

Parallels between tissue repair and embryo morphogenesis

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Summary

Wound healing involves a coordinated series of tissue movements that bears a striking resemblance to various embryonic morphogenetic episodes. There are several ways in which repair recapitulates morphogenesis. We describe how almost identical cytoskeletal machinery is used to repair an embryonic epithelial wound as is involved during the morphogenetic episodes of dorsal closure in *Drosophila* and eyelid fusion in the mouse foetus. For both naturally occurring and wound-activated tissue

movements, JNK signalling appears to be crucial, as does the tight regulation of associated cell divisions and adhesions. In the embryo, both morphogenesis and repair are achieved with a perfect end result, whereas repair of adult tissues leads to scarring. We discuss whether this may be due to the adult inflammatory response, which is absent in the embryo.

Supplemental data available online

Introduction

Building tissues and organs during embryogenesis involves a series of exquisite morphogenetic episodes that are driven by the marriage of regulated proliferative events with a series of precisely orchestrated tissue contractions, foldings and migrations. Slowly, a miniature model of the adult form resolves and subsequent foetal development is largely a matter of growth and remodelling phases. Once established, adult tissues are homeostatically maintained by a balance of cell death and replacement, and most tissues remain in this dynamic but fairly dormant state for the entire life of the organism. But a dramatic reawakening of the tissue building machinery is required if the organism is wounded, in order to replace missing tissues and repair the wound. Recent studies have revealed significant parallels between how tissues are built during development and how they are rebuilt during tissue repair episodes. This review outlines these parallels, focusing in particular on shared signalling cassettes and cytoskeletal machineries that drive epithelial migrations. It also discusses how studies of morphogenesis have shed light on the ways that cell:cell adhesions and cell division might be regulated as tissues move and knit together in a wound situation. In the embryo, wound healing is not accompanied by an inflammatory response and the final repair is perfect without a scar, unlike in the adult. We discuss the link between inflammation and scarring, and how studies of embryo healing might guide us in designing new wound healing therapies.

The biological basis of wound healing

Whenever an organism sustains an injury, especially to its outer protective skin layer, it must act rapidly to repair the wound to prevent further blood and tissue loss and infection. Damage to adult mammalian skin and other tissues generally leads to the rapid plugging of the defect with a fibrin-rich clot.

Subsequently, after a delay period of several hours, the epidermal layer is repaired by the migration of keratinocytes from the cut edges and from the amputated remains of any cut appendages, including hairs or sweat glands (Fig. 1). From these free edges, a sheet of keratinocytes sweeps forward across a provisional matrix of fibronectin, vitronectin and other matrix molecules at the interface between the wound dermis and the fibrin clot. Cells within the front few rows extend lamellipodia and alter their integrin expression; specifically, they upregulate fibronectin/tenascin- and vitronectin-binding integrins, and relocalise their collagen/laminin-binding integrins so that the epidermal sheet can attach down and drag itself forwards over the wound substratum (reviewed by Grinnell, 1992; Martin, 1997; Werner and Grose, 2003). The deeper connective tissue is replaced by activated fibroblasts at the wound edge that proliferate and then migrate into the wound bed to form a granulation tissue (so named because of its granular appearance due to massive invasion by capillary networks), which contracts to aid in closing the wound margins.

This concerted effort by epidermal and connective tissue layers is accompanied by a robust inflammatory response, consisting largely of the production of neutrophils and then macrophages, which emigrate from the rich capillary network within the granulation tissue. These cells kill invading microbes, and mop up cell and matrix debris; they are also a rich source of growth factors and cytokines that possibly coordinate the various cell behaviours – cell migration, proliferation, matrix synthesis and so forth – that lead to tissue repair (Martin, 1997; Werner and Grose, 2003). An inevitable consequence of adult tissue repair is fibrosis and scarring, which leaves densely packed bundles of collagen within the healed connective tissue.

Tissue repair in the mouse embryo involves largely the same

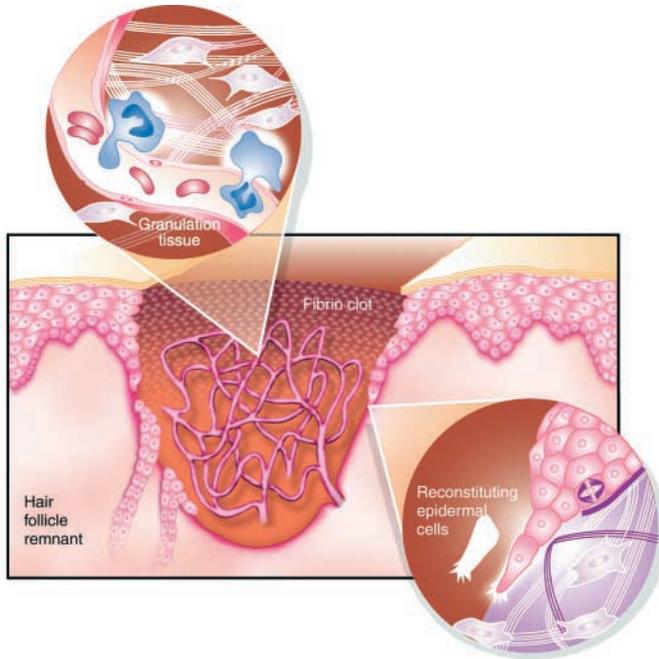


Fig. 1. The cellular players in the healing of a skin wound. The wound is first 'plugged' with a fibrin clot, which is infiltrated by inflammatory cells, fibroblasts and a dense plexus of capillary vessels. The epidermis migrates forwards from the edges of the wound and from the cut remnants of hair follicles. Neutrophils and macrophages (blue) emigrate from the wound capillaries into the wound granulation tissue where they kill microbes, engulf cell and matrix debris, and release signals that act on the host wound tissues. Image modified, with permission, from Martin (Martin, 1997).

tissue movements as in the adult, although on a much smaller scale, but only at late foetal stages is healing accompanied by an inflammatory response (Hopkinson-Woolley et al., 1994; Cowin et al., 1998). Prior to these stages, inflammation is absent and the embryo is capable of essentially perfect, near regenerative repair, with no resulting scar. Wound healing, even in the embryo (Fig. 2), is a complex process involving the coordination of several cell behaviours from several different cell types, and for each stage of wound repair there are

fundamental cell biology issues that still need resolving (see Box 1).

Developmental models of wound healing

A number of naturally occurring morphogenetic events involve tissue movements similar to those required for wound healing. Two of the clearest of these, both of which involve closure of epithelial holes, are dorsal closure in the *Drosophila* embryo and *C. elegans* ventral enclosure.

Dorsal closure in *Drosophila*

Near the end of the complex and intricately orchestrated cell and tissue movements of *Drosophila* gastrulation, including the extension of the germband over the dorsal surface and its subsequent retraction, a large hole is left behind on the dorsal surface of the embryo. An extra-embryonic membrane consisting of large flat cells – the amnioserosa – covers this dorsal hole (Fig. 3A-D). The process of bringing together the two epithelial edges over the amnioserosa to close the hole and form a seamless dorsal midline is known as dorsal closure. The dorsal hole is elliptical or eye shaped, and closure proceeds from the anterior and posterior ends (or canthi) of the opening towards the middle. The integrated efforts of three groups of cells are required for proper closure: the dorsalmost row of ectodermal cells defining the perimeter of the epithelial sheet, termed the leading edge (LE) cells; the more ventral epithelial (VE) cells; and the exposed amnioserosa (AS). Dorsal closure has been described as taking place in four phases (for detailed descriptions, see Harden, 2002; Jacinto et al., 2002b). The first phase, initiation (Fig. 3A), begins just prior to the completion of germband retraction, with the two opposing epithelial sheets moving slowly towards one another as a consequence of amnioserosal cell contraction. The trigger(s) required to start the dorsal closure process are not known, but probably include a combination of chemical and mechanical cues, including dorsoventral patterning information and mechanical stresses generated by germband retraction.

During the second phase, epithelial sweeping (Fig. 3B), leading edge cells accumulate actin and myosin just beneath the cell membrane at their dorsalmost (apical) edge. This F-actin accumulation forms a contractile cable, which pulls the leading edges of the epithelial sheets taut (Jacinto et al., 2002a)

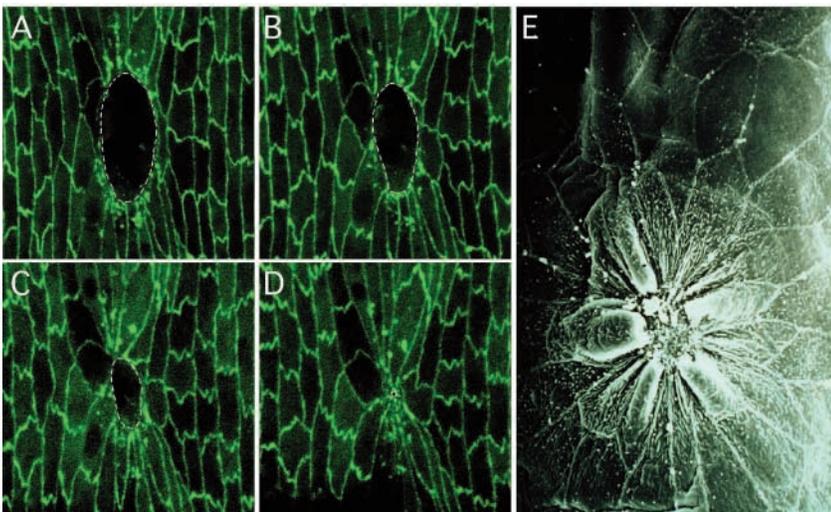


Fig. 2. Imaging wound re-epithelialisation in *Drosophila* embryos and zebrafish larvae.

(A-D) Images taken at ~30-minute intervals from a movie of a laser wound (broken lines) made to the ventral epithelial surface of a *Drosophila* embryo expressing α -catenin-GFP. Cell shape changes and rearrangements of neighbour:neighbour relationships are apparent, but no cell division occurs during the brief repair period. (E) A scanning electron micrograph view of a similar wound in a zebrafish larva, showing how contraction of the leading edge cells causes the wound margin to 'scrunch up' as it is drawn forwards by the action of the purse-string. (A-D) Courtesy of Will Wood; (E) courtesy of Katie Woolley.

Box 1. Some of the key unanswered questions of wound repair

- What are the precise cues that regulate activation of the migratory and proliferative machinery of re-epithelialisation?
- How are adhesions between neighbouring cells modified to allow fluid movement within the epithelium?
- What are the ‘contact-inhibition’ cues that shut all of this machinery down when the wound is closed?
- How are the epidermal fronts bonded together to form a strong seam where wound edges meet one another at the endpoint?
- What are the ‘start’ and ‘stop’ cues that govern fibroblast proliferation, migration into and then subsequent contraction of the wound bed?
- Why does adult wound repair always leads to fibrosis of the healed connective tissue?
- How is the inflammation response – the signals that draw leukocytes to wounds and others that may repel them – activated and resolved?
- How best can the inflammatory response be modulated therapeutically in order to modify the quality of repair?

and drives LE cell apical constriction (i.e. a ‘purse-string’ mechanism, Fig. 4A). The LE cells also begin to elongate along the dorsoventral axis. The continuous sheet of cells located ventral to (back from) the leading edge cells also begin to elongate along the dorsoventral axis. The combined actions of these cell shape changes draw the opposing epithelial sheets dorsally, towards one another.

As the epithelial sheets come into close proximity at the anterior and posterior ends of the opening, the third phase, zippering (Fig. 3C), begins. Filopodia from cells on the opposing epithelia meet and begin to interdigitate. Along with continued contraction of the LE cell actin cable and of the amnioserosal cells, interactions between opposing filopodial and lamellipodial protrusions appear to aid in drawing the two epithelial sheets towards one another and zipping them together.

The final phase, termination (Fig. 3D), produces the seamless midline. During this phase, filopodia regress and their transient adhesions are converted into permanent adhesions with the formation of adherens junctions (see Box S1 at <http://dev.biologists.org/supplemental>). As with the signal(s) required to start the dorsal closure process, those signal(s) necessary to stop the forward movement of the epithelial sheets and prevent overgrowth are currently unknown.

Ventral enclosure in *C. elegans*

Gastrulation in *C. elegans* involves a complex interplay of cell shape changes and cell migrations within the 60 cells comprising the dorsoposteriorly located hypodermis (the epidermis). At the start of gastrulation, the hypodermis is arranged in three rows of 10 hypodermal cells towards the left of the dorsal midline, and a mirror image three rows on the right. Radiating from the dorsal midline, these three cell rows are referred to, respectively, as dorsal hypodermis, lateral seam hypodermis (LSH) and ventral hypodermis (VH). In a process similar to *Drosophila* germband extension, the two dorsalmost rows of cells, the dorsal hypodermis, intercalate to form a single row of cells (‘dorsal intercalation’; Fig. 3E). The stretching of the hypodermis over the ventral surface of the

embryo to form a seamless ventral midline is known as ventral enclosure, and closely resembles *Drosophila* dorsal closure. The integrated efforts of three morphologically distinct cell types are required for proper ventral enclosure: the VH, the LSH and the neuronal cells that form the ventral pocket (VP) over which the ventral hypodermal cells migrate. Ventral enclosure has been described as taking place in three steps (Fig. 3F-H) (Williams-Mason et al., 1997; Chin-Sang and Chisholm, 2000; Simske and Hardin, 2001). The first step, leading cell migration (Fig. 3F), begins just prior to the completion of dorsal intercalation with the two anteriormost ventral hypodermal cells (‘leading cells’) elongating along the dorsoventral axis. These cells produce filopodial extensions at their medial tips that help to draw the hypodermis circumferentially, extending down past the equator of the embryo.

During the second step, leading cell junction formation and fusion (Fig. 3G), the anterior pair of leading cells meet at the ventral midline followed by the rapid formation of adherens junctions for the anteriormost pair and cell fusion for the posterior pair. With the fusion of these leading cells, the remaining posterior ventral hypodermal cells become wedge shaped and elongate along the dorsoventral axis, closing a ventral gap that is called the ventral pocket. F-actin becomes concentrated in the leading edges of these migrating cells, forming an actin cable.

In the third step, ventral pocket enclosure (Fig. 3H), the ventral hypodermal cells lining the ventral pocket contract and migrate over the underlying neuronal cells to close the ventral hole. This contraction is believed to result from actin cable contraction at the leading edges of these ventral hypodermal cells using a purse-string mechanism. As the opposing ventral hypodermal cells meet, adherens junctions assemble and a seamless ventral midline forms.

Model organism paradigms

Genetic screening, genetic epistasis, cell biology, live imaging, molecular and biochemical approaches in these two model organisms have together revealed several of the structural and signalling molecules involved in these morphogenetic episodes. The genetic tractability of both flies and worms has allowed genetic screens to identify mutants that fail to undergo proper dorsal closure or ventral enclosure (Table 1).

The characterization of both fly and worm morphogenetic events and mutants has been greatly aided by recent advances in live imaging. The use of GFP fusion constructs, in particular actin-GFP, has yielded time lapse imaging of the normal processes, allowing the exact sequence of cell shape changes and tissue movements to be determined (Jacinto et al., 2000; Kiehart et al., 2000; Simske and Hardin, 2001; Dutta et al., 2002). These studies have highlighted the role of actin-based structures, such as filopodia and lamellipodia (the morphological features of which are not always fully preserved during fixation protocols), in cell contact and adhesion. Live imaging in combination with laser ablation is also providing a way of systematically addressing questions concerning the contribution of different cells and tissues, and the forces that are required to drive dorsal closure and ventral enclosure (Kiehart et al., 2000; Hutson et al., 2003; Williams-Masson et al., 1997).

Although dorsal closure and ventral enclosure do both

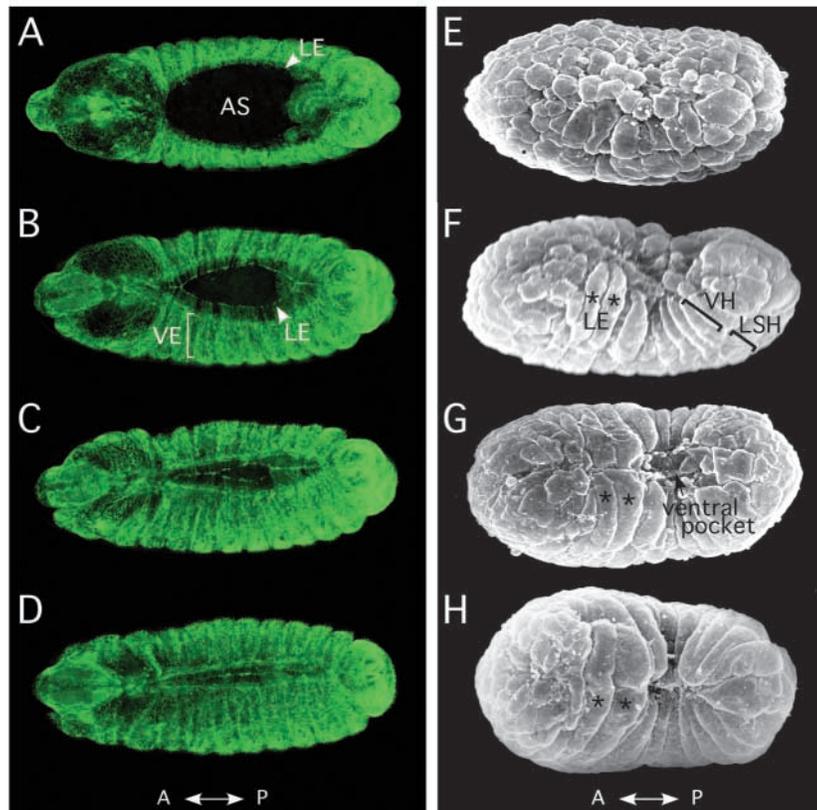


Fig. 3. Epidermal hole closure as part of natural morphogenetic episodes. *Drosophila* dorsal closure and *C. elegans* ventral enclosure. (A-D) Confocal micrographs of the dorsal surface of successively older *Drosophila* embryos expressing α -catenin-GFP that depict the four phases of dorsal closure: (A) initiation; (B) epithelial sweeping; (C) zippering; and (D) termination. LE, leading edge epidermis; AS, amnioserosa; VE, ventral ectoderm. (E-H) Scanning electron micrographs of the ventral surface of successively older *C. elegans* embryos similarly depicting dorsal intercalation and the three phases of ventral enclosure: (E) dorsal intercalation; (F) leading cell migration; (G) leading cell junction formation and fusion; and (H) ventral pocket enclosure. Leading edge cells (LE) are marked with an asterisk. LSH, lateral seam hypodermis; VH, ventral hypodermis. Anterior is towards the left in all images. (A-D) Courtesy of Sarah Woolner; (E-H) courtesy of Jim Priess.

superficially resemble re-epithelialisation of a wound hole, there are several key differences, including the fact that wound repair is initiated by tissue damage, whereas morphogenetic episodes are not. Clearly, these epithelial movements do not model all aspects of the repair process, such as inflammation or connective-tissue contraction and fibrosis, although the epithelial amnioserosa does contract during dorsal closure and so may mirror wound contraction to a certain extent.

The wound repair tool kit in embryos

Wounding skin triggers a cascade of events that leads to re-epithelialisation of the defect and contraction of underlying wound connective tissues. Early studies in the chick embryo showed that re-epithelialisation occurs not by lamellipodial crawling of cells as in adult skin healing; rather, migrating epithelial fronts sweep forward over a mesenchymal substrata in a purse-string-like manner (Fig. 2, Fig. 4A) (Martin and Lewis, 1992), just as discussed above for dorsal closure in flies and ventral enclosure in worms. Transmission electron microscopy indicates that leading edge cells remain adherent to the underlying basal lamina, which is drawn along with the epithelial sheet as it moves forward, in contrast to the adult situation where leading edge cells leave the old basal lamina behind and deposit new matrix after they migrate forwards (McCluskey et al., 1993). A thick cable of actin is apparent in the leading edge of basal marginal cells encompassing the wound, and contraction of this cable almost certainly provides the force that draws the epidermal wound edges together (Martin and Lewis, 1992). Indeed, when new assembly of filamentous actin is blocked by cytochalasin D or by loading cells with the Rho GTPase blocker, C3 transferase, wounds fail

to re-epithelialise (McCluskey and Martin, 1995; Brock et al., 1996). As well as a filamentous actin cable, other components of the contractile machinery, including myosin II, are also assembled. These include proteins – for example E-cadherin – that enable the intracellular cable to link to neighbouring cells via adherens junctions (Brock et al., 1996).

In chick and mouse embryos, assembly of the actin cable is so rapid (visible in leading edge cells within just two minutes of wounding) (Martin and Lewis, 1992; McCluskey and Martin, 1995) that it would seem that at least the early stages of cable formation must be due to re-deployment of existing actin, myosin and junctional proteins. RHO activity is essential for assembly of the wound-induced actin purse-string, whereas analogous RAC-blocking experiments fail to interfere with the wound response. Together, these results indicate that RHO is indeed the master switch that mediates purse-string assembly at the embryonic wound margin (Brock et al., 1996).

Actin networks are used repeatedly to mould embryonic tissues during organogenesis (see Box 2), and wound purse-strings are not simply restricted to embryonic epithelia. Studies in the adult rabbit eye suggest that small corneal lesions are drawn closed by an analogous actin purse-string, but when this is disrupted by α -catenin blocking antibodies, epithelial migration defaults to a more ‘adult-like’ lamellipodial crawling mode (Danjo and Gipson, 1998). Similarly, in vitro studies in the gut epithelial cell line Caco2^{BBE} show that wounds can be closed by purse-string motility (Fig. 4B) (Bement et al., 1993). In all likelihood, most adult simple epithelia use the purse-string mechanism for closing small wounds, and size probably does really matter here, because in the Caco2^{BBE} studies, smaller wounds close by purse-string contraction, larger

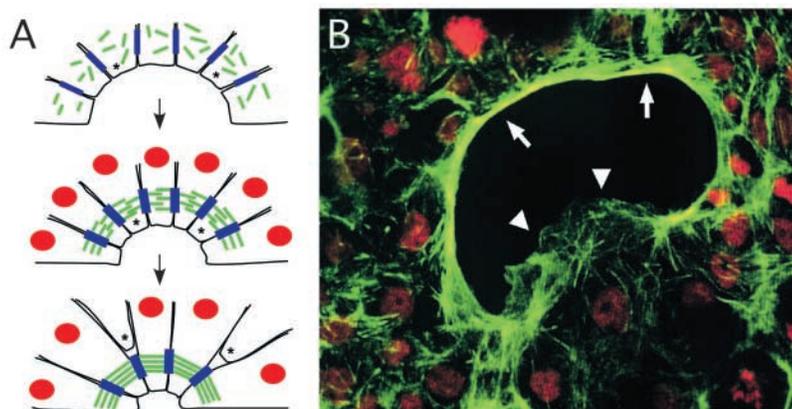


Fig. 4. Lamellipodial crawling versus purse-string closure of an in vitro epithelial wound. (A) A temporal series that illustrates how the contractile actin purse-string acts to draw a wound epidermis closed. The individual actin filaments (green bars) anchor to adherens junctions (blue rectangles) formed between adjacent cells. Contraction of the actin cable in each cell leads to apical cell constriction and reduced wound circumference. As wound closure proceeds, some cells are squeezed out of the front row such that fewer epithelial cells remain in the front row. The remaining cells form new adherens junctions and apical actin cable contraction continues until the contralateral cells meet and fuse. Asterisks indicate cells that will be lost from the leading edge; nuclei are red. (B) Repair of wounds made in monolayers of the gut epithelial cell line Caco2^{BBE} is achieved by lamellipodial crawling or actin purse-string contraction, or a combination of both. In this wound, one group of leading-edge cells is being drawn forwards by contraction of an actin cable (arrows), as occurs during embryonic repair; while other cells are clearly extending lamellae (arrowheads) and crawling forwards, as occurs during repair of an adult skin wound [image courtesy of Jane Brock; reproduced, with permission, from Jacinto et al. (Jacinto et al., 2000)]. Green staining is fluorescein isothiocyanate/phalloidin-tagged filamentous actin; red staining is the nuclear dye 7AAD.

wounds close by crawling and some middle size wounds use a combination of both these strategies (Fig. 4B) (Bement et al., 1993). How cells ‘read’ the mechanical cues that direct which of these modes of motility to adopt is still unclear. Nevertheless, they are probably able to detect the different forces exerted upon them at the various angles of curvature around a wound, through differential tensions on cell adhesions and on the actin stress fibres within them.

More recent studies using transgenic ActinGFP-expressing *Drosophila* embryos, wounded by a laser beam, have allowed live confocal imaging of the actin machinery as it assembles and draws the wound epithelium closed (Wood et al., 2002). In one regard at least, this wound hole closure process differs from dorsal closure because cells can be observed withdrawing from the epithelial margin and shuffling back into submarginal rows (Fig. 2; Fig. 5A); this loss of leading edge cells appears not to occur during dorsal closure except at the zipper fronts. Concomitant with the assembly of an actin cable at the wound margin, dynamic filopodial protrusions are also seen extending from leading edge epithelial cells, just as during *Drosophila* dorsal closure (Fig. 5A-D). These protrusions occasionally make transient contacts with the substratum ahead of them, but show no sign of actively adhering and tugging the epithelium forwards. Genetic approaches using either small GTPase loss-of-function mutants or dominant-negative transgenes for *Rho1* and *Cdc42* have provided a means to analyse in real time the

functions of actin cable and filopodia respectively. In *Rho1* mutants, a cable fails to assemble but, after a lag phase of several hours, cells compensate for the absence of a wound purse-string by tugging on their immediate neighbours using the exuberant filopodia and lamellae that they assemble in place of the cable (Wood et al., 2002). These actin-rich protrusions enable a wound to close even in the absence of a cable by means of numerous foci where cells zipper together, but when a cable is present these filopodia are not necessary, at least during the early phase of healing. Rather, the key role of filopodia in this context appears to be for epithelial fusion (Fig. 5B,D,E). Blocking the activity of CDC42, and consequently the assembly of filopodia, using a dominant-negative transgene, does not hinder the rate of epithelial wound closure, but it does dramatically block the final knitting together of the wound edges as they meet one another, so that these wounds never completely close (Wood et al., 2002).

JNK signalling drives epithelial sheet movements

One signalling pathway that appears pivotal in *Drosophila* dorsal closure is the JUN kinase (JNK) cascade, which leads to AP1 activation in leading edge epithelial cells; the same pathway is concomitantly downregulated in the amnioserosal substratum that covers the dorsal hole (Reed et al., 2001). AP1 activity in leading edge cells leads to induction of at least two downstream genes, *decapentaplegic* (*dpp* – a TGF β family member) and the dual specificity phosphatase *puckered* (*pu*), which negatively feeds back on JNK and thus operates as a ‘brake’ on this signal (Martin-Blanco et al., 1998). Mutants in each component of the JNK signalling hierarchy all fail to close the hole during dorsal closure, as do *kayak* (previously known as *dFos*) mutants and mutants in *thick veins*, one of the DPP receptors (reviewed by Harden, 2002; Jacinto et al., 2002b), but it is still not apparent precisely what the key cell targets of this signal are and the resulting important cell behaviours.

Wounding of adult flies also triggers AP1 activation, as revealed by expression of PUC and flies mutant in *kayak* show retarded healing (Ramet et al., 2002). Rather intriguingly, eyelid closure, which occurs late in mammalian embryogenesis and looks, at least superficially, remarkably similar to *Drosophila* dorsal closure, is also absolutely dependent on JNK signalling. Two recent studies show that tissue specific knockout of JUN in the epithelium of foetal mice leads to a failure of eyelid closure, and these mice are born with open eyelids, whereas their siblings have closed eyes until 10 days after birth (Zenz et al., 2003; Li et al., 2003). One of these lines of mice also exhibits subtle defects in wound healing (Li et al., 2003).

Although AP1 activation during morphogenetic episodes appears to be entirely JNK dependent, tissue repair is a response to a traumatic intervention and so it would not be strange if the primary signals for AP1 activation were

Table 1. Examples of mutations associated with *Drosophila* dorsal closure or *C. elegans* ventral enclosure*

	Dorsal closure	Ventral enclosure	References
Architectural components			
Cytoskeletal proteins	<i>zipper</i> (non-muscle myosin)	<i>nmy-2</i> (non-muscle myosin) Arp2/3 complex WASP	Young et al., 1993; Shelton et al., 1999 Sawa et al., 2003 Sawa et al., 2003
Membrane proteins	<i>coracle</i> (protein 4.1 homologue) <i>Yurt</i> (protein 4.1 homologue)		Fehon et al., 1994 Hoover and Bryant, 2002
Cell junction proteins	<i>shotgun</i> (E-cadherin) <i>armadillo</i> (β-catenin) <i>fasciclinIII</i>	<i>hmr-1</i> (E-cadherin) <i>hmp-2</i> (β-catenin) <i>hmp-1</i> (α-catenin) <i>jac-1</i> (p120 catenin)	Tepass et al., 2001; Costa et al., 1998 Grevengoed et al., 2001; Costa et al., 1998 Woods et al., 1997; Costa et al., 1998 Pettitt et al., 2003
Integrins	<i>myspheroid</i> (β subunit) <i>scab</i> (α subunit)		Brown, 1994 Stark et al., 1997
Motors		<i>zen-4</i> (kinesin-like)	Powers et al., 1998; Raich et al., 1998
Extracellular matrix	Type IV collagen <i>pericardin</i> (type IV collagen)		Borchiellini et al., 1996 Chartier et al., 2002
Signalling pathways			
DPP (TGFβ)	<i>decapentaplegic</i> (TGFβ) <i>thick veins</i> (TGFβ type I receptor) <i>punt</i> (TGFβ type II receptor) <i>mothers against dpp</i> (R-smad) <i>medea</i> (co-smad)		Padgett et al., 1987 Affolter et al., 1994 McEwen et al., 2000 Hudson et al., 1998 Wisotzkey et al., 1998
Wingless	<i>armadillo</i> (β-catenin)	<i>apr-1</i> (APC homologue)	McEwen et al., 2000; Hoier et al., 2000
JNK	<i>hemipterous</i> (Jun kinase kinase) <i>basket</i> (Jun kinase) <i>Jun</i> (JUN) <i>kayak</i> (FOS) <i>misshapen</i> (Ste20 kinase) <i>puckered</i> (phosphatase) <i>canoe</i> (PDZ protein) <i>Sac1</i> (lipid phosphatase)		Glise et al., 1995 Riesgo-Escovar et al., 1996 Glise and Noselli, 1997; Hou et al., 1997 Zeitlinger et al., 1997 Su et al., 1998 Martin-Blanco et al., 1998 Takahashi et al., 1998 Wei et al., 2003
RAS	<i>anterior open/yan</i> (ETS domain)		Riesgo-Escovar and Hafen, 1997
Notch	Notch (transmembrane receptor)		Zecchini et al., 1999
Ephrin/Eph		<i>vab-1</i> (Eph receptor) <i>vab-2</i> (ephrin)	George et al., 1998 Chin-Sang et al., 1999
Other	<i>ZO-1</i> (PDZ/guanylate kinase) <i>ribbon</i> (BTB/POZ) <i>discs large</i> (PDZ/guanylate kinase)	<i>mab-20</i> (semaphorin) <i>xrn-1</i> (5'-3' exoribonuclease)	Takahashi et al., 1998; Roy et al., 2000 Blake et al., 1998; Newbury and Woollard, 2004 Perrimon, 1988
Rho GTPases/effectors			
GTPases	<i>Rho1</i> , <i>Cdc42</i> , <i>Rac1/Rac2/mtl</i>		Harden et al., 1999; Magie et al., 1999; Magie et al., 2002 Genova et al., 2000; Hakeda-Suzuki et al., 2002
Effectors	<i>myoblast city</i> (DOCK180) PKN DPAK		Erickson et al., 1997; Nolan et al., 1998 Lu and Settleman, 1999 Harden et al., 1996
Other	<i>disembodied</i> (ecdysteroid biosynthesis) <i>Nmt</i> (N-myristoyltransferase)		Chavez et al., 2000 Ntwasa et al., 2001

*For additional examples and recent reviews see: Glise and Noselli, 1997; Stronach and Perrimon, 1999; Noselli and Agnes, 1999; Harden, 2002; Simske and Hardin, 2001; Chin-Sang and Chisholm, 2000.

somewhat different here. Indeed, studies of in vitro scrape wounds indicate that AP1 activation after wounding is triggered, at least partially, not by JNK signalling, but by sublethal mechanical damage to the very front row cells. This mechanical damage leads to a Ca²⁺ influx, and a subsequent wave of purine signalling that, in turn, leads to further AP1 activity in undamaged neighbouring cells several rows back from the leading edge (Klepeis et al., 2001). Clearly, this AP1 activity has the potential to coordinate and prime the leading rows of cells for some activity necessary for immediate migration or later filling in of the gap and, if the signal is blocked, then there is a clear slowing down of the in vitro migration/repair process. Recent studies in fish keratocytes and rat epithelial cells suggest that the role of JNK during migration may be at least partially mediated via

phosphorylation of the focal adhesion adaptor, paxillin (Huang et al., 2003), but in vivo the signals may operate in a paracrine way also on adjacent tissues. Indeed, wounds in mouse and rat embryos show a similarly rapid, but transient, activation of AP1 in the front few rows of epithelial cells (Martin and Nobes, 1992). These immediate-early signals may operate as kick-start activators by triggering TGFβ1 expression in wound epithelial cells, which subsequently release this growth factor into the adjacent wound mesenchyme, directing this tissue to contract (Martin et al., 1993), just as TGFβ directs fibroblast contraction of collagen gels in vitro (Montesano and Orci, 1988). Although the precise role of the JNK signal in these various complex processes remains unclear, many epithelial migrations, be they naturally occurring morphogenetic episodes or artificially activated events, seem to be influenced by JNK signalling.

Box 2. Shaping organs with actin networks

Several complex morphogenetic episodes in vertebrates are dependent on contraction and constraining forces exerted by actin networks. Classic studies in the chick heart show an asymmetric arrangement of actin bundles preceding the right handed bending and rotation of the heart tube, indicating that an actin driven contraction is at least partially responsible for heart tube folding (Itasaki et al., 1989; Itasaki et al., 1991). The developing pharyngeal pouches have a similar network of actin filaments in those groups of endodermal cells that form the cornerstones of the pharyngeal invaginations, but here it appears that constraint rather than contraction is crucial (Quinlan et al., 2004). Neural tube closure also is at least partially dependent on an actin network that resides just beneath the apical plasma membrane of neural plate cells as the tube is folding. Cytochalasin blocking experiments have shown that, at least for the cranial region of the neural tube, active actin polymerisation/contraction is essential for folding (Morris-Kay and Tocket, 1985; Ybot-Gonzalez and Copp, 1999). Now, the first regulators of these morphogenetic actin networks are being uncovered. For example, a novel actin-binding protein, *shroom* (*Shrm*), is localized to the apical margin of those neural plate cells that will constrict to form the hinge cells during neural tube closure, and *Shrm* mutants have anencephaly (Hildebrand and Soriano, 1999). Moreover, ectopically expressing *shroom* in epithelial sheets causes these cells to undergo concerted contraction and for the sheet to fold. It appears that *shroom* regulates these actin constrictions via the small GTPases, RAP and RAS, although it is still not clear what governs precisely which cells will express *Shrm* and how *Shrm* protein is directed to its apical location in neural plate cells (Haigo et al., 2003). Soon, we will know more about the signalling machinery controlling this and other complex morphogenetic processes, and some of what we learn may also apply to wound-mediated actin assemblies.

Cell movements and adhesions in wound repair

Currently, it is still not possible to observe directly the migration of epithelial cells as they heal an adult skin wound. Nevertheless, tracking the movements of virally tagged cells in a skin culture model that has been wounded to simulate *in vivo* repair indicates that the simplest model, whereby a coherent sheet of cells is dragged forward by its motile front row, is probably far from true. Rather, there seems to be much shuffling of cell positions, and a general free-for-all in the leading edge (Garlick and Taichman, 1994; Danjo and Gipson, 2002). Indeed, the expression of integrins that enable traction for crawling is upregulated in at least the front ~10 rows of cells, as well as upwards into cell layers above the basal layer (Hertle et al., 1992). During this period, there is tight regulation of cell division in the front rows of the leading epidermis (see Box 3). *In vitro* studies of epithelial monolayers, in which lamellipodial crawling is the primary mode of motility, show that closure is not only achieved by activities restricted to front row cells, as the blocking of RAC signalling, and thus crawling, in only these cells does not prevent repair. To halt closure, the front four or five rows of cells must all be prevented from crawling by disabling their RAC activity (Fenteany et al., 2000). Time-lapse studies of wounds repairing *in vitro* and *in vivo* in the *Drosophila* embryo have revealed how cells move relative to one another, changing their neighbour:neighbour

relationships (Bement et al., 1993; Wood et al., 2002) (see http://www.nature.com/ncb/journal/v4/n11/supinfo/ncb875_S1.html).

Similar cell shufflings occur during vertebrate gastrulation, and signals that regulate and enable this fluidity in the gastrulating epithelium have now been identified. It seems that a key role for actinin during *Xenopus* gastrulation may be to soften cadherin-based adhesions between epithelial cells (Brieher and Gumbiner, 1994). If these signals are countered by exposure to cadherin-activating antibodies, then high adhesivity is restored, the cell rearrangements of convergent extension fail and consequently gastrulation is blocked (Zhong et al., 1999; Gumbiner, 2000). Cell matrix interactions may also feed into this regulation of cell:cell adhesion. Blocking integrin:fibronectin signalling appears to block the rearrangement of cells during convergent extension, and this may define zones, perhaps domains rich in fibronectin, in which cell:cell shuffling within an epithelium is permitted (Marsden and DeSimone, 2003).

Several further ways in which adhesion competence may be modulated within an epithelial sheet are now becoming clear from cell biology studies. For example, one consequence of scatter-factor/MET signalling, which directs cells to break free from an epithelial sheet, is activation of a novel E-cadherin-binding protein, Hakai, which ubiquitinates the E-cadherin complex, leading to its endocytosis (Fujita et al., 2002). Phosphorylation of adherens junction components is clearly key to their function and it seems that some of this regulation will turn out to be via the Pez phosphatase, which is localised to adherens junctions in sheets of coherent epithelial cells. Cells transfected with dominant-negative Pez constructs show increased β -catenin phosphorylation and loosening of junctions leading to enhanced epithelial sheet spreading *in vitro* (Wadham et al., 2003). Adherens junctions may not be the only junctions that are labile in migrating epithelia; studies of desmosomes, which are anchor points for intermediate filaments, rather than for actin, show that their adhesivity rapidly flips from being Ca^{2+} independent to being Ca^{2+} dependent in a wounded epithelial monolayer. This change in desmosome state spreads in a wave mediated by protein kinase C α from wound margin cells to cells many rows back from the wound edge (Wallis et al., 2000).

The biomechanics of wound repair

There is no doubt that many of the cues directing the various cell behaviours that contribute to wound closure, or to any morphogenetic episode, will be chemical signals, released by one cell population and operating on another. For example, some of the growth factors released by degranulating platelets at the wound site are known to have potent effects on the native epidermal and fibroblast wound tissue cell lineages, and to assist in directing cell migrations, contractions and so forth. However, it is now becoming clear that mechanical signals are also likely to provide crucial cues. Simply disrupting the natural tissue tensions by wounding might provide an activating trigger. Cells stretched along the free epithelial edge as a wound initially gapes may be mechanically stimulated to organise their actin in alignment with the force of stress, thus setting up the purse-string that subsequently drives epithelial closure. *In vitro* studies in fish keratocytes have shown that physical tugging on cells can result in the rapid reorganisation

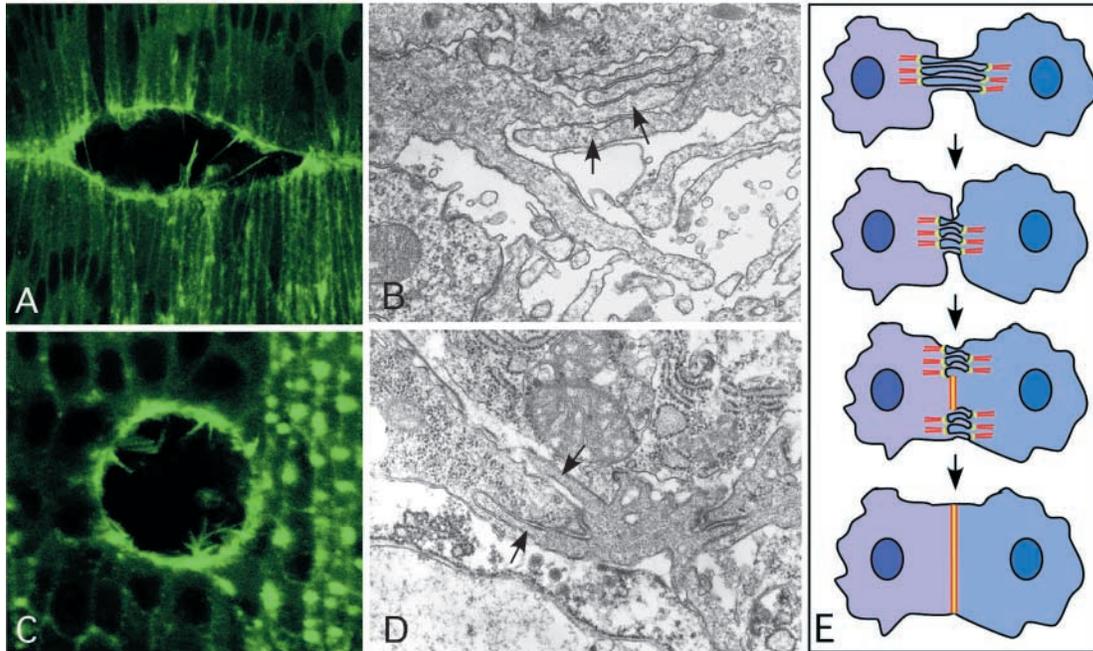


Fig. 5. Parallels between *Drosophila* dorsal closure and wound healing. (A) Confocal micrograph of a dorsal closure stage *Drosophila* embryo expressing GFPactin to reveal the actin cable and filopodial protrusions that drive dorsal closure. (B) A transmission electron micrograph section cut through the zippering zone shows how the filopodia of opposing epithelial cells (arrows) interdigitate and prime the formation of adhesions between the two epithelial fronts. (C,D) Equivalent images from laser wounds in similarly staged embryos that show how opposing epithelial fronts (arrows in D) are knitted together using the same actin-based machineries as for dorsal closure. (E) A temporal series that illustrates how filopodial interdigitation is believed to prime the assembly of mature adherens junctions. Adjacent cells extend filopodia towards each other, which interdigitate, with actin (red), catenins and cadherins (yellow) localizing to the filopodial tips and points of contact. The filopodia then shorten, drawing the cells together. This filopodial zippering is propagated to the edge of the cell resolving into mature junctions. (A-D) Courtesy of Will Wood; (E) courtesy of Craig Magie.

of actin filaments along the direction of force (Kolega, 1986). Presumably, a small GTPase switch transduces this mechanical signal, and, indeed, RHO can be activated mechanically in endothelial cells in the absence of the standard growth factor signals (Li et al., 1999).

In adult tissue repair, there is some evidence to indicate that mechanical forces are, in part, responsible for the conversion of normal wound fibroblasts into myofibroblasts at a wound site. These myofibroblasts closely resemble smooth muscle cells with their expression of α -smooth muscle actin and the capacity for generating strong contractile forces. The signals triggering this transformation from fibroblast to myofibroblast are believed to be a combination of growth factors, including TGF β 1, as well as mechanical cues that are related to the forces resisting contraction (reviewed by Grinnell, 1994).

There is now good evidence to indicate that during morphogenesis of the fly embryo, mechanical, stretching and pushing cues can direct transcriptional events in cells. If gastrula-stage fly embryos are squashed in their DV axis, within minutes they upregulate Twist throughout their epithelium, rather than, as normal, in only a thin ventral strip (Farge, 2003). Moreover, if the tissues linking the posterior mesoderm to those cells destined to invaginate and form the stomodeum are cut (thus denying them the compression forces they would normally experience), these cells now fail to switch on Twist and no longer invaginate.

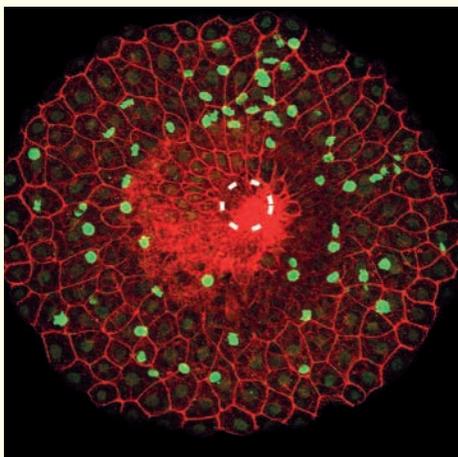
Although it is becoming clearer that mechanical forces may

be key players during both repair and morphogenesis, we know very little about the various tensions and forces operating in each of these scenarios. It is possible to directly measure forces exerted by individual cells in vitro (Wang et al., 2002), but it is clearly much more technically challenging to do likewise with tissues in vivo. One way of visualising the play of tensions within interacting tissues is to release the tension in one location by cutting, and to measure the consequential gape and movement of nearby tissues. This approach has been undertaken for *Drosophila* dorsal closure – using a laser beam to make fine cuts within the amnioserosa and along the leading edge epithelium. These studies show that the contractile amnioserosa and the force-generating mechanisms in the adjacent epithelium make comparable contributions to the advancement of the epithelial leading edge (Kiehart et al., 2000; Hutson et al., 2003). Similar ‘tissue tension geography’ data needs to be gathered for other more complex vertebrate morphogenetic episodes and also for wound healing.

In this regard, labelling small groups of exposed mesenchymal cells at the margin of an embryonic wound allows one to trace mesenchymal movements during the wound closure process. This shows that this tissue contracts to about half its original area by the time the wound has closed, indicating that re-epithelialisation and connective-tissue contraction contribute equally to the wound closure ‘effort’ (McCluskey and Martin, 1995). A similar ratio of tissue

contributions was previously described for repair of adult skin wounds (Abercrombie et al., 1954). In a rather crude mirror of the elegant *Drosophila* tension-cutting experiments already described, it was shown that almost all of the contractile force

Box 3. Cell proliferation in migrating tissues



The requirements for cell proliferation and cell shape changes and migrations that occur during normal development or wound repair place incompatible demands on the cytoskeletal machineries of the cell: cells rounding up to undergo cell division cannot simultaneously undergo the cytoskeletal rearrangements needed to execute the intricate cell shape changes and migrations of gastrulation or wound closure, and yet clearly cell division is needed for growth and replacement of lost tissues. During *Drosophila* gastrulation, this incompatibility is resolved by expression of at least two proteins, Tribbles and Frühstart, which block cells in mitotic domain 10 – the ventral aspect of the embryo where gastrulation occurs – from responding to String (CDC25 homologue), signals that would otherwise direct them to divide (Grosshans and Wieschaus, 2000; Mata et al., 2000; Seher and Leptin, 2000; Grosshans et al., 2003). It is not clear precisely how Frühstart operates, but Tribbles encodes a serine/threonine kinase-related protein that induces degradation of String/CDC25 via the ubiquitin-proteasome pathway (Mata et al., 2000). Neither of these proteins has obvious vertebrate homologues but there are likely to be similar mechanisms in operation during vertebrate morphogenesis and repair. Indeed, during *Xenopus* gastrulation, the dorsal mesodermal cells apparently halt their divisions (Saka and Smith, 2001); and experimental depletion of WEE1, which promotes M-phase entry and cell cycle progression in this tissue, severely disrupts gastrulation (Murakami et al., 2004). A similar block on background levels of cell division is seen in the front few rows of epithelial cells as wounds in zebrafish embryos repair, as shown in the figure (courtesy of Katie Woolley) where phospho histone 3 staining (green nuclei) is apparent only several rows back from the leading edge (broken line).

For small wounds in the embryo and wounds in tissue culture monolayers, repair is so rapid – within a few hours – that there is no time for new cell divisions to ‘kick-in’. However, for large wounds in adult skin where a significant number of cells have been lost, cell replacement is clearly required at some stage. There is a synchronised upsurge in the rate of proliferation at the epidermal wound margin by 12–24 hours (Werner et al., 1994), and this can spread from the wound edges centripetally outwards in a wave-like fashion (Harding et al., 1971).

generated by adult wound connective tissues is delivered by a band of fibroblasts lying within 1–2 mm of the epidermal wound margin, as cutting and removal of the central wound granulation tissue did not alter the rate of wound healing (Gross et al., 1995).

Knitting epithelial edges together

Epithelial fusion is the climax of many morphogenetic episodes and of wound healing. During dorsal closure in flies, the leading edge epithelial cells extend filopodial protrusions that appear to play a key role in bonding the two epithelial sheets together (Fig. 5A,B,E). Filopodia from confronting epithelial cells interdigitate at the zipper front and in the fusion seam, several cell diameters back from the zipper front, these interdigitations resolve to leave mature adherens junctions linking opposing cells (Jacinto et al., 2000). In vitro studies of keratinocytes adhering to one another to form confluent sheets show that these cells too use filopodial interdigitation to prime the formation of adherens junctions (Vasioukhin et al., 2000). Live studies of equivalent filopodial interactions between opposing epithelial leader cells during *C. elegans* ventral enclosure show that α -catenin is pre-localised to filopodial tips, which may aid in assembly of rapid, transient adhesions between filopodia that go on to nucleate the formation of mature junctions (Raich et al., 1999). Evidence that filopodia are pivotal for epithelial fusion during dorsal closure comes from experiments where their assembly is blocked by expressing a dominant-negative mutant form of CDC42 in *Engrailed* stripes of the embryonic fly epithelium. In such cases, fusion of the opposing epithelial sheets in these regions fails (Jacinto et al., 2000). Moreover, these experiments reveal one further role for the filopodia in closure of the dorsal hole. Live imaging of the leading edge in the minutes preceding fusion shows how filopodia scan the opposing leading edge, rather like filopodia from a growth cone sensing for axon guidance cues. If assembly of filopodia is blocked, then the opposing epithelial fronts fail to align properly at the midline, much like a poorly buttoned up waistcoat, indicating that filopodia are, in part, responsible for the cell:cell matching needed to align segments across the midline seam (Jacinto et al., 2000).

It seems unlikely that precise alignment of positional values would occur during repair of an epithelial wound, but as we have already discussed, filopodia play an integral role in finally knitting the wound hole closed (Fig. 5C,D) (Wood et al., 2002). Rather strikingly, it appears that filopodial-mediated fusion probably plays a role in all of the vertebrate developmental fusion events that have been carefully studied to date and may be a universal phenomenon. For example, as the eyelids transiently fuse in late mammalian gestation, filopodial interdigitation can be observed where the opposing lid epithelial cells confront one another (Fig. 6) (Zenz et al., 2003). Indeed, there are several earlier fusion events that occur as the vertebrate face is built that appear to use an almost identical bonding strategy (reviewed by Cox, 2004). Classic studies of the fusions between the medial nasal prominence and the right and left maxillary prominences (primary palate fusion) provide clear evidence of filopodia from both nasal and maxillary epithelial faces; and in the classic CPP (cleft primary palate) chick mutant, they are absent (Yee and Abbott, 1978). Similarly, as the two secondary palatal shelves flip up and over the tongue to make contact with one another, they also express

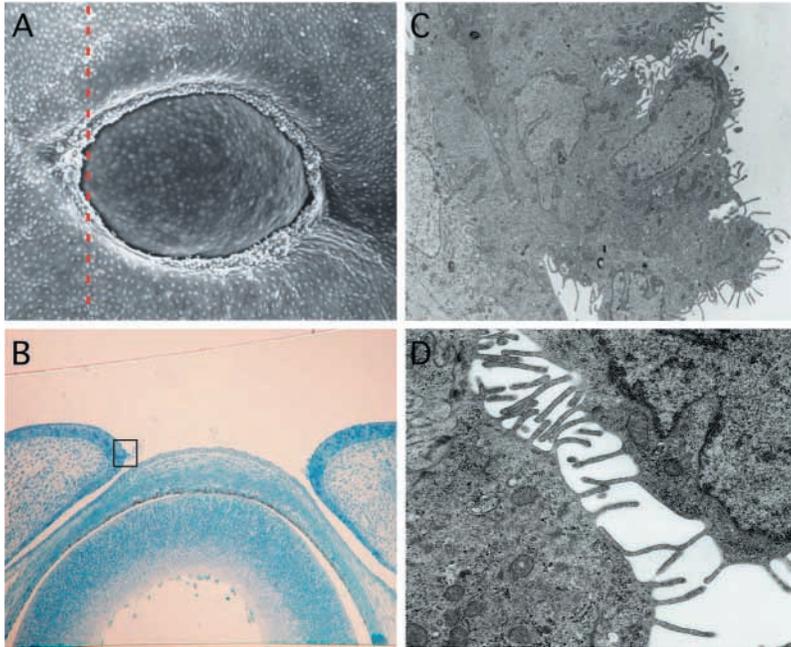


Fig. 6. Eyelid fusion in the mouse. (A) A scanning electron micrograph of the mouse eye at E15, when eyelids are just beginning to advance forwards over the corneal epithelium. (B) A transverse section through the eye taken at the level indicated by the broken line in A. (C) Transmission electron microscopy of the leading edge cells (corresponding to box in B) shows expression of numerous filopodia. (D) When the two eyelids confront one another at the anterior and posterior canthi, the filopodia of opposing epithelial cells interdigitate, just as during *Drosophila* dorsal closure.

exuberant filopodia that appear to bond them together. Possible clues as to the signals regulating assembly of palatal filopodia come from studies of TGF β 3 knockout mice that lack filopodia at the crucial time of contact, thereby failing in palatal fusion such that mice are born with cleft palate (Taya et al., 1999).

In addition to roles for the actin cytoskeleton, recent studies looking at the molecular regulation of vertebrate fusion events are beginning to suggest the involvement of microtubules as well. A human disorder, Opitz syndrome, in which several midline fusion events go awry, is due to lesions in the MID1 gene, which encodes an E3 ubiquitin ligase that targets the microtubule pool of protein phosphatase 2A, and thus may modulate microtubule turnover and dynamics (Schweiger and Schneider, 2003). It will be interesting to discover whether microtubules are also so crucial in wound closure.

Inflammation and chemotactic factors

Beyond a late transition period in foetal development, any tissue damage always results in activation of a robust inflammatory response, whereby largely neutrophils and then macrophages are drawn to the wound site. These cells are attracted by a diverse mix of chemotactic cues, ranging from peptides cleaved from bacterial proteins, to bone fida chemokines released by degranulating platelets, damaged cells and the first patrolling leukocytes arriving at the wound site.

Once at the wound site, the prime role of neutrophils appears to be to kill microbes, while macrophages clear the wound of cellular and matrix debris (including spent neutrophils). It is also the case that both of these leukocyte cell lineages release

a battery of growth factors and cytokines that themselves may supply key tissue repair signals (Rappolee et al., 1988; Hubner et al., 1996). Indeed, a classic series of experiments in the 1970s showed that although antisera depletion of neutrophils from guinea-pig wounds did not significantly perturb tissue repair in sterile conditions, depletion of macrophages with antisera and steroids prevented healing of skin wounds (Simpson and Ross, 1972; Leibovich and Ross, 1975). More recent depletion studies using knockout mouse and other approaches have allowed more direct tests of function for each of the invading cell lineages. Neutrophil knockdown experiments in mice result in repair that is even more rapid than in wild-type healing as long as conditions are sterile, indicating that these cells release signals that are inhibitory to some aspect of the repair process (Dovi et al., 2003). Mice null for Kit W (*Kit* – Mouse Genome Informatics) are deficient in Mast cells and show reduced numbers of neutrophils at a wound site, but otherwise normal repair (Egozi et al., 2003), whereas the PU.1 (*Sfp1* – Mouse Genome Informatics) knockout mouse that lacks both neutrophils and macrophages shows slightly enhanced rates of re-epithelialisation, again indicating that inflammatory cells release signals that are somewhat inhibitory to repair, but are not themselves essential for healing (Martin et al., 2003).

Inflammation and scarring

The inflammatory response at a wound site has clearly evolved to prevent invasion of microbes whenever the skin barrier is broken. However, as embryos can repair wounds perfectly in the absence of an inflammatory response, it is tempting to consider that inflammation may cause some of the unwanted side effects of repair in adult tissues, in particular fibrosis or scarring. This proposal is strengthened by the observation that the transition stage of foetal life when an inflammatory response kicks in, coincides with the earliest stage at which scarring is a consequence of foetal surgery (about E15 in mice and the end of second trimester in human foetuses) (Adzick et al., 1985; Hopkinson-Woolley et al., 1994; Cowin et al., 1998). Beyond this transition period, late foetal and neonatal tissues scar after wounding, but 'macrophageless' PU.1 null neonatal mice appear to repair wounds without a fibrotic response (Martin et al., 2003), indicating that it is not the size of the wound that directs whether it will scar or not, but rather whether it triggers a sustained inflammatory response. One direct consequence of a reduced or absent inflammatory response, whether in the embryo or in a 'macrophageless' neonatal mouse, is a significantly dampened profile of cytokines and growth factors at the wound site, and one of the key growth factors in this regard appears to be TGF β 1. Indeed, there is a growing body of evidence that TGF β 1, and its downstream effector connective tissue growth factor (CTGF) (Igarashi et al., 1996), may be partially responsible for inflammation-mediated fibrosis. When TGF β 1 is mopped up by antibody application or its activity negated in other ways at wound sites in adult rats, repair with reduced scarring occurs (Shah et al., 1992; Shah et al., 1994; Shah et

al., 1995). Further evidence that TGF β signalling may be key in mediating the link between inflammation and fibrosis comes from studies in *Smad3* mutant mice. In these mice, wound keratinocytes, fibroblasts and inflammatory cells all have an impaired capacity for transducing TGF β signals. One consequence of this is a reduced number of inflammatory cells recruited to the wound, and healing, as in the embryo, is again almost scar free (Ashcroft et al., 1999). All these data reveal clear links between TGF β levels at the wound site and subsequent scarring, and indicate that this might be a good therapeutic target for improving tissue repair. What is much less clear is how this growth factor signal is responsible for directing the fibroblast behaviour that leads to an excess of collagen synthesis and its arrangement in bundles, rather than in a basket-weave network as normally found in unwounded skin.

Summary and future directions

There are a number of lessons for better wound healing that can be learned from the embryo. Undoubtedly, there are many parallels between those tissue movements that shape embryos during development and those that are activated upon tissue damage leading to repair of the wound. Indeed, in the embryo it is very likely that tissue damage merely leads to activation of standard morphogenetic machinery so that repair becomes a recapitulation of morphogenesis. Perhaps the embryo ‘reads’ the artificially generated free epithelial edge and the resulting changes in epithelial tensions that arise at a wound site, just as it does any other morphogenetic activation cue, and acts accordingly to close the epithelial hole. The extra complexities of adult wound healing may simply be due to additional processes, such as inflammation, that have evolved to counter infection and cope with the greater size of adult wounds, and of course some of these processes may be more important than the similarities in terms of potential clinical strategies. Equally, there are likely to be aspects of morphogenesis that are not replicated during repair because they are activated or required only in the unique environment of the embryo. It is unlikely, for example, that precise cell:cell matching will occur as wound edges are stitched together, as is the case during *Drosophila* dorsal closure. Furthermore, some morphogenetic episodes that look like wound healing may be misleading. Epiboly in the fish embryo, is a good case in point – while it involves the sweeping forward of a sheet of cells to close a hole, this movement is driven by unique microtubule-based pulling forces generated within the underlying yolk cell (Strahle and Jesuthasan, 1993), in ways that cannot be mirrored in a wound re-epithelialisation scenario.

So far, the parallels we have discussed for morphogenesis and repair have been largely the most obvious ones that occur at the level of epithelial movements. However, there is also likely to be crossover between the signals that guide the directed migrations and subsequent behaviours of inflammatory cells at a wound site, and those signals that guide various migrating cell lineages during normal development. For example, signals whose prime role was believed to be in directing leukocytes to sites of inflammation have now also been shown to guide germ cells from their sites of origin to the primitive genital sites in fish and mice (Doitsidou et al., 2002; Knaut et al., 2003; Molyneaux et al., 2003). In a reciprocal fashion, it seems that white cell migrations might also be

governed by cues previously thought to be the domain of developmental biology, as SLIT, which repels growth cones from crossing the midline during *Drosophila* neural patterning, is repulsive to leukocytes as they attempt to respond to chemotactic cues (Wu et al., 2001). If one wanted to design novel medicines for dispersing inflammatory cells from the wound site, SLIT might be a good candidate for testing.

We suspect that there will also turn out to be parallels at the level of wound fibroblasts and of mesenchymal cells in the developing embryo, particularly during episodes of mesenchymal ‘condensation’ (which precedes cartilage formation and wherever dermal mesenchymal cells aggregate beneath epidermal placodes to form appendages and glandular structures) (Bard, 1990). These mesenchymal condensations resemble the aggregations of previously dormant fibroblasts recruited to wound granulation tissue and many are associated with TGF β and BMP signals, just as both these growth factor cues are believed to be crucial activators of wound fibroblast migrations and contractions. It will be interesting to discover how far such parallels can take us, particularly in understanding how connective tissues are able to undergo physiological contractions without the inevitability of fibrosis.

In hindsight, it is not surprising that many of the tools used to repair and rebuild tissues turn out to be old tools that the embryo used to build those tissues in the first place. For the future, we need to glean which aspects of our detailed understanding of how an embryo is built will be useful in guiding us to better control the cell behaviours of repair in a clinical scenario. As more is learned about the genetics of morphogenesis, not just in flies and worms, but also in some of the more complex vertebrate episodes (as highlighted in Box 2), there will be more clues for repair aficionados.

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