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Apoptotic Force and Tissue Dynamics During *Drosophila* Embryogenesis

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Abstract

Understanding cell morphogenesis during metazoan development requires knowledge of how cells and the extracellular matrix produce and respond to forces. We investigated how apoptosis, which remodels tissue by eliminating supernumerary cells, also contributes forces to a tissue (the amnioserosa) that promotes cell-sheet fusion (dorsal closure) in the *Drosophila* embryo. We showed that expression in the amnioserosa of proteins that suppress or enhance apoptosis slows or speeds dorsal closure, respectively. These changes correlate with the forces produced by the amnioserosa and the rate of seam formation between the cell sheets (zipping), key processes that contribute to closure. This apoptotic force is used by the embryo to drive cell-sheet movements during development, a role not classically attributed to apoptosis.

Morphogenesis is a biomechanical process whereby individual cells and cohorts of cells produce and respond to forces to generate the complex form of a developing multicellular organism (1). Dorsal closure during *Drosophila* embryogenesis (Fig. 1, A to C, fig. S1, and movie S1) is an example of a robust morphogenetic process that also is a model system for biological and biophysical investigations of epidermal fusion and wound healing (2–6). During dorsal closure, two lateral epithelial cell sheets advance to progressively cover an eye-shaped opening that is transiently occupied by the amnioserosa, an extraembryonic tissue. The dorsal-most row of cells in each epithelial sheet constitutes a distinct tissue, known as the leading edge of the lateral epidermis, which contains an actomyosin-rich cable or supracellular “purse string.” As closure progresses, the leading edges approach each other and form seams at each canthus in a process known as zipping (7), in which a canthus is the structure at the anterior- and posterior-most ends of the dorsal opening. Dorsal closure is the consequence of synchronized cellular forces and processes. Active forces are generated by nonmuscle myosin II in each purse string and in the amnioserosa (8), and by a resistive force in the lateral epidermis (2,4). Synchronization is a consequence of an emergent property that correlates the rate of closure with zipping (5,6).

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Materials and Methods

SOM Text

Figs. S1 to S4

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Apoptosis, or programmed cell death, has been extensively investigated [reviewed in (9)] and is an integral part of dorsal closure (10) and other developmental processes. Of particular relevance is the stereotypic sequence of events that characterizes apoptotic cells in epithelia, where delamination and apoptosis must be precisely coordinated so that dying cells are removed without compromising either the transepithelial or planar integrity of the tissue. In Madin-Darby canine kidney cell monolayers, an actomyosin ring forms within the apoptotic cell and a supracellular actomyosin purse string forms in the neighboring cells that surround this apoptotic cell (11). Contraction of these purse strings is reported to drive cell extrusion. In *Drosophila*, apoptosis occurs during stages 11 to 16 of embryogenesis [(12) and supporting online material (SOM) text]. After dorsal closure, the components of the amnioserosa cells are recycled through apoptosis (10). In addition, Kiehart *et al.* (2) observed that 13 out of 110 amnioserosa cells constricted their apical surfaces and dropped out of the epithelial plane and into the interior of the embryo during dorsal closure, suggesting the possibility of apoptosis. Here we show that apoptosis provides one-third to one-half of the net force that drives closure. This feature is in addition to the known roles of apoptosis, such as in eliminating supernumerary cells and maintaining homeostasis.

Through-focus (Z-stack) confocal images of dorsal closure in wild-type embryos that express the F-actin reporter [green fluorescent protein (GFP) fused to the actin-binding domain of moesin (GFP-moe) (2)] indicate that a subset of amnio-serosa cells exhibits the hallmarks of apoptosis (Fig. 2). These include the constriction of their apical surfaces, their extrusion inward from the amnioserosa cell sheet, subsequent blebbing, and cell fragmentation. Five to seven cells that neighbor each apoptotic cell are distorted as part of this process (upper right-hand frame of Fig. 2A), thereby taking on a rosette geometry (13,14). These neighboring cells elongate toward the apoptotic cell to maintain a continuous dorsal surface as the apoptotic cell is extruded; contraction of both the apoptotic cell and the apoptotic supracellular purse string in the neighboring cells (11) may contribute to these cell shape changes, by which the surface area of these nearest-neighbor cells decreased by $27.3 \pm 8.6\%$ ($N = 52$ cells) relative to a $14.2 \pm 4.4\%$ ($N = 58$ cells) reduction in control amnioserosa cells ($P < 0.05$, SOM text). Figure 2B also indicates that the next-to-nearest-neighbor cells are distorted and are pulled toward the apoptotic cell. These observations demonstrate that the vast majority of the amnioserosa cells are directly influenced by the apoptotic process and exceed by a factor of 5 to 7 the number that actively undergo apoptosis before the completion of closure. This raises the possibility that the apoptotic process contributes to the force that favors closure. To test this hypothesis, we used the bipartite GAL4-UAS system, which allows tissue-specific and temporally specific gene expression (15), to inhibit or induce (enhance) apoptosis in the amnioserosa and then investigated the effects on the kinematics and dynamics of closure.

We inhibited the apoptotic process in amnio-serosa cells only, by using a strong driver (c381-GAL4) to express an anti-apoptotic caspase suppressor (*p35*) responder [(16), hereafter referred to as AS-p35]. We induced (enhanced) apoptosis only in amnioserosa cells, by using a weaker driver, c825-GAL4 (17), to express a pro-apoptotic (*grim*) responder [(18), hereafter referred to as AS-grim]. These embryos also express GFP-moe to provide contrast for in vivo confocal microscopy. Complete genotypes and our choice of drivers are accounted for in (19). None of amnioserosa cells in AS-p35 embryos exhibited the hallmarks of apoptosis (movie S2), and the number of apoptotic cells increased in AS-grim embryos relative to controls. We measured the occurrence of apoptosis in amnioserosa cells and defined the rate of apoptosis, $R_{\text{apoptosis}} = n/\tau$, where n is the number of amnioserosa cells exhibiting the hallmarks of apoptosis observed during a time τ (SOM text). $R_{\text{apoptosis}}$ was increased from $5.5 \pm 2.1 \times 10^{-3} \text{ s}^{-1}$ ($N = 6$ embryos) in controls (19) to $16.3 \pm 2.9 \times 10^{-3} \text{ s}^{-1}$ ($N = 6$ embryos) in AS-grim embryos, and no apoptosis ($0.0 \pm 0.0 \times 10^{-3} \text{ s}^{-1}$, $N = 6$ embryos) was observed in AS-p35 embryos (Fig. 1D).

We observed three significant kinematic and dynamic consequences when apoptosis was either inhibited or induced. First, we quantified the rate of closure $v_{\text{native}} = dh/dt$ by measuring h as a function of time t , where h is the maximum distance from a purse string to the dorsal midline (fig. S2). v_{native} is the result of the net force exerted by the amnioserosa, lateral epidermis, and purse string on a leading-edge cell (4,5). v_{native} is reduced from 6.3 ± 0.6 nm/s ($N = 5$ embryos) in controls to 3.6 ± 1.1 nm/s ($N = 6$ embryos) in AS-p35 embryos and increased to 10.2 ± 0.8 nm/s ($N = 6$ embryos) in AS-grim embryos. Plots of H , the height from purse string to purse string at the maximum opening (fig. S2), versus t are well approximated as linear in all cases (Fig. 1, A to C, and E; and movies S3 to S5).

Second, mechanical jump experiments (4,5) were used to quantify changes in σ_{AS} , the force per unit of length produced by the amnioserosa on the leading edge under experimental conditions that either inhibit or induce apoptosis in the amnioserosa. A laser microbeam was used to rapidly dissect the amnioserosa away from one of the leading edges (the edge-cut protocol) [Fig. 3A and movie S6 (19)]. With the release of σ_{AS} , the leading edge recoils away from the dorsal midline to a turning point and closure eventually resumes. The initial recoil velocity, v_{recoil} , is directly proportional to σ_{AS} (SOM text). Taking the ratio of the measured $v_{\text{recoil_AS-p35}}$ (or $v_{\text{recoil_AS-grim}}$) to $v_{\text{recoil_GAL4 control}}$ yields the ratios

$$\frac{\sigma_{\text{AS_AS-p35}}}{\sigma_{\text{AS_GAL4 control}}} \approx \frac{v_{\text{recoil_AS-p35}}}{v_{\text{recoil_GAL4 control}}}, \quad \frac{\sigma_{\text{AS_AS-grim}}}{\sigma_{\text{AS_GAL4 control}}} \approx \frac{v_{\text{recoil_AS-grim}}}{v_{\text{recoil_GAL4 control}}} \quad (1)$$

where the GAL4 control genotype represents several distinct genotypes (19). v_{recoil} was 2373 ± 60 nm/s in controls ($N = 7$ embryos), 1727 ± 287 nm/s in AS-p35 embryos ($N = 5$ embryos, $P < 0.05$), and 3141 ± 246 nm/s in AS-grim embryos ($N = 6$ embryos, $P < 0.05$). Thus, in apoptosis-suppressed AS-p35 embryos, σ_{AS} is reduced by $29 \pm 9\%$, whereas in apoptosis-enhanced AS-grim embryos, σ_{AS} is increased by $32 \pm 7\%$, relative to controls. This indicates that about one-third of the force produced by the amnio-serosa in wild-type embryos is attributable to apoptosis.

Third, we quantified changes in zipping due to inhibiting or inducing apoptosis by evaluating the change in the seam lengths $w_{\text{A}}(t)$ and $w_{\text{P}}(t)$ at the anterior (A) and posterior (P) canthi, respectively (fig. S2). Figure 4A plots the length $w_{\text{A}}(t) + w_{\text{P}}(t)$. Zipping is slower in AS-p35 embryos and faster in AS-grim embryos, relative to controls. Previously, we showed that [SOM text (5)]

$$\frac{dw_{\text{A}}}{dt} + \frac{dw_{\text{P}}}{dt} = \frac{k_{\text{z,A}}}{\tan\theta_{\text{A,R}}(t) + \tan\theta_{\text{A,L}}(t)} + \frac{k_{\text{z,P}}}{\tan\theta_{\text{P,R}}(t) + \tan\theta_{\text{P,L}}(t)} \quad (2)$$

where $k_{\text{z,A}}$ and $k_{\text{z,P}}$ are the zipping rate constants; $\theta_{\text{A,R}}$, $\theta_{\text{A,L}}$, $\theta_{\text{P,R}}$, and $\theta_{\text{P,L}}$ are angles defined by the dorsal midline and the segments of the purse strings that meet at each canthus; and R and L refer to the right and left sides of the essentially bilaterally symmetric embryo (fig. S2). Figure 4B and table S1 compare $k_{\text{z,A}}$ and $k_{\text{z,P}}$. At the anterior canthus, $k_{\text{z,A}}$ decreased by $40 \pm 23\%$ when apoptosis was inhibited. In contrast, at the posterior canthus, $k_{\text{z,P}}$ increased by $73 \pm 34\%$ when apoptosis was increased. Additionally inhibition of apoptosis decreased $k_{\text{z,A}}$ to $k_{\text{z,P}}$, and enhancement of apoptosis increased $k_{\text{z,P}}$ to $k_{\text{z,A}}$.

To investigate why changes in apoptosis might influence the zipping rate at each canthus, we quantified the distribution of $R_{\text{apoptosis}}$ in control, AS-p35, and AS-grim embryos (Fig. 4C). In controls, apoptosis occurs about five times more frequently in the anterior and middle thirds of the dorsal opening, relative to the posterior third. In AS-p35 embryos, there is a uniform absence of apoptosis throughout the dorsal opening. In AS-grim embryos, the occurrence of apoptosis is enhanced and distributed uniformly throughout the dorsal opening. Taken together, these observations indicate that the symmetry properties in the rate of apoptosis correlate with those of zipping (table S2).

Given these three kinematic and dynamic consequences, we considered next the correlations between normalized plots of v_{native} (Fig. 1F), σ_{AS} (Fig. 3B), and k_z (Fig. 4D) versus $R_{\text{apoptosis}}$ (SOM text). These figures demonstrate that v_{native} , σ_{AS} , and k_z are each strongly correlated with $R_{\text{apoptosis}}$ and are mutually correlated.

We conclude that during dorsal closure, delamination of the apoptosing amnioserosa cells produces forces that both facilitate cell extrusion and promote closure. This apoptotic force significantly contributes to σ_{AS} , and thus the dynamics of closure changed when the rate of apoptosis was altered. Our observations suggest directions for future research. It is well known that the disruption of cell-matrix interactions induces apoptosis in epithelial cells (20). Thus, we hypothesize that tension and apoptosis may contribute to a positive feedback mechanism that serves as a force regulator or rheostat.

Our results raise the possibility of a dynamic role for apoptosis in other morphogenic processes. Indeed, an apoptotic force has been proposed as part of the epithelial strand-pull theory in hair follicles (21). Although not all apoptotic processes are related to force generation, we anticipate that apoptotic forces may be important for epithelial fusion in processes such as the development of the adult abdomen of *Drosophila* (22). Moreover, we cannot rule out the possibility that apoptotic forces contribute to the tissue-sculpting processes that drive processes such as digit individualization and joint formation. An apoptotic force may also play a beneficial role during wound healing (11,23) as a source of mechanical tension that promotes tissue reconstruction. We propose that evolution efficiently uses all possible sources of forces for morphogenesis, and that apoptosis in the amnioserosa is one such force that is co-opted to help drive dorsal closure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Dorsal closure in control and apoptotically altered GFP-moe-expressing embryos. Confocal fluorescent images of (A) control, (B) AS-p35, and (C) AS-grim embryos are shown (AS, amnioserosa; LE, lateral epidermis). Anterior is to the left in all figures. Scale bar, 50 μm . (D) $R_{\text{apoptosis}}$ for control (black), AS-p35 (white), and AS-grim (gray) embryos; error bars indicate SD. A single or double asterisk indicates that the value is significantly different from the control value at $P < 0.05$ and $P < 0.01$, respectively. (E) Plot of $H(t)$ as a function of time for embryos that were shown in (A) to (C), respectively. (F) Correlation between v_{native} and $R_{\text{apoptosis}}$ (each normalized by the average value for control embryos as indicated by overbars, correlation coefficient $r = 0.941$).

Fig. 2.

Fate of apoptotic amnioserosa cells and the distortion of the surrounding cells in wild-type embryos. **(A)** Confocal sections through GFP-moe-expressing embryos, where rows indicate depth below the surface and columns indicate time (as shown). Accumulation of F-actin is evident on the surface of one amnioserosa cell at time 0 s (arrow). Contraction occurs between 0 and 1500 s (arrows). Cell extrusion is evident at all depths at 1500 s (arrows). Blebbing is evident 7.90 μm below the surface at 2100 s (arrow). Scale bar, 10 μm. **(B)** Color-enhanced reproduction of the surface images from (A). The red cells are pulled toward the apoptotic cells (white), rearrange their neighbor-neighbor configurations, and fill the gap. The blue cell does not change shape as dramatically but is distorted and pulled toward the apoptotic cell. **(C)** Schematic representation indicating the region of the amnioserosa imaged in (A) (not to scale).

Fig. 3. Experimental determination of σ_{AS} . **(A)** Edge-cut protocol of a wild-type embryo expressing GFP-moe: Shown are the location of the edge cut (dashed line), which commenced at $t = 0$ s, and the morphology at the turning point. Scale bar, 50 μ m. **(B)** Correlation between σ_{AS} and $R_{apoptosis}$ (each normalized by the average value for control embryos, $r = 0.997$). Points are average values for control (black), AS-p35 (white), and AS-grim (gray) embryos normalized by the average value for control embryos as indicated by overbars; error bars indicate SD.

Fig. 4.

Apoptosis contributes to the zipping rate. **(A)** Plot of total seam length $w_A + w_P$ for controls (black), AS-p35 (white), and AS-grim (gray) for the embryos shown in Fig. 1, A to C. **(B)** Histogram of $k_{z,A}$ and $k_{z,P}$ and **(C)** spatial distribution of $R_{\text{apoptosis}}$ in control (black), AS-p35 (white), and AS-grim (gray) embryos (* $P < 0.05$; ** $P < 0.01$); error bars indicate SD. The dorsal opening was segmented by taking thirds of the canthus-to-canthus distance [inset in (C)]. **(D)** Correlation between k_z and $R_{\text{apoptosis}}$ [each normalized by the average value ($k_{z,A}$ and $k_{z,P}$ or $R_{\text{apoptosis_Ant}}$ and $R_{\text{apoptosis_Pos}}$) for controls as indicated by the overbars, $r = 0.802$].