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Review

Drosophila dorsal closure: An orchestra of forces to zip shut the embryo

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ABSTRACT

Dorsal closure, a late-embryogenesis process, consists in the sealing of an epidermal gap on the dorsal side of the *Drosophila* embryo. Because of its similarities with wound healing and neural tube closure in humans, it has been extensively studied in the last twenty years. The process requires the coordination of several force generating mechanisms, that together will zip shut the epidermis. Recent works have provided a precise description of the cellular behavior at the origin of these forces and proposed quantitative models of the process. In this review, we will describe the different forces acting in dorsal closure. We will present our current knowledge on the mechanisms generating and regulating these forces and report on the different quantitative mathematical models proposed so far.

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1. Introduction

Epithelial fusion is a fundamental process occurring during animal development and wound healing (Schoenwolf and Smith, 1990; Jacinto et al., 2001). *Drosophila melanogaster* dorsal closure (DC) has become a classic model system to study the mechanisms regulating epithelial fusion because of its similarities with wound healing. Since the first characterization of the process in early 90s (Young et al., 1993), an extensive description of the cellular movements occurring during DC has been gathered.

DC is a late-embryogenesis process that occurs after the retraction of the germ band. Germ band retraction leaves an epidermal gap on the dorsal side of the embryo, covered by an extraembryonic tissue, the amnioserosa. The term DC refers to the closure of this dorsal gap: the two epidermal flanks converge dorsally, without any proliferation of cells, while the amnioserosa tissue decreases in size (amnioserosa cells are shrinking and a subset will delaminate during the process; the whole tissue will eventually be removed after the end of DC). The epidermal layers progressively fuse at the two canthi (corners) of the eye-shaped opening, resulting in a topologically simply-connected*, scarless epidermal layer (Fig. 1-A).

A main pathway regulating DC is the JunKinase (DJnK)/Dpp pathway. The activity of this pathway, encoded by the gene basket (*bsk*), is required for the dorsalward progression of the epidermal layers (Hou et al., 1997; Kockel et al., 1997; Riesgo-Escovar and Hafen, 1997). During DC, the JnK pathway is upregulated in the first row of

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* A simply-connected (or 1-connected) space is a space in which any closed curve can be shrunk to a point. As an example, the Euclidian R^2 space is simply-connected.

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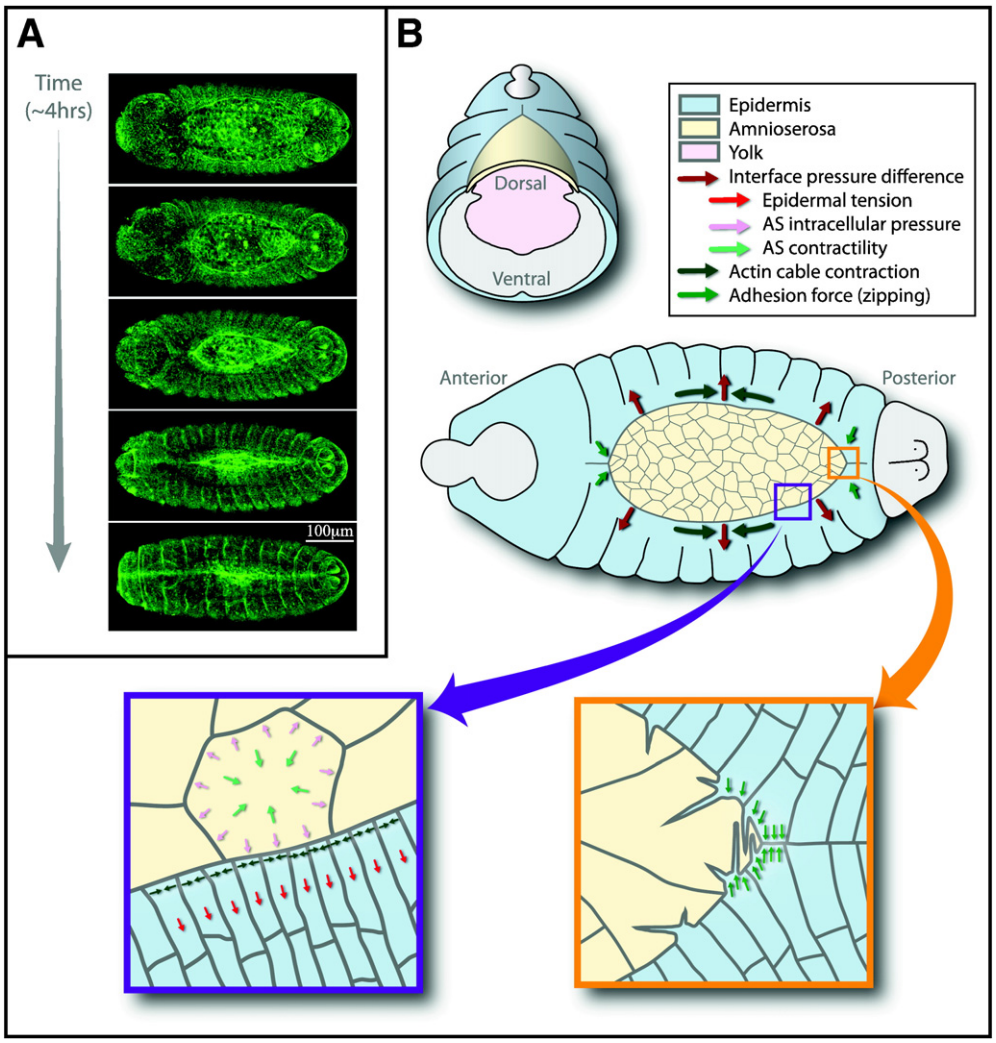

Fig. 1. Forces acting during dorsal closure. (A) Time sequence of an embryo expressing non-muscle Myosin 2 tagged with GFP (sqh-GFP). The dorsal gap, covered by the amnioserosa tissue, is sealed within the time frame of a few hours. Scale bar: 100 µm. (B) Schematics showing embryo geometry at the onset of dorsal closure and the different forces acting throughout the process. Arrows in green represent forces that promote DC, whereas those in red indicate forces that need to be overcome during the process. An interface pressure difference (dark red arrows) acts orthogonally to the cable and comprises an epidermal tension (red arrows) and AS intracellular pressure (pink arrows) opposing the movement, as well as AS contractility (light green arrows) promoting closure. This pressure difference is counteracted by an actomyosin cable surrounding the amnioserosa that generates tangential tension (dark green arrows). Zipping occurs at the two canthi of the opening, adding a supplementary local force to the process (green arrows). The close up view on the bottom left shows the three forces that make up the pressure difference (AS cell contraction, AS intracellular pressure and epidermal tension) as well as the tensile force generated by the actin cable. The close up view on the bottom right shows the activity of lamellipodia and filopodia close to the zipping point.

epidermal cells, referred to as the leading edge cells, and downregulated in the amnioserosa tissue (Reed et al., 2001). The activity of JnK acts on the molecular reorganization of proteins and promotes the formation of a supra-cellular actomyosin cable at the epidermis-amnioserosa interface.

With the emergence of transgenic *Drosophila* strains expressing GFP tagged proteins and of new microscopy techniques in early 2000s, a comprehensive description of the morphogenetic events occurring during DC was built, encompassing the localization of molecular players, respective cell and tissue behaviors and a characterization of the relevant forces. In this review, we outline the different forces acting during the process, report our current knowledge of their molecular origin and discuss the prevailing models describing the mechanics of the process.

2. Dorsal closure: an orchestra of forces

The process of DC encompasses the closure of a ~30,000 µm^2 epidermal gap on the dorsal side of the embryo. To achieve this, several force-generating mechanisms, occurring in different tissues and tissue interfaces, are combined in the process.

A difference in pressure between the epidermal and amnioserosa tissues arises from the contribution of several different forces. A signature of this pressure difference is the curvature of the interface between the two tissues (described in Section 3).

First, the amnioserosa (the tissue covering the gap) exerts active contractile forces, pulling on the two edges of the epidermis (Fig. 1-B) (Kiehart et al., 2000). Actomyosin dependent amnioserosa contractile forces are essential to the process, and disruption of amnioserosa contractility leads to a failure in dorsal closure and to a dorsal open phenotype (Scuderi and Letsou, 2005; Caussinus et al., 2012). This contraction is resisted by the intracellular pressure generated by the body of the AS cells. In addition, an overall tension arises from the epidermis encompassing active contractile and passive forces emanating from the body of the epidermal cells (Fig. 1-B). The contribution of these last epidermal forces is demonstrated by laser dissection of the leading edge cells along the epidermis-amnioserosa boundary resulting in a persistent retraction of the epidermal layer (Hutson et al., 2003).

In addition to these different forces originating in the amnioserosa and epidermis, tensile forces are present along the leading edge of the epidermis (Fig. 1-B). These forces are generated by a supra-cellular actomyosin cable that forms around the dorsal opening (Kiehart et al., 2000; Hutson et al., 2003). The actomyosin cable contracts and shortens the leading edge, much like a purse string, bringing the two epidermal sheets together. This contraction is inhomogeneous along the cable and most of it occurs at the zipping points (Peralta et al., 2008).

Finally, once the two layers are close enough they contact each other via sheet- and finger-like protrusions, known as lamellipodia and filopodia respectively (Fig. 1-B). These contacts develop into adhesion sites at the canthi of the opening, as the epidermal layers 'zip' together. How the adhesion forces generated during zipping contributes to whole-scale DC dynamics is still unclear.

Overall, the combination of these different forces (amnioserosa contraction, actin cable tension and zipping all counteracting intracellular amnioserosa pressure and epidermal tension) drives the process of DC. In the following sections of this review, we will detail each of these force generators and their contribution to the process.

3. The actin cable: a supracellular force generator

A key feature of DC is the formation of a supra-cellular actomyosin cable in the epidermal leading edge cells surrounding the amnioserosa tissue (Fig. 2-C) (Young et al., 1993; Kiehart et al., 2000). This cable forms at the onset of DC within each epidermal cell and connects from cell to cell via adherens junctions. These lateral junctions include E-cadherin and integrin-mediated adhesions (Narasimha and Brown, 2004). Similar structures occur in wound healing and their formation is also JnK-dependent (Bosch et al., 2005). In embryos mutant for Jnk (*bsk*), the leading edge cells do not form an actin cable and DC does not complete, leading to a dorsal open phenotype (Homsy et al., 2006). JnK may also play a role in the contraction of AS cells as recent works have shown that genetic suppression of the cable does not inhibit the completion of closure but rather results in scarring. In these two studies, the extent to which actin cable removal affects closure dynamics is different, but overall the kinetics appear slower (Ducuing and Vincent, 2016; Pasakarnis et al., 2016).

The supracellular actin cable generates contractile forces within each cell along the leading edge, resulting in a tension that propagates over a long-range at the amnioserosa-epidermal interface (Figs. 1 and 2-C). These contractile forces are regulated by Rho-GTPases and local modulation in rho activity leads to defects in the integrity of the cable (Jacinto et al., 2002).

The reason contractile forces tangential to the opening shape can promote progression of the epidermis towards the dorsal pole arises from a simple physical principle described by Laplace and Young in the 19th century. The radius of curvature of the interface between two fluids directly depends on the difference in pressure between these two fluids and on the surface tension of the interface (Fig. 2-A). It is important to note that the origin of the surface tension or pressure difference, whether it be from passive or active forces, does not affect this description. Therefore, the Young-Laplace relationship is well suited to the description of biological systems. In the "two-dimensional" (due to the single epidermal sheet topology) case of DC, the forces applied to the interface by the amnioserosa and epidermis, are balanced by the resultant orthogonal force from the contraction of the actin cable (proportional to T/R, with T the tension generated by the actin cable and R the radius of the opening).

Laser dissection probing tension in the actin cable (Saias et al., 2015) has shown that it increases steadily over time (Fig. 2-B). This increase correlates with an augmentation of myosin concentration in the cable and therefore supports the view that actin and myosin accumulate at the AS-epidermis interface in a cable structure to build up tension. Consistent with this view, the radius of curvature of the opening has been shown to remain constant during most of closure; thus, changes in closure contributing forces generated by the cable only depend, proportionally, on the increase in interface tension over time (Fig. 2-B). Towards the end of DC it has been reported that the radius of curvature decreases strongly (Saias et al., 2015; Peralta et al., 2007), therefore increasing the contribution of the cable (Fig. 2-A and B). Interestingly, at this stage, the myosin levels and lateral tension in the cable appear to plateau (Fig. 2-B). We speculate that at this stage, additional active mechanisms associated with zipping could regulate the radius of curvature of the opening to continue increasing the contribution of the actomyosin cable. We will discuss this possibility in more detail in the following sections while examining the mechanisms that underlie zipping.

4. The amnioserosa: a force generating apoptotic tissue

A central player of DC is the amnioserosa (AS) tissue that bridges the epidermal gap. After specification, this tissue undergoes a series of morphological rearrangements during germ band elongation and retraction (Lacy and Hutson, 2016). The AS appears to have a predominant mechanical role during these stages, applying forces essential for proper epidermal movements (Lynch et al., 2013). In the context of DC, many studies have characterized the mechanical contribution of the AS tissue to the process (Young et al., 1993; Hutson et al., 2003; Saias et al., 2015; Franke et al., 2005; Solon et al., 2009).

The actomyosin cytoskeleton of the AS is an essential machinery to generate forces that counterbalance the epidermal tension and promote DC (Young et al., 1993; Hutson et al., 2003; Saias et al., 2015). A main actor is the molecular motor non-muscle myosin 2, which generates forces by acting on the cortical and medial actin meshwork. Suppression of myosin 2 in the amnioserosa tissue totally impairs DC and leads to an open-phenotype (Caussinus et al., 2012). High-resolution live imaging of myosin 2 in the AS cells during DC shows dynamic propagating waves of contractions (Fig. 3-B) (David et al., 2010; Blanchard et al., 2010). Myosin 2 accumulates locally at the medial array of the AS cells and generates a local contraction (Fig. 2-C), this contraction then propagates within the cell as a contractile wave (Fig. 3-B). Each individual wave of contraction results in a global contraction of the cell, generating regular cell shape oscillations over time (Fig. 3-B) (Solon et al., 2009; Blanchard et al., 2010). Neighboring AS cells preferentially oscillate in antiphase: while one cell is contracting its apical surface area, its direct neighbors are typically expanding theirs. Over the process of DC, these pulsed contractions dampen sequentially from the outermost layer of AS cells to the most dorsal ones, the outermost layer finally sliding under the epidermis (Solon et al., 2009; Sokolow et al., 2012). It has been proposed that these cellular pulsations promote the progression of DC in coordination with the actin cable at the leading edge, in a ratchet like manner (Solon et al., 2009). In this model, the pulsatile activity of the AS tissue would directly influence the shortening of the actin cable resulting in a dorsalward progression of the epidermis.

A peculiarity specific to the AS tissue is that it becomes apoptotic during DC (Saias et al., 2015; Reed et al., 2004; Shen et al., 2013). After the process of DC, the dying AS cells are engulfed by the hemocytes sitting right underneath the epidermis. As a consequence of apoptosis, a subset of the AS cells delaminate from the AS tissue by quickly reducing their apical area prior to the completion of DC (Fig. 3-C). A small amount of these cells delaminate in the early stages of DC (~5% of the cells in the first hour of DC) while more delaminations are observed in the last hour of DC (~10%) (Kiehart et al., 2000; Saias et al., 2015). AS cell delamination is suppressed when apoptosis is inhibited and closure rate is slower; conversely, an overexpression of apoptotic genes (such as *hid* or *reaper*) results in faster dynamics (Muliyil et al., 2011; Toyama et al., 2008). Together this suggests that apoptosis can exert an effective force promoting DC. Using a caspase activity marker, recent studies highlighted that early stages of the apoptotic program were activated, not only in a subset of AS cells, but in the entire tissue at the onset of closure (Saias et al., 2015; Shen et al., 2013). The progression of the

apoptotic program therefore appears to take place during DC in the entire tissue, apoptosis being completed in most cells after DC, as stained by acridine orange (Reed et al., 2004). In addition to some cells modulating the mechanics of the process by delaminating from the epithelial sheet, all AS cells also generate an effective contractile force by decreasing their individual volume through the progression of the apoptotic program (Saias et al., 2015). Cell volume decrease is a hallmark of apoptosis and depends on ion fluxes, particularly potassium ions (Bortner and Cidlowski, 2002; Saias et al., 2015). During DC, this reduction in volume promotes leading edge progression via a decrease in the apical AS surface area without significant increase in the apico-basal length of the AS cells (Fig. 3-A).

The overall force contribution of the AS cells during DC therefore has multiple components: i) a cortical surface tension with myosin 2 contractile pulses promoting closure, ii) a resisting force, arising from the volume of the AS cells (which is reduced by the decrease in cell volume

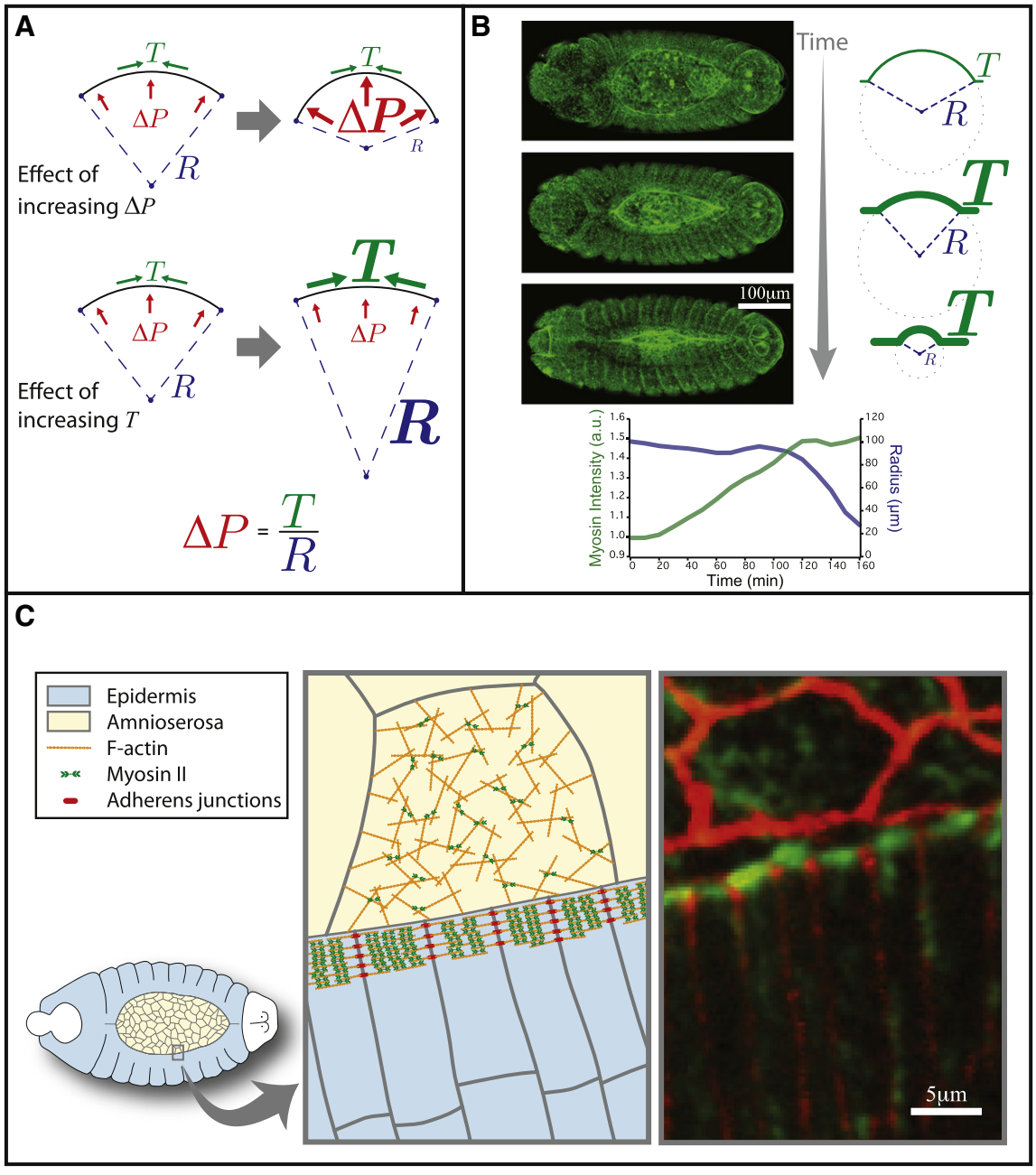

Fig. 2. Force generated by the actin cable. (A) Schematics depicting the Laplace law for the interface between two liquids. The radius of curvature R is dependent on the ratio of the line tension at the interface T (or surface tension for a 2D interface), with the difference in pressure between the two liquids ΔP (with units of force per length, or force per area for a 2D interface). An increase in the pressure difference, at constant interface tension, results in a reduction of the radius of curvature of the interface (top), while an increase in interface tension leads to an increase in curvature radius, when pressure difference is constant (bottom). (B) During dorsal closure, tension in the cable and myosin concentration increase linearly until reaching a plateau. During this tension increase, the curvature radius remains approximately steady. The average radius of curvature decreases in late DC, when the plateau of tension is reached (bottom: the graph shows an example taken from an embryo). The three images are the same as images 1, 3 and 5 from Fig. 1 and show an embryo expressing myosin tagged with GFP at 3 stages of DC. (C) Schematics showing the architecture of the actin cable. The actin cable is connected from cell to cell at the leading edge of the epidermis. Similarly to focal adhesions, assemblies of actomyosin filaments are bound by integrin and cadherin mediated adherens junctions across the cell-cell borders, giving the actin cable its supra-cellular nature. The image on the right shows the area near to the LE-AS interface in an embryo expressing E-cadherin tagged with tomato fluorescent protein (red) and myosin tagged with GFP. The green actomyosin cable can be seen clearly at the interface within the LE cells (image courtesy of Angughali Sumi).

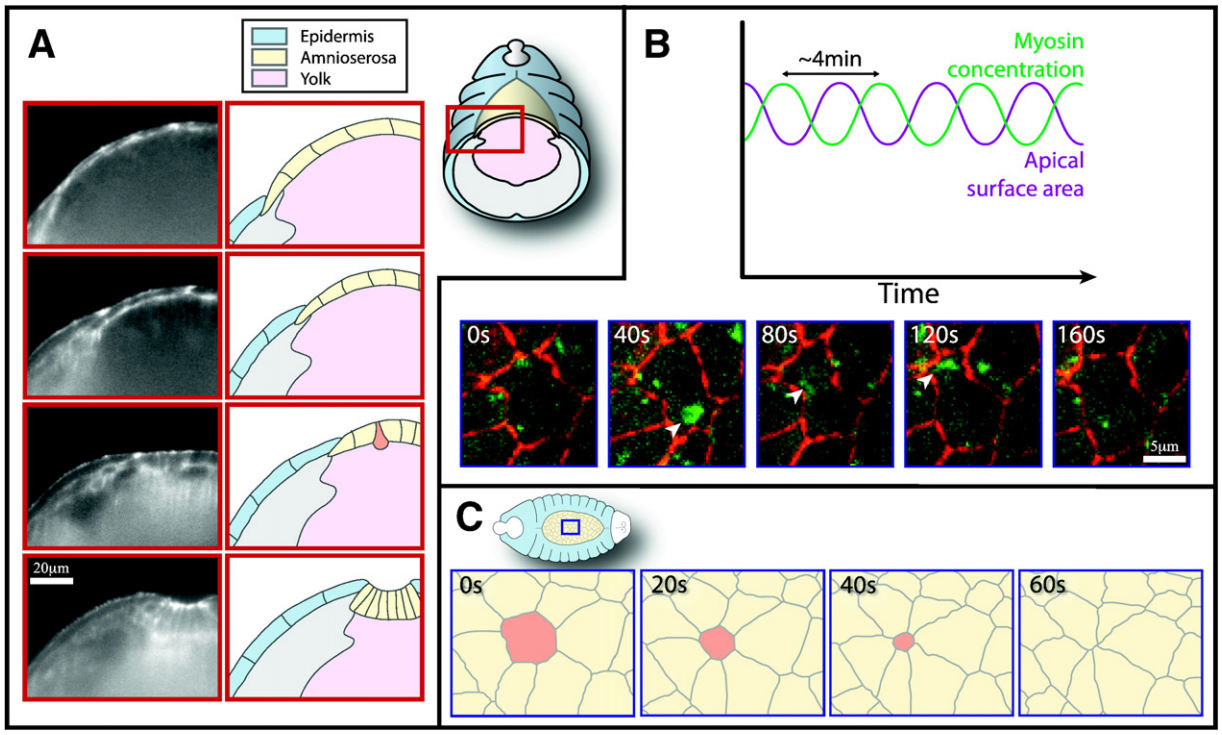

Fig. 3. Forces generated by the amnioserosa tissue. (A) Contraction of the AS tissue during DC. On the left, a transverse section of the AS tissue in an embryo expressing sqh-moe-GFP, performed with SPIM (images courtesy of Laure Saias and Jim Swoger) and on the right, related schematics showing a section of the tissue. During early closure, the tissue contracts with a decrease in individual cell volume and without significant apico-basal elongation. During later stages of DC, cells do elongate basally and actin and myosin accumulate apically. At these stages, individual cell delaminations occur (depicted in the third schematic, but not visible from the images). (B) (Top) Graph sketching the behavior of an individual cell's apical area and myosin levels over time during DC. Area and myosin concentration oscillate nearly antiphasically (the shift being a consequence of cortex turnover) with a periodicity of about 4 min. (Bottom) Time-lapse showing an AS cell expressing sqh-GFP and Tomato-Ecadh. Oscillations of a single cell correspond to the propagation of a local myosin contraction through the cell (arrows) (images courtesy of Angughali Sumi). (C) Schematics adapted from AS tissue segmentation showing the rapid delamination of an individual AS cell as a result of apoptosis. The apical cell surface shrinks to zero in approximately 1 min.

due to apoptosis, thus promoting contraction), and iii) a sporadic effective force promoting closure coming from the delamination of a subset of cells (Fig. 3-A). In addition, the cortical tension of the AS cells, which appears to be constant in the early stages of the process, seems to increase significantly during late stages of DC, promoting the invagination of the tissue (Fig. 3-A) (Hutson et al., 2009; David et al., 2013). It is interesting to note the dual contribution of the AS cells, that generate an apical surface tension resulting from the activity of the actomyosin meshwork and a resisting pressure due to the volume of each AS cell. We will discuss later in the model section of this review the interplay between these two components.

5. Epidermal sealing: a zip made of filopodia and lamellipodia

At the end of DC, the two-epidermal sheets have completely fused, leaving no scar. This sealing is achieved by a progressive zipping, taking place at the two canthi of the opening (Fig. 1). Some studies indicate that epidermal zipping could contribute a significant driving force to DC dynamics (Hutson et al., 2003; Peralta et al., 2007). However other work reported minimal changes in epidermal convergence in the absence of zipping (Jankovics and Brunner, 2006), and as such the extent of its role in closure progression lacks consensus. In this section, we will describe our knowledge on the mechanisms driving zipping and its contribution to closure.

Leading edge cells show a fascinating protrusive activity during DC. Flat pancake-like protrusions (lamellipodia), and finger like protrusions (filopodia), are polarized towards the dorsal side of the embryo in a similar way to those of migratory cells (Jacinto et al., 2000). At DC stage, the epidermis is segmented and its sealing requires a perfect matching of epidermal compartments (such as engrailed or patched). The epidermal protrusions play a direct role in the compartment recognition at the moment of final adhesion to ensure the perfect matching between opposing epidermal segments. Modulation of filopodial morphology by expression of the rho-GTPase cdc42 in the engrailed gene expression pattern (laterally striped) can lead to mismatches in segment adhesion (Jacinto et al., 2000). A high-resolution dynamical analysis of filopodia indicates guidance cues that orient the filopodia towards opposing segments of the same identity (Millard and Martin, 2008). The mechanisms and molecular players involved in the segment recognition, and the precise role of the filopodia are still unclear. What is known is that the epidermal protrusions are essential to zipping.

In contrast with the protrusions described in cell migration (Mattila and Lappalainen, 2008), in addition to the actin filaments, the leading edge protrusions contain microtubule bundles (Jankovics and Brunner, 2006). This microtubule core confers on the filopodia a high temporal persistence and a length of several microns (Fig. 4-A). Disruption of microtubules, either by pharmacological treatment or by overexpression of the microtubule severing protein spastin, induces a complete disruption of protrusions and an absence of zipping (Jankovics and Brunner, 2006). During DC, the microtubule network aligns along the dorso-ventral axis in the leading edge cells and this alignment is thought to be responsible for the polarized generation of protrusions necessary for segment matching and zipping. A recent study performed using 3D tomography reconstruction also identified the involvement of microtubules in the maturation of adhesions during zipping, and proposed that forces generated by these microtubules could aid strengthening of the adhesions (Fig. 4-B) (Eltsov et al., 2015). Apposing leading edge cells were observed to begin adhesion apically by reaching over more than one AS cell, requiring rearrangement of AS-epidermal adhesions. Simultaneously, AS cells near to the canthi were observed to undergo rapid apical constriction and high rates of ingression, which have been proposed to augment zipping by drawing the adhering cells closer.

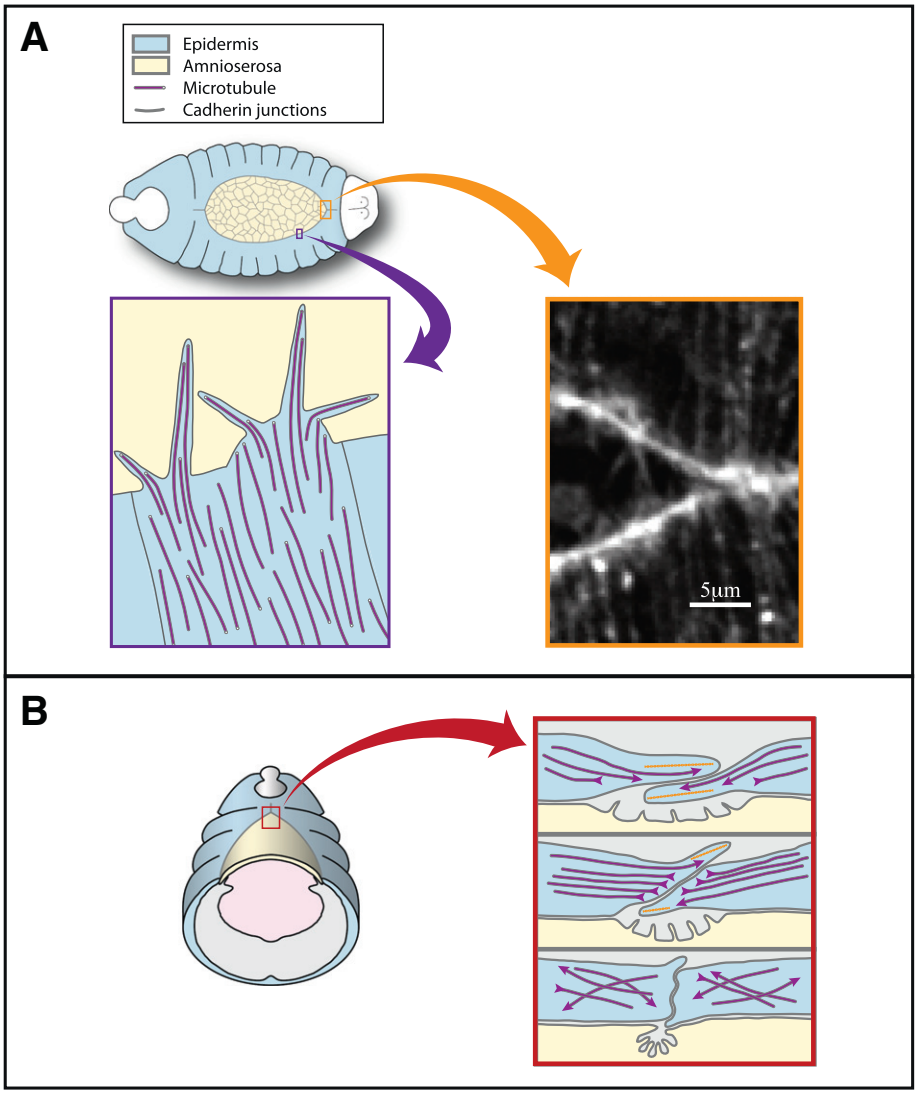

Fig. 4. Epithelial sealing by zipping. (A) Zipping involves the protrusive activity of lamellipodia and filopodia. On the left, the schematic depicts the protrusions emitted by the leading edge cells over the amnioserosa tissue. The protrusive activity depends on the microtubule network that realigns apically in the leading edge cells. On the right, a snapshot of a moesin-GFP (staining the actin network) expressing embryo showing filopodial activity close to the zipping point. (B) Schematics adapted from (Eltsov et al., 2015) showing zipping and subsequent epidermal adhesion. The microtubules protrude in the lamellipodia and serve as a scaffold to stabilize the adhesion zone. After the adhesion zone has extended, the microtubule network reorganizes in the epidermal cells.

Later the bound epidermal cells square off basally to complete the seal (Eltsov et al., 2015; Lu et al., 2016).

Does zipping contribute to forces driving DC? As previously mentioned, it is not clear whether zipping contributes to force generation in whole-scale DC, however, some observations suggest that it could play a significant role. For instance, embryos with impaired zipping show a significant reduction in closure rate during the late stages of DC (Jankovics and Brunner, 2006). This difference in closing rate could result from an increase in the radius of curvature of the opening in non-zipping embryos. As a result, the tension generated by the actin cable would be converted less into an orthogonal force along its length promoting closure (as a consequence of Laplace law). Zipping could then act indirectly as a promoting force by controlling the actin cable curvature radius during the last stages of DC (Fig. 2-A and B). Indeed, towards the end of the process, the tension in the actin cable plateaus and the radius of curvature decreases (Fig. 2-B). The reduction in radius could be the direct consequence of zipping occurring at both canthi, either by manipulating the AS pressure or by driving the system out of equilibrium. Supporting this idea, we observe a systematic deviation from the cable's circular arc geometry at both canthi, suggesting they are pulled together from a distance. In this way, closure may occur as a result of changes in the steady states, imposed by the Laplace equality, due to progressive zipping. However, additional studies are necessary to fully evaluate the contribution of zipping in DC.

6. Biophysical models of DC: from a coarse grained description to individual cell behavior

As experimental methodologies allowed the deciphering of the main players acting during DC, several biophysical models have been developed to provide a quantitative description of the process. In this section, we will review some of these models and highlight routes for future model development.

The emergence of laser dissection techniques brought about the first assessments of the role of each cellular player to the mechanics of DC (Hutson et al., 2003). This permitted the development of quantitative mathematical models encompassing the contribution of each of the forces in the process. The first description was based on the principle that the closure dynamics depend on an imbalance of forces from different origins: a tension arising from the actin cable, the contraction of the

AS tissue and a tension generated by the epidermis (Fig. 5-A) (Hutson et al., 2003). In this model, the contribution of the cable is described by the Young Laplace law (Fig. 2-A), and a zipping term is empirically implemented as the result of the interaction between two circular arcs with a constant zipping rate (Fig. 5-A). This simple mathematical description facilitated the quantification of the different force contributions. Particularly, it highlighted the concerted role of the amnioserosa tissue and actin cable. A derivation of this model was later developed to include the asymmetry in the opening shape and the rate of zipping at opposing canthi (Peralta et al., 2007). In this case, the curvature of the actin cable is still approximated by two arcs of a circle.

Two extensions of this model have also been developed to consider a less-constrained geometry of the opening, which can deviate from two circular arcs depending on local forces (Layton et al., 2009; Almeida et al., 2011). These two last models are conceptually similar with slight differences on the integration of zipping and the AS contribution. While

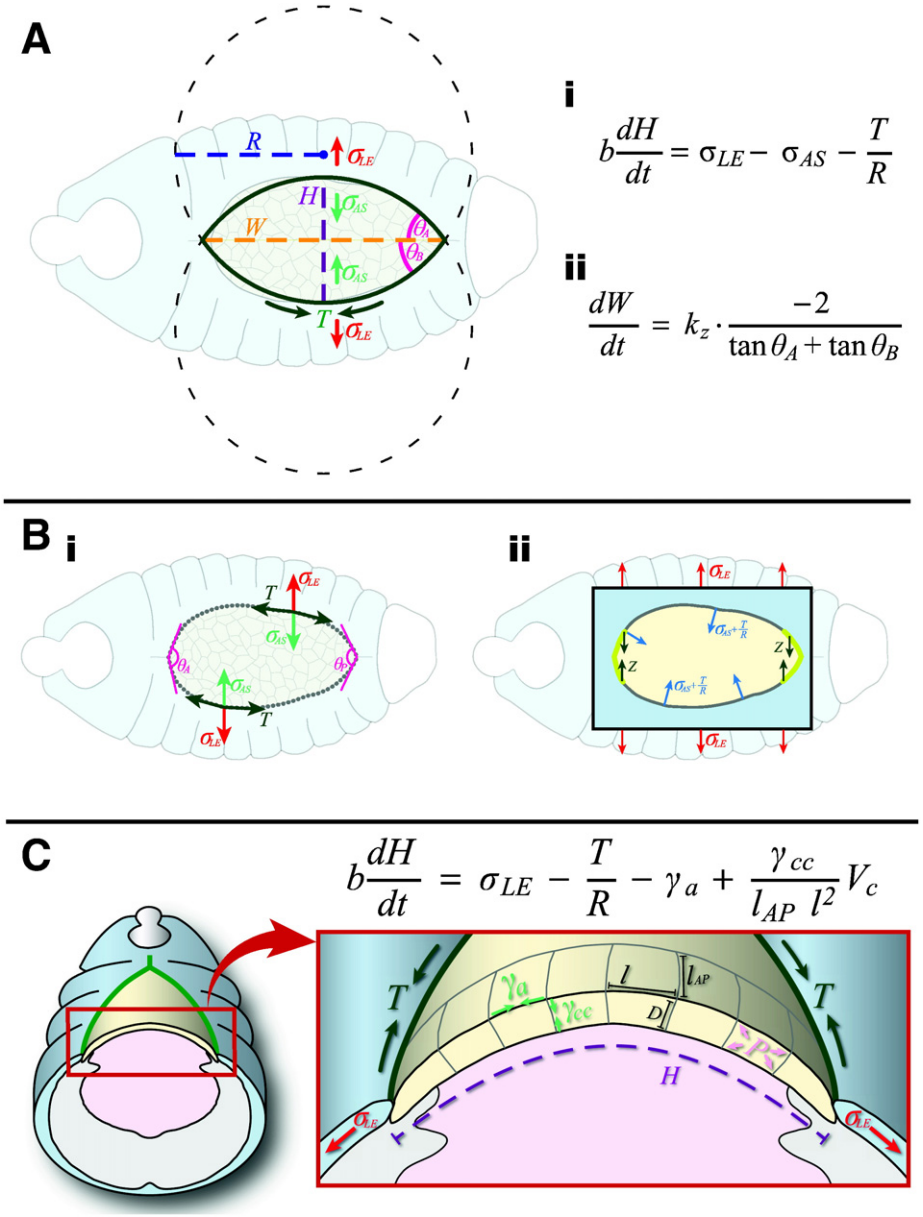

Fig. 5. Biophysical descriptions of DC. (A) Hutson et al. (2003) present a first generation model for DC. The opening shape is simplified to the symmetrical case of two overlapping circular arcs and as such the progress of closure can be represented by two, one-dimensional descriptions: (i) The rate of change of the opening height (H), multiplied by a friction coefficient (b), is given by the force balance between the closure promoting contributions of the actin cable (T/R) & amnioserosa contraction (σ_{AS}) and the tension in the epidermis antagonistic to closure (σ_{LE}). (ii) The opening width (W) decreases at a velocity given by the product of an empirically derived zipping rate constant (k_z) and a function of the half angles at the canthi (θ_A and θ_B). (B) The second generation of DC models, of which there are two versions, moved to a fully 2D description in which the opening shape is not constrained to the symmetrical two-arc geometry. (i) Layton et al. (2009) achieve this by applying the force balance (from panel A-i) to points distributed along the leading edge, giving a rate of change of H_i (the local opening height) for each point. The zipping rates at the two canthi are again described using a zipping rate constant (k_z) but depend on the angles at each canthus separately (θ_A and θ_P). (ii) Almeida et al. (2011) achieve their non-symmetrical 2D model by considering the deformation fields of the epidermal sheets. The two sheets (amnioserosa and epidermis) are considered to be linearly elastic, isotropic and homogenous, and together fill a rectangular domain around the opening. Deformations along the upper and lower bounds of the domain result from the tension in the epidermis (σ_{LE}), whereas those along the leading edge are the consequence of amnioserosa contraction and cable tension ($\sigma_{AS} + T/R$). Zipping is emulated by applying an additional vertical adhesion force (Z) to regions of the LE within a given distance (twice the average length of filopodia) from the opposing LE (represented by the yellow strip). (C) Saias et al. (2015) return to a 1D description using the equation in (Ai), however within this paradigm they explore the effects of the 3D geometry of the tissue. The contractile contribution of the amnioserosa (σ_{AS}) is separated into two components: *i*) the active contractile force from the AS cell cortices (γ_a), and *ii*) a pressure term (P) resulting from the ratio of the cells' apico-basal tension (γ_{cc}) along the length D and distortion; given by the relationship between the cell's volume (V_c) and its dimensions along the apical plane (l and l_{AP}).

one model still considers an ad hoc zipping rate for each canthus (Layton et al., 2009), the other associates, a constant force to sections of the cable that lie within a given distance (twice the typical filopodial length) of the opposite leading edge (Almeida et al., 2011); these sections are consistently found near the canthus (Fig. 5-B). In their current state, these models assume a constant contribution of the different forces over time; however some experimental evidence points towards temporal variation of these forces (for instance the actin cable tension, as discussed in Section 2). In addition, these models are limited to two dimensions and do not consider the 3D behavior of cells during the process.

Recently, a model was implemented to attempt to include the contribution from the 3D architecture of the AS cells (Saias et al., 2015). In this work, the geometry of the opening is still approximated by two symmetrical circular arcs, with an experimentally measured curvature radius. Zipping is not directly included (it is set by the radius of curvature), but the 3D shape of the AS tissue and of the embryo is taken into account (Fig. 5-C). The time evolution of the different tensions is also considered, and reduces the required number of free parameters to recapitulate the kinetics of shape changes during DC. A natural extension of these descriptions would therefore aim to combine the experimental variations of the different tensions, the 3D geometry of the process and a physical description of zipping.

This series of models does not describe individual cell behavior and treats the tissue as a continuous homogeneous material. A few models have also been developed to describe single cell behavior, particularly AS cell pulsations and LE fluctuations (rather than the overall closure dynamics) (Solon et al., 2009; Wang et al., 2012; Dierkes et al., 2014; Jayasinghe et al., 2013). In these models AS cell contraction is generated by an active force on an elastic material, modeling the contraction of myosin on the cortical actomyosin meshwork. In order to generate regular periodic pulses, the active force was either considered as dependent on the stretch applied to the cell (Solon et al., 2009; Jayasinghe et al., 2013) or on the turnover of the binding/unbinding myosin motors (Wang et al., 2012; Dierkes et al., 2014). The latter shows that coupling turnover of a contractile molecule with an elastic material is sufficient to generate regular oscillations. It is interesting to note that, in these models considering cortex turnover, an emergent property of the turnover of contractile molecules is a recovery of the active tension following stretch occurring with a certain time delay: the turnover time. This time delay is essential to generate sustained oscillations. In this respect, these models are similar to the previous models where stretch dependent force requires a time delay to generate oscillations. Recent experimental evidence points towards an essential role of the turnover of actin and myosin in the regulation of these contractions (Jodoin et al., 2015), however the precise regulation of these pulsed contractions still remains unclear. It is also not clear whether these contractions actively contribute to the progression of DC, and additional experimental studies are required to allow the design of biophysical models connecting cellular behavior to global tissue movements.

7. Discussion

In this review, we have detailed our current understanding on the coordination of forces acting during the process of dorsal closure. Several sources of forces, requiring both global and local control, interplay to ensure epidermal sealing, rendering dorsal closure a popular model system, which could help understand many developmental processes and diseases, e.g. spina bifida and cleft palate. Similarly to wound healing, a supracellular cable forms to generate tensile forces that, as a result of cable curvature, translate to a 'closing pressure'. Apoptosis plays a central role in modulating the mechanics of the AS tissue during the process and such a mechanical role for apoptosis is likely to exist in other developmental contexts such as the sculpting of the mammalian brain (Buss et al., 2006) and heart (Barbosky et al., 2006). The presence of whole-tissue apoptosis itself brings interesting questions as to how such a process may be coordinated on such a large scale. Also, epithelial zipping appears to be a well-conserved mechanism to generate de novo cell-cell adhesion (Hashimoto et al., 2015). Dorsal closure therefore encompasses many fundamental mechanisms of epithelial remodeling.

While we are now at the stage where a good description of the mechanics of the process has been achieved, many fundamental questions remain unaddressed. The mechanical role of the AS cell pulsations remains hotly debated. No clear evidence for the function of such visually striking, and energetically costly dynamic behavior, has been forthcoming. Such pulsations appear to be an intrinsic property of many animal cells (Martin et al., 2009; Rauzi et al., 2010; Maitre et al., 2015; Bergert et al., 2015; Ruprecht et al., 2015). One possible explanation is that their role is simply to generate noise in the system. Such noise generation could, through an as yet undescribed mechanism, help the system to reach its final state in much the same way that cycles of cooling and warming are necessary for glassy materials to approach a state of minimum potential. As an analogy for this, consider filling a box with rocks: adding noise to the system by shaking the box can allow the rocks to rearrange and find their minimum potential, causing them to occupy less space. The difference being that, in this system, the rocks would be actively generating the noise. At the cellular level, these pulsations could allow fast cellular plasticity to adapt to the changing environment. In this way, AS cells may be able to remodel their adhesions easily. Indeed, AS cells are adhering to the yolk sac via an ECM layer and this adhesion is an essential component necessary for AS contraction (Narasimha and Brown, 2004). The mechanical contribution of AS cell-yolk interactions could be multiple: they may provide frictional forces to counteract the epidermal tension, while adhesion site rearrangement could facilitate fast AS cell shape remodeling. Also, the yolk dynamics may have a significant contribution via this adhesion, and further studies would be necessary to identify their role in the process. The influence of zipping has also been largely overlooked. The interplay between the tension generated by the actin cable and the adhesion occurring at the canthi, presents itself as an interesting starting point for future studies. Finally, extrinsic forces coming from concomitant processes, such as spiracle formation or head involution, are likely to influence the force balance, and result in some of the observed asymmetries in dorsal closure. It is particularly notable that the onset of epidermal spreading during head involution, coincides with an acceleration in zipping (Peralta et al., 2007). Why would the embryo combine so many different forces to coordinate DC? One obvious reason lies in robustness: a level of redundancy exists between these different force generating mechanisms, that together can ensure closure even when one is partially missing (Hutson et al., 2003).

The holy grail for understanding the orchestration of dorsal closure, and indeed many morphogenetic events, would be to complete the mechanistic loop: to combine our knowledge of the signaling machinery, known to play an important role in DC (such as JnK), with the activation of different force-generating mechanisms, and, in turn, to reveal the mechanical feedback onto both cellular signaling processes and the force generation itself. Notably, the actin cable formation and apoptosis activation are concomitant; JnK is known to be involved in cable regulation, but how? Also, how could mechanical cues, in response, affect JnK signaling? What is the signal that activates apoptosis? Is there regulatory feedback between the machinery regulating actin cable formation and apoptosis? These are questions that will need to be addressed to properly understand how the different tissues present in DC are able to self-organize in a way that leads to such a highly reproducible sequence of events.

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