Drosophila morphogenesis: tissue force laws and the modeling of dorsal closure

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Dorsal closure, a stage of *Drosophila* development, is a model system for cell sheet morphogenesis and wound healing. During closure, two flanks of epidermal tissue progressively advance to reduce the area of the eye-shaped opening in the dorsal surface, which contains amnioserosa tissue. To simulate the time evolution of the overall shape of the dorsal opening, we developed a mathematical model, in which contractility and elasticity are manifest in model force-producing elements that satisfy force-velocity relationships similar to muscle. The action of the elements is consistent with the force-producing behavior of actin and myosin in cells. The parameters that characterize the simulated embryos were optimized by reference to experimental observations on wild-type embryos and, to a lesser extent, on embryos whose amnioserosa was removed by laser surgery and on *mysospheroid* mutant embryos. Simulations failed to reproduce the amnioserosa-removal protocol in either the elastic or the contractile limit, indicating that both elastic and contractile dynamics are essential components of the biological force-producing elements. We found it was necessary to actively upregulate forces to recapitulate both the double and single-canthus nick protocols, which did not participate in the optimization of parameters, suggesting the existence of additional key feedback mechanisms. [DOI: 10.2976/1.3266062]

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Dorsal closure takes place midway through *Drosophila* embryogenesis. It represents a well-characterized model for cell sheet morphogenesis during development and wound healing in systems that include vertebrates [reviewed in Harden (2002) and Jacinto *et al.* (2002)]. It offers the opportunity to map how cells produce, respond to and transmit forces, and keys to understanding the mechanisms of morphogenesis (Keller *et al.*, 2003). *Drosophila* embryos are shaped like irregular prolate ellipsoids, which are approximately 450 μm long and have a nearly circular cross section 150 μm in diameter. An opening in the dorsal surface of the epidermis, referred to as the “dorsal opening,” is shown in Fig. 1(A). It follows the morphogenetic stage called germ band retraction. It is shaped roughly like a human eye, bounded by two arcs intersecting at two canthi (a canthus

is a corner of the eye). The dorsal opening contains the “amnioserosa,” a cell sheet monolayer with large flat polygonal cells. The epidermis flanking the amnioserosa, referred to as the “lateral epidermis,” is composed of smaller cuboidal-to-columnar cells. Along each flank, a single row of the dorsal-most lateral epidermal cells, called the leading edge cells, lies over the outer most row of amnioserosa cells and constitutes a genetically distinct tissue (designated mitotic domain 19 by Foe, 1989). Contacts between this row of leading edge cells and the outermost amnioserosal cells mediate adhesion between these two tissues and their relationship remains virtually unchanged through the course of closure (see Fig. 1 in Rodriguez-Diaz *et al.*, 2008). An actomyosin rich, supracellular cable, or “purse string” runs through these cells sufficiently close to the leading edge that we use the position of the purse

Figure 1. Confocal fluorescent images of native and perturbed dorsal closure in embryos that express GFP-moe. As described elsewhere (Kiehart *et al.*, 2000), such embryos are considered wild-type unless they carry additional mutations [as in (F)]. Nevertheless, they carry the *sGMCA* transgene that encodes GFP-moe and the *mini-white^{wt}* transgene for eye color, a genetic marker used in the selection of germ line transformants. They are also homozygous for *white* (eye color) at the endogenous *white* locus. They can be maintained as a homozygous stock and undergo embryonic development normally. (A) An image of the dorsal surface of a closure staged embryo showing key biological tissues and structures (AS, amnioserosa; LE, lateral epidermis). Time-lapsed images of: (B) a native (i.e., not laser-perturbed) embryo; (C) an embryo with laser cuts to multiple locations designed to compromise the force contributed by the amnioserosa (amnioserosa-removal protocol); (D) an embryo with alternating laser cuts near each canthus to compromise zipping (double-canthus nicking protocol); (E) an embryo with repeated laser cuts compromising zipping near only the posterior canthus (single-canthus nicking protocol); and (F) an embryo homozygous for a null mutation in *myospheroid*, which encodes the β_{PS} integrin subunit. Black lines in (B)–(E) indicate the targeting of laser incisions; elapsed time commences with the first image taken in (B)–(F). (B)–(F) are modified from Fig. 1 in Hutson *et al.* (2003) such that anterior is to the left in all figures. A scale bar in (A) is 50 μ m and in B (bottom panel) is 50 μ m [it applies to all panels in (B)–(F)].

string (which we visualize experimentally through a fluorochrome bound to F-actin) as an indication of the position of the leading edge. Laser microsurgery experiments show that dorsal closure [see Figs. 1(A) and 1(B)] is driven by active cell shape changes in each tissue as described below (Kiehart *et al.*, 2000; Hutson *et al.*, 2003; Rodriguez-Diaz *et al.*, 2008; Ma *et al.*, 2009; Solon *et al.*, 2009). The purse strings shorten, largely through contractions near the canthi [see below and Peralta *et al.* (2008)], and cells of the lateral epidermis elongate toward the dorsal midline. At the same time, cells of the amnioserosa actively change shape anisotropically as their apical surfaces contract to help draw the lateral epidermal sheets together (Kiehart *et al.*, 2000; Jacinto *et al.*, 2002; Gorfinkiel *et al.*, 2009; Solon *et al.*, 2009). Recent experiments demonstrate that cell shape

changes in the amnioserosa are contributed by forces produced near cell junctions and distributed over the apical cytoskeleton (Ma *et al.*, 2009). In addition, our experimental observations indicate that the predominant cellular movement in this stage of dorsal closure occurs normal to the dorsal midline, so we also implement this observation as an assumption of the model (unpublished observations). When brought into close proximity, presumably by forces from the supracellular purse string and the amnioserosa, the two flanks of the lateral epidermis are “zipped” together at the canthi as filopodia and lamellipodia from opposing leading edges interdigitate, and a seam is formed (Jacinto *et al.*, 2000; Bloor and Kiehart, 2002; Gorfinkiel *et al.*, 2009). Ultimately, the dorsal surface is covered by a continuous epithelium that appears seamless. The entire process takes 2.5 h to 3 h at room temperature. When an embryo is gently flattened against a coverslip, most of closure can be followed in a single optical plane and embryonic development proceeds unperturbed until hatching.

Closure is perturbed by mutations in over 60 genes, indicating that the proteins responsible for closure include transcription factors, components of signaling pathways, cytoskeletal elements, adhesion molecules, and components of the extracellular matrix (Harden, 2002; Jacinto *et al.*, 2002). Of particular interest for us is the subset of genes that encode proteins that produce and/or transmit force(s) for closure.

A detailed structural mechanism by which forces are produced by the cytoskeleton has yet to be determined for dorsal closure. Indeed, it is likely that different structures are responsible for cell shape change in the leading edge (with its supracellular purse string) and in the amnioserosa (where the cytoskeleton is arranged in the form of belts at the level of the adherens junctions and as a meshwork in apical arrays). Regardless, experimental evidence identifies nonmuscle myosin II (whose heavy chain is encoded by *zipper*) as essential for force production in both the contractile purse string and the amnioserosa (Young *et al.*, 1993; Wood *et al.*, 2002; Franke *et al.*, 2005). Franke *et al.* (2005) investigated individual cells or small clones of cells that had substantially reduced myosin function using a transgenic mosaic approach. They demonstrated that nonmuscle myosin II is required for both maintenance of tension and active contractility in both the leading edge and the amnioserosa. They also demonstrated that cells depleted of nonmuscle myosin II fail to incorporate properly into the seam, i.e., this research established that nonmuscle myosin II is required for zipping. This conclusion is consistent with the analysis of purse string shortening during the course of closure that demonstrates that net loss of purse string length occurs at the canthi as part of the zipping process (Peralta *et al.*, 2008). A number of genes whose products are associated with the cytoskeleton directly modulate the morphology of arrays of F-actin and their dynamics have also been shown to contribute to dorsal closure (e.g., the barbed end, actin-capping protein antagonist, enabled (Gates *et al.*, 2007); or additional biological

motors such as myosin VI (Lin *et al.*, 2007); or myosin XV [Liu *et al.*, 2008].

Of additional interest are genes that encode cell surface receptors that mediate adhesion to other cells, such as the cadherins (Gorfinkiel and Martinez-Arias, 2007) or echinoid (Laplanche and Nilson, 2006; Lin *et al.*, 2007), or to the extracellular matrix, such as the integrins (Brown *et al.*, 2000; Hutson *et al.*, 2003; Narasimha and Brown, 2004). Mutations in *myospheroid*, *inflated*, and *scab*, which encode the β_{PS} , α_{PS2} , and α_{PS3} subunits of the cell adhesion/cell signaling transmembrane integrin proteins, compromise both zipping at the canthi and the integrity of the junction between the amnioserosa and the lateral epidermis, and cause dorsal closure to fail [Fig. 1(F)]. In addition, such mutations or mutations in interacting proteins cause delayed formation of the canthi, slowed closure, defects in adhesion between the amnioserosa and the yolk, defects in adhesion between the peripheral amnioserosa cells and the leading edge cells, aberrant actin rich purse strings, and alterations in the pattern of contraction of amnioserosa cells (Stark *et al.*, 1997; Kadrmas *et al.*, 2004; Narasimha and Brown, 2004; Reed *et al.*, 2004; Wada *et al.*, 2007; Gorfinkiel *et al.*, 2009).

To investigate the forces responsible for the movements of closure described above, we and others have used a variety of laser microsurgery protocols and compared laser-perturbed closure to native closure (Kiehart *et al.*, 2000; Hutson *et al.*, 2003; Homsy *et al.*, 2006; Peralta *et al.*, 2007, 2008; Rodriguez-Diaz *et al.*, 2008; Thayil *et al.*, 2008; Toyama *et al.*, 2008; Solon *et al.*, 2009). Here, closure is referred to as “native” when it is not laser-perturbed; thus, native closure can take place in wild-type or mutant embryos. The experiments indicated that four processes play an essential role in native closure. These are (1) tension caused by the actin and myosin complex of the purse strings; (2) tension caused by actin networks in individual amnioserosa cells; (3) resistance to stretching of the lateral epidermis; and (4) zipping at the canthi. Interestingly, there is redundancy in the four processes—none of them is absolutely required for closure. For example, closure proceeds at native rates even when zipping is inhibited; it also completes itself successfully when the integrity of either the purse string or the amnioserosa is compromised (Kiehart *et al.*, 2000; Hutson *et al.*, 2003; Rodriguez-Diaz *et al.*, 2008). On the other hand, repeated perturbation (designed to deter healing) of two or more contributing processes blocks closure.

To better understand these observations, Hutson *et al.* (2003) used biophysical reasoning to identify a Newtonian force balance equation [Eq. (1), below] at low Reynolds number that describes the forces acting on the ventral-most point [symmetry point, see Fig. 2(A)] of the purse string and provided a first generation quantitative model for closure

Figure 2. Geometry and forces of dorsal closure. Labeled schematics are of (A), the entire dorsal opening, and of (B), a segment of leading edge/purse string.

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

σ_{LE} is the magnitude of the normal force per unit length that the lateral epidermis applies to the leading edge and its purse string, σ_{AS} is the corresponding magnitude of the normal force per unit length applied by the amnioserosa, T is the tension of the purse string, and κ is the curvature of the purse string. The right side of the equation models the drag force, with dh/dt being the velocity of the symmetry point (h is the distance from the symmetry point of a leading edge to the dorsal midline) and b is an effective drag coefficient.

Hutson *et al.* (2003) used laser surgery to determine relative force magnitudes. The rapid removal of the mechanical contribution of a particular process (tissue) with a laser microbeam constitutes a so-called “mechanical jump experiment” [Fig. 1(C), see also Hutson *et al.* (2003)]. We used the immediate response to the loss of a single process in such mechanical jump experiments to generate a force ladder that describes the relative magnitude of the forces that are due to each process [detailed in Hutson *et al.* (2003); refined in Peralta *et al.* (2007); and considered further in Toyama *et al.* (2008)]. This assessment of the relative magnitudes of the contributing forces demonstrates that when zipping at one canthus is compromised by the laser microbeam [single-canthus nick protocol, see Fig. 1(E)], the zipping rate at the other canthus is upregulated (potentially through effects on the amnioserosa). Similarly, when zipping at both canthi are compromised [the double-canthus nick protocol, see Fig. 1(D)], the force produced by the amnioserosa is upregu-

lated (Peralta *et al.*, 2007). In addition, the upregulation of both σ_{AS} and k_z promote the completion of closure on schedule. These observations point to the redundancy of individual processes as one means of achieving the overall resilience of dorsal closure.

Along with Eq. (1), Hutson *et al.* (2003) proposed two additional model equations. We present them in the simplified context of a dorsal opening that has both anterior/posterior and left/right symmetry without foregoing essential mathematical insights [Fig. 2(A)]. In this case, the distances from the dorsal midline to either symmetry point (leading edge point at maximum opening) are identical and referred to as h . Similarly, the four canthus angles are identical and are referred to as θ . The first equation reflects the observation of Hutson *et al.* (2003) that the speed of closure u at a symmetry point is constant for most of the duration of native closure. This specifies the distance h from a symmetry point to the dorsal midline as a function of time (t), in terms of u equal to the absolute value of dh/dt and the initial position of the purse string $h(0)$,

$$h(t) = h(0) - ut. \quad (2)$$

The second equation is an empirical law of zipping,

$$\frac{dW}{dt} = -\frac{k_z}{\tan \theta}, \quad (3)$$

where W is the canthus-to-canthus distance [Fig. 2(A)] and k_z is the zipping rate constant. Hutson *et al.* (2003) make the additional assumption that the purse string retains the shape of a circular arc throughout the process. Both this and the assumed constancy of the speed of the symmetry point are consistent with experimental observations that hold for most of the duration of native closure. Circular arc geometry and eye morphology break down late in closure (tens of minutes before the end of closure) and closure completes itself edge to edge. Equations (2) and (3) also hold prior to failure for defects in closure that characterize homozygous *myospheroid* mutant embryos (which lack zygotic expression of β_{PS} integrin).

Substituting $\tan \theta \approx 2 \tan(\theta/2)$, which is approximately satisfied for most of the range of dorsal closure (Hutson *et al.*, 2003; Peralta *et al.*, 2007), gives the following equation:

$$\frac{dW}{dt} \approx -\frac{k_z}{2 \tan \frac{\theta}{2}}. \quad (4)$$

The geometric observation, $\tan(\theta/2) = 2h/W$, following from the circular arc leading edge assumption, allows Eq. (4) to be further reduced as follows:

$$\frac{dW}{dt} \approx -\frac{k_z W}{4h(t)}. \quad (5)$$

The system of Eqs. (2) and (5) is now solvable by Eq. (6)

$$W(t) = W(0) \left[1 - \frac{ut}{h(0)} \right]^{k_z/4u}, \quad (6)$$

where $W(0)$ is the initial canthus-to-canthus distance.

The constant speed of closure u was obtained by linear regression of experimental measurements. The constant of proportionality k_z was determined for a simulated embryo by optimizing the fit between the *in vivo* geometry of native closure and the global geometry predicted by Eq. (6). The model reproduced the evolution of the leading edge geometry to a good approximation, in regimes of dorsal closure in which its assumptions hold, validating the phenomenological Eq. (3) or Eq. (4) for the zipping rate (see Fig. 4 in Hutson *et al.*, 2003; Peralta *et al.*, 2008).

We name this a first generation model. It was extended by Peralta *et al.* (2007), who provided a detailed description of anterior/posterior asymmetry during closure. They replaced Eq. (3) with two equations, one for each canthus, setting the velocity of the canthus equal to a quantity similar to the right side of Eq. (3). We do the same in our work below, thus having the capability to explicitly account for asymmetries in dorsal closure as a consequence of laser perturbation. Peralta *et al.* (2007) determined that the zipping rate constant at the anterior canthus is 50% larger than that at the posterior canthus during wild-type native closure.

Accounting for the mechanical jump experiments within the first generation model is problematic. The model's assumption of constant closing speed is strongly violated in these experiments, which cause a change in direction of motion of the leading edge [Fig. 1(C)]. Indeed, following amnioserosa removal, the leading edge rapidly recoils ventrally (i.e., away from the dorsal midline) until it reaches a turning point, where it reverses its direction of motion and resumes closure to completion (Hutson *et al.*, 2003; Peralta *et al.*, 2007). Our second generation model, described below, relates mathematically the movements of the leading edge to a number of tissue force laws. It aims to explain experimental protocols, including those for which the first generation model cannot account. In the absence of a quantitative understanding of canthus dynamics, it retains the kinematic zipping law of the first generation model. The second generation model adjoins to the kinematic zipping law, a force balance equation similar to (1) at each point of the leading edge. The resulting system of equations has a unique mathematical solution. Given initial values for the tissue positions and forces, this set of equations provides the tissue positions and forces on the leading edge at any time during closure. The various parameters that the model equations contain are determined by optimizing the fit between simulated closure and experimental data.

FORMULATION OF A SECOND GENERATION MODEL

In this section, we provide a detailed explanation of how we formulate model equations that specify the contractile and

Figure 3. Dorsal closure in simulated embryos using the straight-line force-velocity relationship. Time evolution of (A) a native embryo; (B) an embryo without any force contribution from the amnioserosa; (C) an embryo with zipping inhibited at both canthi; (D) an embryo with zipping inhibited only at the posterior canthus; and (E) a *myospheroid* mutant embryo, simulated by reduced zipping rates at both canthi. These simulations correspond to experimental observations shown in panels (B)–(F) in Fig. 1. Time progresses from top to bottom in 630 s steps for all simulated embryos. Each column extends for 3780 s.

the elastic properties of the amnioserosa and of the purse string. We use these equations, together with the force balance and the zipping law, to link the movements of dorsal closure with the forces that are responsible for these movements, a feature absent from the purely kinematic first generation model. Readers who are not interested in how the model was formulated can skip from here to the Results section. Nevertheless, we think that the rationale behind the model provides important insight into the significance of the model, especially our derivation of a force-velocity relationship.

This second generation model, like its predecessor, applies to the bulk of closure that can be imaged in a single optical plane and is therefore two-dimensional. A linear law is prescribed for elastic tissue behavior (Hooke's law). The mathematical description of the contractile forces follows from biophysical considerations of the behavior of biological force-producing elements and avoids a detailed force production mechanism at the molecular level, which is still not understood. The description is consistent with forces that are generated by arrays of actin and myosin in each contributing cell type (Young *et al.*, 1993; Franke *et al.*, 2005). The forces are, in turn, transmitted through the mechanical circuits formed by the cells, their junctions with both one another and their junctions with the extracellular matrix. Given initial conditions that match observed experimental geometries, the model embodied in this system of equations (developed in detail below) simulates the time evolution of the leading edge to closure (Figs. 3 and 4), recapitulating five distinct experimental protocols (Fig. 1). In the model equations, we employ parameters that quantify properties of the individual tissues and processes involved. Direct measurement of these parameters is either extremely difficult or beyond present

Figure 4. Simulated embryos using the hyperbolic force-velocity relationship. As in Fig. 3, time evolution of (A) a native embryo; (B) an embryo without any force contribution from the amnioserosa; (C) an embryo with zipping inhibited at both canthi; (D) an embryo with zipping inhibited only at the posterior canthus; and (E) a *myospheroid* mutant embryo, simulated by reduced zipping rates at both canthi. These simulations correspond to experimental observations shown in panels (B)–(F) of Fig. 1. Time progresses from top to bottom in 630 s steps for all simulated embryos. Each column extends for 3780 s.

technology. To evaluate the parameters we conduct a search process within parameter space that optimizes the fit between experimentally observed geometries [Figs. 1(B)–1(F)] and their model-simulated counterparts.

The formulation of the second generation model proceeds conceptually as follows. (a) We set up a coordinate system and name the forces involved in the model. (b) We introduce equations that describe the behavior of biological elements that have both elastic and active, contractile properties as force producers for closure, and we apply it to the two players that drive closure, the amnioserosa and the purse string. (c) We introduce the force balance equation at each point of the purse string. (d) We implement the phenomenological law for zipping of Hutson *et al.* (2003) at the canthi. (e) The equations obtained in (b), (c), and (d) form a system that simulates the time evolution of the position of the leading edge and of the forces acting on it during closure. (f) For each of six experimental data sets, the system is initialized such that the height of the exposed amnioserosa [see *H* in Fig. 2(A)] was 58 μm , indicating that we selected embryos at mid closure stage. The system is then solved numerically. (g) The values of the model parameters are determined by the requirement that the model recapitulates various aspects of dorsal closure optimally, based on a proposed penalty function. Significantly, the optimization of parameters for the simulated embryos is based primarily on native dorsal closure in six wild-type embryos. Two more experimental protocols (removal of the amnioserosa and native mutant closure) are used only to set geometric constraints, i.e., to set bounds on *h*. The remaining two protocols, laser-nicking at one or two canthi, are not considered at all in parameter optimization and allow us to test the model's predictive capabilities for the simulation of novel experimental conditions. We now describe these steps in detail.

Figure 5. A schematic for a biological force-producing element. Each element consists of an elastic element (i.e., a spring) in series with two elements, a viscous element (dash-pot) and a force-generating element (schematic of bipolar myosin filaments bridging antipolar actin filaments) in parallel with one another.

Coordinates and forces

Coordinates: The position of every point along the length of the purse string is defined through a pair of Cartesian coordinates (x, h) , where the x -axis lies along the dorsal midline and h is the distance from that midline. Let $x_{\text{ant}} = x_{\text{ant}}(t)$ be the x -coordinate of the anterior canthus at time t [left in Figs. 1(A) and 2(A)] and $x_{\text{post}} = x_{\text{post}}(t)$ be the x -coordinate of the posterior canthus at time t . The position of the purse string is then specified by the function $h = h(x, t)$ in which x takes values between $x = x_{\text{ant}}(t)$ and $x = x_{\text{post}}(t)$. We chose the origin $x = 0$ to be the midpoint between the two canthi at the initial time, i.e., the symmetry point, $h(0, t)$. We stress the fact that this model places no restriction on the shape of the purse string.

Forces and an assumption on movements: To derive a set of model equations, we partition the amnioserosa into infinitely narrow strips of tissue, each of which generates and transmits a mechanical force along its length that pulls on the leading edge and the purse string in the direction normal to the dorsal midline [Fig. 2(A)]. We label the tension (force per unit width of the strip) $\sigma_{\text{AS}} = \sigma_{\text{AS}}(x, t)$. We denote by σ_{LE} the corresponding tension of the lateral epidermis, which opposes closure. We represent it by an average value, neglecting its space-time variation and thereby casting it into a normalizing role. Thus any changes in σ_{LE} will scale through the other forces. While this is a significant assumption, it has the desirable effect of limiting the number of model parameters and may be justified by experimental evidence that σ_{LE} is the force least responsive to perturbation (Kiehart *et al.*, 2000; Tokutake, 2003; Rodriguez-Diaz *et al.*, 2008).

Modeling the forces of the amnioserosa and of the purse string

We model tissue as consisting of biological force-producing elements that are comprised of an elastic element in series with a contractile element which is in parallel with a viscous element (a dashpot, Fig. 5). Comparable force-producing elements have been used in modeling muscle [see, e.g., Keener and Sneyd (1998) and Colombelli *et al.* (2009)]. Moreover, as described above, we expect the contractile behavior of such idealized force-producing elements to be consistent with that of myosin and filamentous actin in each tissue, which we previously showed drives contractility in both the leading edge and the amnioserosa.

Our modeling of the biological force-producing elements is based on an account of the energy expended as forces are produced to both maintain tension and drive contraction. In addition to the chemical energy required for the mechanical work of contraction, tissue must utilize chemical energy to maintain a force without producing an overall movement—consider the “work” required to hold a large stack of books at arms length. We call this type of work isometric, although it is produced at all stages of contractile operation, not only in isometric equilibrium. We focus on the total power input for each biological force-producing element that contributes to closure, P_c (i.e., we consider only the rate at which energy is utilized for closure and disregard the energy input that is required to keep the cells alive completely independent of closure). We equate P_c with the sum of three terms, dynamic or mechanical power output (power that contributes to work that causes actual movement), plus force-generating static power output (power that contributes to isometric force production), plus dissipation (power lost to inefficiency of the force-producing element and viscous drag encountered during movement). In sum, P_c can be viewed as the rate at which force-producing elements utilize energy for closure [e.g., through hydrolysis of adenosine 5'-triphosphate (ATP)].

The dynamic power output, i.e., the rate at which each force-producing element provides mechanical work, is given by pv , where the force p is the external load and v is the contractile speed at which the load is transported. We take the isometric power cost to equal βp , where the proportionality constant β defines the power cost of maintaining one unit of force. We take the rate of energy losses to dissipation to be proportional to the contractile speed v , i.e., equal to av , where a is a dissipation constant. The input/output power balance is given by the following equation:

$$P_c = pv + \beta p + av. \quad (7)$$

Two regimes can be considered to explore the consequences of Eq. (7): time-independent or time-dependent. When each of the three terms on the right-hand-side of Eq. (7) is independent of time (i.e., constant), then P_c also is independent of time and the system is at steady state. The first term on the right-hand-side is pv , where p is the external load and v is the contractile speed of the force-producing element, which should be compared to $u = dh/dt$ [see Eq. (2)]. During native closure, u has been experimentally observed to be constant, and thus it is reasonable to assume that v is well approximated as constant with respect to time during native closure. It is also reasonable to assume that the external load is well approximated as a constant during native closure; thus, the overall term pv is constant. The assumption that p and v are each constant with respect to time has the consequence that the second term βp and the third term av also will be constant with respect to time since both β and a are constants. To summarize our rationale, in native closure it is reasonable to

assume that P_C is constant because the terms that sum to it are also reasonably assumed to be constant. We preserve the assumption of constant P_C in all simulations, including those of laser or genetically perturbed closure. In these latter cases, redistribution of power consumption among the three terms occurs. These simulations prove to be in basic agreement with experimental observations, suggesting that this assumption is acceptable to a first approximation. Furthermore, constant power is often an efficient condition for energy utilization (MS Hutson and GS Edwards, unpublished).

We note that when P_C is independent of time, we recover an expression for the isometric force, p_0 (the force at $v=0$), where p_0 equals P_C/β . Moreover, solving Eq. (7) for v , we obtain a hyperbolic force-velocity relation at steady state [see Fig. 6(A)],

$$\frac{\beta(p_0 - p)}{p + a} = v. \quad (8)$$

This equation is in a form identical to the force-velocity relationship established empirically by Hill (1938) in the context of muscle (see Keener and Sneyd, 1998; Colombelli *et al.*, 2009).

Indeed, as described below, we find that a more general time-dependent equation is required to accommodate all of the experimental conditions we attempt to model. We refer to this more general equation as the time-dependent Hill law and adopt it as the basis for our modeling. Thus the time-dependent Hill model [Eq. (9)] balances the no-longer-equal left and right-hand-sides of Eq. (8) with an additional derivative term, yielding the following:

$$-\zeta \frac{dp}{dt} + \frac{\beta(p_0 - p)}{p + a} = v. \quad (9)$$

Note that when p is time-independent, the derivative term vanishes, i.e., the more general equation includes the possibility of a steady state solution. The first (derivative) term in Eq. (9) describes elastic components of closure. Omitting the second (hyperbolic) term in Eq. (9) obtains $\zeta dp = dl$, where $dl = (-v)dt$ represents elastic deformation (the minus sign is necessary for consistency: dp and dl are tensile force and deformation, while v is contractile velocity). The coefficient ζ is identified as the elastic compliance. Although developed in the context of muscle, the Hill model (Hill, 1938; Huxley, 1957a; Huxley and Simmons, 1971) follows from principles that do not require the ordered geometry of striated muscle. Indeed, the real organization of the interdigitating thick and thin filaments that comprise the structure of the sarcomere was not revealed until nearly two decades after Hill's formulation (e.g., Huxley, 1957b). We contend that the Hill model is readily applicable to the actin and myosin assemblies of nonmuscle tissue and provide a schematic of the mechanical circuit for a force-producing element within the Hill formalism in Fig. 5. Now the challenge is to model each

Figure 6. (A) Schematic of two hypothetical force-velocity relationships. (B) and (C) The model optimized, straight-line (solid line), and hyperbolic (dashed line) force-velocity relationships are shown for σ_{AS} (panel B) and T (panel C).

tissue that contributes force production to closure in terms of such force-producing elements.

Dynamic law of the amnioserosa: We treat the dorsal opening not as a collection of amnioserosa cells, but instead as parallel strips of amnioserosa tissue. Here, each strip constitutes a biological force-producing element whose force contribution to closure is embodied in Eq. (9), is of infinitesimal width, dx , and extends from one leading edge to the opposing leading edge at a given value of x [Fig. 2(A)].

We note that recent treatments of amnioserosal contractility in closure focus on investigating and modeling force contributions from individual cells in the amnioserosa (Gorfinkel *et al.*, 2009; Solon *et al.*, 2009). The forces $p = \sigma_{AS} dx$, $p_0 = \sigma_{AS,0} dx$, and $a = a_{AS} dx$ in Eq. (9) were determined from the corresponding stresses σ_{AS} , $\sigma_{AS,0}$, and a_{AS} . Note the subscript 0 in $\sigma_{AS,0}$ refers to the isometric force produced by the amnioserosa, i.e., the force produced at zero velocity. Similarly the compliance ζ is given by $\zeta = \zeta_{AS}/dx$, where ζ_{AS} is compliance of a strip of unit width. Inserting these expressions into Eq. (9) and letting $\beta = \beta_{AS}$, we obtained Eq. (10),

$$\zeta_{AS} \frac{\partial \sigma_{AS}}{\partial t} + \beta_{AS} \frac{\sigma_{AS} - \sigma_{AS,0}}{\sigma_{AS} + a_{AS}} = \frac{\partial h}{\partial t}. \quad (10)$$

Dynamic law of the purse string: The case for modeling with a muscle equation is even stronger here. Indeed, upon visualization with resolution provided by the light microscope, the purse string displays a remarkably sarcomere-like distribution of both myosin II and α -actinin (Franke *et al.*, 2005; Rodriguez-Diaz *et al.*, 2008). The biological force-producing element here is the infinitesimal length of a purse string in a leading edge that is in contact with a single biological force-producing element of the amnioserosa. Labeling this length ds , we obtain

$$ds = \sqrt{dx^2 + dh^2} = \sqrt{1 + h'^2} dx \quad (11)$$

where $h' = \partial h / \partial x$. We utilize Eq. (9) with $p = T$, $v = \partial(ds) / \partial t = [\partial(1 + h'^2)^{1/2} / \partial t] dx$, $\zeta = \zeta_T dx$, and $\beta = \beta_T dx$ to obtain the following:

$$\zeta_T \frac{\partial T}{\partial t} + \beta_T \frac{T - T_0}{T + a_T} = \frac{\partial \sqrt{1 + h'^2}}{\partial t}. \quad (12)$$

T_0 is the isometric force, i.e., the tension in the purse string when its rate of contraction is zero, a_T is a dissipative parameter, β_T is resistance to force production per unit midline length, and ζ_T is compliance per unit midline length.

Force balance

In order to continue with the development of our dynamic model of dorsal closure, we developed an equation that describes the balance of forces normal to the dorsal midline at every point of the purse string [see Fig. 2(B)]. At some fixed time, the normal component of the purse string tension T at the point (x, h) of the purse string is $T(x) \sin \alpha(x)$. Hence, the normal force component on the infinitesimal piece of purse string from x to $x + dx$ due to its tension is $T(x + dx) \sin \alpha(x + dx) - T(x) \sin \alpha(x)$. Divided by dx , this yields, in the limit $dx \rightarrow 0$, the partial derivative of $T(x) \sin \alpha(x)$ with respect to x . This is the stress on the purse string normal to the dorsal midline at point (x, h) due to the purse string tension. In order to highlight the similarity with Eq. (1), we define the coefficient K_{PS} by the following:

$$K_{PS} = \frac{-1}{T} \frac{\partial(T \sin \alpha)}{\partial x}. \quad (13)$$

The force balance equation, or more precisely, the balance of stresses on the purse string at point (x, h) , then becomes the following equation:

$$\sigma_{LE} - \sigma_{AS} - TK_{PS} = b \frac{\partial h}{\partial t}. \quad (14)$$

At the symmetry point, K_{PS} assumes a purely geometric interpretation and is equal to κ , the curvature. Indeed, here at the symmetry point $\sin \alpha = 0$ and $\cos \alpha = 1$, the left-hand-side of Eq. (13) reduces to $T d\alpha/dx$, which equals $-T\kappa$, thus, reproducing Eq. (1). The coefficient K_{PS} is positive except under certain experimental conditions [e.g., double-canthus nick protocols, see Fig. 1(D), Hutson *et al.* (2003), and Peralta *et al.* (2007)], where the purse string is concave up. Inertial terms have been neglected in the low Reynold's number environment of dorsal closure (Purcell, 1977).

Equation (14) provides the rate of change in h , in terms of the geometry and forces that enter its left-hand-side, both sides evaluated at time t . To be able to update the shape of the leading edge, we need to obtain similar equations for the other evolving quantities of the model, namely, the stress from the amnioserosa σ_{AS} , the tension of the purse string T and the position of the canthi x_{ant} and x_{post} . In order to show that Eqs. (10) and (12) can be used to update σ_{AS} and T , we first eliminate the $\partial h / \partial t$ from Eqs. (9) and (14) (solve one for this quantity and insert it into the other) to obtain the following:

$$\begin{aligned} \frac{\partial \sigma_{AS}}{\partial t} = & -\frac{1}{\zeta_{AS}} \beta_{AS} \frac{\sigma_{AS} - \sigma_{AS,0}}{\sigma_{AS} + a_{AS}} \\ & + \frac{1}{\zeta_{AS} b} (\sigma_{LE} - \sigma_{AS} - TK_{PS}). \end{aligned} \quad (15)$$

Similar algebraic manipulations yield a formula for the rate $\partial T / \partial t$. Performing the differentiation on the right-hand-side of Eq. (12), we obtain the following equation:

$$\zeta_T \frac{\partial T}{\partial t} + \beta_T \frac{T - T_0}{T + a_T} = \frac{T_0 h'}{\sqrt{1 + h'^2}} \frac{\partial h'}{\partial t}. \quad (16)$$

Taking the derivative of Eq. (14) with respect to the variable x , and recalling the constancy of σ_{LE} and the definition [Eq. (13)], we obtain the following:

$$-\frac{\partial \sigma_{AS}}{\partial x} + \frac{\partial^2(T \sin \alpha)}{\partial x^2} = b \frac{\partial h'}{\partial t}. \quad (17)$$

We finally derive the desired equation for the rate of change of the tension T by eliminating $\partial h' / \partial t$,

$$\frac{\partial T}{\partial t} = -\frac{1}{\zeta_T} \frac{\beta_T(T-T_0)}{T+a_T} + \frac{1}{\zeta_T b \sqrt{1+h'^2}} \times \left(-\frac{\partial \sigma_{AS}}{\partial x} + \frac{\partial^2(T \sin \alpha)}{\partial x^2} \right). \quad (18)$$

Clearly the system of Eqs. (15) and (18) is essentially equivalent to systems (10) and (12).

Canthus kinetics

In order to model the zipping process, we first adopted a zipping law similar to the one introduced by [Peralta et al. \(2007\)](#). For the anterior canthus, we denote the zipping rate as dx_{ant}/dt and set it equal to $k_z/2 \tan \theta_{\text{ant}}$, as determined empirically, where θ_{ant} is the angle that the purse string makes with the dorsal midline and the proportionality constant k_z is the zipping rate constant (thus $dx_{\text{ant}}/dt = k_z/2 \tan \theta_{\text{ant}}$). To first order, symmetric zipping constants were used successfully in [Hutson et al. \(2003\)](#) and for simplicity we assume that they are equivalent for the two canthi. Similarly, for the posterior canthus, $dx_{\text{post}}/dt = k_z/2 \tan \theta_{\text{post}}$, where θ_{post} is the angle at the posterior canthus. Anterior/posterior asymmetry, observed (even in native closure) and investigated by [Peralta et al. \(2007\)](#), may still manifest itself in the model due to asymmetry in either the initial conditions or introduced by laser perturbation

$$\frac{dx_{\text{ant}}}{dt} = \frac{k_z}{2 \tan \theta_{\text{ant}}}, \quad (19)$$

$$\frac{dx_{\text{post}}}{dt} = \frac{k_z}{2 \tan \theta_{\text{post}}}. \quad (20)$$

The system of equations that describe the second generation model and its analytical solution

The second generation model of dorsal closure consists of the three evolution partial differential Eqs. (10), (12), and (14) and the two ordinary differential Eqs. (19) and (20). We refer to the system of these five equations as the second generation model system. From given initial values, the model system enables tracing the time evolution of the overall geometry of the dorsal opening and of the forces acting on each point of the two purse strings that define the dorsal opening. Solving the system requires the calculation of five unknowns for all x and t . These are the positions of the canthi, specified by the functions $x_{\text{ant}}(t)$ and $x_{\text{post}}(t)$, the shape of the purse string, specified by the curve $h=h(x,t)$; the stress of the amnioserosa, specified by $\sigma_{AS}=\sigma_{AS}(x,t)$ and, finally, the tension of the purse string, specified by $T=T(x,t)$. Each of the five equations provides the rate of change of one of the five unknowns. The equations are coupled, i.e., the expression of the rate of change of each unknown involves other unknowns. Thus, the evolution (or updating) must be done simultaneously for the five unknowns. The system of equations is mathematically well posed, i.e., it possesses a unique solution

$[x_{\text{ant}}(t), x_{\text{post}}(t), h(x,t), \sigma_{AS}(x,t), T(x,t)]$ that depends stably on the initial data given by the functions $[x_{\text{ant}}(0), x_{\text{post}}(0), h(x,0), \sigma_{AS}(x,0), T(x,0)]$.

Purse string geometry sets the initial conditions in midstage dorsal closure

At the end of germ band retraction, morphogenesis of the epithelium stalls transiently, then resumes with the onset of dorsal closure. Once closure begins, the stalled leading edges or purse strings accelerate toward the dorsal midline until a constant rate of closure, which characterizes the bulk of closure, is achieved. Our second generation model applies to this, the bulk of closure, during which dh/dt is constant and is ~ 6 nm/s. The initial values of x_{ant} , x_{post} , and h were determined experimentally for each of six model embryos, for which our data sets spanned approximately 4000 s of closure ($dh/dt = 6.41 \pm 0.75$ nm/s). The embryos were staged by restricting further analysis to values of h ranging from $29 \mu\text{m}$ to $5 \mu\text{m}$, where the simulated time t was set to zero at the $h=29 \mu\text{m}$ value for each embryo. However, in general, the model applies to larger values of h . Subsequent time in each experiment was simply elapsed time from $t=0$.

The initial conditions, required for obtaining numerical values for the five evolving quantities, were selected judiciously. We took σ_{AS} and T to be initially constant along the purse string and we set the initial relative forces $\sigma_{LE}:\sigma_{AS}(x,0):T(x,0):\kappa(x,0)$ to be 500:380:120, consistent with force ladder measurements ([Hutson et al., 2003](#); [Peralta et al., 2007](#)) at the symmetry point (κ is the purse string curvature at this point). The most current force ladder ranged from 510:380:130 to 490:380:110 in [Peralta et al. \(2007\)](#).

We solved the model Eqs. (10), (12), (14), (19), and (20) numerically, as summarized in Fig. 7. The equations were first discretized in space using a finite difference approach with ~ 100 grid points, then the resulting equations were integrated in time using the Euler method. Note that Eqs. (10) and (12) each contains two time-derivative terms. To advance the model solution by one time-step, we first updated h by solving Eq. (14); the new h values were then used to estimate $\partial h/\partial t$ and $\partial(1+h'^2)^{1/2}/\partial x$ and to update the remainder of the solution values. All calculations were performed using MATLAB.

The simulated embryo: determination of the parameters through optimization

The model system of equations involves ten parameters: four each for the amnioserosa and the purse string and one each for zipping and viscous drag. These are the resistances to force production β_{AS} and β_T , the compliances ζ_{AS} and ζ_T , the isometric stress $\sigma_{AS,0}$ and the isometric tension T_0 , the dissipation parameters a_{AS} and a_T , the zipping rate constant k_z , and the viscous drag coefficient b . We reduced the number of parameters prior to numerical optimization by referencing the experimentally determined value for k_z ([Peralta et al., 2007](#)) and by considering two set values for a_{AS} and a_T , to be dis-

Figure 7. A schematic of the optimization algorithm. It includes (A) user provided bounds for each parameter (Table III) and user provided estimates within the bounds to seed the initial (B) estimate of the parameter set. The optimization of the parameter set is built around two computational loops. The (B)–(J) outer loop refines the parameter set. When the (J) penalty function converges, the algorithm (K) reports the optimized parameter set. The (E)–(I) inner loop iterates through developmental time. Given (B) either the initial seed or an updated estimate for the parameter set, the algorithm then (C) seeds initial values for h (corresponding to the first confocal image of staged embryos) and σ_{AS} and T (both determined by the force ladder). The next step is to (D) calculate spatial rates of change for the variables h and ds in order to use (E) model equations (14), (19), and (20) to calculate the location of the leading edges. The next steps are to (F) calculate temporal rates of change in h and ds , (G) update the spatial rates of change, and then (H) use model Eqs. (10) and (12) to update the values for σ_{AS} and T . The decision step (I) in effect steps this process throughout developmental time (from computational time 0 to $t_{5\mu m}$). Once the time progression loop has been completed, the next decision step (J) tests the convergence of the penalty function ϕ . The parameter optimization loop is repeated until convergence of the penalty function.

cussed further in the following paragraph. Each of the remaining seven parameters were numerically optimized to simulate dorsal closure in a native wild-type embryo.

We conjectured that the salient, qualitative features of dorsal closure would be only mildly sensitive to the values of the dissipative-loss parameters a_{AS} and a_T . Note, these are constants introduced by the Hill formulation and account

for viscous drag in the force-producing elements. They determine the hyperbolic shape of the force-velocity relationship in Eqs. (10) and (12) and change the concavity of the curves shown in Figs. 6(B) and 6(C). To explore the consequences of this conjecture, we tested two bounds for a_{AS} and a_T . We chose an upper bound equal to $20 \sigma_{LE}$ and found that it yielded the essentially linear relationship shown in Figs. 6(B) and 6(C), where larger values for a_{AS} and a_T would not lead to further meaningful differences in the shape of the force-velocity curve. We next chose a value 40 times smaller ($a_{AS} = a_T = 0.5 \sigma_{LE}$), an unrealistically small dissipative value given that the experimentally obtained force ladders indicate that σ_{LE} is expected to be 500 fold greater than bv . This lower bound resulted in the hyperbolic force-velocity curve shown in Figs. 6(B) and 6(C). Given the geometry resulting from the two values for a_{AS} and a_T , we labeled the first relationship straight line and the second hyperbolic.

To carry out the optimization for the remaining seven parameters, we introduced ϕ , a penalty (or cost) function. This function penalizes the deviation of the simulated geometry or of the simulated stresses from experimental values based on time-lapsed confocal images of closure. The cost function is the sum of the five expressions, (21a)–(21e). The values of h in these expressions are simulated values corresponding to the protocol indicated on the right of each expression. h_{exptl} in (21a) refers to the associated experimental values of h . The values are considered only at the symmetry point $x=0$, thus, the simplified notation $h(t)$ is used in place of $h(0, t)$. The numerical values that lead off each expression were chosen to balance the contribution of each of the five expressions to the penalty function ϕ , where expressions (21a)–(21e) sum to give Eq. (21) and each is developed below.

$$\phi = 100 \left[\frac{1}{n_{final}} \sum_{n=0}^{n_{final}} \left(\frac{|h(t_n) - h_{exptl}(t_n)|}{h_{exptl}(t_n)} \right) \right] \quad (21a)$$

$$+ 10 \left(\frac{\max[\sigma_{AS}(x, t_{5\mu m})]}{\min[\sigma_{AS}(x, t_{5\mu m})]} - 1 \right) \quad (21b)$$

$$+ 100(\max[1.8h(t_{cut}) - h(t_{turning}), 0]) + \max[h(t_{turning}) - 2.2h(t_{cut}), 0]) \quad (21c)$$

$$+ 100(\max[h(t_{check}) - h(t_{cut}), 0]) + \max[0.5h(t_{cut}) - h(t_{check}), 0]) \quad (21d)$$

$$+ 100(\max[0.6h(t_0) - h(t_{5\mu m}), 0]). \quad (21e)$$

The first expression (21a) compares the simulated value for the height of the symmetry point to its experimental counterpart at each time during native closure. At each time t_n , the difference between $h_{simulated}(=h)$ and $h_{experimental}(=h_{exptl})$ is divided by h_{exptl} to yield the fractional difference between simulated and experimentally observed values of h , and the absolute value is taken to prevent cancellation. We sum the devia-

Table I. The optimized parameters based on the straight-line force-velocity relationship. These values are based primarily on dorsal closure in six native wild-type embryos (see text).

Parameter	b (σ s/ μ m)	$\sigma_{AS,0}$ (σ)	β_{AS} (μ m/s)	ζ_{AS} (μ m/ σ)	T_0 (σ μ m)	β_T (1/s)	ζ_T (1/ σ μ m)
Data Set 1	1.88	0.95	1.90×10^{-3}	14.23	0.50	0.110	9.85×10^3
Data Set 2	3.41	0.80	2.40×10^{-3}	10.40	0.38	0.164	1.00×10^4
Data Set 3	1.10	0.81	1.70×10^{-3}	9.09	0.75	0.066	1.14×10^4
Data Set 4	1.44	0.87	4.00×10^{-3}	13.61	0.81	0.055	9.14×10^3
Data Set 5	0.94	0.91	2.30×10^{-3}	15.85	0.81	0.064	1.06×10^4
Data Set 6	1.81	0.45	9.66×10^{-4}	12.33	0.58	0.095	1.02×10^4
Average	1.76	0.80	2.21×10^{-3}	12.58	0.64	0.092	1.02×10^4
Standard deviation	0.89	0.18	1.01×10^{-3}	2.51	0.18	0.041	7.69×10^2

tions over all times for closure and divide by the number of time steps because the value of n_{final} varies from embryo to embryo and because this makes the weighting of this expression comparable to the four remaining expressions. This expression thus integrates the absolute value of the fractional error between simulation and experiment over the duration of closure.

The motivation for the second expression, (21b), is to require that σ_{AS} in the simulation of native closure is approximately uniform along the anterior/posterior axis as suggested by experimental evidence (Peralta *et al.*, 2007). $\max$ and $\min$ are mathematical functions that in this implementation returns the maximum or minimum value for $\sigma_{AS}(x)$ at the final observation time, $t_{5\mu\text{m}}$, taken when h is 5 μ m, considering all possible values for x . In this expression, the value 1 was subtracted from the ratio of the maximum and the minimum values of $\sigma_{AS}(x)$ at $t_{5\mu\text{m}}$, so that when $\sigma_{AS}(x)$ is constant, the ratio is 1 and there would be no penalty associated with expression (21b).

The third expression, (21c), consists of two terms that compare the simulated extent of recoil from an amnioserosal removal protocol to typical experimental values for the turning point and penalizes recoil that is either too small or too big. Our choice of a penalty that is substantially more lenient than expression (21b) gives us insight into the predictive capability of the model. In the simulation, the laser surgical removal of the amnioserosa is treated by setting $\sigma_{AS}(x)$ to zero at t_{cut} . The turning point t_{turning} occurs when recoil ceases and $h(t)$ reaches its maximum value (after which, closure resumes). Experimentally, we determined that the height of the turning point is between 1.8 and 2.2 times greater than $h(t_{\text{cut}})$. In this implementation in MATLAB, the $\max$ function compares the difference between $1.8h(t_{\text{cut}})$ and the h at the turning point [the first term in (21c)] to zero, returning the larger value. The second term in (21c) compares the difference between h at the turning point and $2.2h(t_{\text{cut}})$ to zero and again returns the larger value. Thus, these two terms set lower and upper bounds for h at the turning point and imposes a penalty when the simulated value for $h(t_{\text{turning}})$ falls outside of that specified range.

The fourth expression, (21d), like (21c) above, is based on the ability of simulations to correctly track the progres-

sion of closure after the surgical removal of the amnioserosa. (21d) tracks the turning point by setting bounds on h as it decreases once closure resumes. Experimentally we observe that 2,000 s after t_{cut} values for h range between $h(t_{\text{cut}})$ and $0.5h(t_{\text{cut}})$. The construction of (21d) parallels that of (21c) and penalizes values for $h(t_{\text{check}})$ that are either too big or too small. Here, t_{check} is given by $t_{\text{cut}} + 2,000$ s.

The fifth expression, (21e), compares the simulated progression of closure in homozygous *mysospheroid* mutant embryos, which lack zygotically encoded β_{PS} integrin and fail to complete closure. Experimentally we found that in homozygous, *mysospheroid* mutant embryos closure is characterized by reduced zipping and rates of closure (dh/dt) that vary from 60 to 95% of wild-type until closure ultimately fails when the connection between amnioserosa and the leading edge of the lateral epidermis is ripped apart. We simulate the mutant phenotype by setting $k_z = 0$ and impose a penalty if h progresses to a value of less than 60% of the initial value of h measured at a time when native, wild-type embryos reach $h = 5$ μ m [$t_{5\mu\text{m}}$, as in (21b)].

The steepest descent method (Arfken, 1985) was used to minimize the cost function by varying parameter values. The optimization procedure for the cost function (performed in MATLAB with the mathematical function *fmincon*) was terminated if the difference between a given iteration and the one that preceded it was less than 1×10^{-6} .

It should be noted that only three of the five experiments summarized in Figs. 1, 3, and 4 contributed to the determination of the parameters of the simulated embryos. The optimization of the seven parameters (Tables I and II) was largely due to fitting the time progression of experimental measurements of native closure [Figs. 3(A) and 4(B)]. In addition, the penalty function included three sets of bounds established by two more experiments: the expectation that following the removal of the amnioserosa, the simulated embryo would both recoil and yet still close [Figs. 3(B) and 4(B)], and the expectation that the elimination of zipping in the *mysospheroid* mutant would cause closure in the simulated embryo to fail [Figs. 3(E) and 4(E)]. However, these penal-

Table II. The optimized parameters based on the hyperbolic force-velocity relationship. These values are based primarily on dorsal closure in six native wild-type embryos (see text).

Parameter	b (σ s/ μ m)	$\sigma_{AS,0}$ (σ)	β_{AS} (μ m/s)	ζ_{AS} (μ m/ σ)	T_0 (σ μ m)	β_T (1/s)	ζ_T (1/ σ μ m)
Data Set 1	5.40	0.95	2.31×10^{-5}	19.83	0.51	0.0024	9.85×10^3
Data Set 2	3.47	0.68	2.64×10^{-5}	15.54	0.88	0.0011	1.17×10^4
Data Set 3	3.04	0.61	3.46×10^{-5}	13.82	0.59	0.0017	1.10×10^4
Data Set 4	4.63	0.88	2.37×10^{-5}	16.49	0.91	0.0013	9.74×10^3
Data Set 5	2.75	0.89	3.91×10^{-5}	18.97	0.82	0.0017	1.06×10^4
Data Set 6	1.34	0.59	1.06×10^{-5}	17.19	0.78	0.0013	9.85×10^3
Average	3.44	0.77	2.62×10^{-5}	16.97	0.75	0.0016	1.05×10^4
Standard deviation	1.44	0.16	9.98×10^{-6}	2.21	0.16	0.0005	7.92×10^2

ties were more qualitative bounds as distinguished from fitting the time progression of the dorsal opening during wild-type native dorsal closure. We emphasize that in the initial estimation of parameters and the building of the penalty function for the simulated embryo, the double- and single-nicking protocols [Figs. 3(C), 3(D), 4(C), and 4(D)] were not used at all. In addition, it was necessary to upregulate σ_{AS} in the simulated embryo in order to recapitulate both the double- and single-canthus nicking protocols. The capability of the simulations to recapitulate the various protocols highlights the predictive power of the second generation model.

RESULTS

We generated a system of differential equations [Eqs. (10), (12), (14), (19), and (20)] that model the geometry of the dorsal opening and the tissue forces that contribute to closure. We specify the geometry of the dorsal opening by the location of the leading edge of the lateral epidermis and the fluorescent supracellular purse strings that it includes. The parameters of the model were determined from native dorsal closure based on measurements taken from time-lapsed records of six wild-type embryos that were neither surgically nor genetically manipulated (i.e., they were native wild-type embryos). In addition, time-lapsed records of laser-perturbed and mutant embryos also influenced the choice of parameters through the penalty function ϕ [Eq. 21 = the sum of expressions (21a)–(21e)]. We investigated, in parallel, a high dissipative-loss (straight line) force-velocity relationship and a low dissipative-loss (hyperbolic) force-velocity relationship, as summarized in Tables I and II, respectively. Parameter space was chosen to ensure that each parameter was contained well within the range surveyed when the overall penalty function was minimized (Table III). A comparison of the average values for the parameters in Tables I and II indicate the major differences between the optimization of the straight line, and hyperbolic cases are the values for the resistance provided by the proportionality constants β_{AS} and β_T , which differ by factors of ~ 57 and ~ 84 , respectively. Inspection of Eqs. (10) and (12) indi-

cates that β_{AS} and β_T compensate for the values a_{AS} and a_T in our parallel consideration of the force-velocity relationships.

Simulations of control and experimental embryos modeled with straight line or hyperbolic force-velocity curves are shown in Figs. 3 and 4, respectively. Simulations of native embryos exhibit normal closure [Fig. 3(A) and 4(A)] and were used to assign initial values to the model parameters, then to refine them [expressions (21a) and (21b) of the penalty function ϕ]. Simulations of embryos from which the amnioserosa had been removed [Figs. 3(B) and 4(B), respectively], and simulations of homozygous *myospheroid* mutant embryos [that lack zygotic expression of the β_{PS} integrin adhesion receptor, Figs. 3(E) and 4(E), respectively] contributed to the selection of values for parameters through the penalty function [expressions (21c)–(21e)]. Simulations of double- and single-canthus nicked embryos (3C, 4C and 3D, 4D, respectively) establish the usefulness of the model for predictions under experimental protocols that did not contribute to assigning parameter values. These simulations can be compared directly to closure in panels from time-lapsed videos of experimentally manipulated embryos, as shown in Figs. 1, 8, and 9. For reference, a time-lapsed video of native closure appears as [Supplemental Video 1](#). Time-lapsed numerical simulations of each experimental protocol appear as [Supplemental Videos 2–6](#).

Table III. Constraints on parameter values during the optimization process.

Parameter	Unit	Straight line	Hyperbolic
b	σ s/ μ m	$[1 \times 10^{-1}, 1 \times 10^1]$	$[1 \times 10^{-1}, 1 \times 10^1]$
$\sigma_{AS,0}$	σ	$[1 \times 10^{-1}, 1 \times 10^1]$	$[1 \times 10^{-1}, 1 \times 10^1]$
β_{AS}	μ m/s	$[9 \times 10^{-4}, 9 \times 10^{-2}]$	$[9 \times 10^{-6}, 9 \times 10^{-4}]$
ζ_{AS}	μ m/ σ	$[2 \times 10^0, 2 \times 10^2]$	$[2 \times 10^0, 2 \times 10^2]$
T_0	σ μ m	$[1 \times 10^{-1}, 1 \times 10^1]$	$[1 \times 10^{-1}, 1 \times 10^1]$
β_T	1/s	$[5 \times 10^{-3}, 5 \times 10^{-1}]$	$[5 \times 10^{-5}, 5 \times 10^{-3}]$
ζ_T	1/ σ μ m	$[1 \times 10^3, 1 \times 10^5]$	$[1 \times 10^3, 1 \times 10^5]$

Figure 8. Comparison between simulations and experimental data for a native embryo. (A)–(C) Plots of key quantitative measures of simulated dorsal closure based on straight-line (solid line) or hyperbolic (dashed line) force-velocity relationships are compared with experimental data (open circles). (A) The distance between the symmetry point and the dorsal midline, h ; (B) the contour length of one of the two purse strings, L ; and (C) the canthus-to-canthus distance, W , are plotted versus time. (D) Simulated embryos using the straight-line (red) and the hyperbolic force-velocity relationship (yellow) are superimposed on confocal fluorescent images of native embryo that express GFP-moe.

Parameter sensitivity results

The mathematical model is a system of five equations that contains ten parameters. Eight are specified for either the amnioserosa or the purse string and serve to characterize the stress σ_{AS} , the force T , the elastic coefficients ζ_{AS} and ζ_T , the multipliers β_{AS} and β_T (so-called contractile coefficients) that define the power cost for preventing movement in the isometric regime, and the dissipation constants a_{AS} and a_T that shape the force-velocity relationship. Two additional parameters are for zipping k_z and for viscous drag b .

In our simulations, the parameter k_z is fixed according to experimentally obtained values. Two additional parameters,

a_{AS} and a_T , were investigated at values that defined high dissipative-loss and low dissipative-loss regimes in the force-velocity relationship. Thus, the numerical analysis was used to optimize the seven remaining parameters.

It is useful to identify parameters to which model predictions are particularly sensitive. To achieve this, we sequentially varied by $\pm 10\%$ the individual parameters in the simulated embryo arising from the averaged results for six embryos, while keeping other parameters at their values obtained in the optimization. Separate simulations were performed for the straight line (Table IV) and hyperbolic (Table V) force-velocity relationships. The effects on model

Figure 9. Comparison between simulations and experimental data for an amnioserosa-removal protocol. (A) Simulations of the amnioserosa-removal protocol based on purely elastic (gray line), purely contractile (gray dashed line), or combined elastic and contractile (black line) models compared with experimental data (open circles). Simulation of native embryo (black dashed line) is shown as a reference. Both purely elastic and purely contractile models fail to show good agreement with experimental data. The model based on both elastic forces and actively contractile forces account for experimental data during the bulk of closure. (B) Simulated embryos using two examples of combined elastic and contractile models, where the straight-line (red) and the hyperbolic force-velocity relationship (yellow) are superimposed on confocal fluorescent images of an embryo following the amnioserosa-removal protocol.

results were assessed by the following three measures that are intrinsic features of the penalty function. (1) The time required to attain wild-type native closure. (2) The maximum h measured at the symmetry point in an amnioserosa-removal experiment, i.e., the turning point. (3) The time to attain closure in amnioserosa-removal experiment. (4) The failure of closure in *mysospheroid* mutant embryos.

The parameters for which model results are the most sensitive include the elastic compliances ζ_{AS} and ζ_T , the proportionality constant β_T , and the isometric force T_0 . With a 10% variation in these most sensitive parameters, the native closure time t_{close} varied by no more than 4.8%, the height of the turning point h_{max} varied by no more than 6.5%, and the amnioserosa-removal closure time $t_{closure@ASremoval}$ varied by no more than 6.5%. For the less sensitive parameters, 10% variations in parameter values had consequences of less than 0.2%. These results suggest that our model is only mildly sensitive to variations of the model parameters near their optimized values.

Table IV. Parameter sensitivity study for the straight-line force-velocity relationship. The effects of $\pm 10\%$ variation in individual parameters are assessed by three measurements: (1) the time required to attain wild-type native closure, $t_{close@native}$; (2) the maximum h measured at the symmetry point in an amnioserosa-removal experiment, i.e., the turning point h_{max} ; and (3) the time to attain closure in amnioserosa-removal experiment, $t_{closure@ASremoval}$.

Parameter	Change (%)	Native t_{close}	AS removal	
			h_{max}	t_{close}
b	+10	100.02	98.90	100.05
	-10	99.95	101.19	99.92
$\sigma_{AS,0}$	+10	99.93	100.00	100.00
	-10	100.07	100.00	100.00
β_{AS}	+10	99.95	100.00	100.00
	-10	100.05	100.00	100.00
ζ_{AS}	+10	96.39	99.83	99.92
	-10	104.16	100.18	100.08
T_0	+10	95.82	94.27	94.28
	-10	104.80	106.46	106.87
β_T	+10	97.28	95.23	96.11
	-10	103.12	105.33	104.52
ζ_T	+10	102.67	105.62	104.00
	-10	97.13	94.15	95.82

Model embryo performance

The simulated embryos were able to recapitulate wild-type native closure and the partial advance to and eventual failure of closure in the *mysospheroid* mutant embryo that lacks wild-type function of the β_{PS} -integrin subunit of the integrin cell adhesion receptor. Moreover, when σ_{AS} is upregulated, the

Table V. Parameter sensitivity study for the hyperbolic force-velocity relationship. The effects of $\pm 10\%$ variation in individual parameters are assessed by three measurements, as described in Table IV.

Parameter	Change (%)	Native t_{close}	AS removal	
			h_{max}	t_{close}
b	+10	100.10	98.55	100.11
	-10	99.90	101.58	99.89
$\sigma_{AS,0}$	+10	99.98	100.00	100.00
	-10	100.02	100.00	100.00
β_{AS}	+10	100.00	100.00	100.00
	-10	100.02	100.00	100.00
ζ_{AS}	+10	97.23	99.84	99.94
	-10	102.87	100.17	100.06
T_0	+10	96.50	95.47	94.97
	-10	104.02	105.05	105.97
β_T	+10	97.27	96.08	96.15
	-10	103.09	104.33	104.43
ζ_T	+10	102.60	104.86	104.02
	-10	97.20	94.92	95.80

model predicted the general shape of the purse string and the eventual closure from one side for the single-nicking protocol. Similarly, provided σ_{AS} is upregulated, the simulations properly predicted the outcome of simulated double-canthal nicking protocols: indentations formed in the purse strings, causing a change in the concavity of the leading edge and the establishment of contact between the two dorsally moving lateral epidermal flanks near the middle of the dorsal opening. It is important to recall that the single- and double-nicking protocols did not contribute to either the initial selection of the seven parameters nor did they contribute to the penalty function we used to further refine those parameters. We describe these results in more detail below.

Native closure: The positions of the two purse strings in wild-type native closure is shown in Fig. 1(B), and simulations are shown for the straight line and hyperbolic force-velocity relationships in Figs. 3(A) and 4(A), respectively and in Fig. 8(D). Similar to the experimental observations [Fig. 1(B)], the model canthi approach each other along the dorsal midline and the purse strings approach the model midline at a nearly constant speed. Moreover, closure progresses at a rate consistent with experimental observations: experimental values and simulations of the time evolution of the height at the symmetry point, h , the canthus-to-canthal distance, W , and the contour length of one of the two purse strings, L , are all plotted in Fig. 8. All three plots show good agreement with the experimental data using either form of the force-velocity relationship, as do overlays of simulations and experimental data [Fig. 8(D), see also Fig. 4 in Hutson *et al.* (2003)]. Recall that parameters were selected so as to optimize such agreement.

Amnioserosa removal: In this set of simulations, the amnioserosa of an embryo was rapidly removed, i.e., $\sigma_{AS}(x, t)$ was set uniformly to 0 at the time t_{cut} , while the other parameters of Table I or Table II were unchanged. As observed in the *bona fide* laser surgery experiments, the leading edge/purse string of the simulated embryo rapidly recoiled away from the dorsal midline until it reached a turning point, after which closure resumed. The evolution of the geometry of the dorsal opening is shown in Fig. 1(C) (experimental), Fig. 3(B) (simulated straight-line force-velocity relationship), Fig. 4(B) (simulated hyperbolic force-velocity relationship), and Fig. 9(B) (overlays of simulations and experimental data). The evolution of the height at the symmetry point h is also shown in Fig. 9. Recall that this surgical protocol only contributes to refining the parameters for closure through expressions (21c) and (21e) of the penalty function, ϕ . For the first 1200 s after the surgical removal of the amnioserosa, the simulations fit the experimental data well [Figs. 9(A) and 9(B)]. Interestingly between 800 s and 2,000 s after surgery, the rate is a compromise between experimental data for resumption of closure after amnioserosal removal and simulations from a purely contractile model. After 2000 s, the simulated rate of closure (the slope of h versus t) precisely tracks

the rate of native closure (but is offset due to the delay introduced during the recoil response to amnioserosa removal). To more accurately simulate the amnioserosal removal experiment, the model will need to be refined.

Native homozygous *myospheroid* mutant closure: Homozygous *myospheroid* mutant embryos are characterized by strong defects in zipping during native closure, followed by the failure of closure mediated primarily by the ripping apart of the boundary between the amnioserosa and the leading edge of the lateral epidermis, near the anterior end of the dorsal opening (Hutson *et al.*, 2003; Homsy *et al.*, 2006; Peralta *et al.*, 2007). Other defects caused by mutations in integrin subunits or interacting proteins included delayed formation of the canthi and slowed closure, defects in adhesion between the amnioserosa and the yolk cell, defects in adhesion between the peripheral amnioserosa cells and the leading edge cells, aberrant actin rich purse strings, and alterations in the pattern of contraction of amnioserosa cells (Stark *et al.*, 1997; Kadrmas *et al.*, 2004; Narasimha and Brown, 2004; Reed *et al.*, 2004; Wada *et al.*, 2007; Gorfinkel *et al.*, 2009). Here, we simulated the closure behavior of the *myospheroid* embryo simply by constraining the movement of the canthi, i.e., the positions of the canthi (x_{ant} and x_{post}) were fixed. Thus, our model attempts to address the lack of zipping phenotype that characterizes these mutants. We believe this phenotype is most relevant to modeling closure with tissue level granularity, at least until failure of closure that occurs when the boundary between the amnioserosa and the leading edge rips apart. Clearly, the other phenotypes listed above are likely to contribute. It is worth noting that inhibition of zipping alone caused a significant delay in simulated closure such that when native wild-type embryos were fully closed, $h(t)$ of the simulated *myospheroid* mutant embryos were only partially closed [Figs. 3(E) and 4(E)]. Simulation results are shown for the straight line and for the hyperbolic force-velocity relationships in Figs. 3(E) and 4(E), respectively, and can be compared to experimental data in Fig. 1(F). Recall that analysis of these mutants contributes to refining the parameters for closure through expression (21e). We suggest that to a first approximation, defects in zipping can explain the *myospheroid* phenotype until the onset of closure.

Laser nicking protocols: We simulated laser-nicking at both canthi and at a single-canthal. Experimentally, when the amnioserosa near a canthus is nicked by a laser, the local amnioserosa retracts away from the canthus and zipping is compromised (Hutson *et al.*, 2003; Peralta *et al.*, 2007). To simulate the effects of laser-nicking, we fixed the location of the nicked canthus, i.e., we constrained the position of x_{post} (and also x_{ant} for double-canthal nicking) and we set the amnioserosa force $\sigma_{AS}(x, t)$ to 0 for values of the absolute value of x that are less than or equal to 16 μm from the laser-perturbed canthus (single-nicking) or canthi (double-nicking).

Double-nicking: Double-canthal nicking simulations are shown for the straight line and the hyperbolic force-velocity

relationships in Figs. 3(C) and 4(C), respectively, and can be compared to experimental data in Fig. 1(D). In both cases, the model predicted a local but significant increase in the height h of the leading edge near the nicked canthi, where the closure-driving force σ_{AS} vanished. The local recoil, though relatively small in magnitude, has a significant impact on the shape or curvature κ of the leading edge. However, the simulated embryo failed to close, asymptotically reaching a minimal value for h at $\sim 4.5 \mu\text{m}$, and did not exhibit any indentation as seen experimentally. Consequently, the simulated embryo was modified by increasing σ_{AS} , where the value for this upregulation was chosen to ensure the simulated embryo closed in a time consistent with experimental observations. This upregulated, simulated embryo [Figs. 3(C) and 4(C)] reproduced the indentation in the experimental double-nicking protocol [Fig. 1(D)]. The local change in curvature associated with the indentation caused $T\kappa$ to oppose closure rather than to promote closure as in native dorsal closure, which is overcome by the upregulation of σ_{AS} , allowing dorsal closure to complete (Hutson *et al.*, 2003; Peralta *et al.*, 2007). In this implementation, σ_{AS} was increased uniformly in Eqs. (10) and (14) by a value equal to $0.9\sigma_{LE}$, when the two leading edges closed to a distance of $\sim 58 \mu\text{m}$. It is important to note that when σ_{AS} was boosted in this simulation, none of the ten optimized parameters were altered (the optimized parameter $\sigma_{AS,0}$, the isometric force, is not altered and should be distinguished from σ_{AS} , the force that is altered). Recent experiments of Peralta *et al.* (2007) indicate that σ_{AS} was upregulated by $37 \pm 12\%$ as a consequence of the double nicking protocol, an experimental value ~ 3.2 fold less than the simulated value (based on force ladder considerations).

Single-nicking: The simulation of single-canthus nicking experiments is summarized in Figs. 3(D) and 4(D) for the straight-line case and the hyperbolic force-velocity relationships respectively and can be compared to experimental data shown in Fig. 1(E). While the simulated embryo did close for this protocol, the time of closure exceeded that observed experimentally by a factor of 1.5. Similar to the previous case, the simulated embryo was modified by upregulating the value for σ_{AS} by $0.9\sigma_{LE}$ in the remaining amnioserosa so that the dorsal closure completed on time (again, $\sigma_{AS,0}$ remained unaltered). During these simulations with the upregulated embryo, the canthus angle θ increased in the neighborhood of the nicked canthus and decreased near the un-nicked canthus. Since the zipping rate is inversely proportional to $\tan \theta$, a smaller angle would increase the zipping rate. Peralta *et al.* (2007) assessed the single-nicking experiments, indicating that two factors contribute to an increased zipping rate at the unperturbed canthus in the single-nicking protocol. First, a smaller angle increases the zipping rate through the $\tan \theta$ dependence, consistent with a local upregulation in σ_{AS} , and second, the zipping rate constant also is upregulated (Peralta *et al.*, 2007).

The apparent requirement to upregulate σ_{AS} in the simulated embryos to account for the double-canthus nicking protocol and the single-canthus nicking protocol needs to be consid-

ered in light of recent experimental results. Peralta *et al.* (2007) speculated that the upregulation of k_z was mediated by the upregulation of σ_{AS} local to the canthus. Furthermore, Toyama *et al.* (2008) experimentally characterized a mutual correlation between k_z , σ_{AS} and the rate of apoptosis of amnioserosa cells, characterizing an apoptotic force. Finally, it stands to reason that if contributing forces are large compared to the net force that drives closure, regulation must play an important role in balancing the forces so that progress toward closure is inexorable and dh/dt remains constant.

Failure of a purely elastic and of purely contractile model

In view of these results and in view of the well-established necessity of both actin and myosin II for closure, the second generation model presented here establishes the combination of contractile and elastic behavior described by Hill's force-velocity relationship, Eq. (9) (Hill, 1938; Keener and Sneyd, 1998) as a viable basis for investigating a set of tissue force laws for dorsal closure. To that end, we investigated two natural limits of the Hill relationship corresponding to elasticity and contractility, respectively, by attempting to identify optimal parameter sets that simulate experimental observations under conditions that allow only elastic or only contractile forces. We evaluated a purely elastic or purely contractile model by eliminating the second term and the first term, respectively, on the left sides in Eqs. (10) and (12), i.e., β_{AS} and β_T were set to zero for the purely elastic model, whereas ζ_{AS} and ζ_T were set to zero for the purely contractile model. We then optimized model parameter sets using the procedure described previously but now based on five parameters.

Both models produced reasonable predictions for wild-type dorsal closure. However, simulations of neither the elastic limit nor the contractile limit alone appropriately reproduced a key experimentally manipulated dorsal closure process. Specifically, the purely contractile model failed to achieve substantial recoil after the removal of the amnioserosa (Fig. 9), and the purely elastic model predicted a recoil that attains a maximum height that is 320% higher than experimental observation. As a consequence, the purely elastic model failed to predict dorsal closure within the biologically relevant time interval. Such results suggest that both tissue elasticity and active contractility play an important role in dorsal closure. Interestingly, the results presented in Fig. 9 suggest that recoil can be driven largely by elastic forces, but contractile forces are necessary to prevent excessive recoil and to promote recovery from the turning point. Thus the time-dependent Hill equation, with both elastic forces and actively contractile forces, are necessary for this model to recapitulate dorsal closure behavior during an amnioserosa-removal experiment.

Recoil and drag

Dissipative loss and damping are both at play in this system. In the Hill model it is represented by the dissipative-loss parameter a . In the force ladder it is represented by the Stokes drag, $b dh/dt$. In the end, the recapitulation of the amnioserosal removal protocol was insensitive to our high dissipative-loss (a_{AS} and a_T set to an upper bound of $20\sigma_{LE}$) and low dissipative-loss (a_{AS} and a_T set to a lower bound of $0.5\sigma_{LE}$) extreme values for a . As an aside, during native dorsal closure the applied forces σ_{LE} , σ_{AS} , and $T\kappa$ are large compared to the drag forces by two to three orders of magnitude ($\sigma_{LE}:\sigma_{AS}:T\kappa:b dh_{native}/dt \approx 500:380:120:1$) and each is large by a similar extent to the net force that drives closure [Hutson *et al.* (2003) and refined by Peralta *et al.* (2007)]. Nevertheless, even though the values of dh_{native}/dt are small during native closure (~ 6 nm/s), viscosity and the drag that results are large compared to inertial forces, i.e., the system is at a low Reynolds number (Purcell, 1977). The removal of the amnioserosa eliminates the term σ_{AS} from Eqs. (1) and/or Eq. (14), resulting in an initial dh_{recoil}/dt of ~ 2.3 $\mu\text{m/s}$ (as compared to $dh_{native}/dt \sim 6$ nm/s), causing the drag force $b dh_{recoil}/dt$ to momentarily increase by more than two orders of magnitude. As the purse string stretches during the recoil, the stress term $T\kappa$ that promotes closure increases quickly (both curvature κ and elasticity in the purse string tension T increase, contractility of T may increase as well) and the magnitude of the speed $|dh/dt|$ drops. At the turning point, $T\kappa$ balances the stress σ_{LE} , recoil ceases, the recovery stage begins, and closure resumes.

DISCUSSION

We have developed a second generation mathematical model to investigate the forces that account for the dynamics of dorsal closure. In the model, we applied a set of force laws based on the time-dependent Hill equation, which addresses both elastic and contractile contributions to the dynamics of closure. Model parameters were identified by means of an optimization procedure that uses primarily data from wild-type dorsal closure, with additional bounds reflecting the amnioserosa-removal protocol and dorsal closure in *myospheroid* mutants. In general the models simulate experimental data and confirm that both elastic and contractile forces contribute to closure. Nevertheless, our model is underdetermined—precisely how the force laws and the parameters map on to the real mechanical and force-producing properties of biological tissues that they are meant to model is not clear. Further comparisons between experiment and the model will be required to evaluate their precise relationship(s).

The simulation of the amnioserosal removal protocol captures the recoil, the turning point, and the resumption of closure quite well (Fig. 9). However, it fails to capture a characteristic transition in the experimental data that slows recovery at the later stages. To date we have anchored parameters in time throughout native closure and throughout those

experimental protocols that were used to refine the values of the parameters. We have not allowed the parameters to vary during the course of the simulation. Such time-dependent change(s) might well allow better fits to the data and could shed light on the biological mechanisms that contribute to completing closure in laser-perturbed experimental animals.

Simulations of the single- and double-canthus nicking protocols require us to increase the net dorsalward force on the leading edge by a discontinuous upregulation of σ_{AS} essentially at the time of the laser ablation (we added a constant to σ_{AS} in Eqs. (10) and (14) but left the optimized parameters unchanged). This increase is consistent with the upregulation of σ_{AS} , as observed experimentally (Peralta *et al.* 2007; Toyama *et al.* 2008). Nevertheless, the biological mechanism(s) associated with the increase remains open to interpretation. Alternatively, relaxing the constraints to allow for one or more time-varying parameters in the simulation of the single and double-canthus nicking protocols might achieve comparable or better fits. As our understanding of the relationship between the mathematical nature of the force laws in the time-dependent parameter regime and real changes in the geometry and mechanical properties of the dorsal opening improves, we expect to be further informed as to the biological mechanisms responsible for dorsal closure.

Our model does not provide a definitive dynamical explanation of dorsal closure. We consider it as an early member of a hierarchy of models that will presumably become more and more determined as new data become available. We expect subsequent generations of models to provide simulations that more stringently recapitulate closure as the force laws are refined and new features are introduced in response to model testing through new experimental protocols. Judged by what it seeks to accomplish, which includes implementing dynamic tissue relations and allowing arbitrary purse string shapes, the present model, although significantly more complex than the first generation model, remains quite simple. This research suggests that further investigations of the force-velocity relationship and other force laws will be an important ingredient in understanding dorsal closure with applications to tissue dynamics in general.

There is considerable uncertainty in the geometric structure and in the operation of the chemical and biomolecular processes that effect dorsal closure. Our model proposes a muscle type mechanism and a simple kinetic zipping mechanism that is intended as an average of the molecular, cellular, and tissue properties that characterize closure. We view our model as consistent with the collective behavior of polar actin filaments or filament bundles functioning in concert with myosin (presumably in the form of bipolar filaments) that we know experimentally are required for closure.

We recognize that it is certainly possible that the biological processes that play an important role in dorsal closure have been neglected in the second generation model. Ques-

tions remain even within the realm of the basic modeling assumptions. For example, signaling and the possible existence of a “throttle” that upregulates the rate at which different active molecular processes contribute to closure are certainly required to simulate closure for the single- and double-canthus nicking protocols. The biological elastic and contractile elements proposed in the model may change their operating point drastically as tissue is ablated, which is one interpretation of the boost in σ_{AS} that was used in order to recapitulate the single- and double-canthus nicking protocols. It is reasonable that the tissues involved likely sense the ablation and, through some sensing and/or signaling mechanism(s), respond by recruiting more force-producing elements and/or adjusting the “throttle” on the existing ones. Including such effects in more sophisticated models of closure or models that address related processes, such as wound healing, may be necessary.

The absence of experimental means for direct measurement of the forces is a further difficulty. Ideally, a mathematical model of physical or biological processes is constructed based on established physical relationships. Model parameters are determined or measured experimentally; and using those parameter values, the model is used to make predictions, which are then compared with experimental observations. In regard to establishing a mathematical relationship between force and movement in dorsal closure tissues, one must consider that typically the changing geometry of the tissue is accurately measurable with current tools; information pertaining to the magnitudes of the tissue forces has been obtained through the response of the geometry, specifically through measurement of the recoil following tissue laser perturbation. In the absence of techniques to directly measure the majority of model parameters, an optimization procedure was used to identify parameter sets that best match a range of experimental protocols. A challenge we must contend with in the optimization is that, in addition to the unavailability of measurements for each model parameter, the valid ranges for many of these parameters are also uncertain. This implies that the optimization problem is only partially constrained and the parameters that we identified may possibly represent a local and not a global optimization. The likelihood of this is increased by the large number of model parameters that are allowed to vary in the optimization procedure. However, the relative insensitivity to model parameters is reassuring. More importantly, it is reassuring that not all protocols are used in the optimization; successful recapitulation in the unused protocols demonstrates the predictive capability of the model. This second generation model is a key step in a modeling hierarchy that aims to provide a definitive account of dorsal closure dynamics.

Our model, which describes closure at the cell sheet or tissue level of granularity, is generally compatible with two recent models that address aspects of dorsal closure. Solon *et al.* (2009) proposed force laws to account for the early stages of dorsal closure, prior to the onset of zipping, with

cellular granularity. They model the purse strings as an elastic cable with time-dependent force constants, which couples elastically to the periphery of the amnioserosa tissue. Both elastic and contractile forces model an amnioserosa cell, where cell junctions provide mechanical connection between amnioserosa cells. To account for the experimentally observed cell oscillations, the contractile force initially is oscillatory but then can become a sigmoidal, tension dependent contraction force. A comparison of Solon’s approach with the one presented here reveals two distinctions. First, Solon’s time-dependent use of oscillatory and sigmoidal force laws and use of time-dependent force constants are an alternative set of laws as compared to our use of elasticity and contractility based on a force-velocity relation. Second, Solon provides insight into the early stages of dorsal closure prior to the onset of zipping. In contrast, our model addresses the behavior of the entire dorsal opening and includes the contribution of zipping. It applies specifically to the mid-to-late stages of closure when it can be imaged in a single focal plane, but also is likely to apply to earlier stages, once zipping commences, following the formation of the anterior and posterior canthi.

In addition, our model is compatible with a recent study also focused on the amnioserosa by Gorfinkel *et al.* (2009). They used semi-automated image analysis to estimate local strain rates and evaluate the kinematics of closure with cellular granularity. They describe a slow phase of closure that occurs at its onset, prior to when our model is relevant. During this phase of closure apical contraction of amnioserosal cells at the posterior end predominates. They also observe a fast phase of closure where cells all along the anterior-posterior axis undergo apical contraction and zipping occurs. This fast phase corresponds to the stage of closure covered by our model. Like Solon *et al.* (2009), they observe a gradient of contraction with marginal (more ventral) cells contracting earlier than cells located closer to the dorsal midline. Moreover, they show that *mysospheroid* mutations perturb the dynamics of amnioserosa contraction.

FUTURE CONSIDERATIONS

Here we modeled cell sheet morphogenesis based on derived, force-velocity relationships that also characterize striated muscle contraction (Hill, 1938). In the contractile purse string, the periodic arrangement of actin, myosin and α -actinin are remarkably sarcomere-like, at least at low resolution (Rodriguez-Diaz *et al.*, 2008). No such arrangement of these proteins is observed in the amnioserosa, yet the model still applies. We surmise that our modeling efforts will be applicable to those movements whose kinematics and dynamics have distinct similarities to dorsal closure, including ventral closure in nematodes (*Caenorhabditis elegans*, Martin and Parkhurst (2004), Pellegrino *et al.* (2009), and references therein); and wound healing (Martin and Parkhurst, 2004), eye closure (Martin and Parkhurst, 2004;

Xia and Karin, 2004), and epiboly (Koppen *et al.*, 2006) in vertebrates.

Furthermore, the model itself can be improved, for example, in the recapitulation of latter stages of dorsal closure in the amnioserosal removal protocol (Fig. 9). Improvements will be sought within the framework of the current model; for example, we can modify and/or expand the penalty function to demand parameters that provide closer fits to experiments. We can also consider refined force laws that will constitute a new generation of models designed to accommodate nonlinear elastic, plastic, or rheological properties of the tissues and of the force-producing elements. Clearly, of value is our ability to map terms in the model's equations to the experimental properties of tissues in closure. As new mutants are investigated and as new laser surgical protocols are implemented (for example the spaceship cuts of Rodriguez-Diaz *et al.*, 2008), the ability of the model to recapitulate tissue responses to laser or genetic perturbations will allow us to further refine the algorithms that we use to explain wild-type dorsal closure. Finally, our model points to measurements that we should make experimentally, including the determination of the magnitudes of the force-producing processes, the compliances, the dissipation and drag coefficients, and more direct insights into the mechanism(s) for zipping.

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NOMENCLATURE

Independent variables

- x : position along the dorsal midline
- x_{ant} : position of the anterior canthus along the dorsal midline
- x_{post} : position of the posterior canthus along the dorsal midline
- t : time

Dependent variables

- α : angle between tangent of purse string to dorsal midline
- $h(x, t)$: distance from dorsal midline to leading edge
- σ_{AS} : amnioserosa force per unit length
- σ_{LE} : lateral epidermis force per unit length
- T : purse string tension
- θ : angle between purse string at canthi to dorsal midline

Parameters

- $\zeta_{\text{AS}}, \zeta_T$: elastic coefficients
- $\beta_{\text{AS}}, \beta_T$: contractile coefficients
- $\sigma_{\text{AS},0}, T_0$: isometric forces per unit length
- a_{AS}, a_T : dissipation constants
- b : viscous drag coefficient
- k_z : zipping constant

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