ABSTRACT Embryonic tissues heal wounds rapidly and without scarring, in a process conserved across species and driven by collective cell movements. The mechanisms of coordinated cell movement during embryonic wound closure also drive tissue development and cancer metastasis; therefore, embryonic wound repair has received considerable attention as a model of collective cell migration. During wound closure, a supracellular actomyosin cable at the wound edge coordinates cells, while actin-based protrusions contribute to cell crawling and seamless wound healing. Other cytoskeletal networks are reorganized during wound repair: microtubules extend into protrusions and along cell–cell boundaries as cells stretch into damaged regions, septins accumulate at the wound margin, and intermediate filaments become polarized in the cells adjacent to the wound. Thus, diverse cytoskeletal networks work in concert to maintain tissue structure, while also driving and organizing cell movements to promote rapid repair. Understanding the signals that coordinate the dynamics of different cytoskeletal networks, and how adhesions between cells or with the extracellular matrix integrate forces across cells, will be important to elucidate the mechanisms of efficient embryonic wound healing and may have far-reaching implications for developmental and cancer cell biology.

INTRODUCTION
The ability of embryonic tissues to rapidly repair damage has fascinated scientists for more than a century. Embryos repair wounds with little to no inflammation or scarring, in a process conserved from fruit flies to humans. Collective cell migration drives the efficient sealing of lesions in the embryonic epidermis. Similar coordinated cell movements contribute to wound closure in adult tissues, and are critical for tissue morphogenesis and cancer cell migration. Thus, studying embryonic wound repair provides an opportunity to understand the biochemical and mechanical signals that cells use to integrate their behaviors during tissue development and in human disease (Friedl and Gilmour, 2009).

The study of embryonic wound closure offers several experimental advantages over other morphogenetic processes. First, the wound healing response is well defined in time, with a clear beginning and end. Therefore, it is possible to register multiple experiments based on the time of wounding, and the progress in wound closure can be measured based on the size of the wound. Second, if wounding assays are conducted in early embryos, breathing and muscle contraction do not interfere with live microscopy, and the process can be visualized without imaging artifacts. Third, embryonic wound repair occurs within a relatively short timescale (typically under one hour in most species), which facilitates the analysis of a greater number of samples, thus increasing the statistical power of the results. The ability to conduct many experiments within short timescales enables exploration of results that may otherwise be missed due to their variability, including the effects of wound size and geometry on the dynamics of repair (Davidson et al., 2002; Abreu-Blanco et al., 2012; Wyczalkowski et al., 2013) or the subtle phenotypes caused by some genetic and pharmacological treatments (Zulueta-Coarasa et al., 2014). Thus, embryonic
between different cytoskeletal components may be a critical factor during embryonic wound closure, and we propose that cross-talk between different cytoskeletal networks plays a role. Cytoskeletal networks include actin, microtubules, septins, and intermediate filaments. Cytoskeletal networks are anchored at adherens junctions (AJs), which maintain epithelial cell connections (Mege and Ishiyama, 2017), providing a physical link between the cytoskeletons of neighboring cells. Here, we review the roles that different cytoskeletal networks play during embryonic wound closure, and we propose that cross-talk between different cytoskeletal components may be a critical factor for cells to generate and bear the mechanical stresses associated with the rapid, coordinated cell movements that drive embryonic repair.

**A CONTRACTILE ACTOMYOSIN CABLE FORMS ALONG THE WOUND MARGIN**

Upon wounding, the cells immediately adjacent to the wound polarize their cytoskeleton. Both actin and myosin accumulate at the interface with the wounded cells, forming a supracellular cable around the wound (Figure 1, A and B). Actomyosin polarization and supracellular cable assembly as mechanisms of wound closure were first demonstrated in the chick embryo (Martin and Lewis, 1992; Brock et al., 1996), and subsequently in mouse (McCluskey and Martin, 1995), frog (Davidson et al., 2002), fruit fly (Kiehart et al., 2000; Wood et al., 2002), and zebrafish (Hunter et al., 2018) embryos, as well as in cnidarians (Kamran et al., 2017), adult worms (Xu and Chisholm, 2011), and the adult mouse cornea (Danjo and Gipson, 1998). Notably, human intestinal epithelial cells also repair damage through the assembly of an actomyosin cable around the wound, both in culture (Bement et al., 1993) and in vivo in patients with inflammatory bowel disease (Russo et al., 2005), suggesting that the mechanisms of wound closure observed in the embryo may represent an ancient, general repair mechanism of epithelial monolayers.

Cell–cell junctions play an important role in maintaining tissue integrity and organizing the cytoskeleton during wound closure. In the Drosophila embryonic epidermis, the actomyosin cable forms following the disassembly of the AJs along the wound edge, in a process driven by Src-dependent endocytosis (Hunter et al., 2015, 2018; Matsubayashi et al., 2015), and maintained through transcriptional down-regulation of E-cadherin (Carvalho et al., 2014). Blocking endocytosis or overexpressing the core AJ component E-cadherin prevents the formation of the actomyosin cable, and down-regulating E-cadherin while endocytosis is partially blocked rescues actin assembly around the wound (Hunter et al., 2015; Matsubayashi et al., 2015). These results suggest that AJ internalization from the wound edge precedes cable formation (Figure 2). However, the reasons why AJs must be disassembled to form an actin cable are not understood. One possibility is that removal of AJ complexes provides physical space for the polymerization of actin networks (Carvalho et al., 2014). Alternatively, AJ components may need to be recycled to form specialized junctional structures that promote actin assembly around the wound (Matsubayashi et al., 2015). Finally, it is also plausible that AJ disassembly is an efficient mechanism to “break down” the preexisting actin cortex and generate short actin filaments and monomers for the assembly of wound-associated actin networks (Hunter et al., 2015). Experiments using photoconversion approaches and superresolution microscopy to track changes in the localization of AJ components and AJs in the wound edge contribute to the formation of a contractile cable in a zebrafish embryo expressing nonmuscle myosin light-chain 12 (the myosin II regulatory subunit) tagged with GFP. (C) Immunofluorescence staining in a Xenopus embryo showing microtubule (green) alignment perpendicular to the wound edge (arrowheads). β-Catenin (magenta), an AJ component, is present in all cell–cell boundaries with the exception of the wound edge (dotted line). (D) The septin subunit Sept7 accumulates both at the wound edge and along cell–cell boundaries perpendicular to the wound edge. (A–D) Scale bars, 20 µm. (C, D) Reprinted with permission (Shindo et al., 2018).
The supracellular cable originates from punctate AJs that form around the edge of the wound (Figure 2) and may serve as anchor points for the cable (Danjo and Gipson, 1998; Matsubayashi et al., 2015). The mechanisms of assembly of the discrete AJs at the wound edge, and whether they are the only sites where the actin cable is anchored, remain unclear. Furthermore, the role of other cell–cell adhesive structures during wound repair has not been established. Recent work demonstrates the importance of occluding junctions, which primarily control transport of molecules across the epithelium. Loss of occluding junctions results in altered tissue mechanical properties, abnormal cellular rearrangements around the wound, and defective actomyosin dynamics at the wound edge, thus delaying wound closure (Carvalho et al., 2018). These results highlight the importance of investigating the contributions of different adhesive structures to cytoskeletal organization during embryonic wound repair.

The actomyosin cable around wounds is a dynamic, contractile structure that coordinates cells through mechanical signals transmitted by AJs. The cable forms in a Rho-dependent manner (Brock et al., 1996; Wood et al., 2002; Abreu-Blanco et al., 2012) and encircles the wound. However, the cable is not homogeneous in composition, with segments of increased actin and myosin localization and others with reduced actomyosin levels (Figure 3, green and orange) (Bement et al., 1993; Zulueta-Coarasa and Fernandez-Gonzalez, 2018). Actomyosin heterogeneity drives efficient wound closure by allowing the staggered contraction of different segments of the wound edge, thus minimizing the resistance they encounter as they reduce their length. When a segment of the wound edge contracts, myosin is rapidly recruited to the neighboring, stretched segments in a process that involves mechanically gated ion channels (Zulueta-Coarasa and Fernandez-Gonzalez, 2018). In addition, tension stabilizes myosin in the segments of the wound edge that are actively contracting, thus providing a ratcheting mechanism indispensable for rapid wound closure (Kobb et al., 2017). The mechanisms by which tension stabilizes myosin at the wound edge, and the channel or channels that facilitate myosin recruitment to the wound margin, remain unknown.

Actin-based protrusions could also mediate proper cell matching after wound repair, as evidenced by the role of protrusions in other morphogenetic processes. Dorsal closure, for example, is the sealing of a discontinuity in the epidermis of the Drosophila embryo that employs several of the same mechanisms as wound repair. Dorsal closure is driven by the collective movements of the cells in the two epidermal sheets flanking the discontinuity. Protrusions originating from the cells at the leading edge of each epidermal sheet knit the epidermis together, maintaining the proper alignment of gene expression patterns across the seam (Jacinto et al., 2000). Additionally, in Drosophila heart development, two rows of cardiac progenitors meet to form the primitive heart tube, and Cdc42-dependent protrusions are required for the two rows of migrating progenitors to line up properly (Zhang et al., 2018). The mechanisms that facilitate recognition of matching cells through filopodial interactions are not completely understood, although patterned expression of adhesion molecules may facilitate cell pairing. It is therefore possible that during wound closure,
Disrupting microtubules (Tetley et al., 2018). While the actomyosin cable provides collective guidance cues that coordinate cell movements, protrusive activity may promote cell shape changes and the exchange of neighbors to locally fluidize the tissue, dissipating active stress and facilitating cell migration into the wound. The model suggests that cells rely on different modalities of migration to minimize the duration of wound closure depending on the mechanical properties of the tissue, and the area and shape of the wound (Staddon et al., 2018), consistent with experimental findings (Vedula et al., 2015; Begnaud et al., 2016). Recent work demonstrated that the ability of cells to exchange neighbors accelerates wound closure (Razzell et al., 2014; Tetley et al., 2018), although actin-based protrusions have not yet been implicated in the cellular rearrangements associated with wound repair. Thus, both the actomyosin cable and actin protrusions are important for wound healing, and their relative contributions may depend on physical tissue properties and wound morphology.

OTHER CYTOSKELETAL ELEMENTS CONTRIBUTE TO EPITHELIAL WOUND REPAIR

Microtubules, septins, and intermediate filaments have not been as intensely studied as the actin cytoskeleton during embryonic wound closure. In Drosophila embryos, microtubules are present within filopodial protrusions at wound edges (Abreu-Blanco et al., 2012), where they may provide pushing force and facilitate the transport of adhesion receptors or guidance cues along the protrusion (Figure 3, cyan). In addition, in Xenopus embryos, microtubules form bundles along cell cortices perpendicular to the wound edge (Figure 1C) (Shindo et al., 2018). Disrupting microtubules results in reduced elongation of the cells adjacent to the wound edge, suggesting that microtubule bundles may stabilize the lateral edges of cells as they elongate into the wounded region (Shindo et al., 2018).

Septins have been implicated in cell migration in a variety of cell types, and further investigation may reveal a conserved role in wound healing. Septins can interact with both actin filaments and microtubules, as well as the cell membrane. Notably, Sept7, which is a subunit of septin heterooligomers, localizes to the lateral cell edges and to the wound edge in Xenopus embryos (Figure 1D) (Shindo et al., 2018). Sept7 is required for the reorientation and organization of microtubules during wound closure, as well as for contraction of the actomyosin cable around the wound (Shindo et al., 2018), suggesting that septins may coordinate the rearrangements of different cytoskeletal networks during embryonic wound repair (Figure 3, magenta).

The role of intermediate filaments in wound closure is not well understood. In cell culture models of wound healing using human epithelial cells, vimentin is up-regulated in the cells at the leading edge (Figure 3, red), and reduction of vimentin expression reduces migration speed (Gilles et al., 1999). Indeed, both vimentin and keratins continue to be implicated in regulating cell motility. The interaction of cell–matrix adhesions with intermediate filaments in Caenorhabditis elegans provides mechanosensitive cues that promote epithelial morphogenesis (Zhang et al., 2011). Therefore, intermediate filaments and the hemidesmosomal adhesion structures that they interact with may prove necessary for cell migration during embryonic wound repair.

CONCLUSION

The cytoskeleton as a whole is indispensable for successful wound healing, providing the necessary structural support and contractile
forces to drive forward movement and coordinate cell behaviors. Although we currently have a general idea of how actomyosin structures contribute to wound closure, the specifics of the formation, regulation, and maintenance of these structures remain unclear. For example, the actomyosin cable may be anchored at AJs at cell–cell boundaries around the wound, but a single filament cannot bridge the gap between these punctate adhesion structures. Actin-binding proteins, such as filamin or actinin, could cross-link multiple shorter filaments together. Alternatively, proteins that bind both actin filaments and the cell membrane, such as ERM family proteins or septins, may provide anchor points between AJs. Additionally, the role of actin-based protrusions is not well characterized in vivo, with respect to whether they form cell–matrix or cell–cell adhesions that assist in driving forward migration, or if protrusions “zip” the gap between two cells. Finally, we have only begun to investigate the role of other cytoskeletal networks that may influence the structural stability of the tissue during wound closure. The cross-talk between different cytoskeletal components is likely important in bearing the mechanical loads that cells experience during wound healing. Uncovering the role of the cytoskeleton in coordinating cell movement for wound repair will contribute to the broader understanding of complex morphogenetic processes during embryonic development and how collective cell behaviors contribute to the spread of disease.

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