate formation, heart closure, and epiboly (Ray & Niswander 2012, Heisenberg & Bellaiche 2013). These movements do not precisely parallel closure; nevertheless, many of the key cell shape changes and signaling cascades are recapitulated in closure. In each case, flanking epidermal sheets migrate and/or change their overall shape to form a continuous epithelium. Finally, several movements originally thought to involve the migration of single cells frequently entail the migration of cell clusters or sheets, e.g., the migration of metastatic cancer cells and of neural crest cells (Martin & Wood 2002, Belacortu & Paricio 2011, Heisenberg & Bellaiche 2013, Pocha & Montell 2014, Cai et al. 2016).

Dorsal Closure in a Nutshell

At the onset of dorsal closure, the overall shape of the embryo is an irregular prolate ellipsoid. The germ band consists of a ventral, lateral, and dorsal epithelium topologically shaped much like the hull of a canoe or kayak (Turner & Mahowald 1979, Tomer et al. 2012, Keller 2013). A dorsal hole or opening in the epithelium is filled by a one-cell-thick sheet of thin squamous cells: the amnioserosa. The large, flat, initially isodiametric cells of the amnioserosa are easily distinguished from smaller, elongated cells that characterize the lateral epidermis. The amnioserosa, which fills the dorsal hole, contracts to provide force(s) for closure as its cells ingress below the advancing epidermal cell sheets and undergo apoptosis. During closure, the two lateral epidermal cell sheets include prominent Actomyosin-rich purse strings or cables near their leading edges that also contribute forces for closure. Initially, in Kiehart et al. (2000), the purse string was referred to in the singular, but the morphology of the canthi, the corners of the eye-shaped opening, suggests that two purse strings, one in each leading edge, contribute to closure. Together, the applied forces drive the circumferential elongation (or stretching?) of the lateral epidermis dorsally until the two flanking cell sheets meet in a process devoid of both cell proliferation and convergent extension. Zipping (aka zippering) at the canthi ensues such that first a seamed

epithelium and then a seamless epithelium is formed. Topologically, the canoe-shaped epithelium morphs into a submarine (Figure 1 and Supplemental Video 1, reproduced with permission from Tomer et al. 2012).

Why Is Dorsal Closure a Key Model System?

Several key features of closure establish its utility as a model system. GFP-tagged proteins are used to follow morphogenetic events, which occur mostly within 15 μm of the surface of the embryo (e.g., Edwards et al. 1997, Jacinto et al. 2000, Kiehart et al. 2000, Oda & Tsukita 2001). Fortunately, living *Drosophila* embryos can be imaged using high-resolution laser scanning microscopy, spinning disk confocal microscopy, or light sheet microscopy for hours, indicating that phototoxicity is not a major problem. The ability to image living embryos allows for a detailed description of cell shape changes and tissue movements, i.e., the kinematics of morphogenesis (e.g., Edwards et al. 1997, Jacinto et al. 2000, Kiehart et al. 2000, Keller 2013, Chen et al. 2014, Stegmaier et al. 2016). Sophisticated methods for automated image segmentation applied to time-lapsed records of closure in 3D readily provide digital representations of the 4D kinematics of closure (e.g., Blanchard et al. 2009, Wells et al. 2014, Barbier de Reuille et al. 2015, Heemskerk & Streichan 2015, Stegmaier et al. 2016, Zuo & Tomasi 2016). The favorable topology and optical qualities of closure, coupled with a wide variety of fluorescent contrast agents and segmentation methods, make closure amenable to biophysical analysis using laser or mechanical probes (e.g., Kiehart et al. 2000, Hutson et al. 2003, Solon et al. 2009, Saias et al. 2015). From such studies, biophysical, mathematical, and computational models can be generated and then validated or rejected (e.g., Peralta et al. 2007, Rauzi et al. 2008, Layton et al. 2009, Ma et al. 2009, Meghana et al. 2011, Fischer et al. 2014). Systematic investigation of closure using laser perturbations to introduce mechanical jumps probes the biomechanical mechanisms of closure and provides understanding of how cytoskeletal forces and remodeling of adhesions lead to the cell shape changes and movements that characterize morphogenesis (i.e., that establish the underlying dynamics of closure). Pharmacological analyses further probe the molecular mechanisms via microinjection (e.g., Jankovics & Brunner 2006) or via application to embryos removed from their vitelline envelopes (Mateus & Martinez Arias 2011). Classical genetic methods and more modern, molecular genetic methods coupled with a relatively small genome (~15,000 genes encoded by $\sim 1.5 \times 10^8$ bp of haploid genome) make *D. melanogaster* among the most genetically tractable metazoan model organisms for gene discovery and manipulation (Jürgens et al. 1984, Nüsslein-Volhard et al. 1984, Wieschaus et al. 1984, Campos et al. 2010, Jankovics et al. 2011, Rousset et al. 2017). Collectively, FlyBase [the database of *Drosophila* genes and genomes (<http://flybase.org>); Attrill et al. 2016] and the literature currently identify more than 140 dorsal closure genes, mutations in which result in defects during closure. Most dorsal closure genes encode proteins that define cytoskeletal structure and function, that participate in adhesion, or that contribute to signaling pathways. New dorsal closure genes are being identified at a remarkable rate. In addition, most have homologs known to participate in morphogenesis and wound healing in vertebrates. Finally, the *Drosophila* research community includes innovators in developing new strategies to manipulate gene expression and protein function. For example, most recently, a new method to rapidly deplete embryos of GFP-tagged proteins was applied to closure for proof of principle and was followed by a detailed study

(described below) that calls into question key models for the molecular mechanisms of closure (Caussinus et al. 2012, Pasakarnis et al. 2016).

Dorsal Closure Today

Excellent reviews provide global summaries of dorsal closure (Harden 2002, Jacinto et al. 2002b, Gorfinkiel et al. 2011, Hayes & Solon 2017). Relatively recent reviews also focus on specific aspects of closure, including signaling events (Rios-Barrera & Riesgo-Escovar 2013), the amnioserosa (Lacy & Hutson 2016), oscillations and contraction (Martin & Goldstein 2014, Gorfinkiel 2016), planar cell polarity (Munoz-Soriano et al. 2012), adherens junctions (Harris 2012, Roper 2015), embryonic patterning and the cytoskeleton (Harris et al. 2009), and numerous cytoskeletal components (Harris et al. 2009). Other reviews address closure's relationship to wound healing (Heisenberg 2009, Belacortu & Paricio 2011, Razzell et al. 2014, Begnaud et al. 2016); to other cell sheet movements (Pocha & Montell 2014); and to head involution, a temporally overlapping morphogenetic process (VanHook & Letsou 2008). Here, we focus on the kinematics and dynamics of various cell shape changes and movements required for closure with respect to the molecular and biophysical interplay within and between cells and tissues that accounts for closure. We conclude that dorsal closure will remain an exciting, valuable, and informative model system for years to come.

MORPHOGENESIS DURING CLOSURE

We consider four somewhat overlapping stages of closure. The preclosure stage includes various cell shape changes and movements during germ band retraction (GBR) and the gap period that ensues. Both set the stage for closure. The onset-of-closure stage begins when the leading edge of the lateral epidermis begins concerted displacement toward the dorsal midline. The bulk-of-closure stage follows, during which the eye-shaped dorsal opening shrinks dramatically. Finally, the endgame-of-closure stage creates a seamed and then seamless epithelium as the dorsal hole is closed and the amnioserosa cells ingress and undergo apoptosis (a process that starts earlier in closure). Dorsal closure requires approximately 2.5–3 h to complete (at 25°C).

Preclosure: Setting the Stage for Closure

Key cell shape changes and movements set the stage for dorsal closure and occur before and during GBR (Schock & Perrimon 2002, Lacy & Hutson 2016). During germ band extension (before GBR), cell intercalations and elongation, asymmetric distributions of polarity and cytoskeletal components, contraction of the Actomyosin cytoskeleton, and active remodeling of adherens junctions and cell membranes through endocytic and exocytic processes contribute to the morphogenetic movements that set the stage for closure (reviewed in Gorfinkiel 2016, Lacy & Hutson 2016, Kong et al. 2017). During GBR, the germ band retracts posteriorly, the amnioserosa makes intimate contact with the exposed yolk (Narasimha & Brown 2004, Reed et al. 2004), and squamous amnioserosa cells assume an isometric morphology (Schock & Perrimon 2002, Pope & Harris 2008, Lynch et al. 2013). Amnioserosa cells also begin to oscillate or pulsate (Solon et al. 2009, Sokolow et al. 2012, Gorfinkiel 2016). The dorsal-most epidermal (DME) cells of the lateral epidermis cells begin to elongate (Young et al. 1993, Glise et al. 1995, Kaltschmidt et al. 2002, Schock &

Perrimon 2002, Morel & Martinez Arias 2004, Houssin et al. 2015), and microtubules in the DME cells align (Kaltschmidt et al. 2002, Jankovics & Brunner 2006). Several of the same players and processes involved in germ band extension and GBR also have important roles in dorsal closure. For example, mutations in the transcriptional regulator *u-shaped (ush)* [the expression of which is activated by Dpp, a ligand of the TGF/BMP superfamily (Frank & Rushlow 1996, Lada et al. 2012)], in the adhesion molecule Integrin, or in the extracellular matrix (ECM) protein Laminin [both Integrin and Laminin are regulated by JNK (Narasimha & Brown 2004, Sorrosal et al. 2010)] lead to failures in aspects of both GBR and dorsal closure.

The role of Integrins in pre-dorsal closure tissue alignment

During GBR, the interactions at the interface between cells and the ECM require the binding of Integrin transmembrane receptors to Laminins and other components of the ECM to maintain both a polarized epithelium and tissue adhesion (Narasimha & Brown 2004, Meghana et al. 2011). As the germ band retracts, the amnioserosa is separated from the yolk by a fluid-filled cavity. Integrins, expressed in the yolk, the amnioserosa, and the lateral epidermis (Narasimha & Brown 2004), mediate interactions between the amnioserosa and the yolk. Initially, such interactions entail exploratory filopodial projections that are unlikely to produce or transmit force, but they may play a role in Integrin-coupled ion channels for signal transduction (Heckman & Plummer 2013). Ultimately, Integrin/ECM interactions drive the close apposition of the amnioserosa with the surface of the yolk with its Actomyosin-rich cortex (Figure 2; see Supplemental Video 2, reproduced with permission from Reed et al. 2004), which is likely to provide structural stability to the otherwise thin and flexible amnioserosa cell sheet. The amnioserosa and the yolk to which it is tightly adhered bulge outward in a remarkably smooth, asymmetric dome: Quantitative analysis indicates that the amnioserosa has uniform and isotropic surface tension (Lu et al. 2016).

Embryo morphology at the onset of closure

During the course of GBR, the posterior end of the embryo, which has extended anteriorly by ~65–70% (to within ~160 μm of the anterior end of the ~450–500- μm -long embryo), retracts, exposing a dorsal hole filled with amnioserosa cells that encompasses ~40% of the circumference of the embryo when measured at a point midway between the anterior and posterior ends of the dorsal opening (Campos-Ortega & Hartenstein 1997, Wieschaus & Nüsslein-Volhard 1998, Lacy & Hutson 2016). At this early stage, the anterior end of the dorsal opening is blunt and lies adjacent to the first thoracic segment (Figure 3), whereas the posterior end is rounded and lies in the eighth abdominal segment. A 1–1.5-h gap follows the end of GBR (which occurs ~9 h and 20 min after fertilization at 25°C). The onset of closure begins with the first, concerted, dorsalward movement of the DME cells that line the perimeter of the exposed amnioserosa. This leading edge perimeter is initially scalloped, but with time, the scalloped edge accumulates F-Actin and Myosin II in purse string structures and resolves itself into a smooth arc, suggesting that Actomyosin contractility plays a key role in the shape transformation of the leading edge of the dorsal opening (Figure 4; panels taken from Supplemental Video 3, from previously unpublished material by R. Montague, V. Williams & D. Kiehart; see also Kiehart et al. 2000).

Five distinct cell types are involved in closure

Five distinct cell types are intimately involved in closure: the underlying yolk and, from ventral to dorsal around the circumference of the embryo, the lateral epidermis, the DME cells, the peripheral-most amnioserosa (PAS) cells, and the cells of the bulk of the amnioserosa. The ventral epidermis plays little or no direct role in closure (Kiehart et al. 2000). The five cell types are distinct from each other, both transcriptionally and behaviorally. The DME cells are identified as cells in mitotic domain 19 [and therefore acquire a unique identity early, at ~4 h into development (Foe 1989)]; uniquely express a Gal4 driver (Glise & Noselli 1997); and are characterized by an Actomyosin-rich, supracellular purse string or cable that runs parallel to their leading edges. The DME cells also respond to various signaling gradients along the A/P axis (JNK, segmentation, and A/P signaling pathways) so that each DME cell is transcriptionally distinct from its neighbor (Perrimon & Desplan 1994, Mallo & Alonso 2013, Rousset et al. 2017). The PAS cells are also transcriptionally and behaviorally distinct from the remainder of the amnioserosa cells [a Gal4 driver is expressed solely in the PAS cells (Wada et al. 2007)].

Integrins mediate the reorientation of the interface between DME cells and PAS cells

Early in closure, there is a dramatic reorganization of the PAS/DME interface, and this event is mediated by Integrins (Figure 2). During GBR, the DME and PAS cells lie juxtaposed to one another in a curved surface, with lateral cell margins between the two cell types oriented perpendicular to the surface of the embryo (Narasimha & Brown 2004). As closure begins, these lateral margins become extended such that the DME and the PAS become reciprocally wedge shaped; consequently, the adhesive surface area shared by the two types of cells is dramatically increased (Figure 2). Typically, each PAS cell lies under three to five DME cells (measured along the A/P axis) and extends approximately the length of the DME cells (measured along a D/V circumference). These two cell types are also joined by adherens junctions (Kaltschmidt et al. 2002). Similarly, the row of DME cells extends approximately over the row of PAS cells. In embryos that lack the zygotic expression of Integrin, these changes fail to occur, the junctions remain perpendicular to the embryo's surface, and adhesion is limited largely to the thin band of adherens junctions mediated by Cadherins (Figure 2). As a result, the normal thickening of amnioserosa cells that occurs as closure progresses fails to transpire (Narasimha & Brown 2004, Reed et al. 2004).

The Onset of Closure

Approximately 1–1.5 h after the end of GBR, the concerted displacement of the leading edge of the lateral epidermis toward the dorsal midline begins concomitantly with purse string maturation and the formation of canthi. The DME cells move toward the dorsal midline because the lateral epidermis elongates circumferentially and sequentially: First the DME cells and then more lateral epidermal cells elongate (Young et al. 1993, Jacinto et al. 2002a). The DME cells do not crawl over a substrate that is the amnioserosa (Figure 5 and Supplemental Video 4, reproduced with permission from Rodriguez-Diaz et al. 2008); instead, they maintain an essentially fixed relationship with the PAS cells as they translate toward the dorsal midline (Wada et al. 2007, Rodriguez-Diaz et al. 2008, Lu et al. 2015). They “migrate over” the amnioserosa only in the sense that, by the end of closure, the DME

cells from both lateral flanks have met at the dorsal midline and the amnioserosa cells have been internalized and have undergone apoptosis.

As closure proceeds, the dorsal opening becomes eye shaped as canthi and seams form at both the blunt anterior end and the rounded posterior end (Figure 3). At the anterior end, the flattened end of the purse string shortens until a canthus and a nascent seam are formed. A purse string morphs into two when the flattened end of the purse string contracts to a point on the dorsal midline. At the posterior end, the gently curved epidermis also morphs into a canthus with a nascent seam. The different morphologies of the two canthi suggest that the mechanisms for their formation differ in detail and are in any event unknown. In addition, closure at the anterior end occurs over the yolk and is impacted by the movements of head involution, which begins during closure (VanHook & Letsou 2008). In contrast, at the posterior end, the hindgut elongates between the yolk and the amnioserosa, and such elongation is coordinated with the morphogenesis of the spiracle mounds (Manning & Krasnow 1993). At approximately the same time at which the canthi form, cellular projections (filopodia and lamellipodia) become abundant all along the leading edge of the DME cells (Jacinto et al. 2000, 2002a; Eltsov et al. 2015). As closure proceeds, the eye-shaped dorsal opening closes through coordinated shrinkage in both the height and width of the dorsal opening as the amnioserosa thickens and amnioserosa cells begin to become internalized (see below).

The Bulk of Closure

Following the formation of the anterior and posterior canthi, zipping of the lateral epidermal sheets coordinates changes in the height and width of the dorsal opening. During the bulk of closure, both the rate of change in the height of the opening and the radius of curvature of the advancing epidermal cell sheets are essentially constant. As the amnioserosa cells continue to oscillate and ingress, the lateral epidermal sheets at either end of the dorsal opening meet at the dorsal midline. An initially seamed epithelium subsequently transitions into a seamless one.

Actomyosin provides forces for closure

In fly, two genes encode nonmuscle Actins (*Act5c* and *Act42A*; Fyrberg et al. 1983), but their function in dorsal closure has not been examined genetically. Single genes *zipper*, *spaghetti squash*, and *Mlc-c* encode the heavy chain, the regulatory light chain, and the essential light chain subunits of the nonmuscle Myosin II hexamer (Kiehart & Feghali 1986, Kiehart et al. 1989, Karess et al. 1991, Edwards et al. 1995). *zipper* null alleles fail in dorsal closure, whereas *spaghetti squash* null alleles survive until the larval stages, suggesting that maternal loads of the regulatory light chain are more extensive than those of the heavy chain (Karess et al. 1991, Young et al. 1993, Jordan & Karess 1997). The function of *Mlc-c* in dorsal closure has not been examined genetically. Multiple spliceforms of *zipper* encode nine heavy chain variants [FlyBase (the database of *Drosophila* genes and genomes; <http://flybase.org>), Mansfield et al. 1996]. Native Myosin forms small bipolar filaments that are assumed to be essential for Actomyosin function (Kiehart & Feghali 1986, S.L. Liu et al. 2008, Ricketson et al. 2010, Vasquez et al. 2016). Recent in vitro characterization of fly nonmuscle Myosin II function demonstrates that its Actin-activated ATPase activity and in

vitro motility are comparable to those of vertebrate nonmuscle Myosin II paralogs (Heissler et al. 2015, Vasquez et al. 2016).

Regulation of F-Actin assembly, Myosin II filament formation, and Actomyosin function are essential for choreographing the various contractile events that culminate in closure. Force production for normal closure requires the function of at least three distinct Actomyosin assemblies: the purse string segments in the DME cells and the junctional belts and medioapical arrays in the amnioserosa cells. The supracellular purse string near the leading edges of the DME cells consists of contractile elements in individual DME cells that are linked to each other by adherens junctions to provide structural integrity and a mechanical circuit (Kiehart et al. 2000, Franke et al. 2005, Gorfinkiel & Martinez-Arias 2007, Harris 2012). Cells in the amnioserosa are characterized by junctional belts of Actomyosin, transiently formed medioapical arrays of Actomyosin, and Actin-rich lamellipodia and filopodia, which are most prominent at cell junctions. Laser dissection has established that all three of these Actomyosin structures are under tension (Kiehart et al. 2000, Hutson et al. 2003, Ma et al. 2009, Solon et al. 2009, Jayasinghe et al. 2013, Saravanan et al. 2013, Fischer et al. 2014, Saias et al. 2015) and that tension in each structure appears to increase as closure proceeds. Cells in both tissues (and, as far as we know, in all other tissues in the fly) also have a thin Actomyosin cortex that characterizes virtually all animal cells, but whether these generic cortical structures play a unique role in closure is not known. The mechanisms by which these various Actomyosin arrays are coordinated in space and time to contribute to seamless closure have been under intense study.

Purse strings

The tensions of the two contractile purse strings were demonstrated both in laser ablation studies that correlated tension with tissue recoil and in the lack of contractility observed when the purse strings were depleted of Myosin (Kiehart et al. 2000, Hutson et al. 2003, Franke et al. 2005, Rodriguez-Diaz et al. 2008, Wells et al. 2014, Ducuing & Vincent 2016, Pasakarnis et al. 2016). Although the canthi are required to maintain the curvature of the purse strings and the advancing lateral epidermal sheets, closure can still proceed when the canthi are removed by laser microsurgery and the purse strings relax into two nearly parallel fronts (Wells et al. 2014). However, the end stages of closure are slowed, consistent with the possible role of a functional purse string, of zipping at the canthi, and/or of canthus function(s) that helps remove amnioserosa cells for efficient closure. Whether the purse strings function as a ratchet has been the source of some controversy (Hutson et al. 2003, Peralta et al. 2007, Solon et al. 2009, Blanchard et al. 2010, David et al. 2010, Mason & Martin 2011, Sokolow et al. 2012, Wells et al. 2014, Jurado et al. 2016). Significantly, the function of the purse string during dorsal closure is recapitulated in wound healing processes: An Actin-based contractile purse string also forms in the leading edge cells of wound margins in vertebrate systems (Martin & Lewis 1992), and when the formation of the Actin cable is disrupted, closure of the wound is slowed (Wood et al. 2002).

The purse strings are zipped into each canthus and shorten as closure progresses while a small number of cells are added to the leading edge at segment boundaries, potentially to reduce tension and to help avoid patterning defects and ruptures at the segment boundaries

(Peralta et al. 2008, Gettings et al. 2010, Gettings & Noselli 2011). The width of the DME cells in the free leading edge oscillates reversibly about the average value of 2.6 μm (Peralta et al. 2008). These oscillations are reminiscent of the oscillations that characterize amnioserosa cells (see below). Net shortening of the purse string and the width of a DME cell occurs as part of the zipping process, i.e., when a DME cell is zipped into the seam at a canthus. We speculate that the material (i.e., the mechanical) properties of the DME cells change as the DME cells engage in the zipping process in the region of a canthus (Lu et al. 2015).

Two recent studies call into question the contributions of the purse string as a force producer for closure (Ducuing & Vincent 2016, Pasakarnis et al. 2016). In embryos that depend on a GFP-tagged Myosin regulatory light chain for Myosin II function, deGradFP strategies that target GFP-tagged proteins to the proteasome (Causinus et al. 2012) and compromise Myosin function confirm that Myosin II function is necessary for closure. Dorsal closure was blocked when deGradFP strategies were used to acutely deplete Myosin in the amnioserosa, and closure was impaired when Myosin was depleted from the leading edges of the epidermal cells. When Myosin was depleted in the amnioserosa, closure failed, and the data show that the geometry of the dorsal opening is aberrant (canthi did not form; see figure 1*b* of Pasakarnis et al. 2016). Because Myosin depletion in the amnioserosa occurred prior to canthus formation, purse string curvature could not be maintained, and closure based on purse string contractility would not be expected to proceed. It would be informative to study the depletion of Myosin in the amnioserosa after the canthi have formed, thereby inhibiting only one force-producing structure instead of two. Moreover, embryos in which Myosin was depleted only in the leading edge of the lateral epidermis were abnormal: 27% had severe puckers and 41% showed rupture at their anterior ends. In addition, the lack of functional Myosin in the leading edge led to a late onset of closure, to a slower closure rate, and to hyperextended lamellipodial and filopodial projections from the leading edge. Both canthi formation and zipping were also severely disrupted. Overall, the data presented are quantitatively consistent with the purse strings providing ~22–25% of the force for closure in native embryos (Hutson et al. 2003, Peralta et al. 2007). (Here and elsewhere, native refers to embryos that have not been subjected to laser cuts or pharmacological perturbation. Thus, native embryos can be either wild type or mutant.)

In a second study to question the contributions of the purse string, closure in embryos homozygous for mutations in *Zasp52*, a gene whose product is required for normal purse string formation, was also investigated (Ducuing & Vincent 2016). The authors of this study assert that the kinematics of closure were unperturbed in the presence of a disrupted purse string, with defects in the morphology of the leading edge. Nevertheless, upon removal of the amnioserosa with laser dissection, these authors' data indicate that closure with a normal purse string occurs at approximately twice the rate of closure with a *Zasp52* mutant purse string (see figure 3*e,f* in Ducuing & Vincent 2016). The authors also point out that mixer cells can lack a segment of cable, which they interpret as showing that the cable is not essential for the leading edge, at least over short distances. Previous studies established that laser cuts on single DME cells gave rise to limited recoil of the leading edge (see figure 7*b* in Kiehart et al. 2006), with the inference that the overall structure of the lateral epidermis and the amnioserosa can compensate for small lesions in the purse string. Furthermore,

when Myosin is reduced in short segments of the purse string as in transgenic mosaic animals, in which amnioserosa tissue also lacks a full complement of Myosin, the effects on the integrity of the leading edge are much more severe (see figure 3 in Franke et al. 2005). A more detailed analysis of closure in these *Zasp52* mutant embryos is required before the contributions of the purse strings can be discounted. Ultimately, both genetic and physical perturbations of closure are likely to invoke stress responses that may rescue one or another aspect of perturbed closure. Careful analysis of both kinds of lesions will be essential for an accurate understanding of closure's mechanisms.

Amnioserosa: oscillations and ingressions

Active and diverse oscillations or pulsations characterize changes in the apical cross sections of the amnioserosa cells that occur on a timescale that is one to two orders of magnitude faster than closure [period of an oscillation ~200 s versus duration of closure at ~9,000 to 10,800 s (Fernandez et al. 2007, Solon et al. 2009, Sokolow 2011, Wells et al. 2014)]. Fluctuating Actomyosin concentrations in both the junctional belts and the medioapical arrays (Blanchard et al. 2010) are linked to cell- and tissue-level deformations (Gorfinkiel & Blanchard 2011, Gorfinkiel et al. 2011, Martin & Goldstein 2014, Gorfinkiel 2016, Coravos et al. 2017). The pulsed apical cell deformations are also affected by the interplay of Cadherin-based cell-cell adhesions and Integrin-mediated connections with the ECM, the latter of which provides a mechanical coupling of the substrate to the apical contractions and cell deformations, thereby damping cell displacements (Goodwin et al. 2016, 2017). Pulsed apical contractions occur at other developmental stages of *Drosophila* embryogenesis [e.g., germ band extension (Martin 2010)] and across phylogeny [e.g., *Caenorhabditis elegans*, *Ciona intestinalis*, and *Mus musculus* gastrulation (Roh-Johnson et al. 2012, Hashimoto et al. 2015, Samarage et al. 2015)]. Such contractions are considered a conserved feature of cell and tissue remodeling (Gorfinkiel 2016). The regulation of Myosin's ATPase activity and motility through phosphorylation and/or the use of phosphomimetics has been characterized in vitro (Vasquez et al. 2016). Myosin activation through regulatory light chain phosphorylation appears to be essential in amnioserosa cells for their transition from a fluid-like composition to more viscoelastic, solid-like behavior as closure progresses (for more on the regulation of Myosin and the link to emergent cell properties, see Saravanan et al. 2013, Fischer et al. 2014, Machado et al. 2015, Duque & Gorfinkiel 2016, Hara et al. 2016, Vasquez et al. 2016). The asymmetric flows of Actomyosin that characterize planar polarization within the cell via interactions with junctions that contain Cadherin, Catenin, and Integrin also contribute to cell sheet morphogenesis (Martin et al. 2010, Rauzi et al. 2010, Levayer & Lecuit 2013, Munjal et al. 2015).

Amnioserosa cells contribute forces to closure, but the bulk of amnioserosa cells (not including the PAS cells) must also get out of the way of the advancing DME/PAS cell complexes so as not to impede closure. For example, mutations in the receptor tyrosine kinase gene *pvr* lead to dorsal midline closure defects and heart defects that correlate with the inability of the hemocytes to clear the amnioserosa corpses (Garlena et al. 2015). Cells in the amnioserosa, which had started oscillating or pulsating during GBR stages, continue to oscillate as they also begin to ingress (or extrude). These cells thicken (radially), shorten (along a circumference of the embryo perpendicular to its A/P axis), and ultimately alter

their curvature from convex to concave (Figures 2d,e and 5). The cells also lose volume. Such volume loss is triggered by caspase activation at the onset of the apoptotic program in a process dependent on K^+ channels (Saia et al. 2015) and may actively facilitate contraction of the amnioserosa. Likewise, mutations that increase apoptosis rates in the amnioserosa increase closure rates, and conversely, mutations that decrease apoptosis rates decrease closure (Toyama et al. 2008, Muliyl et al. 2011).

All amnioserosa cells eventually ingress into the embryo's interior by one of three distinct categories of ingression processes (Kiehart et al. 2000, Toyama et al. 2008, Sokolow et al. 2012). Approximately 10% of the cells in the bulk of the amnioserosa ingress as individual cells that have increased levels of reactive oxygen species (Kiehart et al. 2000, Muliyl & Narasimha 2014). Amnioserosa cells also ingress near each canthus as a part of zipping (Sokolow et al. 2012). Finally, the marginal cells near each leading edge of the lateral epidermis also ingress (Fernandez et al. 2007, Sokolow et al. 2012). Once closure is complete, all amnioserosa cells undergo apoptosis (Rodriguez-Diaz et al. 2008).

Closure: a coordination of signals and forces

The identification of new players in signaling cascades that regulate closure provides a greater understanding of cross talk between cells within each tissue and between cells in the lateral epidermis and the amnioserosa. This signaling cross talk has established vital roles for junctional complexes, endocytosis, vesicle trafficking, ion channels, regulation of cholesterol and steroid hormone synthesis, and mechanosensing linker proteins that connect the cytoskeleton to the membrane to promote polarized distribution of polarity complex proteins (Gilbert 2004, R. Liu et al. 2008, Bhuin & Roy 2012, Enya et al. 2014, Hunter et al. 2014, Houssin et al. 2015, Jodoin et al. 2015, Wei et al. 2015, Duque & Gorfinkel 2016, Hall & Ward 2016, Jurado et al. 2016, West & Harris 2016, Goodwin et al. 2017, Takacs et al. 2017). Here we highlight a few examples.

Adherens junctions are the prime communicator of forces and tension during tissue morphogenesis (reviewed in Harris 2012, Heisenberg & Bellaiche 2013, Roper 2015). The rapid cellular remodeling that occurs during tissue morphogenesis requires that the adherens junctions be properly positioned and be able to transmit tension, but also be sufficiently labile to allow shape change. Rab-mediated trafficking of adhesion complex proteins and the apicobasal polarity components that help position them (i.e., the polarity protein Crumbs) ensures that these complexes are assembled and maintained in the proper positions during closure (Roeth et al. 2009, Levayer & Lecuit 2013, West & Harris 2016). E-Cadherin-based junctional complexes are required to mechanically connect neighboring DME cells and DME/PAS cells; reduced Cadherin levels result in tears between these cells (Gorfinkel & Martinez-Arias 2007). Mutations in the subunits of the adhesion protein Integrin also generate tears between the DME and PAS cells (Hutson et al. 2003, Narasimha & Brown 2004, Homsy et al. 2005, Peralta et al. 2007), but these tears occur in the later stages of dorsal closure.

Recent studies on the role of the Cadherin binding partner α -Catenin find that α -Catenin that lacks an Actin binding domain leads to defects in purse string formation and to tearing of the DME/PAS interface (Jurado et al. 2016). The interdependency between junctional

complexes and the Actomyosin cytoskeleton has been known for some time (reviewed in Roper 2015). Recent progress has begun to identify adaptor proteins that link junctional complexes to the Actin cytoskeleton, thereby strengthening cell-cell adhesion. Girdin is one such adaptor protein that associates with α -Catenin. Without functional Girdin, the D/V elongation of the DME is delayed, the Actomyosin purse string is wavy, and most of the cells located behind the DME do not elongate (Houssin et al. 2015).

Coupling of Actomyosin and adhesion function during morphogenesis also occurs through the function of Crumbs. Apical localization of Crumbs is needed for proper apicobasal polarity in both epidermal and amnioserosa cells (Harden 2002), and Crumbs directly interacts with the cell membrane/Actin binding linker protein Moesin (Wei et al. 2015). Mutations in *crumbs* that prevent this interaction with Moesin result in a failure in dorsal closure due to the overactivity of Actomyosin function in the amnioserosa that leads to uncoordinated contractions. In conjunction with disrupted adherens junctions, the resultant defects in Actomyosin function lead to failure of amnioserosa tissue integrity (Flores-Benitez & Knust 2015).

Recent work has advanced our understanding of the role that Moesin plays in Cadherin-based adhesion (Duque & Gorfinkiel 2016). The Myosin Light Chain Phosphatase (Mbs) regulates both Myosin and Moesin phosphorylation states, thereby regulating these proteins' dynamics. Constitutively active forms of Mbs can inhibit amnioserosa cell oscillations, but closure still progresses to completion. In contrast, reducing Mbs activity results in tears between the DME cells and the PAS cells and in tears within the amnioserosa at sites of ingression. Furthermore, reducing Mbs activity results in Cadherin-based adhesion defects, likely due to the misregulation of both Myosin and Moesin: Myosin contractility affects Actin turnover, which in turn regulates E-Cadherin recruitment to the membrane, where Moesin interacts with Actin. Thus, additional investigation of the linkage between junctional complexes, polarity proteins, and the cytoskeleton will help to elucidate the coordination of these events during closure.

The role that ion channels play in morphogenesis is another area of growing interest. As discussed above, there is a link between the function of K^+ channels and the loss of amnioserosa cell volume during closure (Saia et al. 2015). Likewise, Ca^{2+} signaling is likely to play a role in tissue remodeling. It is a key player in the regulation of Clathrin-mediated endocytosis during wound healing processes, whereby the removal of E-Cadherin from the wound margin allows for the formation of an Actomyosin cable to close the wound margin (Hunter et al. 2015, Zulueta-Coarasa & Fernandez-Gonzalez 2017). Moreover, release of free Ca^{2+} due to UV-mediated uncaging has been tied to contraction of both the DME and amnioserosa cells, suggesting that Ca^{2+} can cause localized contraction in both cell types. Conversely, the chelation of Ca^{2+} slows closure (Hunter et al. 2014). Furthermore, pharmacological inhibition of mechanically gated channels causes increases in cytoplasmic free Ca^{2+} and Actomyosin contractility and, in the long-term, blocks closure in a dose-dependent manner. Embryos homozygous for mutations in channel subunits, *ripped pocket*, or *dtrpA1* (TrpA1) both cause defects in closure and partially phenocopy pharmacological inhibition. Blocking channels leads to defects in force generation via failure of Actomyosin structures and impairs the ability of tissues to regulate forces in response to laser

microsurgery. These results point to a role for ion channels in closure and suggest a mechanism for the coordination of large forces whose vector sum is the small force that drives closure.

The Endgame of Closure

The two converging, contralateral epidermal sheets are zipped together at the anterior and posterior canthi during a zipping process dependent upon filopodia and lamellipodia (Jacinto et al. 2000, Hutson et al. 2003, Peralta et al. 2007, Millard & Martin 2008, Eltsov et al. 2015). In addition to coordinating the curvature of the purse strings, zipping involves a second remodeling of junctions between the DME cells and the PAS cells, as such junctions are replaced by junctions between pairs of DME cells from matching segments in the leading edges of apposing flanks of the lateral epidermis (see Figure 5f; Jacinto et al. 2000, Hutson et al. 2003, Wada et al. 2007, Lu et al. 2015).

Segment alignment

As the lateral epidermal sheets meet at the canthi, Actin-rich filopodia play a key role in aligning these cell sheets during zipping (Jacinto et al. 2000, Gates et al. 2007, Millard & Martin 2008). How the converging filopodia from each cell sheet communicate and engage to ensure that matching segments align correctly is a process that is not well understood but that likely involves gated channel functions (Heckman & Plummer 2013). When signaling, polarity, adhesion, or cytoskeletal proteins are absent or functionally abnormal in the DME cells, the improper alignment of segments during zipping increases relative to wild type (Jacinto et al. 2000, Franke et al. 2005, Jankovics & Brunner 2006, Laplante & Nilson 2006, Gates et al. 2007, R. Liu et al. 2008, Millard & Martin 2008, Stevens et al. 2008, Gettings et al. 2010, Laplante & Nilson 2011, Pickering et al. 2013, Flores-Benitez & Knust 2015, Rousset et al. 2017, Takacs et al. 2017). For example, defects in Myosin II function can result in segment mismatch, but remarkably, cellular alignment within the mismatched segments is essentially perfect. This finding suggests that Myosin II plays a role in moving segments close to one another but that other mechanisms are responsible for aligning individual partner cells within a segment (Franke et al. 2005). The unconventional MyTH-FERM-containing Myosin XV (MyoXV) homolog, encoded by *sisyphus*, is also required for proper segment alignment (R. Liu et al. 2008). Sisyphus cargoes include adhesion, cytoskeletal, and polarity components (e.g., E-Cadherin) and microtubule components or binding proteins (e.g., α -Tubulin, Katanin-60, EB1, Milton, and aPKC). Thus, MyoXV may function in intrafilopodial trafficking and to coordinate Actin and microtubule dynamics during closure (R. Liu et al. 2008).

Zippering, filopodia, lamellipodia, and the remodeling of cell junctions

Both the DME and the amnioserosa cells have filopodia that appear to be distinct in morphology, in life span, and in the types of Actin elongation factors that they express (Nowotarski et al. 2014). The DME-based filopodia are required in zipping and for proper segment matching through a mechanical interaction and/or a biochemical interaction reflecting the unique transcriptional profile of each DME cell, both within each zipping segment and along the A/P axis (Jacinto et al. 2000, Franke et al. 2005, Jankovics & Brunner 2006, Laplante & Nilson 2006, Gates et al. 2007, R. Liu et al. 2008, Millard & Martin 2008,

Stevens et al. 2008, Laplante & Nilson 2011, Pickering et al. 2013, Flores-Benitez & Knust 2015, Rousset et al. 2017, Takacs et al. 2017). It is not clear whether the filopodia emanating from the PAS and bulk amnioserosa play an active role in the zipping process, in particular at the canthi, or whether they function primarily as a site for extra membrane storage as the apical surfaces of the cells undergo pulsatile contractions. The filopodia that emanate from the amnioserosa may play a role that is analogous to that of cytonemes found during *Drosophila* larval wing disc development (Nowotarski et al. 2014). Cytonemes are specialized long filopodia present in the nonmigratory wing disc cells that mediate a role in contact-dependent Dpp signaling (Kornberg & Roy 2014, Roy et al. 2014). Because JNK and Dpp signaling regulates closure (Glise et al. 1995, Glise & Noselli 1997, Reed et al. 2001, Fernandez et al. 2007, Ducuing et al. 2015), it is interesting to consider a cytoneme-like role for filopodia in the cross talk between the DME and PAS cells during closure.

Two complementary investigations have advanced our understanding of zipping (Lu et al. 2015) and how the filopodia interact (Eltsov et al. 2015). The 3D architectures of the amnioserosa, the DME cells, and the purse strings during zipping were investigated with light microscopy (Lu et al. 2015). The amnioserosa cells constrict apically as they enter the region of a canthus and progressively ingress. These amnioserosa cells continue to constrict even when underlying the canthus and seam. During zipping, the DME cells and the PAS cells, which were previously reciprocally wedge shaped and tightly apposed to one another, shear with respect to one another such that, near the canthus, the leading edges of the DME cells and the purse strings they contain slide over the apical surface of the amnioserosa to meet along the dorsal midline. Subsequently, the apposing DME cells come together at their apical edges and then square off basally to form a lateral junction as the purse strings move basally and begin to disassemble. The DME/PAS interface is remodeled twice during closure: The DME/PAS interface transitions from a squared-off to a wedged morphology during early closure and transitions from a wedged to a squared-off morphology during the zipping process. Adhesion between the two apposing leading edges occurs at this lateral junction (Figure 5f).

Electron microscopy also correlated the morphology of apposing leading edge cells with cytoskeletal architecture by sectioning through the tissue near a canthus (Eltsov et al. 2015). Filopodial extensions provide the earliest contact between advancing cell sheets, but the penetration of filopodial extensions into adjacent cells was observed only between lateral epidermal cells in one cell sheet or the other. Lamellipodial sheets from two apposing leading edges overlap as they extend from the apical ends of the DME cells to form a single, essentially planar interface with dispersed adhesion sites between the leading edges of advancing DME cells. These overlaps are rich in microtubules, and there is little or no evidence of bundles of Actin filaments (which nevertheless were apparent as part of the F-Actin purse strings). The lamelli subsequently shorten as the junction between two apposing leading edge cells forms. Because of the paucity of F-Actin bundles, these observations suggest a role for microtubules in generating a force that draws the DME cells together. However, branched Actin filament networks, believed to drive lamellipodial extension and to participate in lamellipodial retraction in other systems (Pollard & Borisy 2003), may contribute.

Microtubules may be required in zipping as a generator of force, as a conduit for vesicle or other component delivery during the zipping process, or both. Several other studies support a role for microtubules in normal zipping, through either the injection of colcemid or the expression of the microtubule-severing protein Spastin (Jankovics & Brunner 2006, Almeida et al. 2011). In *dpp* mutant backgrounds, disorganized microtubule bundles also correlate with aberrant zipping (Fernandez et al. 2007), and mutations in the F-Actin/microtubule cross-linker *short stop* disrupt filopodia formation and subsequent zipping (Takacs et al. 2017).

Zippering is the first step in establishing a seamed and then a seamless dorsal epithelium. In the end stages of closure, once the leading edges come to within 10–30 μm of one another, final closure is edge to edge as new bridges form between the advancing DME cells along the entire width of the dorsal opening, and bidirectional zipping from multiple contact points completes the formation of a seam (Peralta et al. 2007, Rodriguez-Diaz et al. 2008, Layton et al. 2009, Wells et al. 2014). Adhesions between the DME and PAS cells are broken down and replaced by adhesions between the matching DME partner cells in a process mediated by the Integrin-localized serine/threonine kinase Pak (an effector for the small G proteins Rac and Cdc42) and the Scribbled complex, which in turn is required for the formation of septate junctions (Bahri et al. 2010). Ultimately, once new epithelial adhesions are formed and various features of the formed seam (e.g., residual components of the purse strings) disappear, the dorsal epithelium becomes seamless, dorsal closure is complete, and embryogenesis continues.

BIOMECHANICS OF CLOSURE

Much progress has been made in understanding the biomechanics of dorsal closure. Mechanics (and biomechanics) entails the characterization of both kinematics, which describes and quantifies the movement of subcellular structures, cells, and tissues, and dynamics, which describes the forces responsible for driving those movements. As applied to dorsal closure, biophysical mechanisms are expressed mathematically as sets of equations, which can be validated or falsified by comparing them to time series of segmented, time-lapsed images. The material properties of the subcellular structures, cells, and tissues involved play a key role in determining how a tissue responds to applied forces (Keller et al. 2003). An important advance in the study of dorsal closure is the biophysical modeling of experiments that probe the dynamics of both cells and tissues with a combination of laser dissection and genetic manipulation (Kiehart et al. 2000, Hutson et al. 2003, Peralta et al. 2007, Toyama et al. 2008, Ma et al. 2009, Solon et al. 2009, Jayasinghe et al. 2013, Saravanan et al. 2013, Dierkes et al. 2014, Fischer et al. 2014, Wells et al. 2014, Machado et al. 2015, Saias et al. 2015). These studies have characterized the biomechanical properties of dorsal closure in terms of viscoelasticity, contractility, and strain. Studying the kinematics of closure at the molecular, cellular, and tissue levels elucidates the coordination of the biomechanical processes and the emergent properties that lead to closure.

The oscillations or pulsations of the amnioserosa cells during GBR have been studied quantitatively (Solon et al. 2009, Azevedo et al. 2011, Sokolow et al. 2012, Lynch et al. 2013). Asymmetric zipping rates at the canthi have also been quantified (Peralta et al. 2007).

Quantitative analyses of DME elongation and the kinetics of purse string formation are largely anecdotal: More detailed, quantitative characterizations of each have great potential as open research questions.

Native Closure Is the Consequence of Four Biomechanical Processes

Four biomechanical processes contribute to closure: (a) Amnioserosa oscillations and ingression coupled with apoptosis promote closure, (b) tension in the curved Actomyosin-rich purse strings promote closure, (c) zipping at the canthi coordinates amnioserosa dynamics and contracts and maintains the curvature of each purse string, and (d) elastic and/or contractile forces in the lateral epidermis oppose closure. Several biophysical and mathematical models have been proposed to account for how these forces drive closure (Hutson et al. 2003, Peralta et al. 2007, Layton et al. 2009, Solon et al. 2009, Almeida et al. 2011, Wang et al. 2012, Jayasinghe et al. 2013, Dierkes et al. 2014, Saias et al. 2015, Gorfinkel 2016, Hayes & Solon 2017). These studies shape our understanding of the emergent properties of the forces provided by lateral epidermis and the amnioserosa and how they are coordinated.

These four processes have been characterized in embryos encased within their vitelline envelope, where turgor pressure pushes the embryo against the inside of the eggshell (see, e.g., figures 2–8 in Wieschaus & Nüsslein-Volhard 1998). In addition, the amnioserosa bulges into the perivitelline space as a smooth, asymmetric dome, and the purse strings can indent the embryo surface. These observations suggest a pressure internal to the embryo (Lu et al. 2016). Moreover, loss of amnioserosa cell volume may be essential for force production in the amnioserosa (Saias et al. 2015). These observations beg the question as to whether turgor pressure is a fifth force that either facilitates or impedes closure. On one hand, embryos can be peeled out of their eggshell and proceed through closure, suggesting that compromising turgor does not block closure (Mateus & Martinez Arias 2011). On the other hand, there is precedent for increases in hydrostatic pressure triggering a biochemical response that generates a counter force—a mechanochemical signal is sufficient to maintain epithelial tension and geometry of the egg chamber (Koride et al. 2014).

Closure requires a nonzero net force on each DME cell, which is well described by Newton's second law at low Reynolds number (Hutson et al. 2003). As depicted in Figure 6, the vectors σ_{ASds} (the force produced by the amnioserosa on the purse string) and σ_{LEds} (the force produced by the lateral epidermis on the purse string) point toward and away from the dorsal midline, respectively. Symmetry of the dorsal hole dictates that, at the maximum dorsal opening, the direction of both σ_{ASds} and σ_{LEds} be perpendicular to the dorsal midline. But in general, there is some uncertainty as to these vectors' precise directions, which depend on location along the remainder of the leading edge. The kinematics of DME cell movement suggests that σ_{ASds} is directed largely perpendicular to the dorsal midline and that the precise direction of σ_{LEds} may be influenced by the shape of the DME cell at that location. Thus, determining the precise direction of the vectors that describe the sum of forces is complex. However, Newton's second law applied to a segment of the leading edge at a symmetry point is reliably given by the scalar equation

$$\sigma_{LE} - \sigma_{AS} - T\kappa = -b \frac{dh}{dt}. \quad 1.$$

T is the tension in a purse string, κ is the curvature of a purse string, h is a purse string's distance (at the symmetry point) from the dorsal midline, and $b \frac{dh}{dt}$ is the viscous drag.

Empirical observations indicate that the rate at which each leading edge moves toward the dorsal midline (dh/dt) is well approximated as constant during the bulk of closure. From the available data (see supplement to Hutson et al. 2003 and refined values in Peralta et al. 2007), force ladders that compared the magnitude of the forces contributed by the lateral epidermis, the amnioserosa, and the purse strings with the drag force were determined (Peralta et al. 2007). The direction of T is tangent to the arced leading edge. T is the largest of the applied forces, but because it is resolved in the direction of closure, it contributes only one-fourth to one-third of the total force required for native closure. To be clear, the purse strings contribute to closure because they are curved, contractile bands that produce a force resolved in the direction of movement. The force ladders also indicate that each of the applied forces responsible for closure is much larger (by two to three orders of magnitude) than the net force that drives closure.

Zippering as a biomechanical process

Given the role that zipping plays in closure, a quantitative model for dorsal closure requires two equations. The zipping process is well approximated by a rate equation for the width W of the dorsal opening, where H is the maximum height of the dorsal opening at the symmetry point and k_z is an empirically determined rate constant:

$$\frac{dW}{dt} = \frac{-k_z}{\tan(\theta_A/2) + \tan(\theta_B/2)} = \frac{-k_z W}{2H}. \quad 2.$$

Since this first-generation model of dorsal closure was proposed in 2003 (Hutson et al. 2003), additional models have addressed forces that are at locations other than the symmetry points (Layton et al. 2009, Almeida et al. 2011), and one model takes into consideration that changes in the volume of the amnioserosa cells can modulate forces (Saías et al. 2015). These models are nicely summarized in a recent review (Hayes & Solon 2017). Several other biophysical models that account for the balance of these forces that drive the completion of closure have also been proposed and are discussed elsewhere (Solon et al. 2009, Wang et al. 2012, Jayasinghe et al. 2013, Dierkes et al. 2014, Lu et al. 2015, Machado et al. 2015, Gorfinkiel 2016, Hara et al. 2016, Lu et al. 2016, Hayes & Solon 2017, Yu & Fernandez-Gonzalez 2017). A future review will compare models for molecular-, cellular- and tissue-level forces (A. Aristotelous, J.M. Crawford, G.S. Edwards, D.P. Kiehart & S. Venakides, manuscript in preparation).

A remarkable feature of zipping is that during native closure it proceeds at a rate that maintains a near constant curvature of the purse strings. Perturbation of zipping can result in

a cigar-shaped dorsal opening (Hutson et al. 2003, Jankovics & Brunner 2006, Peralta et al. 2007). Interestingly, in contrast to the upregulation of anterior zipping that is a consequence of laser perturbation of posterior zipping (Peralta et al. 2007, Toyama et al. 2008), anterior zipping in embryos mutant for the *Abd-B* HOX family segmentation gene (where posterior zipping is genetically inhibited) is decreased (Rousset et al. 2017). Understanding the mechanism by which specific laser and genetic manipulation influences the movements of closure at sites far from the site of such perturbation is an open research question.

In a recent analysis, a thermodynamic model of zipping describes junction remodeling as two apposing DME cells square off and form new adhesions at the canthi, a process that occurs during seam formation (Lu et al. 2015). The mechanism for zipping at the posterior canthus is based on adhesion dynamics, whereas the mechanism for zipping at the anterior canthus includes both adhesion and amnioserosa dynamics. These observations provide further evidence that the canthi, although similar in overall structure, are clearly different in dynamical detail. In addition, the ends of the purse strings bend basally during the zipping process, and such behavior suggests a canthus-localized force that has yet to be characterized.

Amnioserosa forces

Several studies have suggested that Actomyosin-based apical contractions and ingression events in the amnioserosa are the main force generating processes for closure (Kiehart et al. 2000, Hutson et al. 2003, Franke et al. 2005, Solon et al. 2009, Blanchard et al. 2010, David et al. 2010, Sokolow et al. 2012, Ducuing et al. 2015, Ducuing & Vincent 2016, Pasakarnis et al. 2016). Myosin powers the apical contractions of amnioserosa cells (Franke et al. 2005), and recent studies have shown that cycles of phosphorylation and dephosphorylation are required for normal Actomyosin-based oscillations (Azevedo et al. 2011, Saravanan et al. 2013, Fischer et al. 2014, Duque & Gorfinkiel 2016, Vasquez et al. 2016). During germ band extension in the epithelial cells, Myosin kinases (including Rok) and the Mbs of a Myosin phosphatase(s) are colocalized along with Myosin to the apical surfaces of the oscillating cells, leading to contraction (Munjal et al. 2015). Contraction then leads to an increase in tension and viscosity in the cortex that in turn provides negative feedback. It is interesting to consider that such a mechanism may be an emergent property of the amnioserosa oscillations as well. Additional genetic studies provide further insight into the tissue-level forces that contribute to closure. For example, a constitutively active form of Mbs eliminates cell oscillations, but closure proceeds to completion, whereas a reduction in Mbs levels generates tears between the DME/PAS interface and within the amnioserosa at sites of ingression events (Duque & Gorfinkiel 2016).

The overall material properties of the amnioserosa cells also depend upon Myosin phosphorylation (Ma et al. 2009, Fischer et al. 2014, Machado et al. 2015). Over the course of closure, the amnioserosa solidifies, as indicated by a decrease in the recoil power-law exponent (Ma et al. 2009). The effective stiffness doubles at the onset of net tissue contraction, and laser ablation demonstrates that, although junctional stress increases twofold, medial stress increases fourfold. Thus, the force generated by contraction of the

amnioserosa originates from both medioapical Actomyosin arrays and junctional belts (Ma et al. 2009, Machado et al. 2015).

Adhesion complexes and junctional belts are critical for the proper communication of forces at the tissue scale and maintain the balance of forces within the amnioserosa and at the junction between the DME cells and the PAS cells (reviewed in Harris 2012, Heisenberg & Bellaiche 2013, Roper 2015). For example, mutations in *myspheroid*, which encodes β -PS Integrin, result in amnioserosa contractions that remain in the slow phase of apical contractions that characterize the onset of closure; the contractions do not advance to the fast phase of contraction that is coupled with the engagement of the anterior canthus and that begins after the onset of closure (Gorfinkel et al. 2009, Machado et al. 2015). These mutants also display a slower zipping rate, albeit with morphologically normal filopodia. In another example, α -Catenin, through the mechanosensing protein Vinculin, regulates apical contractions in the amnioserosa by stabilizing Actomyosin and Cadherin foci at the membrane (Jurado et al. 2016). Likewise, junctional stability enhances apical contractions and promotes force production: A lack of α -Catenin results in a compromised purse string, in slower amnioserosa oscillations, and in a decrease in overall closure rate, even though the rates of ingressions increase.

Work on wound healing may also provide insight into the role of mechanosensing proteins in the maintenance of junctional complexes and in the transmission of forces during morphogenesis. Recent work in embryonic wound healing indicates that the force balance at the wound margin is affected by curvature (Zulueta-Coarasa & Fernandez-Gonzalez 2017), possibly through the function of BAR domain-containing proteins that can simultaneously sense or induce membrane curvature and moderate Actin function (Scita et al. 2008). Similar mechanosensing dynamics may occur during closure so that the state of the cytoskeleton can adapt constantly to local changes in the curvature of the amnioserosa during native closure or during closure perturbed by laser or genetic perturbation strategies.

The ingression events that drop individual cells out of the plane of the amnioserosa and out of the way of the advancing lateral epidermal cell sheets are essential for efficient closure. Localized patterns of ingression events are an inherent feature of closure (Kiehart et al. 2000, Fernandez et al. 2007, Sokolow et al. 2012, Lu et al. 2015), with most cells ingressing at the canthi and adjacent to the PAS cells, the so-called marginal cells. Approximately 10% of the cells ingress in the bulk of the amnioserosa (Kiehart et al. 2000, Toyama et al. 2008). Understanding the developmental role of ingression will include identifying the players that can trigger the ingression events, for example, (a) an imbalance in the levels of POSH, a scaffold protein that promotes the assembly of signaling components (including JNK), during closure (Lennox & Stronach 2010, Zhang et al. 2010) and (b) increases in reactive oxygen species that herald the impending ingression (Mulyil & Narasimha 2014). Modifications in the apoptosis pathway that either increase or decrease apoptosis alter the rate of closure (Toyama et al. 2008, Mulyil & Narasimha 2014) and demonstrate the existence of an apoptotic force that is responsible for up to one-third of the net force produced by the amnioserosa (Toyama et al. 2008) during native closure. Nevertheless, other studies do not observe a direct link between ingression and closure rates (Mulyil et al. 2011, Jurado et al. 2016). For example, ingression rates are increased, but closure rates are

decreased, in α -Catenin mutants characterized by altered oscillation rates (Jurado et al. 2016), and conversely, ingression is absent, but closure rates are increased, in mutants of the proapoptotic gene *hid* (Mulyil et al. 2011). These results suggest a complexity in the coordination of the multiple forces that contribute to closure (Kiehart et al. 2000, Hutson et al. 2003, Peralta et al. 2007).

Dorsal Closure Is a System of Redundant Processes

Numerous laser and genetic perturbation experiments have demonstrated that dorsal closure is a collective system that is redundant, resilient, and robust. Laser perturbation of any one of the key processes that promote closure does not result in failure (Kiehart et al. 2000, Hutson et al. 2003, Peralta et al. 2007, Rodriguez-Diaz et al. 2008, Wells et al. 2014). Gross laser dissection of the amnioserosa results in a significant delay of dorsal closure. Upon dissection of the amnioserosa, the leading edges of the DME cells dramatically recoil away from the dorsal midline to a turning point, and then closure resumes, initially at an accelerated rate and eventually at the native rate (see figure 2 in Hutson et al. 2003; see also Peralta et al. 2007). Remarkably, when the laser ablates tissue within the anterior or posterior half of the dorsal opening, the ablated half typically closes faster than the nonablated, native half (Kiehart et al. 2000). Similarly, after laser dissection of a segment of the purse string, a secondary purse string assembles as the tissue recoils (Rodriguez-Diaz et al. 2008). Subsequently, the secondary purse string reaches a turning point, and then closure resumes. Laser perturbation designed to prevent zipping at one canthus leads to the upregulation of the rate of zipping at the other canthus, and closure proceeds at near native rates (Peralta et al. 2007). Laser perturbation designed to prevent zipping at both canthi (by alternatively targeting the amnioserosa adjacent to each canthus) leads to qualitative changes in the shape of the dorsal opening, where the curvature of each leading edge of the lateral epidermis in the central region of the dorsal opening changes sign (flips) so that the tension in each purse string now opposes closure (Hutson et al. 2003, Peralta et al. 2007). Nevertheless, forces in the amnioserosa are upregulated, and closure proceeds at near native rates (Peralta et al. 2007). The upregulation in the zipping rate constants and of the force produced by the amnioserosa correlates with the rate of apoptosis and is attributed to an apoptotic force (Toyama et al. 2008). Furthermore, a double-canthus removal protocol minimizes any contribution from zipping (Wells et al. 2014). In this protocol, the Actomyosin cable assumes a geometry nearly parallel to the dorsal midline such that the Actomyosin cable can contribute little to closure and the amnioserosa promotes closure that proceeds at near native rates until the end stages of closure. The delay at the end of closure may be attributable to a pileup of amnioserosa cells due to an absence of ingression associated with canthi, and consequently, the cells fail to get out of the way of the advancing DME/PAS complex.

Genetic perturbation of the forces from the two tissues likewise does not compromise the completion of closure. When functional Myosin is removed from the purse string, closure still completes (Franke et al. 2005, Pasakarnis et al. 2016); when oscillations are inhibited by blocking the activation of Myosin, closure still completes (Franke et al. 2005, Duque & Gorfinkel 2016); and when apoptosis is inhibited, closure still completes (Toyama et al. 2008, Mulyil & Narasimha 2014).

Genetic studies are beginning to uncover molecular mechanisms that may contribute to the upregulation in the force generated by one tissue when the other is compromised either physically or genetically. There is good evidence for cross talk between the DME cells and the PAS cells (Kiehart et al. 2000, Reed et al. 2001, Fernandez et al. 2007, Zahedi et al. 2008). A functional amnioserosa can promote cell shape changes and coordinated behavior in an otherwise mutant epidermis via the Dpp target *ush* (Lada et al. 2012). The subsequent activation of JNK in these DME cells indicates that JNK signaling may be a mechanical response of the epidermis (see below).

These experimental observations support the view that dorsal closure exhibits emergent properties and is robust, is resilient, and benefits from redundancy when perturbed. Closure is robust. It continues despite internal and external perturbations without fundamental changes in the original system; e.g., closure occurs at native or near native rates when laser surgery removes cells that normally contribute to closure (Kiehart et al. 2000, Hutson et al. 2003, Peralta et al. 2007, Wells et al. 2014). Closure is also resilient. The method of closure can adapt in response to challenges such that closure continues: For example, closure occurs even when cells are abnormally shaped and lack critical components of the Actomyosin cytoskeleton, as in mutant *wingless* (*wg*) embryos (McEwen et al. 2000, Kaltschmidt et al. 2002, Morel & Martinez Arias 2004). Closure is redundant at the cellular and molecular levels. When zipping is compromised or the contribution of the Actomyosin cable to closure is reduced or eliminated, the forces produced by the amnioserosa are upregulated (Hutson et al. 2003, Peralta et al. 2007). Key molecules are redundant in that in some cases multiple genes encode two or more similar protein products and loss of only one gene is insufficient to block closure. Thus, three Src-like, non-receptor tyrosine kinases that function upstream of JNK signaling and three Rac-like genes that encode small GTPases can regulate Actin function and contribute to closure in other ways (Tateno et al. 2000, Woolner et al. 2005). Compromising one Src-like gene or one Rac-like gene does not perturb dorsal closure. Because dorsal closure is robust and resilient, previous screens (e.g., for embryonic lethals or wound healing screens) have failed to recover genes that nevertheless disrupt dorsal closure in substantial ways (Jürgens et al. 1984, Nüsslein-Volhard et al. 1984, Wieschaus et al. 1984, Campos et al. 2010). Screens that analyze not only the outcome of closure but the kinematics and dynamics of closure (Jankovics et al. 2011, Rousset et al. 2017) will continue to provide insight into the genes required for closure and the genetic basis of this robustness and resilience.

Closure is also the result of emergent properties: As with cell sheet morphogenesis in vertebrates, native closure requires biological organization and function at several length scales and timescales. Yet we know very little about how organization and function emerge from a lower level of organization and about how systems of components at each level influence the organization or function of systems at other levels (Peralta et al. 2007, Gorfinkiel et al. 2011). Two examples of emergent properties of dorsal closure are as follows. First, each of three applied forces that sum to drive dorsal closure is two to three orders of magnitude larger than the net force responsible for dorsal closure (Hutson et al. 2003, Peralta et al. 2007). Nevertheless, during the bulk of closure, the DME cells move inexorably toward the dorsal midline at near linear rates (Hutson et al. 2003). Regulatory mechanisms must maintain precise ratios of contributing forces such that a small net force

drives dorsal closure in the right direction. Second, in native closure, zipping rates are tuned to maintain purse string curvature as the dorsal hole shrinks, and defects in zipping cause cigar-shaped dorsal openings [e.g., in Integrin-defective mutants and colcemid-injected embryos (Hutson et al. 2003, Jankovics & Brunner 2006)].

None of the forces described above are a prerequisite for the completion of dorsal closure. Nevertheless, they contribute to native closure. Moreover, some combination of these tissues and forces must remain intact for successful closure. Exactly how an embryo “knows” that one or another force for closure is compromised remains a mystery. What generates the signals that indicate that one force or process is perturbed, resulting in the upregulation of another force or process? What receives those signals and mediates an adequate and timely response, through either increased apoptosis or some other mechanism, is also unknown. We speculate that one possible mechanism is mechanosensitive membrane proteins that respond to changes in tissue tension (e.g., Hunter et al. 2014, Jurado et al. 2016).

WHAT TRIGGERS CLOSURE?

What mechanistically might a trigger (or triggers) for dorsal closure look like? Is the trigger for closure transcriptional or physiological? A good example of a transcriptional trigger for morphogenesis is *nullo*, whose transcription, just in time, is required for cellularization in early *Drosophila* development (Wieschaus & Sweeton 1988). Another example is transcription of components of the heart gene-regulatory network that specifies the transcription/migration interface in heart precursors of *Ciona* (Christiaen et al. 2008).

Alternatively, closure may be triggered physiologically. The early developmental programs and morphological transitions that are initiated upon fertilization of an egg provide an example of physiologically catalyzed onset of morphogenesis. Models for how arrays of Actin and Myosin are regulated to drive tissue morphogenesis may well be provided by their functions in cell migration, cytokinesis, and smooth muscle or striated muscle contraction, each of which is an example of cellular morphogenesis (i.e., cell shape change or movement) and none of which require just-in-time transcription. All of these processes are triggered physiologically, as are early events in wound healing (Cordeiro & Jacinto 2013). For example, cytoplasts isolated from human neutrophils migrate much like their parent neutrophils, even responding appropriately to chemotactic gradients (Huang et al. 1991). These cytoplasts are cell fragments that contain no nucleus. Thus, the complex, directed cell shape changes that characterize cell migration and chemotaxis can occur in the complete absence of transcription. Of course, appropriate transcriptional programs are required to provide cells with the components they need to assemble the molecular machines that drive closure or other morphogenetic processes. A physiological trigger would indicate that there is no just-in-time transcription.

Signaling for Closure

Do any of the signaling pathways that are known to regulate closure serve as its trigger? A complex ensemble of signaling pathways are required for closure and include small GTPases in the Rho superfamily, non-receptor tyrosine kinases, the Wg/Wnt pathway, the Notch pathway, the JNK pathway, the BMP/Dpp pathway, and the insect steroid hormone

Ecdysone (reviewed in Harden 2002, Gilbert 2004, Woolner et al. 2005, VanHook & Letsou 2008, Harris et al. 2009, Belacortu & Paricio 2011, Gorfinkiel et al. 2011, Munoz-Soriano et al. 2012, Rios-Barrera & Riesgo-Escovar 2013). To our knowledge, the precise time at which each of these pathways must be active to effect normal closure is not clear.

Although Wg/Wnt signaling is well characterized, is highly conserved, and plays roles in tissue morphogenesis, polarity, and patterning, this pathway is unlikely to be the trigger for closure. Severe mutations in *wg* result in embryos that complete closure even though they have a very weak Actomyosin cable, and the cells in the lateral epidermis, including the DME cells, have dramatically altered morphology—they are elongate along the A/P axis (McEwen & Peifer 2000, Kaltschmidt et al. 2002, Morel & Martinez Arias 2004). The remarkably abnormal shapes of lateral epidermal cells in *wg* embryos, and the ability of such embryos to complete closure, suggest that *wg* is not a trigger for closure.

Is JNK the Trigger?

The JNK pathway consists of conserved serine/threonine kinases that phosphorylate c-Jun and other transcription factors required in development. Mutations in JNK signaling pathways lead to defects in closure. The JNK signaling pathway is a potential trigger for the onset of dorsal closure; its components are localized at the zone of activity at the interface between the epithelial and amnioserosa cells prior to the onset of closure. JNK activity is required for dorsal closure, and JNK signaling pathways are upregulated when the function of one of the two tissues is compromised.

Numerous upstream activators and downstream effectors of JNK signaling have been identified in studies on both dorsal closure and wound healing (e.g., Jasper et al. 2001, Lennox & Stronach 2010, Sorrosal et al. 2010, Zhang et al. 2010, Belacortu & Paricio 2011, Rios-Barrera et al. 2015). Nevertheless, when JNK function is required is not known, and the single signal or signals that activate JNK function have not been identified. Studies in a variety of systems suggest that JNK activation may involve several inputs, including growth factors, polarity cues, mechanical stretching, and the release of ATP and/or Ca^{2+} (Kushida et al. 2001, Ramet et al. 2002, Igaki et al. 2006). It is interesting to speculate that several inputs are integrated to effect the JNK signaling required for closure and that, as a consequence, there is no one trigger for closure. Downstream effectors of JNK can also feed back and negatively regulate JNK activity [e.g., Puckered and Scarface (Martin-Blanco et al. 1998, Rousset et al. 2010, Sorrosal et al. 2010)]. JNK regulation also occurs at the transcript level; two examples are the activity of a 5′–3′ exonuclease encoded by *pacman* and a long, noncoding nuclear RNA encoded by the *acal* locus, with both having effects on closure (Grima et al. 2008, Rios-Barrera et al. 2015).

Recently identified functions of JNK signaling include the regulation of apoptosis (Adachi-Yamada et al. 1999), mixer cell identification and segmental boundary lineage switching (Gettings et al. 2010), Rab-mediated vesicle transport (Thomas et al. 2009), and apicobasal polarity (Llense & Martin-Blanco 2008). The JNK target Dpp works in concert with Ack family tyrosine kinases to regulate the transcription of nonmuscle Myosin II in both the amnioserosa and DME cells (Shen et al. 2013). JNK and Dpp are also part of a feedforward loop that regulates the expression of proteins that interact with the cytoskeleton (Ducuing et

al. 2015), including Jaguar, a Myosin VI homolog that associates with the cell adhesion molecule Echinoid to regulate cell shape changes during closure (Lin et al. 2007). Expression of *ush* may mediate communication between the amnioserosa and the DME; Ush is required in the amnioserosa to initiate JNK activation in the DME cells, Ush is required in both the amnioserosa and the DME for the formation of the Actomyosin purse string, and a tissue-specific rescue of mutant *ush* demonstrates the critical role that the amnioserosa plays in closure (Lada et al. 2012). Mutations in *ush* result in premature apoptosis of the amnioserosa during GBR (Frank & Rushlow 1996).

A recent study provides key insights into JNK signaling as a key regulator of closure and its function in stress response as an activator of apoptosis (Beira et al. 2014). Dpp signaling suppresses JNK-induced apoptosis in the dorsal regions of the lateral epidermis, including the DME cells. The Dpp pathway transcriptional repressor *schurri* represses the proapoptotic gene *reaper*, and conversely, in knockdowns of Dpp or in *schurri* mutants, *reaper* expression is upregulated in dorsal tissue. Live imaging of *schurri* mutant embryos is consistent with defects induced by increased apoptosis in dorsal epidermal tissues. It is interesting to speculate that a balance between JNK-activated apoptosis and cell shape change lies at the heart of dorsal closure's resilience and robustness. According to this model, in response to genetic or laser-induced stress, the upregulation of apoptosis could provide an apoptotic force(s) that upregulates the tension provided by the amnioserosa. Unfortunately, key experiments that define activation of the JNK signaling pathways as the trigger for closure are lacking.

CONCLUSION

Studies on the kinematics and dynamics of dorsal closure in wild-type, mutant, laser-perturbed, and pharmacologically interrogated embryos lead to an appreciation of simple cell sheet movements that are robust and resilient. Some of closure's success in the face of genetic adversity or compromising cells and dissecting tissues with laser microbeams is due to redundancy at the molecular, cellular, and tissue levels. Nevertheless, a substantial part is due to emergent properties that we do not adequately understand. How the embryo recognizes defects in one or another biomechanical process, and then modifies existing processes in the absence of fundamental changes (thereby depending on robustness) or modifies its mode of operation (thereby depending on resilience) to execute the aesthetically pleasing and beautifully orchestrated cell movements and interactions that constitute closure, remains a mystery. Dorsal closure promises to provide a rich field for experimental inquiry and insights into chordate morphogenesis for years to come.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Overview of *Drosophila* embryogenesis from extended germ band to completion of dorsal closure. (*a–d*) Low-resolution confocal images of a *Drosophila* embryo expressing a protein that fuses GFP to the Actin binding domain of *Drosophila* Moesin (GFP-Moe-ABD), which labels F-Actin (the embryo is otherwise essentially wild type; U.S. Tulu & D.P. Kiehart, unpublished). (*a'–d'*) Schematics traced from panels *a* to *d*. Red tissue is amnioserosa. (*a, a'*) Extended germ band stage. (*b, b'*) End of germ band retraction. Canthi are not yet formed. Arrows depict the direction of movement of the leading edge of the lateral epidermis. Forces from either flank are equal; arrows from the far side of the embryo depicted are shorter because their origins lie behind the bulging amnioserosa. (*c, c'*) Canthi have formed (ACa and PCa denote anterior and posterior canthus, respectively), and the position of the purse strings (PS) near the leading edge of the advancing dorsal-most epidermal cells is shown. (*d, d'*) Late closure. The lateral epidermal sheets have met at the dorsal midline; the amnioserosa has been internalized; and a seam remains, as the purse strings have not yet completely disassembled.

Figure 2.

Integrins mediate close attachment of the amnioserosa (AS) to the surface of the yolk and the rearrangement of the interface between the dorsal-most epidermal (DME) and peripheral-most amnioserosa (PAS) cells. (a) The AS as it adheres to the surface of the yolk during four stages of germ band retraction (redrawn with permission from Reed et al. 2004). The yolk is in red, the germ band is in blue, and the AS is in black. (b) Micrographs show successive stages of the AS (arrow) adhering to the surface of the yolk (reproduced with permission from Reed et al. 2004). (c) Schematic showing the rearrangement of the relationship between the lateral epidermis (blue) and the AS (yellow) at the DME/PAS interface (arrowhead) in wild-type embryos. (d) Schematic drawn from panel e showing the interface between the AS (yellow) and the lateral epidermis (blue) at the DME/PAS interface (arrowhead) in wild-type (upper panel) and Integrin mutant (bottom panel) embryos. The AS, the DME cell, and the rest of the lateral epidermis do not adhere properly to the yolk in Integrin mutant embryos (double arrow). (e) Micrograph (reproduced with permission from Narasimha & Brown 2004) from which panel d was drawn. The position of the DME cell is shown.

Figure 3.

Shape of the dorsal opening and canthus formation. Confocal micrographs of living embryos that are essentially wild type other than carrying a ubiquitin-driven transgene that encodes GFP-Cadherin. Anterior is left and posterior is right in all panels. (*a*) At the end of germ band retraction, the dorsal hole has a blunt anterior end and a rounded posterior end. (*b–d*) Canthus formation at the anterior end. (*e–g*) Canthus formation at the posterior end. Panel *a* is from one embryo, and panels *b* through *g* are from another embryo. Seams that are obvious in embryos labeled for F-Actin or Myosin are not so obvious in GFP-Cadherin

embryos (see the beginning of anterior seam formation in panel *c* and compare it to the formed, posterior seam shown in Figure 4d). Images were provided by Stephanie Fogerson.

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Figure 4.

The leading edge accumulates F-Actin to form the supracellular purse string in the dorsal-most epidermal (DME) cells and is transformed from scalloped to smoothly curved. Shown are confocal images of a *Drosophila* embryo expressing a protein that fuses GFP to the Actin binding domain of *Drosophila* Moesin (GFP-Moe-ABD), which labels F-Actin (the embryo is otherwise essentially wild type). Posterior is to the left and anterior is to the right in all panels. (a) Near the end of germ band retraction, but before the onset of dorsal closure. The leading edge of the lateral epidermis is scalloped, and a few DME cells have begun to accumulate F-Actin (arrowheads show accumulation). (b) Just prior to the onset of closure,

F-Actin has begun to accumulate (*arrowheads*). (*c*) By the onset of closure, F-Actin has accumulated in virtually all cells. (*d*) A robust purse string (*arrowhead*) has formed. The leading edge also accumulates Myosin in a pattern akin to bars on a string (not shown; see Young et al. 1993, Franke et al. 2005). Previously unpublished images provided by R. Montague, V. Williams & D.P. Kiehart.

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Figure 5.

Dorsal-most epidermal (DME) cells remain associated with peripheral-most amnioserosa (PAS) cells throughout the duration of closure. (a–e) Micrographs depict the morphogenesis of the amnioserosa (AS) from mid- to late closure. The AS is brightly labeled with a protein that fuses GFP to the Actin binding domain of *Drosophila* Moesin (GFP-Moe-ABD), which labels F-Actin and whose expression is driven by a mostly AS-specific promoter/enhancer complex (Gal4-C381). The arrows in panel a depict the location of the leading edges of the DME cells. Some cells in the lateral epidermis also weakly express the F-Actin label GFP-Moe-ABD. Panels a–e reproduced with permission from figure 1 (panels g, h, i, j, and l) in Rodriguez-Diaz et al. (2008). (f) Schematic of a cross section of the DME, PAS, and bulk of

the AS during closure. The progression of closure (early is at the *top*) and corresponding tissue arrangements are shown in successive time points during closure. The schematic illustrates the relationship between the DME cells and the PAS cells (cells labeled 1 and 9, respectively), which remain tightly associated with one another as dorsal closure proceeds. The cells in the bulk of the AS (cells labeled 2 through 8) progressively ingress. Red arrows indicate the positions of the purse strings. Panel *f* reproduced with permission from figure 3*b* in Lu et al. (2015).

Figure 6.

Newtonian force vectors. (a) An embryo, midway through closure, labeled with GFP-Cadherin. The white line highlights the curvature of the bulk of the leading edge of the DME cells and the purse string that they contain. (b) Newtonian force vectors for $\sigma_{LE}ds$ (green), $\sigma_{AS}ds$ (blue), and T (red) and $T\kappa$ (red). The vertical arrows are drawn approximately to scale such that $\sigma_{LE} \approx \sigma_{AS} + T\kappa$. The curvature of the leading edges of the DME cells is reproduced from panel a and is shown in black. Micrograph provided by Stephanie Fogerson.