

Wound-induced assembly and closure of an actomyosin purse string in *Xenopus* oocytes

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Background: Both single cells and multicellular systems rapidly heal physical insults but are thought to do so by distinctly different mechanisms. Wounds in single cells heal by calcium-dependent membrane fusion, whereas multicellular wounds heal by a variety of different mechanisms, including circumferential contraction of an actomyosin 'purse string' that assembles around wound borders and is dependent upon the small GTPase Rho.

Results: We investigated healing of puncture wounds made in *Xenopus* oocytes, a single-cell system. Oocyte wounds rapidly assumed a circular morphology and constricted circumferentially, coincident with the recruitment of filamentous actin (F-actin) and myosin-II to the wound borders. Surprisingly, recruitment of myosin-II to wound borders occurred before that of F-actin. Further, experimental disruption of F-actin prevented healing but did not prevent myosin-II recruitment. Actomyosin purse-string assembly and closure was dependent on Rho GTPases and extracellular calcium. Wounding resulted in reorganization of microtubules into an array similar to that which forms during cytokinesis in *Xenopus* embryos. Experimental perturbation of oocyte microtubules before wounding inhibited actomyosin recruitment and wound closure, whereas depolymerization of microtubules after wounding accelerated wound closure.

Conclusions: We conclude the following: actomyosin purse strings can close single-cell wounds; myosin-II is recruited to wound borders independently of F-actin; purse-string assembly is dependent on a Rho GTPase; and purse-string assembly and closure are controlled by microtubules. More generally, the results indicate that actomyosin purse strings have been co-opted through evolution to dispatch a broad variety of single-cell and multicellular processes, including wound healing, cytokinesis and morphogenesis.

Background

The ability to repair physical insults is a critical feature of individual cells and multicellular systems. In both cases, unrepaired breaches have serious consequences: death for single cells and unregulated movement of solutes through tissues. Accordingly, single cells [1] and tissues [2] have rapid mechanisms of wound repair. For single cells, recent work has demonstrated that membrane fusion establishes a barrier to influx of ions and small molecules in response to the exposure of cytoplasm to extracellular calcium [3–5]. For cell layers and tissues a variety of healing mechanisms have been described, including cell migration, contraction of cells underlying wounds, and 'purse string' based healing, wherein circumferential contraction of epithelial cells bordering wounds is driven by a purse string composed of filamentous actin (F-actin) and myosin-II [6–8].

The latter mechanism is morphologically similar to the healing of wounds made in amphibian eggs and embryos that was first described more than 50 years ago. Holtfreter

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[9] reported that cortical wounds undergo circumferential shrinkage until the wound is closed. Healing of wounds in *Xenopus* oocytes and eggs has since been shown to be sensitive to perturbation of the actomyosin cytoskeleton [10–12], leading to the proposal that amphibian oocyte and egg wounds heal by closure of an actomyosin-based purse string, a mechanism similar to that used in cytokinesis in animal cells.

To date, however, localization of F-actin and myosin-II around wounds has not been demonstrated in *Xenopus* oocytes or in any other single-cell system. Furthermore, it has been reported that perturbation of the actomyosin cytoskeleton impairs healing in sea urchin eggs by inhibiting transport or localization of vesicles required for membrane fusion [13]. It is presently unclear, therefore, whether actomyosin purse strings have any role in the healing of single-cell wounds.

The basic biological importance of wound healing and the proposed similarity between wound-induced purse strings

and those responsible for cytokinesis prompted us to re-investigate wound healing in *Xenopus* oocytes. We show first that a circumferential, contractile purse string that is composed of F-actin and myosin-II assembles around wounds and closes them, and then we exploit the strengths of this system to provide insights into mechanisms of purse-string assembly.

Results

Wounding triggers assembly and closure of actomyosin purse strings

Follicle-cell-free oocytes were wounded with glass needles just above the oocyte equator. Oocytes were fixed at increasing times after wounding and then processed for analysis of wound diameter and staining of F-actin and myosin-IIA. Wounds in oocytes that were fixed within 30 seconds of puncture were relatively ragged (Figure 1a). At later time points, however, wounds became more rounded and the diameter decreased progressively, which is consistent with a contractile mechanism of closure. The rounding and decrease in diameter coincided with accumulation of F-actin and myosin-II at the wound margin as seen in both forward facing (Figure 1a) and confocal z-sections (Figure 1b). To determine whether the apparent accumulation of F-actin and myosin-II at wound borders reflected a biological response to wounding rather than a greater access

of actomyosin probes to wound edges, control oocytes were wounded after fixation and then processed for actomyosin labeling. In such controls, there was no apparent increase in F-actin or myosin-II staining at wound edges (Figure 1a).

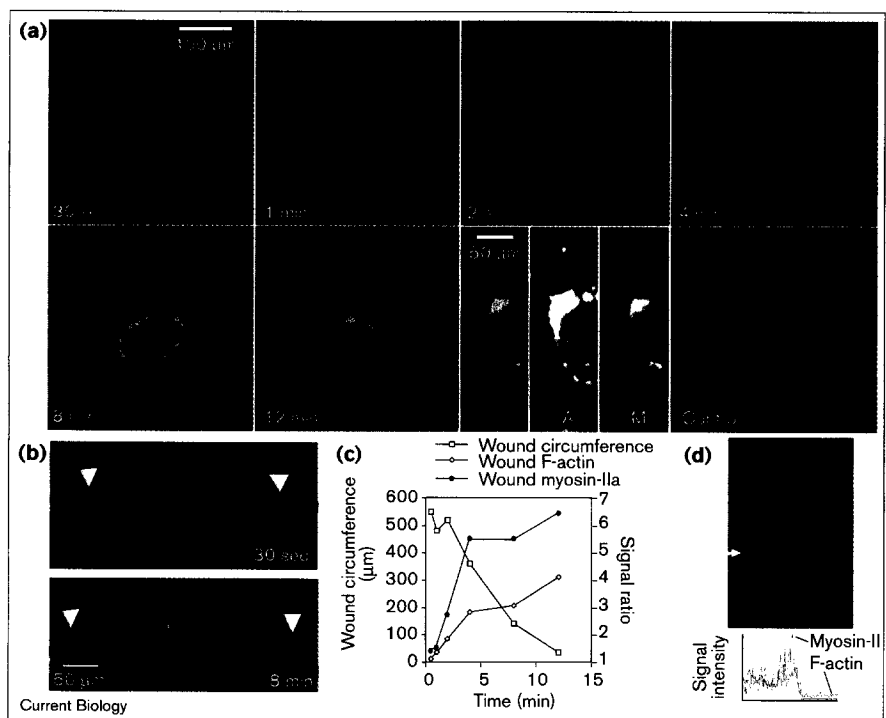
To quantify the accumulation of F-actin and myosin-II around wound borders, mean fluorescence intensities were measured at the wound edge and at regions more than 100 μm away from the wound in the same oocyte and then expressed as the ratio of the two measurements. This approach controls for oocyte-to-oocyte and treatment-to-treatment variation in signal intensity. Quantification of the relative signals for F-actin and myosin-II confirmed that wound closure was correlated with actomyosin accumulation and showed that F-actin and myosin-II accumulation paralleled each other for most of the time course (Figure 1c). Surprisingly, however, inspection of early time points showed that myosin accumulation could occur before F-actin accumulation (Figure 1d). In addition, wounds typically exhibited a bipartite distribution of myosin-II and F-actin, such that myosin-II was concentrated on the inside and F-actin on the outside of the purse string (Figure 1a).

Wound closure is dependent on actomyosin

The correlation between actomyosin recruitment and wound constriction suggested that actomyosin purse

Figure 1

Time course analysis of wound closure in oocytes and actomyosin accumulation around wounds. **(a)** Confocal, double-label images of F-actin (red) and myosin-II (green) distribution at the indicated times after wounding. F-actin and myosin-II are recruited to the wound border coincident with rounding and closure of the wound. High-magnification, split-channel views of the 12 min time point (7th panel) shows that most of the F-actin (A) concentrated towards the outer portion of the ring, whereas most of the myosin-II (M) concentrated towards the inner portion of the ring. In oocytes wounded after fixation (control), no enrichment of F-actin or myosin-II was evident at the wound edges. **(b)** Confocal z-sections showing F-actin (red) and myosin-II (green) at early (30 sec) and late (8 min) times following wounding. Healing was accompanied by accumulation of both F-actin and myosin-II (shown as yellow), which closed the purse string (arrowheads). **(c)** Plot of mean wound circumference (from five or more oocytes at each time point) and the ratios of the signal for F-actin and myosin-II at wound edges compared with areas of the cortex more than 100 μm away from the wound. F-actin and myosin-II signal increased in parallel, except at the earliest time point (30 sec), where the F-actin ratio was still around 1 and the myosin-II signal was 1.4. **(d)** Image of a wound edge (30 sec after wounding) double-labeled for

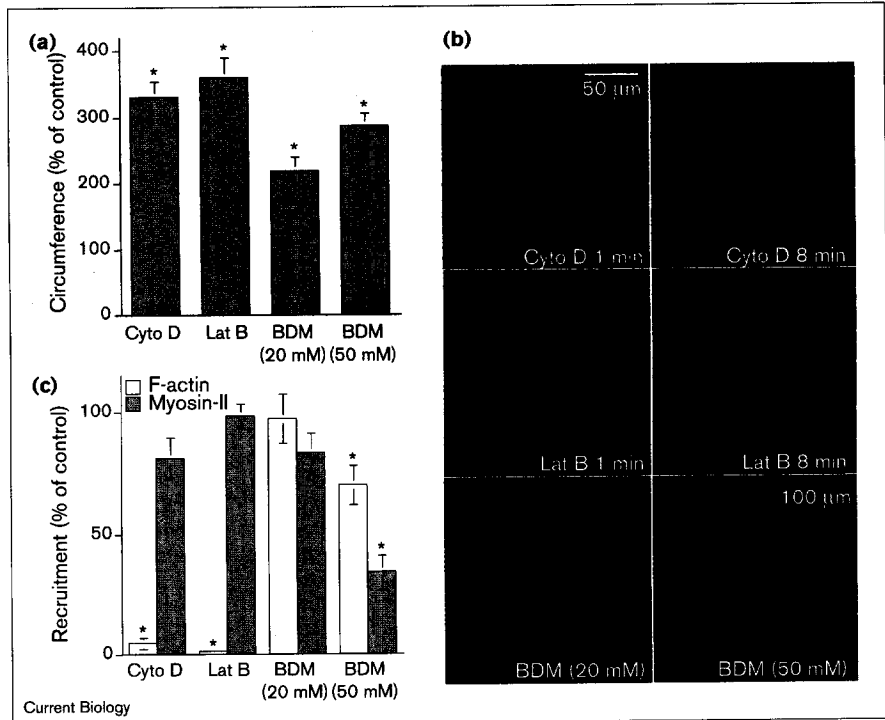


F-actin (red) and myosin-II (green) with a corresponding signal intensity plot. The signal intensity for a line drawn across the wound at

the point indicated by the arrow shows that the myosin-II signal (green line) was higher at the wound edge than the F-actin signal (pink line).

Figure 2

Effects of actomyosin inhibitors on wound closure and purse-string assembly. **(a)** Bar graph showing that a 1 h pretreatment of oocytes with 20 μ M cytochalasin D (Cyto D), 10 μ M latrunculin B (Lat B), or the indicated concentrations of BDM inhibit wound closure. Wound circumferences were measured after 8 min of healing. Results are the mean \pm standard error of the mean (SEM). Asterisks indicate significant differences relative to controls ($p < 0.05$; $n = 3$). **(b)** Confocal micrograph showing actomyosin localization in early (1 min) and late (8 min) wounds following treatment with cytochalasin D or latrunculin B and in 8 min wounds in oocytes treated with 20 or 50 mM BDM. Myosin-II (green) accumulated around wounds in both latrunculin- and cytochalasin-treated oocytes in spite of the lack of F-actin (red) recruitment. Both F-actin and myosin-II were recruited to wound borders in oocytes treated with 20 mM BDM, but in oocytes treated with 50 mM BDM, F-actin and myosin-II accumulated in large cables adjacent to the wound. **(c)** Bar graph showing quantification of F-actin and myosin-II recruitment to wound borders 8 min after wounding of oocytes treated with cytochalasin D, latrunculin B or two different concentrations of BDM. Concentrations and treatments were as above; signal ratio was calculated as in Figure 1. Results are the mean \pm SEM. Asterisks indicate significant differences relative to recruitment in control wounds ($p < 0.05$; $n = 3$).



strings are responsible for wound closure. To confirm this point, oocytes were subjected to perturbation of actomyosin function, wounded and then processed for analysis 8 minutes after wounding. Cytochalasin D, which disrupts actin filaments, prevents wound closure (Figure 2a). Latrunculin B, an agent that disrupts actin filaments by a mechanism distinct from that of the cytochalasins, also prevented healing (Figure 2a). Butanedione monoxime (BDM), a general myosin ATPase inhibitor [14], also inhibited wound closure (Figure 2a). Thus, the assembly of the actomyosin purse string is not merely an epiphenomenon of wounding but is actually required for wound closure.

Myosin-II is recruited to wound borders independently of F-actin

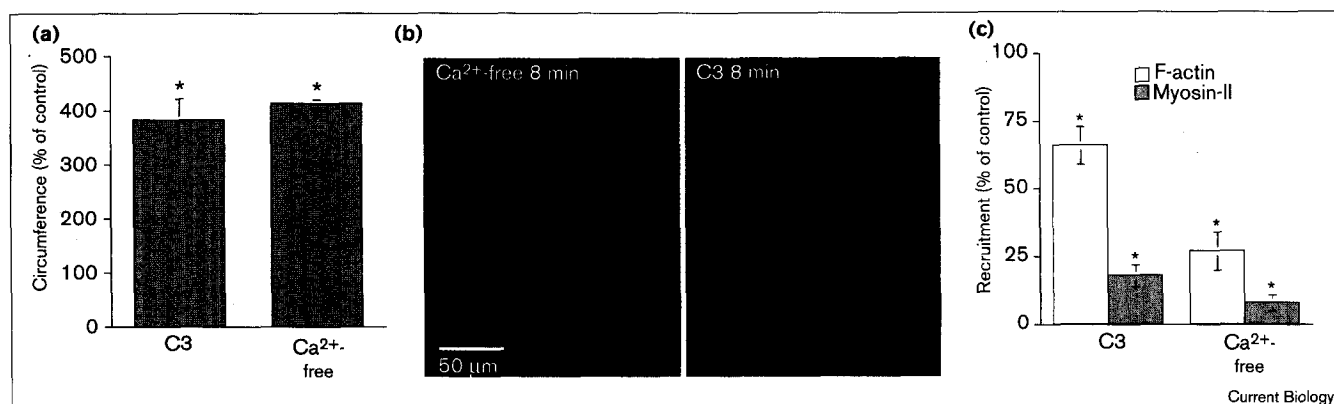
The finding that myosin-II accumulation could be observed in the absence of F-actin accumulation and the observation that F-actin and myosin-II did not precisely colocalize around wounds suggested that myosin-II might not require F-actin for targeting to wound borders. To assess this point directly, oocytes were treated with cytochalasin D or latrunculin B, and then processed for quantification of relative levels of F-actin and myosin-II

at wound borders. On the basis of comparisons of fluorescence intensity between treated and untreated oocytes from the same experiments, cytochalasin B reduced oocyte F-actin by $55 \pm 8\%$, whereas latrunculin reduced oocyte F-actin by $97 \pm 3\%$ (mean \pm standard error of the mean, SEM; $n = 3$). Double-stained images reveal that myosin-II is recruited to wound borders even when F-actin recruitment is prevented by cytochalasin or latrunculin treatment (Figure 2b). Fluorescence quantification confirms this point: cytochalasin D and latrunculin B prevent F-actin accumulation at wound edges but do not prevent myosin-II accumulation (Figure 2c). BDM also failed to prevent recruitment of myosin-II to wound borders at concentrations sufficient to inhibit wound closure (Figure 2c). At higher concentrations, BDM inhibited recruitment of both F-actin and myosin-II, although interpretation of these results was complicated by the formation of large myosin-II aggregates adjacent to wounds (Figure 2b).

Actomyosin purse-string assembly and closure is Rho- and calcium-dependent

Membrane fusion-dependent healing of single-cell wounds requires external calcium [3–5], whereas purse-string

Figure 3



Effects of removal of external calcium and Rho inhibition on wound closure and actomyosin purse-string assembly. **(a)** Bar graph showing that wounding in the absence of external calcium (Ca²⁺-free) or in the presence of C3 exotransferase (C3) inhibits wound closure. Wound circumferences were compared after 8 min of healing. Results are the mean \pm SEM. Asterisks indicate significant differences relative to control wounds ($p < 0.05$; $n = 3$). **(b)** Confocal micrographs showing actomyosin localization 8 min after wounding oocytes in the absence of external calcium (Ca²⁺-free) or in the presence of C3

exotransferase. Neither F-actin (red) nor myosin-II (green) exhibited significant accumulation in the absence of external calcium while some F-actin accumulation is observed in the presence of C3. **(c)** Bar graph showing quantification of F-actin and myosin-II recruitment to wound borders in oocytes wounded in the absence of external calcium (Ca²⁺-free) or the presence of C3 exotransferase relative to recruitment in control wounded oocytes. Calcium-free wounding strongly inhibited recruitment of both F-actin and myosin-II whereas C3 showed greater inhibition of myosin-II recruitment. Results are the mean \pm SEM.

healing of multicellular wounds is sensitive to Rho GTPase inhibition by C3 exotransferase [8]. To test the calcium dependence of oocyte purse-string healing, oocytes were wounded in the absence of extracellular calcium. Removal of external calcium blocked healing (Figure 3a) and prevented recruitment of F-actin and myosin-II (Figure 3b,c). To test whether oocyte purse string healing was dependent on Rho GTPases, oocytes were wounded in the presence of C3 exotransferase. C3 blocked healing (Figure 3a), inhibited myosin-II recruitment and modestly inhibited F-actin recruitment (Figure 3b,c). Purse-string-mediated healing of oocyte wounds therefore has features that are characteristic of healing in both single-cell and multicellular systems.

Wounding triggers microtubule reorganization

The actomyosin dependence and the morphology of wound-induced purse strings were obviously reminiscent of the contractile structures that drive animal cell cytokinesis. Because cytokinetic apparatus assembly is microtubule dependent, we investigated the distribution of microtubules in wounded oocytes. Forward-facing views revealed that wounding resulted in an increase in cortical microtubules in the wounded area within 1 minute (Figure 4a). Over time, microtubules accumulated in large bundles in the wound, and extended radially from the edges of the closing wound (Figure 4a). Confocal z-sections confirmed the apparent increase in microtubules in the wound immediately following wounding and clearly showed the formation of microtubule bundles

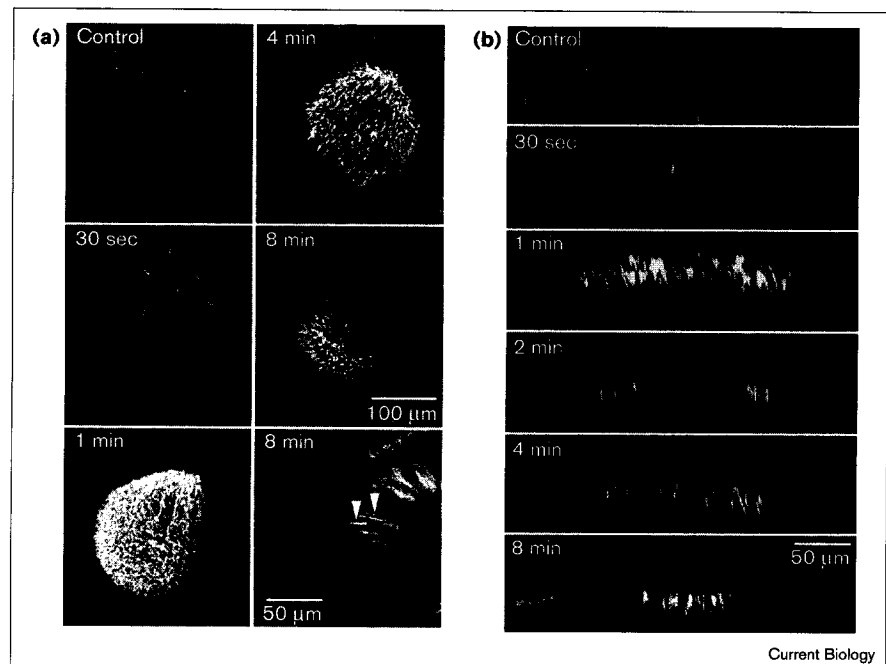
in the interior of the wound (Figure 4b). As the wounds closed, microtubules became progressively restricted to the interior of the closing wound (Figure 4b).

Purse-string healing is both positively and negatively modulated by microtubules

The changes in the microtubule cytoskeleton occurred within the same time-frame as actomyosin accumulation, suggesting that microtubules might modulate wound-induced purse strings. To assess this point, oocytes were pretreated with either nocodazole, to depolymerize microtubules, or taxol, to increase oocyte microtubule levels. Both treatments inhibited healing (Figure 5a) suggesting that microtubule reorganization, which occurs in response to wounding, is important for the healing process. Quantification of F-actin and myosin-II recruitment to wound borders revealed that although both taxol and nocodazole pretreatment dramatically reduced the amount of myosin-II recruited to wound borders, nocodazole pretreatment only slightly inhibited recruitment of F-actin to wound borders (Figure 5b,c). In a second series of experiments, oocytes were wounded, allowed to heal for 2 minutes, and then treated with nocodazole, to depolymerize microtubules. Oocytes were then fixed at 8 minutes and processed for wound measurements and analysis of F-actin and myosin-II recruitment to wound borders. Remarkably, this treatment actually accelerated wound healing (Figure 5a). Analysis of relative levels of F-actin and myosin-II signal at wound borders showed no significant increase in actomyosin recruitment (Figure 5b,c).

Figure 4

Wounding results in the reorganization of cortical microtubules. **(a)** Forward-facing confocal images showing time course changes in cortical microtubules following wounding. Microtubules increased in density in early wounds (30 sec–1 min). Microtubules were gathered into bundles in the wound interior over time. The higher magnification view of the 8 min timepoint shows microtubule bundles (arrowheads) radiating outwards from the closing purse string. **(b)** Confocal z-sections showing time course changes in cortical microtubules following wounding. Microtubule density within the wound increased at early time points (30 sec–1 min). Microtubules formed bundles perpendicular to the plane of the closing purse string; these bundles became progressively restricted to the interior of the purse string as it closed (1 min–8 min). In (a,b), the control shown represents an oocyte wounded after fixation.

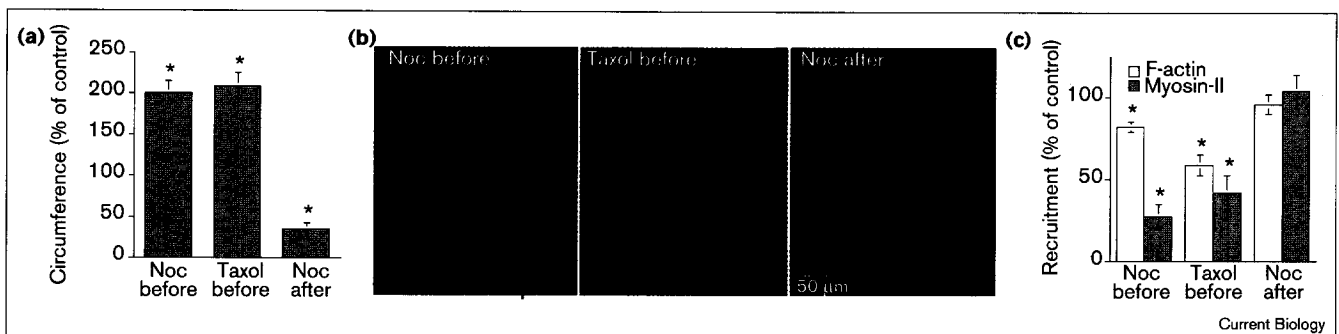


Current Biology

Purse-string closure occurs in early *Xenopus* tadpoles

The similarity of wound-induced purse strings in *Xenopus* oocytes to the wound-induced, multicellular purse strings previously described [6–8], suggested that purse strings might function to heal wounds in both single-cell and

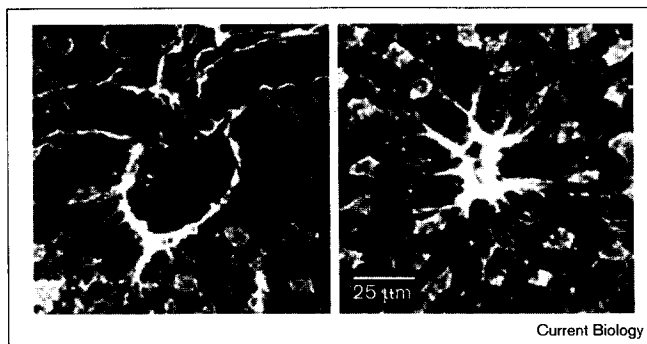
multicellular systems within the same organism. Consistent with this idea, tadpole wounds were healed by a purse-string-based mechanism, as judged by their circumferential constriction and the accumulation of F-actin around wound borders (Figure 6).

Figure 5


Current Biology

Effects of microtubule manipulation on wound closure and actomyosin purse-string assembly. **(a)** Bar graph showing that wounds made in oocytes pretreated for 1 h in 40 μM nocodazole (Noc before) or 20 μM taxol (Taxol before) prior to wounding healed poorly relative to control wounds that had not been pretreated, whereas wounds made in oocytes treated with 40 μM nocodazole 2 min after wounding (Noc after) heal more quickly. Wound circumferences were compared after 8 min of healing. Results are the mean ± SEM. Asterisks indicate significant differences relative to control wounds ($p < 0.05$; $n = 3$). **(b)** Confocal micrograph showing actomyosin localization 8 min after wounding of oocytes pretreated with either nocodazole (Noc before), taxol (Taxol before) or in an oocyte treated with nocodazole after

wounding (Noc after). Nocodazole pretreatment inhibited myosin-II (green) accumulation but had less effect on F-actin (red) accumulation. Taxol pretreatment inhibited accumulation of both F-actin and myosin-II. Treatment of oocytes with nocodazole after wounding had no obvious effect on either F-actin or myosin-II recruitment. **(c)** Bar graph showing quantification of F-actin and myosin-II recruitment to wound borders in oocytes wounded after pretreatment with nocodazole (Noc before), Taxol (taxol before) or treated with nocodazole after wounding (Noc after). Results are the mean ± SEM. Asterisks indicate significant differences relative to recruitment in control wounds ($p < 0.05$; $n = 3$).

Figure 6

Purse strings also form in wounded tadpoles. Confocal micrographs showing F-actin accumulation around wounds made in *Xenopus* tadpoles, a multicellular system. The micrographs show wounds in early (left panel) and late (right panel) stages of healing. F-actin is enriched around wound borders and cells bordering the wound are wedge-shaped, indicative of actomyosin purse-string closure.

Discussion

The results presented here show that single cells can close wounds by assembly and contraction of a circumferential, actomyosin purse string, similar to the multicellular purse strings reported to drive wound closure in embryonic [6,8] and cultured epithelia [7]. This assertion is made on the basis of the following findings: first, F-actin and myosin-II rapidly accumulate around wounds; second, the accumulation of actomyosin coincides with circumferential contraction of wounds; third, purse-string assembly and wound closure are sensitive to pharmacological perturbation of actomyosin; finally, purse-string assembly and wound closure is sensitive to C3 exotransferase, a specific inhibitor of Rho GTPases that inhibits other actomyosin-based contractile processes including stress fiber formation and cytokinesis [15,16].

Recent work using sea urchin eggs and cultured cells has shown that plasma membrane wounds are sealed by a calcium-sensitive, membrane-fusion-based process [1]. What is the relationship between this process and purse-string based closure? Although purse-string-mediated healing is rapid, it is still too slow to prevent an inrush of ions. Further, it is difficult to imagine how a purse string could assemble at an edge that is freely exchanging with the surrounding medium. It is therefore probable that the two mechanisms have complementary roles in oocyte wounds: membrane fusion seals the cell and the actomyosin purse string re-establishes a functional cortex. That is, by stretching pre-existing cortex over the area resealed by membrane fusion, the purse-string mechanism could help to ensure that a plasma membrane with at least some associated cytoskeletal proteins, transporters and other membrane proteins is rapidly restored. Because the half-life of newly synthesized cortical cytoskeletal proteins

is greatly extended in the presence of a pre-existing cortical cytoskeleton [17], such a mechanism would presumably be much more efficient than *de novo* assembly of a cortical cytoskeleton on relatively naked membrane, particularly in the large wounds described here.

Although cellular wound healing is of considerable intrinsic interest, one of our goals has been to develop simple model systems for the analysis of microtubule-actomyosin interactions [18,19]. The wound-induced purse strings are particularly useful in that they bear a number of obvious similarities to the purse strings that execute cytokinesis in animal cells. Besides being comprised of F-actin and myosin-II, wound-induced purse strings are also dependent on players implicated in cytokinesis, including microtubules and Rho GTPases. In contrast to cytokinesis, wherein the cytokinetic ring cannot be detected until it has already assembled, wound-induced purse-strings can be produced on demand, permitting analysis of the early events of purse-string assembly.

One of the surprising findings to emerge from this analysis is the F-actin-independent recruitment of myosin-II to wound borders. On the basis of models of actomyosin-based cortical contraction and cortical flow [20], we expected F-actin and myosin-II to act in lock-step (absolute coordination). At very early times after wounding, however, myosin-II accumulation could be observed even in the absence of F-actin accumulation, and the spatial distribution of myosin-II within purse strings differed from that of F-actin. Most significantly, myosin-II was recruited to wound borders even in the absence of F-actin recruitment following cytochalasin D or latrunculin B treatment. We therefore conclude that wounding results in F-actin-independent recruitment of myosin-II to wound borders; whether a similar mechanism is operative during animal cell cytokinesis is unknown. But myosin-II localizes to the site of septation in budding yeast ahead of F-actin [21,22], whereas myosin-II lacking motor activity [23,24] localizes in the prospective cleavage furrow in *Dictyostelium*. F-actin-independent recruitment of myosin-II might therefore be a general feature of nascent purse strings.

How does this occur? The inhibition of myosin-II recruitment by the Rho inhibitor, C3 exotransferase, suggests at least one promising line of thought: Rho is thought to control actomyosin by either regulation of F-actin via the ezrin/radixin/moesin (ERM) family of F-actin-binding proteins or by increasing the phosphorylation of the regulatory light chains (RLCs) of myosin-II [25,26]. Of these two possibilities, RLC phosphorylation is the better choice because myosin-II recruitment occurs in the absence of F-actin. RLC phosphorylation results in two significant changes in myosin-II-increased actin-dependent ATPase activity, and increased filament formation

[27]. Of these two changes, the former is unlikely to account for the effects of Rho inhibition, again on the basis of the F-actin independence of recruitment. Instead, it is probable that the recruitment mechanism either acts on myosin-II filaments or promotes myosin-II filament formation, or both.

The relationship between microtubules and wound-induced purse strings is also striking, because in spite of the obvious difference in the stimuli for wound healing and cytokinesis, the end result with respect to microtubules is remarkably similar. Following wounding, microtubules form bundles perpendicular to the plane of the purse string, whereas other microtubule bundles radiate outwards from the closing purse string. Likewise, in *Xenopus* embryos, microtubule bundles run perpendicular to the cytokinetic arc, as well as radiating outward from the arc [28]. Indeed, the presence of microtubule bundles perpendicular to the purse string is also reminiscent of cytokinesis in cultured cells, where the cytokinetic apparatus closes on the parallel microtubule bundles of the midbody [29]. Because microtubule reorganization coincides with purse-string assembly and because pretreatment of oocytes with nocodazole or taxol inhibits myosin-II recruitment, the initial stimulus provided by wounding might trigger microtubule reorganization, which then somehow results in myosin-II recruitment to wound borders.

The acceleration of purse-string closure resulting from nocodazole treatment after purse-string assembly parallels work showing acceleration of cortical flow in *Xenopus* oocytes [18] and contractile-ring closure during cytokinesis in cultured cells [30] following microtubule depolymerization, as well as classic studies on echinoderm embryos showing that the spindle apparatus is dispensable following the onset of cytokinesis [31]. Experimental acceleration of wound closure is also of potential clinical significance given that it suggests that wound healing might be facilitated by localized microtubule disruption, if one assumes that mechanisms of purse-string healing in single cells are similar to those occurring in multicellular wounds.

Finally, the wound healing results with oocytes and tadpoles, considered within the context of studies in other systems, suggest that actomyosin purse strings represent a basic biological motility mechanism that has been repeatedly employed during evolution to drive an otherwise diverse set of biological processes. That is, in addition to wound healing in single-cell and multicellular systems and the well-known role of actomyosin purse strings in cytokinesis, multicellular purse strings might be responsible for dorsal closure in *Drosophila* [32] and ventral enclosure in *Caenorhabditis elegans* [33]. For wound healing, it is not difficult to imagine a plausible evolutionary sequence,

because a purse-string-based mechanism for single-cell wounds could be converted to one for multicellular wounds simply by the development of cell-cell attachments, assuming that the same signaling mechanism is employed. The relationships between purse strings in wound healing, cytokinesis, and morphogenesis are less clear, but the results of the present study suggest two areas of overlap. First, Rho GTPases have been implicated in cytokinesis [16,26], multicellular wound healing [8], single-cell wound healing (this report), and dorsal closure in *Drosophila* [34]. Second, microtubules somehow control the positioning and assembly of the contractile actomyosin network in cytokinesis [29], single-cell wound healing (this report), and microtubule motors are required for ventral enclosure in *C. elegans* [35]. Thus, regardless of the system in question, it might be that actomyosin purse-string assembly is triggered and regulated by a restricted set of conditions.

Conclusions

The results show that actomyosin purse strings can heal single-cell wounds and are assembled by Rho-dependent, microtubule-dependent, and F-actin-independent recruitment of myosin-II to wound borders. The results also indicate that actomyosin purse strings represent a fundamental mechanism that has been repeatedly employed throughout evolution to dispatch different biological motility processes.

Materials and methods

Oocyte and embryo acquisition, preparation and wounding

Oocytes were obtained from adult *Xenopus* females and stored in 1× OR-2 (82.5 mM NaCl, 2.5 mM KCl, 1 mM CaCl₂, 1 mM MgCl₂, 1 mM Na₂HPO₄, 5 mM Hepes pH 7.4) containing 5 mg/ml BSA as described previously [18]. Tadpoles were purchased from Nasco (Ft. Atkinson, WI). For wounding, oocytes were positioned on petri dishes with nylon grids (Spectrum) affixed to their bottoms and stabbed at or just above the equator with a capillary tube pulled and broken to a diameter of 150 μm. Tadpoles were first incubated in the anesthetic benzocaine (Sigma Chemical Co.) for 30 min and then wounded as above.

Pharmacological perturbations

Oocytes were preincubated in the agents (nocodazole, taxol, cytochalasin D and latrunculin B from Calbiochem; BDM from Sigma) for 1 h. Nocodazole, taxol, cytochalasin D and latrunculin B were prepared immediately before use from DMSO stock solutions stored at -80°C; BDM was prepared immediately before use from the powder. DMSO at the same final concentration as in the experimentals was used as a control. In experiments where nocodazole was applied after wounding, it was first diluted from the stock into a 10× solution in BSA-OR-2 and then gently pipetted onto healing oocytes. For wounding in the absence of external calcium, oocytes were rinsed 3× in Ca²⁺-free OR-2, rinsed 1× in Ca²⁺-free OR-2 containing 5 mM EGTA, rinsed 2× in Ca²⁺-free OR-2 and then wounded immediately.

C3 exotransferase treatment

To conserve C3 (purchased from Calbiochem), eight oocytes were placed in a droplet of OR-2 on a piece of parafilm in a moist chamber. Excess OR-2 was wicked away and replaced with 100 μl OR-2 containing 200 μg/ml C3, and rapidly wounded.

Confocal fluorescence microscopy

For staining of F-actin and myosin-II, samples were rapidly fixed in sploogee buffer (SB; 80 mM PIPES, pH 6.8, 5 mM EGTA, 1 mM MgCl₂, 0.2% Triton X-100) containing 3.7% paraformaldehyde [36]. Oocytes were fixed for 4–8 h, washed for 8–16 h in Tris-buffered saline + 0.1% NP-40 (TBSN), blocked for 1 h in TBSN + 5 mg/ml BSA (TBSN-BSA), and incubated for 8–12 h at 4°C in TBSN-BSA + 1 U/ml Texas red-phalloidin (Molecular Probes) and 10 µg/ml affinity-purified, rabbit anti-*Xenopus* nonmuscle myosin-IIA antibody [37]. Samples were washed for 12–16 h in TBSN-BSA at 4°C and then incubated for 8–12 h at 4°C in TBSN-BSA + 10 µg/ml Oregon green goat anti-rabbit antibody (Molecular Probes). Oocytes were then washed for 12–16 h in TBSN-BSA and mounted in silicon grease rings.

For microtubule staining, oocytes were fixed in SB + 3.7% paraformaldehyde, 0.25% glutaraldehyde, and 0.5 µM taxol overnight, post-fixed in methanol at –20°C for 24 h, rehydrated in phosphate-buffered saline (PBS), quenched for 8 h in PBS + 100 mM sodium borohydride, and then washed and blocked for 4 h in multiple changes of TBSN-BSA at 4°C. Samples were then subjected to antibody incubations as above, except that the primary antibody was 10 µg/ml monoclonal anti- α -tubulin antibody (DM1A, Amersham) and the secondary was 10 µg/ml rhodamine-anti-mouse antibody (Cappel). Oocytes were then washed for 12–24 h in TBSN, dehydrated in methanol, and then cleared and mounted in 1:2 benzyl alcohol-benzyl benzoate [35].

Image analysis and quantification

Samples were examined on a Biorad 1024 at the Keck Center for Neural Imaging, University of Wisconsin, Madison. Samples were examined with the 20 \times objective, and optically sectioned to a depth of 100–200 µm, to allow visualization of the entire cortical region of the wound and the surrounding cortex. Optical sections were then stacked and merged. Merged images were analyzed without image processing in Adobe Photoshop. For each oocyte, the fluorescence signal at wound borders was quantified by positioning a box at eight positions around the wound border and then measuring the mean red and green signal within the box. The signal for the eight points was averaged and divided by the average signal for eight positions more than 100 µm away from the wound border [7]. All statistical analyses were done using Microsoft Excel. In all cases, a minimum of three oocytes were analyzed for each data point and *n* refers to the number of independent experiments, each using oocytes from a different frog.

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References

- McNeil PL, Steinhardt RA: **Loss, restoration, and maintenance of plasma membrane integrity.** *J Cell Biol* 1997, **137**:1-4.
- Martin P: **Wound healing—aiming for perfect skin regeneration.** *Science* 1997, **276**:75-81.
- Steinhardt RA, Bi G, Alderton JM: **Cell membrane resealing by a vesicular mechanism similar to neurotransmitter release.** *Science* 1994, **263**:390-393.
- Miyake K, McNeil PL: **Vesicle accumulation and exocytosis at sites of plasma membrane disruption.** *J Cell Biol* 1995, **131**:1737-1745.
- Terasaki M, Miyake K, McNeil PL: **Large plasma membrane disruptions are rapidly resealed by Ca²⁺-dependent vesicle-fusion events.** *J Cell Biol* 1997, **139**:63-74.
- Martin P, Lewis J: **Actin cables and epidermal movement in embryonic wound healing.** *Nature* 1992, **360**:179-183.
- Bement WM, Forscher P, Mooseker MS: **A novel cytoskeletal structure involved in purse string wound closure and cell polarity maintenance.** *J Cell Biol* 1993, **121**:565-578.
- Brock J, Midwinter K, Lewis J, Martin P: **Healing of incisional wounds in the embryonic chick wing bud: characterization of the actin purse-string and demonstration of a requirement for rho activation.** *J Cell Biol* 1996, **135**:1097-1107.
- Holtfreter J: **Properties and functions of the surface coat in amphibian embryos.** *J Exp Zool* 1943, **93**:252-323.
- Merriam RW, Christensen K: **A contractile ring-like mechanism in wound healing and soluble factors affecting structural stability in the cortex of *Xenopus* eggs and oocytes.** *J Embryol Exp Morphol* 1983, **75**:11-20.
- Meeusen RL, Bennett J, Cande WZ: **Effects of microinjected *N*-ethylmaleimide-modified heavy meromyosin on cell division in amphibian eggs.** *J Cell Biol* 1980, **86**:858-865.
- Bement WM, Capco DG: **Analysis of inducible contractile rings suggests a role for protein kinase C in embryonic cytokinesis and wound healing.** *Cell Motil Cytoskel* 1991, **20**:145-157.
- Bi G, Morris RL, Liao G, Alderton JM, Scholey JM, Steinhardt RA: **Kinesin and myosin-driven steps of vesicle recruitment for Ca²⁺-regulated exocytosis.** *J Cell Biol* 1997, **138**:999-1008.
- Cramer LP, Mitchison TJ: **Myosin is involved in post mitotic cell spreading.** *J Cell Biol* 1995, **131**:179-189.
- Chrzanowski-Wodnicka M, Burridge K: **Rho-stimulated contractility drives formation of stress fibers and focal adhesions.** *J Cell Biol* 1995, **133**:1403-1415.
- Kishi K, Sasaki T, Kuroda S, Itoh T, Takai Y: **Regulation of cytoplasmic division of *Xenopus* embryo by rho p21 and its inhibitor GDP/GTP exchange protein (rho GDI).** *J Cell Biol* 1993, **120**:1187-1195.
- Nelson WJ, Veshnock PJ: **Dynamics of membrane-skeleton (fodrin) organization during development of polarity in Madin-Darby canine kidney epithelial cells.** *J Cell Biol* 1986, **103**:1751-1765.
- Canman JC, Bement WM: **Microtubules suppress actomyosin-based cortical flow in *Xenopus* oocytes.** *J Cell Sci* 1997, **110**:1907-1917.
- Sider JR, Mandato CA, Weber KL, Zandy AJ, Beach D, Finst RJ, Skobel J, Bement WM: **Direct observation of microtubule-F-actin interaction in cell free lysates.** *J Cell Sci* 1999, **112**:1947-1956.
- Bray D, White JG: **Cortical flow in animal cells.** *Science* 1988, **239**:883-888.
- Lippincott J, Li R: **Sequential assembly of myosin-II, an IQGAP-like protein, and filamentous actin to a ring structure involved in budding yeast cytokinesis.** *J Cell Biol* 1998, **140**:355-366.
- Bi E, Maddox P, Lew DJ, Salmon ED, McMillan JN, Yeh E, Pringle JR: **Involvement of an actomyosin contractile ring in *Saccharomyces cerevisiae* cytokinesis.** *J Cell Biol* 1997, **142**:1301-1312.
- Yumura S, Uyeda TQ: **Transport of myosin-II to the equatorial region without its own motor activity in mitotic *Dictyostelium* cells.** *Mol Biol Cell* 1997, **8**:2089-2099.
- Zang JH, Spudich JA: **Myosin-II localization during cytokinesis occurs by a mechanism that does not require its motor domain.** *Proc Natl Acad Sci USA* 1998, **95**:13652-13657.
- Matsui T, Maeda M, Doi Y, Yonemura S, Amano M, Kaibuchi K, et al.: **Rho-kinase phosphorylates COOH-terminal threonines of ezrin/radixin/moesin (ERM) proteins and regulates their head-to-tail association.** *J Cell Biol* 1998, **140**:647-657.
- Hall A: **Rho GTPases and the actin cytoskeleton.** *Science* 1998, **279**:509-514.
- Bresnick AR: **Molecular mechanisms of nonmuscle myosin-II regulation.** *Curr Opin Cell Biol* 1999, **11**:26-33.
- Danilchik MV, Funk WC, Brown EE, Larkin K: **Requirement for microtubules in new membrane formation during cytokinesis of *Xenopus* embryos.** *Dev Biol* 1998, **194**:47-60.
- Field C, Li R, Oegema K: **Cytokinesis in eukaryotes: a mechanistic comparison.** *Curr Opin Cell Biol* 1999, **11**:68-80.
- Hamilton BT, Snyder JA: **Acceleration of cytokinesis in PtK1 cells treated with microtubule inhibitors.** *Exp Cell Res* 1983, **144**:345-351.
- Hiramoto Y: **Cell division without mitotic apparatus in sea urchin eggs.** *Exp Cell Res* 1956, **11**:630-636.
- Young PE, Richman AM, Ketchum AS, Kiehart DP: **Morphogenesis in *Drosophila* requires nonmuscle myosin heavy chain function.** *Genes Dev* 1993, **7**:29-41.
- Williams-Masson EM, Malik AN, Hardin J: **An actin-mediated two step mechanism is required for ventral enclosure of the *C. elegans* hypodermis.** *Development* 1997, **124**:2889-2901.

34. Harden N, Ricos M, Ong YM, Chia W, Lim L: **Participation of small GTPases in dorsal closure of the *Drosophila* embryo: distinct roles for Rho subfamily proteins in epithelial morphogenesis.** *J Cell Sci* 1999, **112**:273-284.
35. Raich WB, Moran AN, Rothman JH, Hardin J: **Cytokinesis and midzone microtubule organization in *Caenorhabditis elegans* require the kinesin-like protein ZEN-4.** *Mol Biol Cell* 1998, **9**:2037-2049.
36. Gard DL: **Organization, nucleation, and acetylation of microtubules in *Xenopus laevis* oocytes: a study by confocal fluorescence microscopy.** *Dev Biol* 1991, **143**:346-362.
37. Kelley CA, Sellers JR, Gard DL, Bui D, Adelstein RS, Baines IC: ***Xenopus* nonmuscle myosin heavy chain isoforms have different subcellular localizations and enzymatic activities.** *J Cell Biol* 1996, **134**:675-687.

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