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# Embryonic Wound Healing by Apical Contraction and Ingression in *Xenopus laevis*

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We have characterized excisional wounds in the animal cap of early embryos of the frog *Xenopus laevis* and found that these wounds close accompanied by three distinct processes: (1) the assembly of an actin purse-string in the epithelial cells at the wound margin, (2) contraction and ingression of exposed deep cells, and (3) protrusive activity of epithelial cells at the margin. Microsurgical manipulation allowing fine control over the area and depth of the wound combined with videomicroscopy and confocal analysis enabled us to describe the kinematics and challenge the mechanics of the closing wound. Full closure typically occurs only when the deep, mesenchymal cell-layer of the ectoderm is left intact; in contrast, when deep cells are removed along with the superficial, epithelial cell-layer of the ectoderm, wounds do not close. Actin localizes to the superficial epithelial cell-layer at the wound margin immediately after wounding and forms a contiguous "purse-string" in those cells within 15 min. However, manipulation and closure kinematics of shaped wounds and microsurgical cuts made through the purse-string rule out a major force-generating role for the purse-string. Further analysis of the cell behaviors within the wound show that deep, mesenchymal cells contract their apical surfaces and ingress from the exposed surface. High resolution time-lapse sequences of cells at the leading edge of the wound show that these cells undergo protrusive activity only during the final phases of wound closure as the ectoderm reseals. We propose that assembly of the actin purse-string works to organize and maintain the epithelial sheet at the wound margin, that contraction and ingression of deep cells pulls the wound margins together, and that protrusive activity of epithelial cells at the wound margin reseals the ectoderm and re-establishes tissue integrity during wound healing in the *Xenopus* embryonic ectoderm. Cell Motil. Cytoskeleton 53:163–176, 2002. 

**Key words: ingression; apical contraction; actin; purse-string; cell migration**

## INTRODUCTION

Wound healing involves the coordination of cell behaviors and tissue biomechanics to bring about closure and the re-establishment of tissue integrity. Wounds in adult tissues can induce an inflammatory response as well as rounds of cell proliferation. In contrast, wounds in embryonic tissues heal rapidly without the complications of immune system response and cell proliferation [Nodder and Martin, 1997]. Nowhere is this more evident than in the rapid movement of tissues and closure of wounds after microsurgery in amphibian embryos [Townes and Holtfreter, 1955].

The process of wounding and wound repair in embryonic tissues and cell culture models can be broken

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into several distinct phases each accompanied by a set of cellular events. The first event is the wounding and the immediate biochemical response. Mechanically induced wounds result in a tearing of the cell membrane followed by either cell lysis or resealing [Bement et al., 1999; Mandato and Bement, 2001; Steinhardt et al., 1994]. A variety of growth factors can be released from lysed or wounded cells [McNeil et al., 1989]. These growth factors can stimulate growth factor receptors and initiate a calcium wave, activating cells at the site of the wound, as well as more distant cells, through gap junctions [Sammak et al., 1997]. In *Xenopus* embryos, one of the local responses to wounding is the activation of the MAP kinase pathway [LaBonne et al., 1995]. Cells in the *Xenopus* animal cap ectoderm are connected by gap junctions [Paul et al., 1995] that might activate a wound response in cells more distant from the wound site. Additionally, novel gene expression is induced by wounds in early *Xenopus* embryos [Klingbeil et al., 2001].

The cellular response begins after the immediate effects of wounding. Wounded epithelial and underlying mesenchymal cells alter cell-cell and cell-substrate adhesion [Mutsaers et al., 1997; Unger et al., 1998]. Cells at or near the margin of the wound may use mechanosensors to initiate these changes [Riveline et al., 2001]. A wounded epithelial sheet can develop a bundled actin purse-string at the wound margin [Kiehart, 1999; Martin and Lewis, 1992] via activation of Rho GTPase [Brock et al., 1996b]. This structure may serve both to draw the wound closed as well as maintain cohesion between epithelial cells at the wound margin [Danjo and Gipson, 1998; Vasioukhin and Fuchs, 2001]. Activation of the cytoskeleton within cells at the wound site can also cause mesenchymal cells to contract surrounding extracellular matrix [Grinnell, 1994; Nodder and Martin, 1997]. MDCK epithelial cells at the margin of a wounded sheet normally migrate in to fill a wound and require activation of ARF6 [Santy and Casanova, 2001], which activates Rac1 GTPase [Fenteany et al., 2000]. In embryonic limb buds, the deep mesenchymal cells contract as epithelial cells migrate over them from the wound margin [McCluskey and Martin, 1995].

At the conclusion of wound healing, an epithelium covers the wound and tissue morphology is re-established. As the opposite sides of the wound come into apposition, the actin purse-string structure is broken down and converted to cell-cell junctions [Brock et al., 1996b]. Once the embryonic epithelium is resealed, the deep cells reorganize and the original tissue morphology is restored.

Wounds in adult tissues can take days to heal; in contrast, wounded vertebrate embryonic tissues reseal quickly, making mouse, chick, and amphibian models useful for in vivo studies of wound repair. Recent work has focused on intracellular signaling, construction, and disassembly of the actin-based "purse-string" responsible for closing wounds in the chick limb bud epidermis [Martin and Lewis, 1992; McCluskey and Martin, 1995; Nodder and Martin, 1997]. Parallel studies of wound healing in the limb bud epidermis of mutant mice lacking keratin 8 exposed the fact that intermediate filaments may not be required for wound repair [Brock et al., 1996a]. Details on cell migration during wound closure have come principally from studies on teleost epidermis [Fink and Trinkaus, 1988], urodele epidermis [Lash, 1955], and *Xenopus laevis* tadpole tail epidermis [Radice, 1980a,b].

We introduce the animal cap ectoderm of the *Xenopus laevis* embryo as a model system for the study of wound healing. We have analyzed closure of simple excisional wounds in the animal cap ectoderm using low-magnification time-lapse video microscopy. As in other cases of epithelial wounding, an actin purse-string quickly assembles in the epithelial cells at the margin of the wound. However, in our case we have used microsurgical manipulations to rule out a major force-generating role for these actin structures in the early phase of wound healing. Further observations of mesenchymal cells within the wound identify apical contraction and ingression as providing the driving force for wound closure. Once these forces have drawn the epithelial margins of the wound together, protrusive activity of epithelial cells at the margin reseals the epithelium. In this study, we use an integrated approach, combining observations of cell behaviors and microsurgical manipulations of tissue mechanics, to identify the force-generating processes driving closure in a simple excisional wound.

## MATERIALS AND METHODS

### Embryo Culture and Microsurgery

Embryos are obtained from adults by standard methods [Kay and Peng, 1991]. Embryos are grown to stage 9 ½ [Nieuwkoop and Faber, 1967] and transferred to Danilchik's Solution for microsurgery [Keller et al., 1985]. The embryos are then removed from their vitelline envelope with forceps. Microsurgery is carried out with tools made of eyebrow hairs and hair loops mounted with wax in glass pipettes [Keller et al., 1985]. The prospective ectoderm of the animal cap at this stage is two to three layers thick [Keller, 1980]. A single superficial cell layer of the animal cap ectoderm can be removed by making a shallow incision with a fine eyebrow hair and peeling the superficial layer off the deep cell layer. When desired, the superficial and deep layers may be removed with an incision through both layers of the animal cap

ectoderm. A fibronectin substrate was prepared [Ramos and DeSimone, 1996]. Briefly, glass coverslips were cleaned with dilute acid in ethanol then flamed. Human plasma fibronectin (Roche Molecular Biochemicals) was prepared to 20 μg/ml and incubated on glass overnight at 4°C. The substrates were then washed with PBS and blocked with Danilchik's Solution for 1 h prior to receiving explants.

### Histology

Embryos used for confocal analysis of cell shape were injected at the 1 cell stage with 0.5 nL of a 50 mg/mL stock of 10 kDa rhodamine dextran amine (lysine fixable, Molecular Probes, Eugene, OR) in water. Embryos were then fixed in MEMFA, dehydrated in methanol, and cleared in benzyl benzoate and benzyl alcohol (2:1) [Kay and Peng, 1991]. For localization of F-actin, embryos were simultaneously fixed and stained with TRITC-phallacidin (Sigma, St. Louis, MO) in 100% acetone at 0°C for 10 min, then washed three times in PBS. Immediately after the washes, the localization of actin staining was recorded with a laser scanning confocal microscope (Olympus Fluoview).

### Videomicroscopy, Morphometrics, and Statistical Analysis

Low-magnification time-lapses were collected with a Dage (Michigan City, IN) CCD 72 camera mounted on Zeiss (Thornwood, NY) STEMI SV6 stereomicroscope. Video frames were captured using a frame grabber (Scion Corp.) installed in a personal computer (Apple Power Mac 7600) using image processing software (NIH-Image, version 1.62; Wayne Rasband, http://rsb.info.nih.gov/nih-image).

### Low Light Videomicroscopy

Low light time-lapses were collected using a Hamamatsu C2400-008 SIT camera mounted on the camera port of an Olympus IX-70 inverted microscope. Video frames, electronic shutter, camera control, and the collection of video frames were carried out with the Metamorph imaging system (Universal Imaging Corp.). Image stacks were then stored and subsequent morphometric analysis carried out using NIH-Image.

## RESULTS

Between late blastula stage (stage 9 ½) and the early gastrula stage (stage 10), the animal cap ectoderm thins from three to two cells thick [Keller, 1980; Marsden and DeSimone, 2001]. While all layers are referred to as *ectoderm* or *prospective ectoderm* only the superficial, epithelial cell-layer (facing the external medium) is a true epithelium. In contrast, the deep layers that face the blastocoel are mesenchymal. The superficial cells are cuboidal and the deep cells are isodiametric. These stereotyped, distinct layers of cells allow us to make two different types of excisional wounds with eyebrow knives and hairloops. The simplest of these wounds, which we call a *deep wound*, involves removing all the layers, superficial and deep, exposing the inside of the blastocoel. The second of type of wound, which we will call a *superficial wound*, involves the removal of only the superficial, epithelial cell-layer of the ectoderm leaving one to two layers of the mesenchyme-like deep cells. With practice, deep and superficial wounds can be made quickly and precisely in a variety of shapes and sizes. While wounds can be made at later stages, the kinematics of wound healing can be altered by morphogenetic movements. After a preliminary study on the closure of many different wounds, we describe the tissue and cell movements that accompany closure and propose mechanisms responsible for these movements.

Low-magnification time-lapse recordings of wounds closing in the animal cap of late blastula stage embryos show that wounds close quickly when only the superficial ectoderm is removed (Fig. 1A) while wounds do not close when both the deep and superficial layers of ectoderm are removed (Fig. 1B). Subsequent measurements of these wounds quantitate the rate of closure by following the change in the area (Fig. 1C), length (the long axis; Fig. 1A), and width (Fig. 2B) of the wound. By analyzing wounds of varying shapes, several empirical relationships emerge: small wounds close at a faster rate than large ones even when the initial size of the wound is taken into account; and similarly, long narrow wounds close at a faster rate than equally wide square wounds (data not shown).

Wound closure progresses with thickening of the deep cell-layers. We optically sectioned wounds fixed at 0, 15, and 30 min after surface layer excision using confocal microscopy (Fig. 2) to determine the tissue rearrangements and to assess the effectiveness of the excisions. Transverse confocal sections taken immediately after wounding show a single layer of deep cells within the wound margins of the superficial ectoderm (Fig. 2A). Bottle-shaped cells with narrow apices form 15 min after wounding at the newly exposed surface (Fig. 2B and C). After 30 min cells "pile up" within the wound as the margins are brought together (Fig. 2D). These sections show that once the superficial layer is removed, deep cells quickly aggregate under the wound and that some of these cells exhibit bottle-shapes (see arrowheads in Fig. 2C).

Early movements of wound closure do not require a collapse of the blastocoel roof. Since the animal cap ectoderm lies over the fluid-filled space of the blastocoel, one possible explanation for the rapid closure of wounds

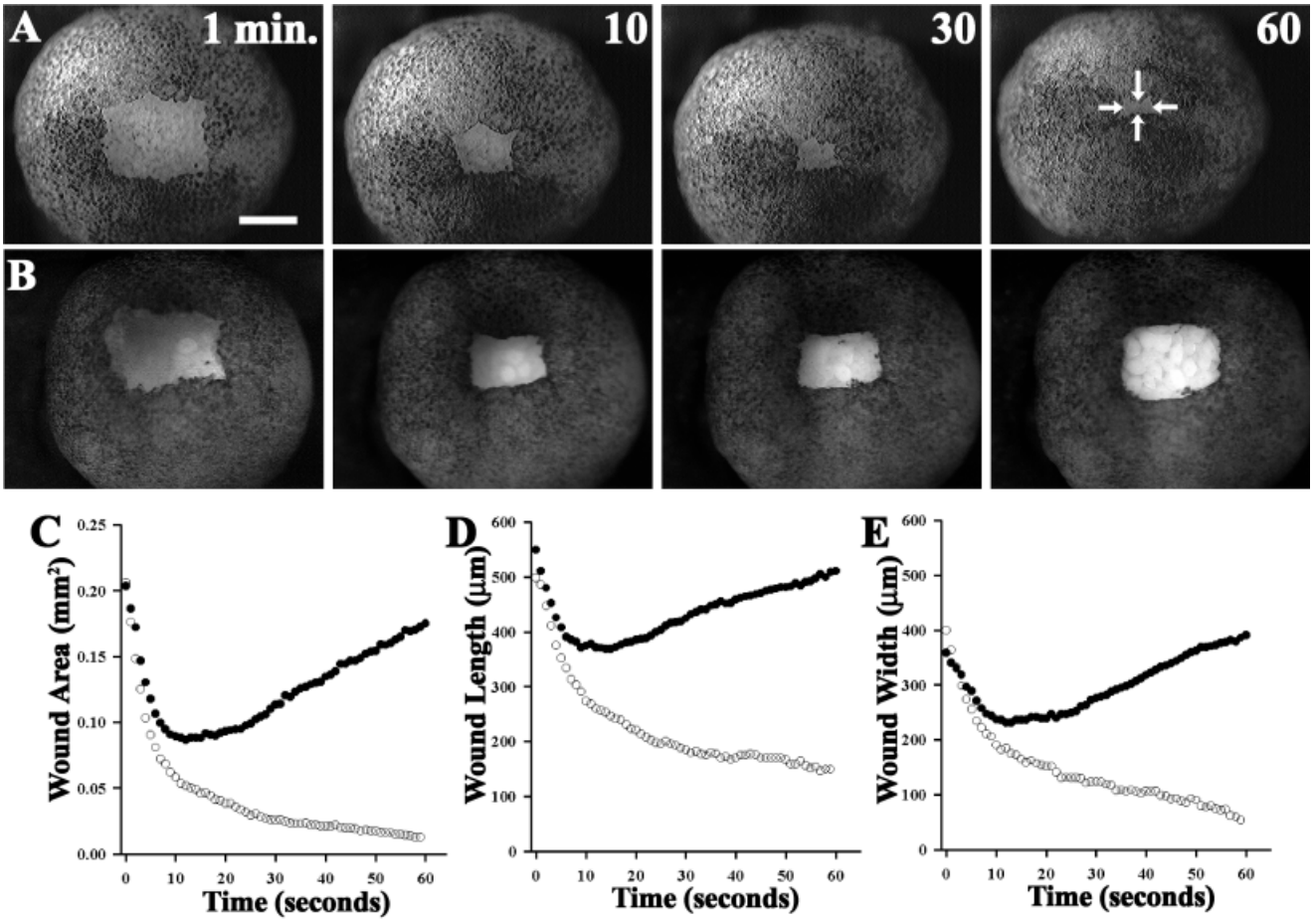



Fig. 1. Superficial wounds close while deep wounds do not. Rates of closure for two types of excisional wounds. **A:** Four frames from a time-lapse recording at 1, 10, 30, and 60 min after only the epithelial layer has been microsurgically removed from a rectangular region in the animal cap of *Xenopus laevis* embryo at stage 9½. **B:** Frames from a time-lapse recording at 1, 10, 30, and 60 min after both the deep and epithelial layers have been microsurgically removed from a rectangular region. Scale bar = 300 μm. **C–E:** Change in the exposed area (C), length (D), and width (E) of the wounds shown in A and B over the course of 60 min (*solid circles* for the deep and superficial excision, *open circles* for the superficial only excision).

in the animal cap ectoderm is that the ectoderm undergoes a "collapse" as the blastocoel shrinks from loss of fluid. To test this hypothesis, we created wounds in an animal cap ectoderm that we had previously removed microsurgically and placed onto a coverslip coated with fibronectin. Wounds in these "flat" ectodermal preparations (Fig. 3A) close rapidly with similar dynamics as wounds in intact animal cap ectoderm (Fig. 3B), eliminating the blastocoel collapse hypothesis.

Another possible explanation for rapid wound closure is that the deep cells try to round up as if they were an isolated tissue fragment. To test this hypothesis, we excised the superficial layer and the deep layer separately and observed the rounding behavior of these tissue fragments (Fig. 3C). Each piece behaved differently; the superficial layer, a true epithelium, rolled up within 50 min. In contrast, the isolated deep layer underwent rounding and aggregation. However, the rate of tissue rounding (Fig. 3D) is much less than the rate of wound closure (Fig. 1D or 1E). Thus, the process of healing superficial wounds is more efficient and organized than simple tissue reaggregation.

Myosin-mediated contraction of a contiguous actin cable has been proposed to drive wound closure in other cases of embryonic wound healing [Brock et al., 1996b; Martin and Lewis, 1992; Nodder and Martin, 1997]. In those cases, a super-cellular actin structure assembles at the margin of a wounded epithelial cell sheet and myosin II is thought to generate tensile forces that draw the margins of the wound closed like a purse-string. We asked whether a similar structure was present surrounding wounds in the animal cap ectoderm. We fixed and labeled wounded embryos with TRITC phallacidin and found intense actin staining at the wound margin as early as 5 min after wounding (Fig. 4A). After 15 min, this sparse actin forms a super-cellular

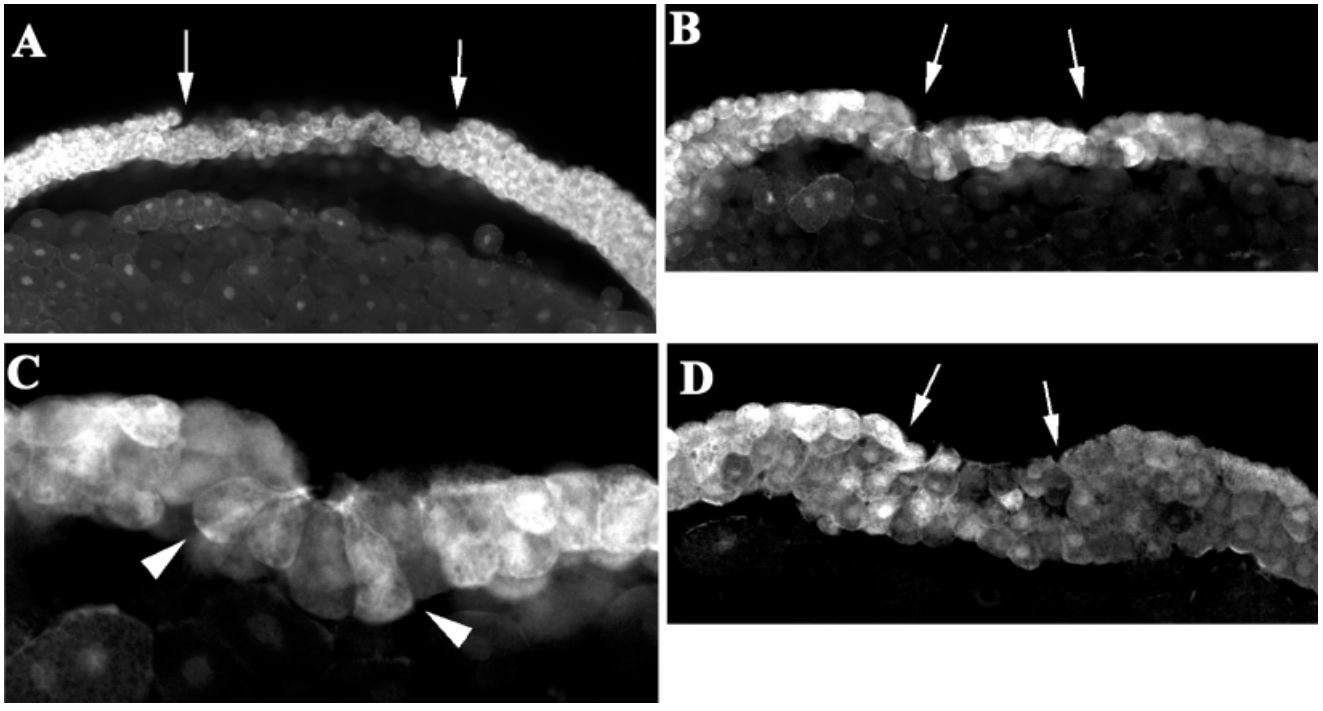



Fig. 2. Closure is accompanied by reorganization of deep-layer cells. Transverse confocal sections across a wound where only the epithelial layer has been removed after 0, 15, and 30 min. **A:** The deep-layer is intact and only one-cell-layer thick immediately after microsurgical removal of the superficial layer (*arrows* indicate edge of the wound). **B:** Within 15 min, the deep cells contract their exposed surface becoming bottle-shaped (**C**) at the margin of the wound (*arrowheads*). **D:** Within 30 min, the wound has contracted more than 50% as the deep-layer becomes two to three cells thick.

line of actin along the margin of the wounded superficial cell layer (Fig. 4B).

A contractile purse-string model for wound closure makes testable predictions of the kinematics and dynamics of wound closure [Kiehart et al., 2000; Mandato and Bement, 2001]. A purse-string structure can close a wound because tension acting tangential to the wound margin pulls out sharp corners in the wound margin, smoothing the wound margin, and pulling the margin over exposed deep cells. Such a model predicts two features of a closing wound. The first prediction is a kinematic one; the wound margin should become round as the wound closes. The second prediction is mechanical, that the wound margin is under tension as the superficial cell sheet is pulled over the deep cells. We set out to test both of these predictions in closing wounds.

First, we tested the kinematic prediction by making wounds of various shapes. In the first of these tests, we made two small wounds, one square (Fig. 4C) and one rectangular shaped (Fig. 4D). With a contractile purse-string, the square wound should form a circle as soon as the actin purse-string is assembled. After 10 min, the square wound closes significantly but remains square with sharp corners. A contractile purse-string should also cause any rectangular wound to round as the purse-string draws the wound closed. For a long rectangular wound (Fig. 4D), we measured the length and width and calculated the changing aspect ratio (Fig. 4E). The rectangular wound becomes even more rectangular as the wound closes (see open circles in Fig. 4E) becoming "slit-like." As a further test, we created triangular excisional wounds (Fig. 4F). Instead of becoming round, a triangular wound heals through a "Y"-shaped intermediate (Fig. 4G). The intermediate shapes of these variously shaped wounds contradict predictions of the purse-string model.

Next, we tested the mechanical prediction that the actin-mediated purse-string is under tension. We made rectangular wounds in the superficial layer of the ectoderm, let the wound heal for 15 min, and made two incisions about 200 μm apart across the wound margin (Fig. 5A). These two nicks across the bundle should allow the contractile bundle between the two sites to "snap-back" in recoil as the tension is released (Fig. 5B). Frames from a time-lapse sequence of such a wound before and up to 30 min after the incisions show no elastic recoil when the margin is cut (Fig. 5C). The length of the margin between the two cuts decreases 5% over 30 min. However, this rate of closure is no different than

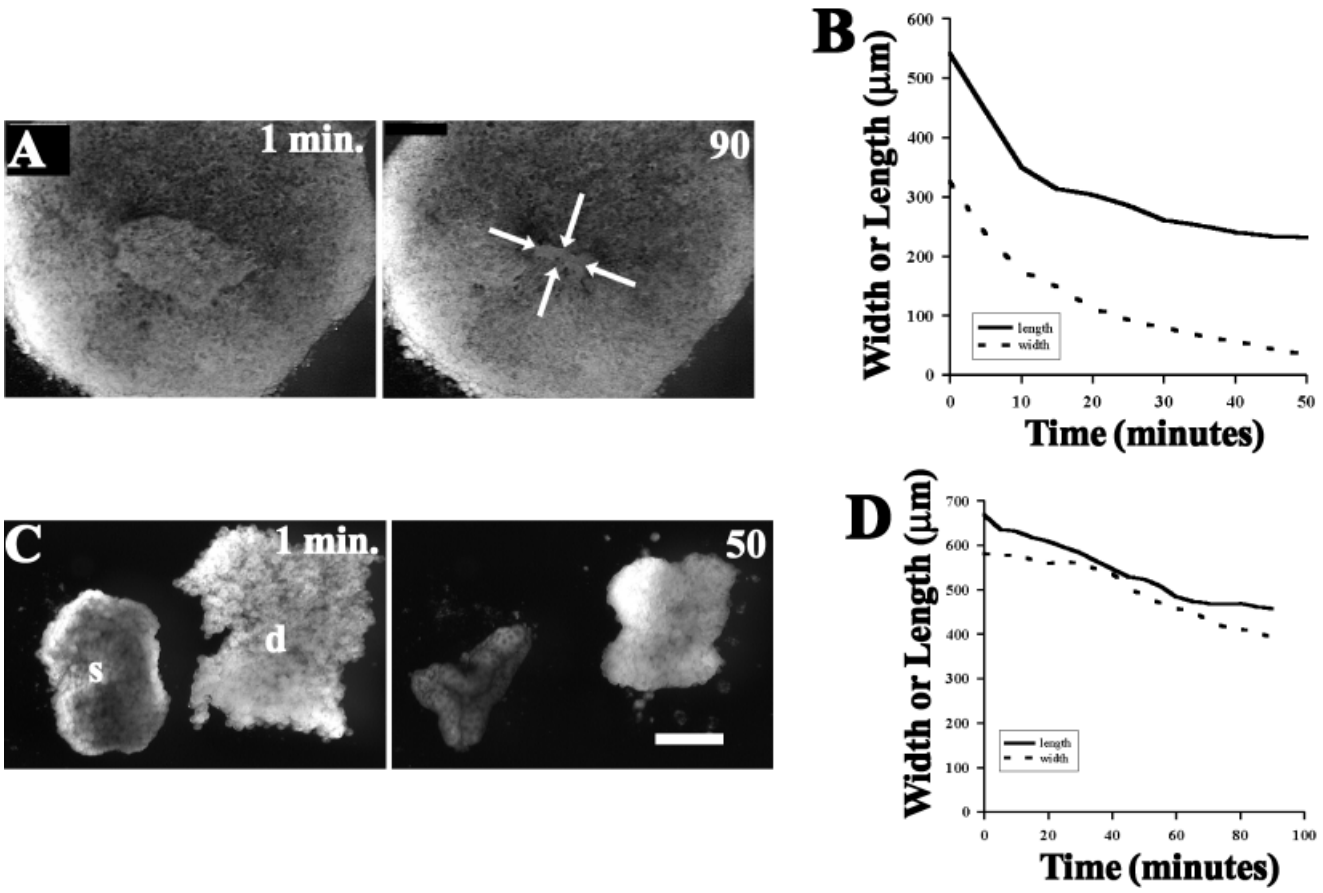



Fig. 3. Wounds in explants and isolated tissues show that the kinetics of wound closure requires two-layers: the superficial, epithelial-like layer and the deep, mesenchyme-like layers of the animal cap ectoderm. **A:** Two frames from a representative time-lapse sequence 1 and 90 min after an excisional wound was made on an animal cap explant plated on a fibronectin coated glass coverslip. Dimensions of the final wound are shown by *arrows*. **B:**Change in the length and width of the wound shown in A. **C:** Frames from a time-lapse 1 and 50 min after deep (d) and superficial (s) layers were separated and excised. Scale bar = 250 μm. **D:** Changes in the length and width of the tissue isolated from the deep cell-layer.

that seen along the undisturbed wound margin. If a contractile actin bundle were generating force, the length of the wound margin between the incisions should have decreased dramatically.

Since there was no clear evidence of a tension-generating purse-string during wound healing, we investigated other cell behaviors within the wound that might be responsible for wound closure. Since actin appeared to be enriched in the apical cortex of exposed deep cells (arrowheads in Fig. 4A), we followed the behaviors of these deep cells. To create scattered labeled populations of deep-layer cells at the late blastula stage, we injected single blastomeres in embryos at the 64-cell stage with a fluorescent lineage tracer. Subsequent cleavage scatters these labeled cells against a background of unlabeled cells. In time-lapse sequences over 45 min, deep cells can be seen contracting their exposed faces (Fig. 6A and A'). Over 60% of the exposed deep cells (26 of 43 cells traced) disappear from the surface (see green cells in Fig. 6A') as the total wound area decreases by 70% (Fig. 6B). Contraction and ingression of deep cells does not appear to proceed in unison but instead occurs stochastically (Fig. 6C) with cells ingressing randomly from various locations in the exposed deep layer throughout the course of the time-lapse.

Embryonic ectodermal cells in *Xenopus* embryos at later tadpole stages develop migratory behavior and reseal wounds quickly [Radice, 1980b]. We decided to look for evidence of epithelial cell migration at the margin of the wound using time-lapse videos of fluorescently labeled cells scattered through only the superficial epithelial cell-layer (Fig. 7A). Throughout most of wound closure cells at the margin show little protrusive activity. Once apposing wound margins are within one to two cell diameters (until 2:45 in Fig. 7B and B') cells at the margin become active, extending lamellae and filopodia across the wound to the opposite margin (2:45 through 2:53 in Fig. 7B). After the wound is resealed, the margin

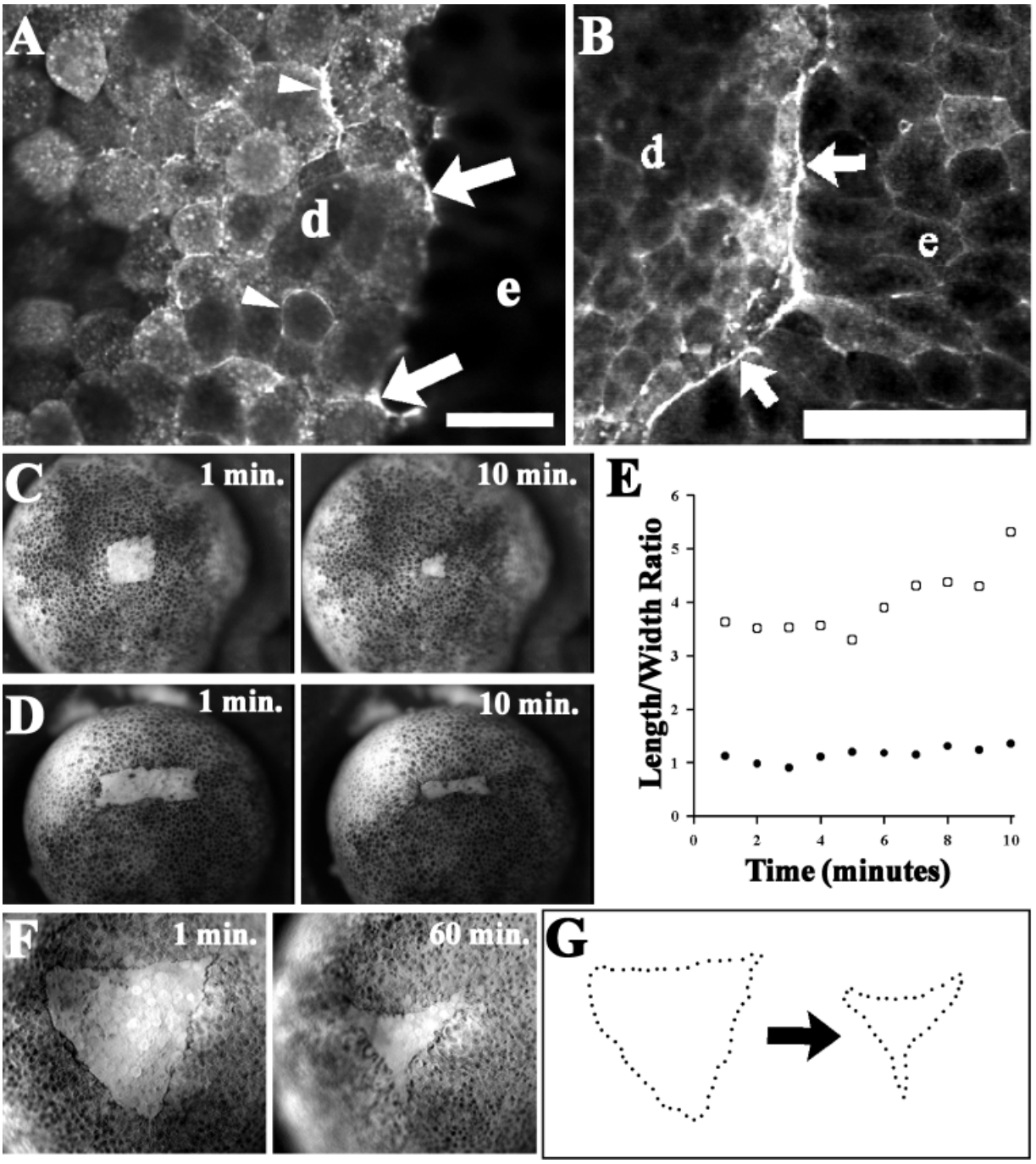



Fig. 4. Actin "purse-string" forms at the margin of the wound but odd-shaped wounds show that the purse-string may not play a role during closure. **A:** F-actin labeled by TRITC-phallacidin is present within the first 5 min at the margin (*arrows*) of the wounded ectoderm (e); actin also appears in the apically contracting deep cells (d) exposed by the wound (*arrowheads*). **B:** Intense actin-staining becomes contiguous between the intact ectoderm (e) and the exposed deep layer (d) as closure progresses (*arrows*). Scale bars in A and B = 50 μm. **C:** Frames from a time-lapse sequence of a square wound at 1 and 10 min after wounding. **D:** Frames from a time-lapse sequence of a long narrow wound at 1 and 10 min after wounding show closure as the rectangle forms a slit. **E:** Graph showing change in the aspect ratios of the wounds shown in C and D. **F:** Frames from a time-lapse sequence of a triangular wound 1 and 60 min after wounding shows closure does not proceed via a circular intermediate shape. **G:** Schematic of the changing shape of the triangular shaped wound shown in F.

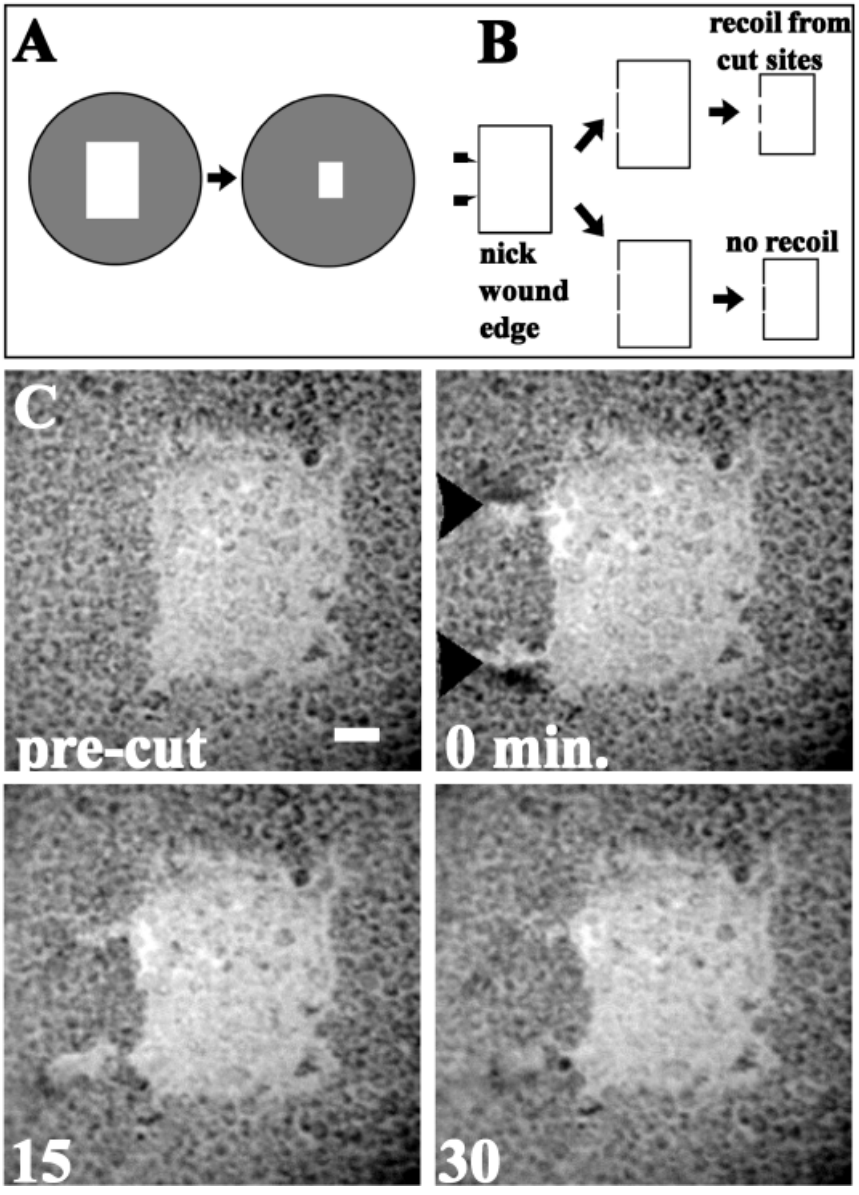


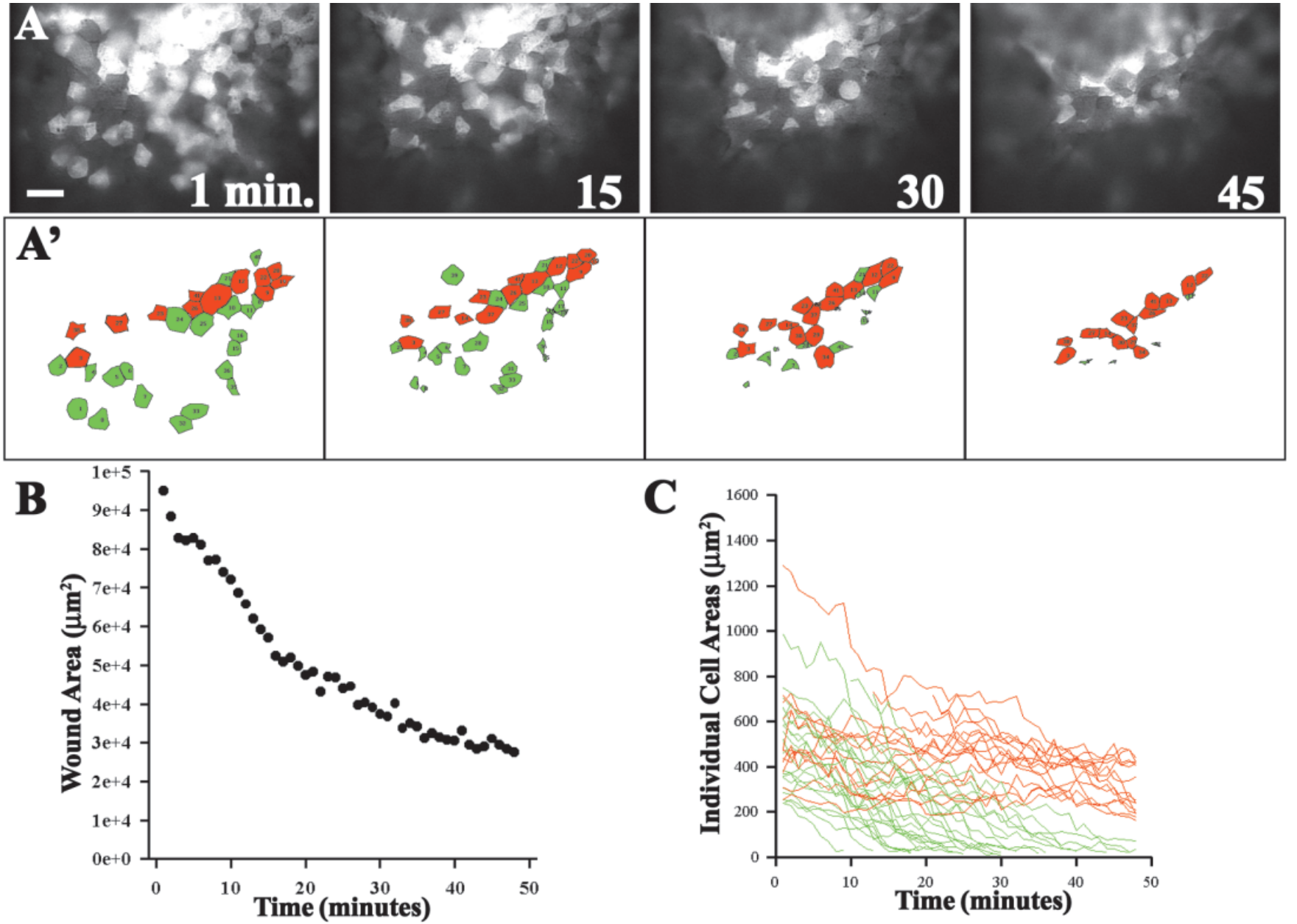

Fig. 5. The actin-rich "purse-string" is not under tension. **A:** A small rectangular wound is cut and allowed to close by 50%. **B:** Possible outcomes after two small cuts are made through the superficial layer at the margin of the wound. The wound margin should recoil from the cut sites if the purse-string created elastic tension in the margin. **C:** Frames from a representative time-lapse before and after two nicks (*arrowheads*) are made in the margin. No recoil is between Scale bar = 50 μm.

cells return to a quiescent state (5:00 in Fig. 7B). While the late phase of wound closure, wound resealing, is accompanied by protrusive activity of the epithelial cells at the wound margin, the early large-scale movements proceed without protrusive activity.

## DISCUSSION

We show here that wounds in the animal cap ectoderm of late blastula stage *Xenopus laevis* embryos close within minutes using deep, mesenchymal cell contraction and ingression, actin purse-string contraction, and epithelial marginal cell sheet migration. We propose that the bulk of these movements are the result of apical contrac-

Fig. 6. Deep cells apically contract and ingress from the surface. **A:** Frames from a representative time-lapse sequence showing apical contraction and ingression of deep cells within the center of a wound. Scale bar = 50 μm. **A':** Outlines of cells in A. Cells that will ingress or contract to less than 100 $\mu m^2$ are shown in *green* and those cells that will not contract to less than 100 $\mu m^2$ are shown in *red*. Within a minute after excision of the superficial ectoderm, the deep cells are intact and roughly isodiametric. After 15 min, all deep cells have contracted and several cells are in the process of ingressing. Thirty minutes after the superficial layer has been removed, several more cells contract and leave the exposed surface of the deep layer. After 45 min, the wound has closed considerably and the majority of traced cells have ingressed. **B:** Area of the entire wound. **C:** Areas of individual cells that ingress or contract to less than 100 $\mu m^2$ (*green lines*), and areas of individual cells remaining exposed during closure (*red lines*).


A
1 min.
15
30
45
A'
B
Wound Area (μm²)
1e+5
9e+4
8e+4
7e+4
6e+4
5e+4
4e+4
3e+4
2e+4
1e+4
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10
20
30
40
50
Time (minutes)
C
Individual Cell Areas (μm²)
1600
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
Time (minutes)

Figure 6.

tion and ingression of deep cells exposed by the wound. While an actin purse-string quickly assembles around the wound margin, experiments fail to find a direct force-generating role for this structure. Once deep contraction and ingression brings opposing margins of the wound together, it is the protrusive activity of superficial epithelial cells at the margin of the wound that complete wound healing.

The mechanics of the animal cap ectoderm and the force generated at the wound determine the course of wound closure. Wounds close because the forces bringing the margins closer exceed the forces pulling the wound apart. While the late blastula stage appears static, there are epibolic movements underway [Keller, 1980]. These movements as well as other tensions within the embryo create an animal cap ectoderm that must be under some degree of tension [Beloussov et al., 1990] at least in the superficial layer [Keller, 1980]. When the superficial layer of ectodermal cells are cut away, the pre-tension in that layer is lost. The wound can close only if forces are applied to overcome the tension pulling the ectoderm apart. Apical contraction and deep cell ingression must generate sufficient forces to pull the wound margins into apposition where cells in the superficial epithelial cell-layer can reseal the ectoderm. When these layers are missing, as in deep wounds, or when the deep cell layer is nicked or wounded (personal observations) the wound will not close.

The presence of an actin purse-string does not always indicate that a contractile purse-string is operating. Actin bundles have been reported in numerous wound healing studies on single cells [Bement et al., 1999; Mandato and Bement, 2001] and embryonic tissues [Martin and Lewis, 1992; Nodder and Martin, 1997] as well as in a number of morphogenetic movements (such as dorsal enclosure during *C. elegans* development [Williams-Masson et al., 1997], bottle cell formation during primary invagination in sea urchins [Nakajima and Burke, 1996], and dorsal closure in Drosophila development [Kiehart et al., 2000; Young et al., 1993]. For wound healing in the Xenopus ectoderm, however, such a contractile structure makes several predictions. Testing these predictions, we demonstrated that although intense actin-staining is present, the resulting structure contributes little to bringing the wound margins into apposition. In contrast, using a set of experiments similar to ours, Mandato and Bement [2001] established a contractile role for an actin purse-string during closure of wounds in the plasma membrane of single cells. Laser ablation studies of dorsal closure in *Drosophila* also identify a contractile role for the actin purse-string found at the margin of the dorsal epidermis [Kiehart et al., 2000]. In some cases, such as closure of wounds in the plasma membrane and dorsal closure, the contractile function of the purse-string is clear. In other cases, such as ours, the role of these actin-rich structures is unclear but might have a mechanical function either "harnessing" forces generated by cell migration and transmitting those forces to the remainder of the epithelial sheet or by "anchoring" the free-edge of the epithelium to the contracting deep cell-layer.

Numerous examples of cell migration following wounding can be found in mature epithelia [Honda et al., 1982], in embryonic epidermis [Radice, 1980a,b], and in cultured cell sheets [Sammak et al., 1997]. While we cannot rule out a role for migration during wound closure we can identify limits to that role. As seen in Figure 6A, the margin of the wound advances only a short distance over the deep cell layer even as the deep layer is contracting. In our case, cell migration does not cover much of the exposed deep cell-layer but instead appears to function as a "zipper" during re-epithelialization. Epithelial cells at the wound margin display protrusive behaviors only late in the healing process, just as the apposing margins of the wound are brought together. These protrusions appear to play a role in the resealing of the ectoderm. Dynamic protrusions have been identified at such "closure events" in several instances of epithelial morphogenesis in invertebrates such as filopodial "priming" during ventral epidermis fusion in *C. elegans* [Raich et al., 1999] and the dorsal epidermis fusion in *Drosophila* [Jacinto et al., 2001].

Apical contraction is generally a cell behavior restricted to epithelial cells bearing apical junctional complexes and sub-apical belts of actin cytoskeleton [Hardin and Keller, 1988; Kam et al., 1991; Kimberly and Hardin, 1998; Owaribe et al., 1981; Sweeton et al., 1991]. Before excision of the superficial layer, deep cells have a mesenchymal character expressing β-catenin and actin appropriately [Fagotto and Gumbiner, 1994; Kurth et al., 1996; Reintsch and Hausen, 2001]. Surprisingly, exposed deep cells appear to assembly structures like sub-apical belts of actin (Fig. 4A and B), apically constrict during wound healing (Fig. 6A), and ingress as if they were bottle cells (Fig. 2C). Questions remain as to the nature of this transition and whether these mesenchymal cells transiently assemble structures more akin to polarized epithelial cells during wound closure.

Tissue movements and cell behaviors active during wound closure are similar to the events of neurulation [Nodder and Martin, 1997]. Apical contraction and re-epithelialization of embryonic wounds parallel movements during neural tube closure in *Xenopus*. In *Xenopus* the prospective epidermis at the margin of the neural plate moves medially as cells in the intervening neurectoderm apically contract to form bottle cells [Davidson and Keller, 1999; Schroeder, 1970]. These epidermal cells detach from the lateral margin of the neural plate

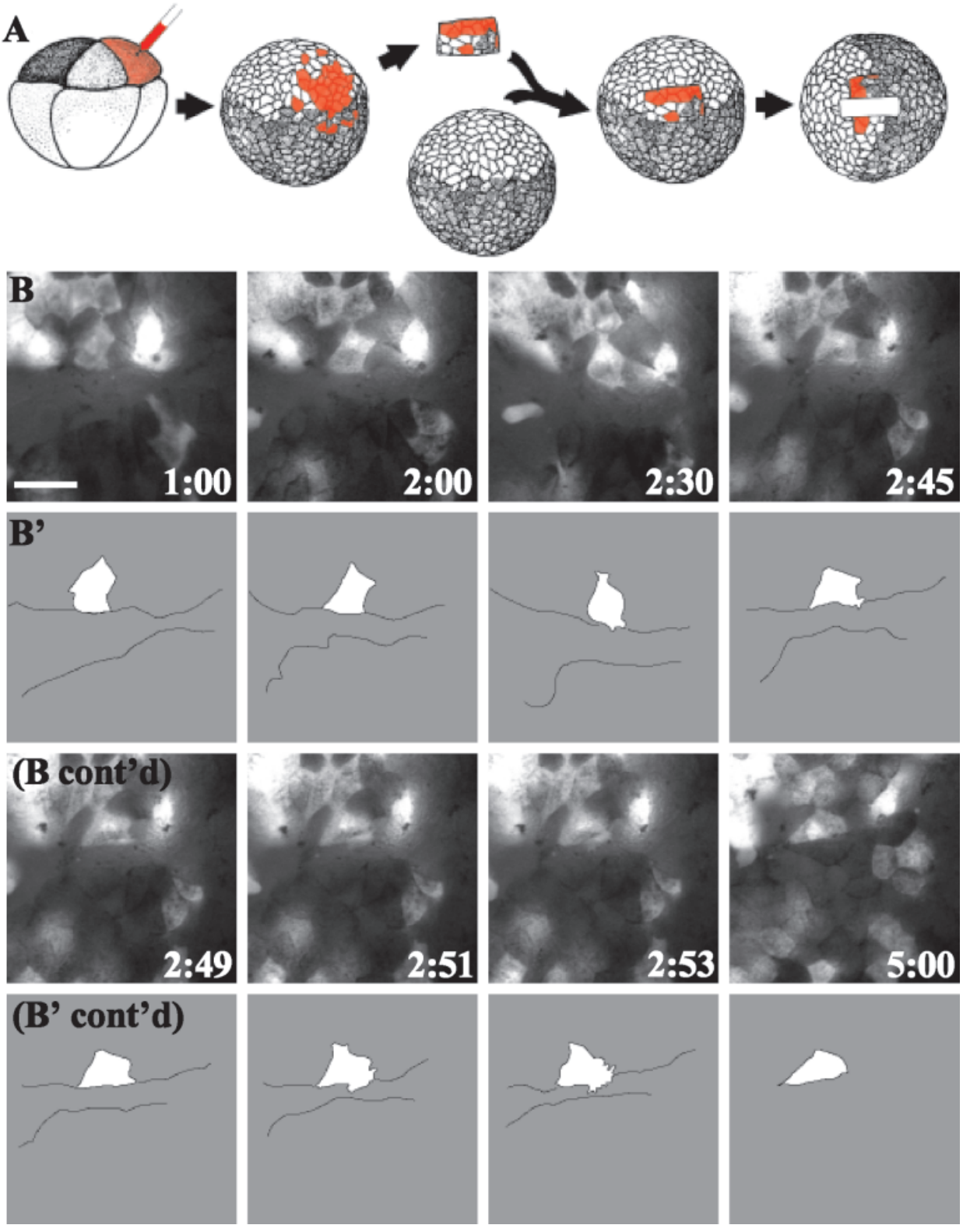


Fig. 7. Cells along the margin do not start protrusive activity until margins close with one cell diameters. Protrusions directed across the wound then accompany resealing of the epithelium. **A:** Schematic of the experiment. A single blastomere is injected with a fluorescent lineage label that produces a patch of scattered labeled cells. A superficially labeled patch of cells is grafted to an unlabeled host embryo and after sufficient healing a rectangular excisional wound is made through the labeled patch. **B:** Frames from a representative low-light epifluorescence time-lapse sequence of a cell at the wound margin. Margin of wound 1 h after excision of the superficial layer shows that the cell is quiescent. After 2 h, apposing wound margins are within two cell diameters. Frames taken at 2-min intervals starting 2 1/2 h after wounding show a rapid series of lamellae as the labeled margin cell begins protrusive activity. Five hours after wounding, the margins have joined and the cell again becomes quiescent. **B':** Drawings of the protrusive cell in B is shown with the wound margin. Scale bar = 50 μm.

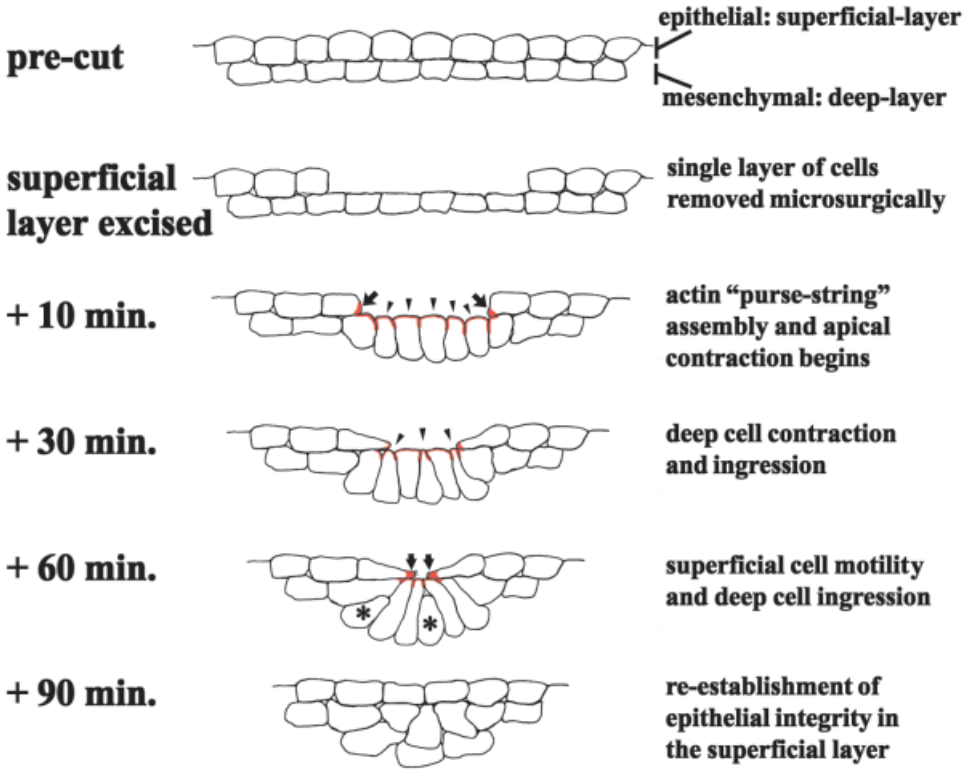


Fig. 8. Steps in embryonic wound healing in the *Xenopus laevis* animal cap ectoderm. An excisional wound can be made that removes only the epithelial cell layer of the two cell-layered animal cap ectoderm. Apical contraction (*arrowheads*) and ingression (*asterisks*) drive rapid reduction in wound size. While an actin purse-string forms early (+10 min; *arrows*), active cell protrusions appear only later in healing (+60 min; *arrows*) and appear to guide resealing of the wound and re-establishment of the epithelium.

and re-attach to epidermal cells on the opposite margin. Once the epidermis fuses, neurectodermal cells take on mesenchymal character as they re-establish the neural tube lumen. While fusion of the epidermis and re-establishment of the neural tube lumen appears similar to wound healing, there is no actin purse-string assembled at the edge of the neural folds in *Xenopus* (LD, personal observations). Cell protrusive activity in apposed neural folds have not yet been characterized.

## CONCLUSION

Here we describe embryonic wound healing in the *Xenopus laevis* animal cap ectoderm. After eliminating a contractile role for the actin-bundle surrounding the wound, we propose that deep cell contraction and ingression drive the immediate closure of the wound. Cell migration and the leading edge actin bundle are implicated in the events of wound "zippering" and re-epithelialization.

Excisional wounds in the animal cap of *Xenopus laevis* embryos provide a model system that can be used to study all the events of wound healing from release of growth factors at the moment of mechanical wounding to the reorganization of tissue architecture following re-epithelialization. The biochemical as well as biomechanical environment of the wound can be altered with exogenously introduced proteins and mRNAs and the use of microsurgery to alter the size and shape of the wound. Video and confocal microscopy can be used to describe the 3-D cell shape changes and tissue movements that accompany healing. Together these approaches can be combined to uncover previously obscured interactions between molecular mediators of force generation and tissue structure and to elevate our understanding of wound healing providing practical tools to improve the speed and effectiveness of the healing process.

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