Genetic Screen in *Drosophila melanogaster* Uncovers a Novel Set of Genes Required for Embryonic Epithelial Repair

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ABSTRACT

The wound healing response is an essential mechanism to maintain the integrity of epithelia and protect all organisms from the surrounding milieu. In the “purse-string” mechanism of wound closure, an injured epithelial sheet cinches its hole closed via an intercellular contractile actomyosin cable. This process is conserved across species and utilized by both embryonic as well as adult tissues, but remains poorly understood at the cellular level. In an effort to identify new players involved in purse-string wound closure we developed a wounding strategy suitable for screening large numbers of *Drosophila* embryos.

Using this methodology, we observe wound healing defects in *fun-related antigen* (encoding DJUN) and *scab* (encoding *Drosophila* αPS3 integrin) mutants and performed a forward genetics screen on the basis of insertional mutagenesis by transposons that led to the identification of 30 lethal insertional mutants with defects in embryonic epithelia repair. One of the mutants identified is an insertion in the *kars* locus, which encodes *Drosophila* β<sub>Heavy</sub>-spectrin. We show β<sub>Heavy</sub>-spectrin (β<sub>Heavy</sub>) localization to the wound edges where it presumably exerts an essential function to bring the wound to normal closure.

WOUND healing is essential to organisms throughout the animal kingdom. It must occur for restoring tissue integrity after injury both during embryonic and adult life. Epithelia, in particular, act as a physical barrier protecting living organisms and their organs from the surrounding environment and have evolved robust mechanisms to ensure their integrity. Simple embryonic epithelial tissues have an extraordinary capacity to reseal small discontinuities very rapidly and efficiently through an epithelial resealing mechanism. This was initially described in the chick embryo (Martin and Lewis 1992), but seems to be conserved across species as it was shown to also occur in mouse, frog, and fly embryos (McCluskey et al. 1993; Davidson et al. 2002; Wood et al. 2002). In all these systems, small epithelial wounds close via the cooperation of three distinct mechanisms: the assembly of an actomyosin purse string in the epithelial cells at the wound margin, the proliferative activity of epithelial cells at the margin, and the contraction and ingress of deep cells when those are exposed (see Jacinto et al. 2001; Martin and Parkhurst 2004; Garcia-Fernandez et al. 2009 for review).

Advances in live imaging of *Drosophila* embryos expressing fluorescent proteins made time-lapse microscopy of the epithelial healing process possible and the exact sequence of cell movements to be determined (Wood et al. 2002). The cells at the wound margin constrict their apical edges through the action of an actomyosin cable that assembles just minutes after wounding and is linked intercellularly through adherens junctions. Concomitant with the formation of the purse string, cells at the wound margin begin to extend actin-rich protrusions. When opposing wound margins come into close proximity, filopodia and lamellipodia from opposing flanks make contact and they appear to pull the wound margins toward one another. For laser-induced oval wounds of ~10 by 20 μm, the entire healing process can be completed in just over 2 hours (Wood et al. 2002).

The signaling cascades that regulate the epithelial resealing process are just beginning to be unraveled, but the known molecular mechanisms appear to be conserved in both vertebrates and invertebrates, namely the involvement of Grainy-head (GRH) transcription factors or the JNK signaling cascade, transduced by JUN/FOS transcriptional complexes (Rame et al. 2002; Li et al. 2003; Ting et al. 2003, 2005a,b; Galko and Krasnow 2004; Mace et al. 2005). In the fly, the expression of some genes at the wound site is dependent on functional GRH
and JUN/FOS dimers (AP1) binding sites in their promoter region (MACE et al. 2005; PEARSON et al. 2009). These observations are consistent with abnormal wound healing in grh or basket/DJ/NK mutants’ larval cuticle and the activation of JNK signaling pathway at wild-type larval wound sites (GALKO and KRASNOW 2004; MACE et al. 2005). Recently, it was suggested that extension of actin-based cellular processes by the wound-edge epidermal cells of Drosophila larvae is dependent on Pvr, a PDGF/VEGF-like receptor, and one of its ligands, Pvf1 (WU et al. 2009). In addition, the Rho family of small GTPases including Rho, Rac, and Cdc42 are known to be critical to mediate the rapid cytoskeleton rearrangements that control cell shape changes (as described above) of wound bordering epithelial cells during closure (reviewed in JACINTO et al. 2001; MARTIN and PARKHURST 2004). The upstream signal activating the cells surrounding the wound is still unknown, but it is established that extracellular signal-regulated kinase (ERK) is phosphorylated upon wounding, an event required at wound sites for a robust response (MACE et al. 2005). Taking together the fact that Drosophila GRH and FOS proteins can be phosphorylated by ERK in vitro (UV et al. 1997; CLAPPONI et al. 2001) and more recent data identifying Stitcher, a receptor tyrosine kinase that also induces ERK phosphorylation as a Grh target, one can envision a Grh-dependent positive feedback loop that could function as an amplification mechanism ensuring efficient epidermal wound repair (WANG et al. 2009).

To gain new insights into the cell biology of epithelial rescaling, we performed a genetic screen using the Drosophila embryo with the aim of finding new genes involved in the regulation of wound healing. For that purpose, we developed a wounding assay that facilitates large-scale screening and validated it by showing that Jra, a mutant in the JNK signaling pathway, and scab, a mutant in an α-integrin isoform, are both required for embryonic wound healing. We then tested 655 piggyBac and P-element insertion mutations (Exelixis) and were able to identify 30 lines with impaired wound healing. One isolated mutant is an insertion in the karst gene, encoding the Drosophila homolog of β-Heavy spectrin. Karst has been previously implicated in cytoskeleton organization and associated with tissue morphogenesis (THOMAS et al. 1998; ZARNESCU and THOMAS 1999) but its precise function has remained elusive. We further show that the Karst protein accumulates around the wound edges in a cable-like manner, where it must play an important function in the healing process.

MATERIALS AND METHODS

Fly strains and genetiques: A total of 655 insertional mutant lines on the second and third chromosomes were picked from the 2100 inserts chosen as single gene tags for the Genome Disruption Project, originally generated by Exelixis Corporation (THIBAULT et al. 2004) and distributed by the Blooming-ton Stock Center. The lines with a wound closure phenotype were remapped by inverse PCR (iPCR) and sequenced, following protocols described at the Berkeley Drosophila Genome Center Web site (http://www.fruitfly.org/about/methods/inverse.pcr.html) and at the Bellen lab Web site (http://flypush.imagen.bcm.tmc.edu/~pscreen/). We confirmed the insertion location as listed in FlyBase for all except 3 of our positive lines. Specifically, we could not determine the insertion locations for lines Eaf110d150, Neu310m169, and side6063. We did not succeed in remapping the Eaf110d150 line by iPCR, but we assume the genomic location is correct since this line failed to complement two different deficiencies in the region (Df(2R)ED1673 and Df(2R)Drk19, stock nos. 9062 and 8888, respectively). The Neu310m169 line, which is listed as an insertion in the gene Neu3 (cytological map location 88C10–88D1, in 3R), was mapped by 5’ primer sets (the 3’ set did not work) to a different location in the end of the 3L chromosome, in gene CG4047. There are no available deficiencies in this region (the last three deficiencies of the chromosome all complemented the line, stock nos. 2587–2589) and this line complements another Neu3 allele (Neu320d136, stock no. 23312) and a deficiency in the Neu3 region (Df(3R)ED1555, stock no. 23714). Therefore, we consider that the line Neu310m169 is not an insertion in the Neu3 region. Finally, the line side6063, which is listed as an insertion in the gene sidestep (cytological map location 97F6–97F10) was mapped by both 5’ and 3’ primer sets to a different location, 86D9. This line fails to complement Df(3R)ED5516 (deletion of 86D8–86E13), confirming our mapping results.

For the pilot screen, the following mutant alleles were used: Jra1 (KOCKEL et al. 1997), pur1 (RING and MARTINEZ ARIAS 1993), shak1 (FERNANDEZ et al. 2000), ike1 (NELLEN et al. 1994), shm1 (ARORA et al. 1995), zf1p (YOUNG et al. 1993), uib-6 (FRANK and RUSHLOW 1996), Phe110k (SPARRING et al. 1999), side6063 (SCHOCK and PERRIMON 2003), Rho110k (MAGIE and PARKHURST 2005), fxs2 (LEKKEN et al. 1998), and Efg41 (later renamed Efg4) (PRICE et al. 1997).

All lethal or semilethal lines were crossed to balancer stocks that drive eGFP under the twist promoter, active at embryonic stages. Specifically, males from original lines were crossed to either Gla/CyO-CTG or Dr/TM3-FTG virgins, depending on insertion site. Flies of the following generation were selected against Gla or Dr and used to start new GFP-balanced stocks, which were then used to collect the screened embryos.

Or5, ubi-DE-Cad-GFP (ODA and TSUKITA 2001), and sGMCA, which expresses the actin binding domain of moesin fused to GFP, labeling filamentous actin (KIEHART et al. 2000) were used as control lines.

Using standard strategies, the following recombinant stocks were generated: CG28130m007, ubi-DE-Cad-GFP/CyO-CTG, CG51980m107, ubi-DE-Cad-GFP/CyO-CTG, CG56400m121, ubi-DE-Cad-GFP/CyO-CTG, and karst1103 sGMCA/TM6b-GFP.

Wounding assay: Two-hour egg collections of GFP-balanced lines (Mutation/CyO-CTG or Mutation/TM3-FTG) taken on standard apple juice agar plates supplemented with yeast extract, were allowed to develop at 18° overnight. Embryos were dechorionated in bleach and typically 40–80 non-GFP (homozygous mutant) stage 15/16 embryos were sorted under UV light. Homozygous mutant embryos were aligned ventral side up, stuck to double-sided tape on a slide, covered with halocarbon oil 700 (Sigma) and a coverslip, and subjected to wounding using a nitrogen laser-pumped dye laser connected to a Zeiss Axiovert 200M microscope (Micropoint Photonic Instruments). After wounding, the coverslip was removed and the embryos were left to develop at 22° for 16 hr before being scored under a dissecting scope for wound closure. The wound healing phenotype was calculated as a percentage of nearly hatching first instar larvae with unclosed wounds over...
the total number of wounded larvae (dead animals were disregarded for this calculation).

**Imaging:** Live embryos were wounded as described above and live imaging was performed using LSM510 Meta confocal system (Carl Zeiss MicroImaging). Images were taken every 3 or 30 min. All images were processed using ImageJ imaging software (National Institutes of Health) and Photoshop (Adobe).

Live wounded larvae were imaged using a DC500 Leica camera mounted on an upright widefield DM5000B Leica microscope under phase contrast conditions.

**Immunohistochemistry of wounded embryos:** Stage 14 Or^x^ embryos were selected and subjected to the wounding assay, except wounds induced were smaller and embryos were allowed to heal in a humid chamber for ∼1 hr before further processing. Wounded embryos were loosened from the tape with forceps and then removed from the oil with a paintbrush dipped in heptane (Sigma) and transferred to a glass vial containing fix mix, 1:1 heptane: 3% FA in PLP (3% formaldehyde, 0.01 NaIO_4, 0.1 m PIPES, pH 7.3, 0.1 m lysine) (modified from Thomas and Kiehart 1994), and incubated on a roller for 40 min at room temperature (RT). Embryos were removed and hand devitilized in PBS (NaCl 137 mM, KCl 27 mM, KH_2PO_4 43 mM, NaHPO_4·2H_2O 47 mM, incubated 1 hr in block (0.3% BSA in PBST, which is 0.3% Triton X-100 in PBS), and then overnight at 4°C with the following primary antibodies diluted in block: m-armadillo at 1:50 (DSHB) and β_H^specific antiserum no. 243 at 1:500 (Thomas and Kiehart 1994). Embryos were then rinsed three times and washed 1 hr in block, incubated 1 hr in secondary antibodies (Molecular Probes), diluted 1:200 in PBST (Alexa 568 anti-rabbit and Alexa 488 anti-mouse or Alexa 488 anti-rabbit and 1 μg/ml Alexa 594-phalloidin), rinsed three times and washed 1 hr in PBST, rinsed in PBS, and mounted in 80% glycerol with 2% Dabco.

**RESULTS**

**Wounding strategy and assay validation:** To identify novel Drosophila strains with wound healing phenotypes, we designed and optimized a wounding assay suitable for a high-throughput screen (see MATERIALS AND METHODS and Figure 1). To verify that the wounding assay is sensitive enough to find genes required for wound healing, we tested candidate genes previously shown to be required either for wound healing or for dorsal closure (DC) in Drosophila (Figure 2). DC is a developmental process involving epithelial sheet movements to close a naturally occurring dorsal hole created when the germ band retracts after its extension. It has previously been reported that this process occurs in a mechanistically analogous way to embryonic wound healing (Jaicinto et al. 2000; Wood et al. 2002). Therefore, we tested members of major pathways that control DC such as Jun-related antigen (fra), puckered (puc), and Src homology 2 ankyrin repeat tyrosine kinase (shark) in the DJNK signaling pathway and thickveins (tkv), schnurri (shm), and zipper (zip) in the TGF-β signaling pathway. Furthermore, we tested additional genes involved in DC such as u-shaped (ush), Epidermal growth factor receptor (Egfr), Protein kinase related to protein kinase N (Pkn), scab (scb), and Rho1, which has also been

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**Figure 1.—Wound healing protocol.** (A) Two-hour egg collections from GFP-balanced lines are left to develop until stage 15/16 and homozygous, non-GFP, mutant embryos are selected under fluorescent light for wounding. (B) Selected embryos are mounted for laser wounding on a microscopic slide with the ventral side up. (C) Embryos are laser wounded on the medial ventral region. (D) After approximately 16 hr in a humid chamber, nearly hatching larvae are screened under the dissection scope for wound closure and percentage of unclosed wounds calculated; arrows point to typical healed (left) and unhealed (right) wounds (see MATERIALS AND METHODS for details).
previously implicated in embryonic wound healing (Wood et al. 2002).

We scored wound healing phenotypes as percentage of unclosed wounds. The negative control OrR displayed 5% of phenotype in our assay while the analyzed DC mutants’ phenotype ranged from 2% (shn1) to 74% (Jra1) (Figure 2). The JNK signaling cascade had been previously demonstrated to be involved in wound healing of larval and adult fly tissues (Ramet et al. 2002; Galko and Krasnow 2004; Boscin et al. 2005). To our knowledge, the Jra1 (null mutation in DJun) mutant phenotype observed here (74% open wounds) is the first report of the importance of the JNK cascade in fly embryonic epithelial wound healing. To define a cutoff threshold for wound healing defects, we performed a statistical analysis on the basis of the maximum likelihood ratio test (LRT). By comparing all mutants in Figure 2 with OrR we conclude that any line with a phenotype >30% would be statistically different from the wild-type situation (5% of open wounds), with a P-value of <0.001 (data not shown). Therefore, we observe a clear wound healing phenotype in Jra1 and scb5J38 (74 and 53%, respectively). The Rho11B phenotype is not above the threshold but is still significantly different from the control strain (P < 0.005), while all the other genes tested had either no phenotype or had severe patterning defects at the embryonic stage at which wounds were made, thus making scoring impossible (Figure 2).

The fact that not all tested DC mutants have a wound healing phenotype suggests that albeit mechanistically similar, each process is distinct in some aspects. In this respect, it is worth mentioning that during DC there is never an actual “hole” to close as the epithelial cells actually remain in contact with an extraembryonic tissue, the amnioserosa (AS), throughout the closure process. It is now well established that AS cells have an active role during DC and it is the fine orchestration of the epithelial and AS tissue contributions that ensures successful closure (Kiehart et al. 2000; Hutson et al. 2003; Scuderi and Letsou 2005).

Taken together, these pilot results demonstrate both the effectiveness and the sensitivity of the wounding assay, allowing us to conclude that we developed a simple and robust method that can be used in a large-scale genetic screen. Next, we performed a wound healing screen using a series of largely uncharacterized, yet molecularly mapped mutant lines from the Exelixis stock collection (Thibault et al. 2004).

**Wound healing screen:** The Exelixis transposon insertion collection is composed of four transposon types: three piggyBac-based transposons and one P-element-based transposon. A subset of this collection likely to disrupt gene function is present at the Bloomington Stock Center. We tested all chromosome 2 and 3 lethal lines in this subset and a few additional viables, for a total of 655 insertional mutant lines (see supporting information, Table S1).

Of the 655 lines analyzed, we observed 30 (4.6% of the total lines screened) that presented a wound closure defect (more than 75 embryos analyzed for each line, Table 1). The total number of mutants with wound

| Allele tested | Molecular function | Cellular processes | Phenotype |
|---------------|--------------------|--------------------|-----------|
| Jra1          | Transcription factor| Stress response, JNK signaling | 74% (n=390) |
| puc100        | Phosphatase        | Morphogenesis, JNK signaling | 3% (n=74) |
| shn1          | Tyrosine kinase    | Cell polarity, JNK signaling | 6% (n=262) |
| tkv2          | TGF-β receptor     | TGF-β signaling     | 3.5% (n=256) |
| shn1          | Transcription factor| TGF-β signaling     | 2% (n=52) |
| zip1          | Myosin heavy chain | Cell movement, Cell shape changes | Unscorable |
| ush2          | Transcription factor| Morphogenesis       | Unscorable |
| Pfy3(773a)    | Serine/threonine kinase | Morphogenesis | 12% (n=42) |
| scab5(3a)     | Integrin           | Cell adhesion       | 55% (n=192) |
| Rho11B        | Small GTPase       | GTPase activity     | 22% (n=473) |
| fa2           | Transcription factor| Stress response, JNK signaling | Unscorable |
| Egfp12        | EGF receptor       | EGF signaling       | Unscorable |

Figure 2.—Dorsal closure mutants with wound healing phenotypes. (A) List of tested alleles, respective molecular function, and cellular process as in FlyBase (Tweedie et al. 2009). Wound healing phenotypes are shown as percentage of total (n) embryos presenting an unclosed wound approximately 16 hr postwounding. Some mutations are “unscorable” due to gross morphological defects either at the wounding stage (15/16) or at the scoring stage (early first instar larvae). (B) Graphic representation of wild-type phenotype (OrR) and the three strongest phenotypes observed. (C–F) wounds in first instar larvae in OrR (C), Jra1 (D), scb5J38 (E), and Rho11B (F).
TABLE 1
Insertional mutants with wound healing phenotypes

| Allele tested | Molecular function                          | Cellular processes                        | Phenotype (%) |
|---------------|---------------------------------------------|-------------------------------------------|---------------|
| *alar*1       | Calcium binding, transmembrane transporter  | Mitochondrial transport                   | 33 (n = 283)  |
| *arc*-p20     | Cytoskeleton component                      | Cell movement, cell shape changes         | 43 (n = 199)  |
| *atg*2         | Unknown                                     | Autophagy                                 | 30 (n = 86)   |
| *dEaf*         | Transcription elongation                    | Stress response                           | 31 (n = 97)   |
| *dUtx*         | Chromatin remodeling                        | Gene Silencing                            | 47 (n = 76)   |
| *gla*          | Splicing                                    | Oogenesis                                 | 60 (n = 221)  |
| *grp*          | Serine/threonine kinase                     | Cell cycle                                | 44 (n = 171)  |
| *gs1-like*     | Glutamate-ammonia ligase                    | Metabolic processes                       | 35 (n = 162)  |
| *kst*          | Cytoskeleton component                      | Membrane structure, cell polarity, scaffolding | 50 (n = 362)  |
| *Pc*           | Chromatin remodeling                        | Gene Silencing                            | 55 (n = 311)  |
| *Ser12*        | Serine protease                             | Proteolysis                               | 44 (n = 212)  |
| *Stam*         | JAK pathway signal transduction adaptor     | JAK-STAT signaling                        | 43 (n = 183)  |
| *CG2813*       | Unknown                                     | Fatty acid β-oxidation                    | 39 (n = 167)  |
| *CG31805*      | Unknown                                     | Immune response                           | 49 (n = 147)  |
| *CG4389*       | Long-chain-3 hydroxyacyl-CoA dehydrogenase  | Unknown                                   | 80 (n = 142)  |
| *CG5198*       | Unknown                                     | Unknown                                   | 33 (n = 114)  |
| *CG6005*       | Unknown                                     | Unknown                                   | 37 (n = 212)  |
| *CG6750*       | Unknown                                     | Unknown                                   | 37 (n = 212)  |
| *CG7627*       | Transmembrane transporter                   | Xenobiotic transporter                    | 31 (n = 282)  |
| *CG9249*       | Hexaprenyldihydroxybenzoate methyltransferase activity | Ubiquinone metabolism                    | 51 (n = 182)  |
| *CG10217*      | Unknown                                     | Unknown                                   | 95 (n = 205)  |
| *CG11089*      | IMP cyclohydrolase activity                 | Purine metabolism                         | 35 (n = 130)  |
| *CG12913*      | Acetylglactosaminyl transferase             | Chondroitin sulfate biosynthesis          | 38 (n = 247)  |
| *CG15170*      | Unknown                                     | Protein metabolism                        | 36 (n = 84)   |
| *CG16833*      | Tubulin-tyrosine ligase process             | Protein metabolism                        | 42 (n = 104)  |
| *CG30010*      | Unknown                                     | Translation regulation                    | 33 (n = 221)  |
| *CG3294*       | Splicing                                    | tRNA aminoacylation for protein translation | 48 (n = 280)  |
| *CG33123*      | Leucine-tRNA ligase                         | tRNA aminoacylation for protein translation | 48 (n = 280)  |

Phenotype refers to percentage of total (n) embryos presenting an unclosed wound ~16 hr postwounding. Molecular function and cellular processes modified from FlyBase (Tweedie et al. 2009) and additional references mentioned in the text.

healing defects is 28 since for two of the lines, Neu3c01955 and sidec00677, the original insertion location was not confirmed (see MATERIALS AND METHODS). A number of these mutant alleles were recombined with fluorescent markers to allow for observation of tissue morphogenesis and wound closure dynamics at the cellular level. In Figure 3, stills taken every 30 min from *fra*, *CG2813*, *CG5198*, and *dUtx* mutations recombined with *ubi-DE-cad-GFP* (which marks adherens junctions and allows for cell boundary visualization), show that all four mutant embryos depicted still have open wounds after 3 hr, whereas in the control embryo (*ubi-DE-cad-GFP*) only a tiny hole remains. These results confirm that our screen successfully uncovered mutants that display wound healing phenotypes in both larger (assay type) and smaller, more experimentally tractable wounds, as the ones depicted in Figure 3. Interestingly, wounds made in these mutants appear to assemble an actin cable as visualized by phalloidin staining (data not shown), suggesting that these mutants must be affecting a process downstream of the initial rapid response of the wound by the proximal epithelial cells.

**A karst mutant with wound healing defects:** One of the isolated wound healing mutants is a previously unstudied allele of *karst* (50% open wounds, n = 362, Table 1). This particular mutant, *karst* (2011), is a P-element-based transposon insertion in the *minus* orientation at nucleotide position 5219 of the *karst* ORF, falling within exon 8. This exon is common to all four predicted karst transcripts (Wilson et al. 2008); therefore, this insertion is likely to disrupt the function of all possible gene isoforms.

The *karst* locus encodes the Drosophila β<sub>heavy</sub>-Spectrin (*β<sub>H</sub>), which is a large F-actin cross-linking protein specific to epithelial tissues (Thomas and Kiehart 1994), with orthologs in various species including...
**Caenorhabditis elegans** and humans (InParanoid eukaryotic ortholog groups). In epithelial cells, \( \beta \text{H} \), together with its binding partner \( \alpha \)-spectrin make up the apical portion of the spectrin-based membrane skeleton (SBMS). This structure is a protein meshwork lying under the plasma membrane and links the cytoskeleton to the plasma membrane, providing resistance to mechanical stress, while at the same time is possibly acting as a scaffold for protein/protein interactions (for review see Thomas 2001). Apart from the structural role, the SBMS is important for maintenance/establishment of the Zona adherens, modulation of the apical membrane area, as well as apical constriction and other contractile actin-ring-based cell morphogenesis occurring during cellularization in Drosophila embryos or body elongation in *C. elegans* embryos (McKeown et al. 1998; Thomas et al. 1998; Thomas and Williams 1999; Zarnescu and Thomas 1999; Williams et al. 2004; Prattis et al. 2005).

The insertion present in the *karst\(^{d1183}\)* mutant is located in segment 15 of \( \beta \text{H} \) (see Thomas et al. 1997, for an explanation of the segment nomenclature). This mutation causes a premature stop codon three amino acids downstream of the insertion site.

**karst\(^{d1183}\) mutants have weaker wound-induced actin cables and less cellular protrusions:** To study the dynamics of wound healing in the *karst\(^{d1183}\)* mutant, this line was recombined with sGMCA (the actin binding domain of moesin fused with GFP, Kiehart et al. 2000) and time-lapse recordings were analyzed. Upon wounding, the actin cable was consistently weaker and appeared fragmented over time (arrows in Figure 4C, i–iii). Cells at the wound margin do not properly elongate toward the center of the wound and many

**Figure 3.—**Wound closure dynamics in control embryos and wound healing candidate mutants. (A) Stills from movies of wounded control embryos (*ubi-DE-Cad-GFP*), (B) *Jra*, (C) *CG2813^G01207\*, (D) *CG5198^G01231\*, and (E) *dUtx^G01221* embryos recombined with *ubi-DE-Cad-GFP*, taken 30 (i), 60 (ii), 90 (iii), 120 (iv), and 180 (v) minutes postwounding (mpw). After 3 hr, only wounds in control embryos are nearly closed (compare panel Av to Bv, Cv, Dv, and Ev).
failed to productively contract their wound marginal edges (compare artificially colored wound marginal cells in mutant to those in control in Figure 4C, i–iii with 4B, i–iii, respectively). In addition, we observed reduced actin-based protrusion activity at the wound edge when compared to wild-type (compare arrowheads in Figure 4C, i and ii with 4B, i and ii). After 2 hr, the wounds were still open while corresponding wounds made to control embryos closed in 1.5 hr (Figure 4C, i–iii and 4B, i–iii). These observations suggest that βH helps to maintain the actomyosin cable while it is contracting and remodeling as the wound closes. The results further suggest that βH facilitates other spatially restricted actin-based dynamics, such as filopodial extension, and may serve to connect the intercellular cues coming from the actin cable to the intracellular responses required to produce a polarized cell shape change, such as cell elongation toward the wound and wound edge contraction.

βH localizes to wound edges: Given that the karstd11183 mutant has a wound healing phenotype, we examined whether the βH protein is present at the right time and place to play a direct role in wound closure. It was already known that βH is expressed in epithelial tissues throughout embryonic development (Thomas and Kiehart 1994) but we wanted to know whether it has an altered expression or localization pattern within wounded epithelium. Using βH-specific antiserum no. 243, which recognizes the N-terminal domain of Drosophila βH (Thomas and Kiehart 1994), we observed that the protein concentrates strongly at wound edges and is present not just at the adherens junction level as
shown by Armadillo staining (Figure 4D, i–iii), but in a cable-like pattern that seems to coincide, at least partially, with the actin cable (Figure 4E, i–iii). This observation is especially interesting because, to our knowledge, the only endogenous proteins previously described to localize to the wound edge in a cable-like manner are actin and myosin.

Taken together with the above mutant phenotypes, these observations suggest that wild-type β1l functions locally to properly form and/or maintain the intercellular actomyosin cable while it is contracting and remodeling during wound closure. β1l function is also required for wild-type intracellular responses such as wound marginal cell edge constriction as well as polarized extension of the wound edge cells toward the wound center. Our results further suggest that β1l can facilitate other spatially restricted actin-based events, such as wound edge filopodia dynamics.

DISCUSSION

Wound assay and pilot screen: Using previously described DC or wound healing mutants we performed a pilot screen to validate our embryonic wounding strategy. The fact that we identified a member of the DJNK pathway (fra/Djun) in our assay is in accordance with other reports that implicate this pathway in wound healing. Specifically, two mutations in components of the DJNK pathway, bsk/DJNK and kay/DJos, were previously shown to have defects in fly larval and adult wound closure, respectively (RAMET et al. 2002; GALKO and KRASNOW 2004). In addition, MACE et al. (2005) described a reporter construct that requires consensus binding sites for the JUN/FOS complex to be activated upon wounding. Interestingly, the authors still observed reporter activation in fra mutants, which suggests that additional signaling pathways are involved in wound closure (MACE et al. 2005).

An apparent discrepancy arose when our assay revealed a phenotype with fra but not with puc mutants, another component of the same signaling pathway. This result might be explained by the fact that Jra and puc function in opposite directions in the DJNK signaling pathway. Puc functions as a pathway repressor, so in a puc mutant the JNK pathway should be less repressed and we could expect to have an opposite effect to a fra mutation. In addition, we note that activation of a puc–lacZ reporter has been shown to occur in larvae, wing imaginal discs, and adult wounds that take 18–24 hr to close, but it is only robustly detectable 4–6 hr post-puncture (RAMET et al. 2002; GALKO and KRASNOW 2004; BOSCH et al. 2005). Embryonic wounds are faster to heal, and even after inflicting a large laser wound on stage 14/15 embryos, we failed to detect activation of the puc–lacZ reporter (assessed in open wounds 3 hr postwounding by immunofluorescence; data not shown). This observation suggests that, in rapidly healing epithelial wounds, the JNK pathway is not activated to high enough levels to trigger auto-inhibition.

The α-integrin scab was never before implicated in embryonic wound healing, but this mutant’s phenotype comes as no great surprise. The first scab mutation was isolated due to its abnormal larval cuticle patterning (NUSSEIN-VOLKHARD et al. 1984). The scab gene encodes for Drosophila α-PS3 integrin, which is zygotically expressed in embryonic tissues undergoing invagination, tissue movement, and morphogenesis (STARK et al. 1997). Integran proteins are involved in cell–matrix interactions and α-PS3 integrin regulation, in particular, mediates zipping of opposing epithelial sheets during DC (HOMSY et al. 2006). Similarly, our observation of a wound defect in scb178 mutants is consistent with a role for α-PS3 integrin in zipping of opposing epithelial cells during the healing process.

A previous study using confocal video microscopy has shown that Rho11B mutants take twice as long to close an epithelial wound when compared to wild type (WOOD et al. 2002). Rho1 was confirmed in our assay to be important for wound healing, although with a weaker phenotype (22% of embryos had unclosed holes). This result shows nonetheless that our assay can be sensitive enough to pick up a “weak” wound healing mutant such as Rho11B, which is still able to heal wounds albeit slower than wild type.

Transposon screen: The genes identified in the screen represent a variety of functions indicating that wound healing is a complex mechanism that requires the participation of many cellular processes. A large class of the candidate mutants are involved in several aspects of gene expression, including factors that regulate chromatin remodeling (dUtx and Pc), elongation (dEaf), splicing (Glo and CG3294), and translation (CG33123) (ZINK and PARO 1989; SCHNEIDER et al. 2004; SMITH et al. 2008; KALIFA et al. 2009; TWEEDIE et al. 2009). These factors are likely needed during wound healing for the induction of a repair transcriptome (COOPER et al. 2005; ROY et al. 2008; STRAMER et al. 2008). Interestingly, JNK signaling-dependent Pc group (PcG) gene downregulation has been observed during imaginal disc regeneration (LEE et al. 2005). In addition, a recent study revealed that PcG methylases are downregulated during wound healing, while counteracting demethylases, Utx and Jmjd3, are upregulated (SHAW and MARTIN 2009). Our results for the Pc and Utx mutants are consistent with these studies and highlight the importance of epigenetic reprogramming in the repair process.

Some of the genes such as arc-p20 and karst probably have a more direct role in the cell shape changes that drive the tissue morphogenetic movements during epithelial repair. The gene product of arc-p20 is a component of Arp2/3, a complex that controls the
formation of actin filaments, and *karst* encodes a component of the spectrin membrane cytoskeleton described in detail below (Thomas and Kiehart 1994; Kunda et al. 2003; Borghese et al. 2006). Also related to morphogenesis, CG12913 encodes an enzyme involved in the synthesis of chondroitin sulfate (Tweedie et al. 2009), which is usually found attached to proteins as part of a proteoglycan, suggesting a predictable contribution of the extracellular matrix in the tissue movements necessary for wound healing.

The epithelium is the first line of defense of the organism against pathogens and tissue integrity. It would thus seem plausible that genes involved in innate immunity could be identified with our screening protocol. Indeed, two of the genes (*Ser12* and *CG5198*) seem to point to the involvement of the immune response in the healing of the laser-induced wounds. *Ser12* is a member of the serine protease family, a class of proteins that has been shown to play a role in innate immunity (De Gregorio et al. 2001; Ross et al. 2003). The *CG5198* gene has no described function in Drosophila so far, but its homolog, CD2-binding protein 2, is involved in T lymphocyte activation and pre-RNA splicing (Kofler et al. 2004; Heinze et al. 2007). Another candidate that might represent a link to immunity is *Alg2*, a gene important for the regulation of autophagy, a process by which cells degrade cytoplasmic components in response to starvation. In Drosophila, autophagy has been linked to the control of cell growth, cell death, and, recently, to the innate immune response mechanism against vesicular stomatitis virus and listeria infection (Scott et al. 2004; Yano et al. 2008; Shelly et al. 2009).

Isolation of an insertion in the *stam* gene points to the involvement of the JAK-STAT signaling cascade in this regenerative process (Mesilaty-Gross et al. 1999). Interestingly, *stam* has been shown to be involved in Drosophila tracheal cell migration and is upregulated following Drosophila larvae infection by *Pseudomonas entomophila* (Vodovar et al. 2005; Chanut-Delalande et al. 2007).

One candidate could be involved in the uptake or export of some important wound signal (*CG7627*) as this gene encodes for a multidrug resistant protein (MRP), part of the ABC transporter superfamily, involved in drug exclusion properties of the Drosophila blood–brain barrier (Tarnay et al. 2004; Mayer et al. 2009).

The kinase encoded by *grapes* is the Drosophila homolog of human Check1 (Chk1) involved in the DNA damage and mitotic spindle checkpoints (Fogarty et al. 1997; Furnari et al. 1997; Zachos et al. 2007). To our knowledge, all the Chk1 literature has focused on its role during the cell cycle. However, the Drosophila late embryonic epithelium is a quiescent tissue, even after wounding (data not shown). Understanding Grapes function in this context is a challenging task that could lead to new paradigms. One hypothesis is that Grapes is involved in tension sensing, as it is in the spindle checkpoint, or may uncover a cellular repair process that could help damaged cells “decide” to either die by apoptosis or participate in the repair process.

The remaining genes with a putative function represent a wide range of general metabolic processes (*aralar1*, *gs1*-like, *CG4389*, *CG9249*, *CG11089*, and *CG16833*), suggesting that healing the epithelium is a highly demanding process (Soehnge et al. 1997; Del Arco et al. 2000; Tweedie et al. 2009).

Finally, we have also selected a significant number of genes that have not yet been studied and do not contain identifiable protein domains (*CG2813*, *CG31805*, *CG6005*, *CG6750*, *CG10217*, *CG15170*, and *CG30010*). At the moment it is not possible to predict the role that these genes may play, but further study may help to identify novel wound healing regulatory mechanisms.

**Possible role for β_H in wound healing:** One of the mutants identified in our transposon screen was *kst*(11183), an insertion in the β_H-spectrin locus. This mutation is likely producing a truncated protein terminating three amino acids into the *P*element insertion (Figure 4A). Other mutations identified in nearby segments 14 (kst14, kst1) and 16 (kst1) lead to the production of a detectable truncated protein (Medina et al. 2002) so it is likely that *karst*(11183) mutation also gives rise to a truncated protein. These mutant forms of β_H lack approximately half of the wild-type protein, including a COOH-terminal PH domain region, which is involved in targeting the protein to the membrane (Medina et al. 2002), thus producing a potential dominant negative form of β_H. However, the *karst*(11183) mutant should still have maternally loaded wild-type protein, as previous studies describe a complete absence of maternal protein only by the third instar larval stage (Thomas et al. 1998). This maternal contribution is likely the main reason that this mutant, as well as the other mutants isolated in our screen, does not have a fully penetrant wound healing phenotype.

We show for the first time that β_H-spectrin localizes to the actomyosin purse string, a supracellular contractile cable that forms rapidly upon wound induction. Live imaging has demonstrated that actin and myosin can accumulate in this cable structure within minutes after wounding (Wood et al. 2002). Unfortunately, due to the size of the β_H gene (>13 kb) cloning and tagging it for live imaging is not possible using standard methods, but our experiments in fixed tissue tell us that β_H can accumulate very rapidly in this cable structure. We have observed β_H accumulation at the earliest time point technically feasible, 15 min postwounding (data not shown). These observations are consistent with previous studies, also in fixed tissue, demonstrating rapid changes in β_H localization during the process of cellularization in Drosophila embryos (Thomas and
Taken together, it is clear that at least the \( \beta_1 \) component of the membrane skeleton is not just a static structural scaffold as the name implies, but rather a dynamic protein capable of responding to or directing changes in cellular dynamics. Our studies suggest that polarized redistribution of \( \beta_1 \) exerts an essential function to facilitate actin-based cellular responses, such as cable accumulation/maintenance and wound edge filopodia dynamics, which are necessary to properly close a wound.

**\( \beta_1 \) as a link between cell membranes and contractile rings:** \( \beta_1 \) has been previously observed in association with actin “rings” during development of Drosophila and *C. elegans* (reviewed in Thomas 2001). Arguably, *C. elegans* provides an example of actin ring function most analogous to our wound edge purse string. During the final stages of *C. elegans* development, cortical arrays of actin in the outer epithelial cells, the hypodermis, dramatically reorganize to form parallel apically localized bundles of circumferential supracellular actin rings (McKeown et al. 1998; Prattis et al. 2005). In this system, sma1, the *C. elegans* ortholog of \( \beta_1 \), also localizes apically to these actin rings. In *sma1* mutants the rings fail to productively contract and begin to disorganize, losing connection to the cell membranes. An additional phenotype observed in these mutants is the inability of cells to change their shape, a process normally “directed” by these contractile rings, the end result being a short worm, a phenotype we see as functionally analogous to an unclosed wound in our system.

In Drosophila, \( \beta_1 \) has been previously implicated in modulating cell shape changes during apical constriction of follicle cells (a process also involving actin rings) and has been proposed to function as a link between cross-linked actin networks/rings and the cell membrane (Thomas 2001). Further studies revealed that the C-terminal domain of \( \beta_1 \) has the ability to directly modulate the apical membrane area by regulating endocytosis (Williams et al. 2004), adding one more tantalizing piece of evidence pointing to the fact that \( \beta_1 \) could be a major player in cell shape changes, not only as a structural link but also by directly modulating the membrane area in response to cytoskeletal clues (or vice versa).

Although we know from previous studies that the actin cable is not absolutely required for wound closure (Wood et al. 2002), the process takes much longer without one. In *Rho1* mutant embryos, cells lacking a cable are able to pull the wound closed using filopodia (Wood et al. 2002). The filopodial defect observed in *karst* mutants, adds another line of evidence to the absolute requirement of these structures for wound closure. In addition to the reduced actin cable accumulation and filopodial dynamics in *karst* mutants (which would lack the C-terminal domain responsible for membrane modulation), we also see a lack of cell shape change in the wound edge cells. Taken together, these data and the published work discussed above, introduce the intriguing possibility that \( \beta_1 \) could be serving as a link between wound edge dynamics and the coordinated cell shape changes usually observed in wild-type wound edge cells. The combination of the proposed ability of \( \beta_1 \) to modulate the apical membrane area as well as cross-link actin and act as an apical membrane-wide scaffold for other interactions, makes \( \beta_1 \) a good candidate to provide the physical link that would coordinate tissue-wide actions, such as supracellular actin cable contraction, with the individual cellular responses, such as cell shape change and polarized filopodia activity.

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Genetic Screen in *Drosophila melanogaster* Uncovers a Novel Set of Genes Required for Embryonic Epithelial Repair

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| Insertional Mutant     | n=  | % holes |
|------------------------|-----|---------|
| 140uf07279             | 33  | 12.1    |
| Aals-met09449          | 61  | 4.9     |
| Aals-pro09660          | 59  | 0.0     |
| abd01907               | 57  | 1.8     |
| abd01908               | 30  | 10.0    |
| Ac00215                | 62  | 1.6     |
| Act00971               | 50  | 6.0     |
| Akap200EP238          | 41  | 0.0     |
| ak01261                | 42  | 11.9    |
| am00332                | 38  | 10.5    |
| Antp06610              | 0   | *       |
| Ant106648              | 206 | 27.7    |
| AP-2003132             | 56  | 3.6     |
| Apc2-03212             | 48  | 8.3     |
| aralar1EP1506          | 283 | 33.2    |
| Arc-p2900819           | 189 | 43.4    |
| Atf512EP15062          | 37  | 0.0     |
| Atf502275              | 49  | 14.3    |
| Atf87C04425            | 92  | 22.8    |
| Atf89PS1007            | 86  | 30.2    |
| Atf7160396             | 0   | **      |
| att-ORFA00114 or att-ORFB00114 | 46  | 15.2    |
| Atch05745              | 49  | 2.0     |
| bch066813              | 213 | 11.7    |
| Gene   | Symbol | p-Value | q-Value |
|--------|--------|---------|---------|
| beat-V | 06401  | 56      | 8.9     |
| Best1  | 007108 | 86      | 14.0    |
| Bif    | 66161  | 66      | 0.0     |
| Bruce  | 00924  | 59      | 16.9    |
| Btk29A | P2157  | 52      | 7.7     |
| c(2)M  | EP2115 | 83      | 0.0     |
| c02493 |        | 25      | 0.0     |
| c05413 |        | 55      | 5.5     |
| c05496 |        | 51      | 0.0     |
| c05504 |        | 40      | 10.0    |
| c07013 |        | 26      | 0.0     |
| Cad96C | 07355  | 65      | 3.1     |
| CAP-D2 | P03381 | 48      | 2.1     |
| Cap-D3 | 02131  | 182     | 26.4    |
| Cap-G  | G0093  | 89      | 18.0    |
| Cap-GP | P2146  | 10      | 0.0     |
| capG   | H01676 | 45      | 8.9     |
| capuH  | 00258  | 96      | 21.9    |
| Cdt1   | HP3346 | 0       | *       |
| cdcl   | 03195  | 31      | 9.7     |
| Cdc8   | 05337  | 57      | 17.5    |
| Cdc12  | H06260 | 48      | 12.5    |
| CG10137| 014516 | 40      | 10.0    |
| CG10139| 016035 | 36      | 2.8     |
| CG10154| 01176  | 57      | 1.8     |
| CG10168| 009185 | 43      | 7.0     |
| CG10195| 506099 | 55      | 0.0     |
| Gene   | Description | Start | End   | Length | Coverage |
|--------|-------------|-------|-------|--------|----------|
| CG10217 |            | 205   | 95.1  |        |          |
| CG10259 |            | 94    | 22.3  |        |          |
| CG10289 |            | 35    | 0.0   |        |          |
| CG10335 |            | 38    | 21.1  |        |          |
| CG10341 |            | 50    | 8.0   |        |          |
| CG10343 |            | 111   | 15.3  |        |          |
| CG10413 |            | 50    | 2.0   |        |          |
| CG10414 |            | 52    | 3.8   |        |          |
| CG10429 |            | 110   | 10.9  |        |          |
| CG10561 |            | 76    | 15.8  |        |          |
| CG10600 |            | 160   | 29.4  |        |          |
| CG10602 |            | 40    | 27.5  |        |          |
| CG10627 |            | 67    | 14.9  |        |          |
| CG10628 |            | 69    | 27.5  |        |          |
| CG10650 |            | 44    | 13.6  |        |          |
| CG10658 |            | 90    | 7.8   |        |          |
| CG10754 |            | 43    | 0.0   |        |          |
| CG10907 |            | 46    | 21.7  |        |          |
| CG11007 |            | 55    | 3.6   |        |          |
| CG11030 |            | 127   | 27.6  |        |          |
| CG11070 |            | 18    | 0.0   |        |          |
| CG11089 |            | 130   | 35.4  |        |          |
| CG11166 |            | 97    | 30.9  |        |          |
| CG11180 |            | 67    | 3.0   |        |          |
| CG11188 |            | 99    | 11.1  |        |          |
| CG11200 |            | 55    | 10.9  |        |          |
| CG11241 |            | 62    | 12.9  |        |          |
| Gene Symbol | Gene ID | Time (h) |
|-------------|---------|----------|
| CG11266 | g07714 | 66 | 13.6 |
| CG11319 | g06271 | 40 | 2.5 |
| CG11419 | g01070 | 39 | 5.1 |
| CG11426 | g00246 | 49 | 4.1 |
| CG11457 | g08265 | 47 | 12.8 |
| CG11486 | EP0109 | 55 | 0.0 |
| CG11490 | g08523 | 59 | 0.0 |
| CG11583 | 01124 | 39 | 0.0 |
| CG11593 | g06001 | 52 | 0.0 |
| CG11839 | g09501 | 55 | 18.2 |
| CG11851 | EP0021 | 112 | 27.7 |
| CG11896 | g08516 | 61 | 0.0 |
| CG11927 | 04401 or mRPS2 | 133 | 22.6 |
| CG11984 | 14981 | 42 | 4.8 |
| CG12063 | g06002 | 104 | 20.2 |
| CG12140 | g05649 | 100 | 29.0 |
| CG12267 | g05999 | 40 | 0.0 |
| CG12314 | 001342 | 143 | 23.1 |
| CG12348 | 001488 | 58 | 3.4 |
| CG12413 | 00268 | 114 | 27.2 |
| CG12753 | 087673 | 69 | 0.0 |
| CG12829 | g02002 | 48 | 8.3 |
| CG12901 | g07656 | 70 | 7.1 |
| CG12934 | 00283 | 247 | 37.7 |
| CG13018 | g05355 | 90 | 27.8 |
| CG13109 | EP0238 | 56 | 8.9 |
| Gene      | Chromosome | Distance |
|-----------|------------|----------|
| CG13131   | 3096310    | 84       |
| CG13377   | 401398     | 47       |
| CG13398   | 403476     | 49       |
| CG13444   | 502566     | 54       |
| CG13466   | 602655     | 42       |
| CG13527   | 704452     | 63       |
| CG13551   | 803979     | 63       |
| CG13594   | 908918     | 57       |
| CG13689   | 100073     | 52       |
| CG13776   | 110248     | 88       |
| CG13792   | 1201301    | 68       |
| CG13993   | 132570     | 35       |
| CG14017   | 145326     | 51       |
| CG14023   | 154290     | 44       |
| CG14057   | 165520     | 52       |
| CG14133   | 1785716    | 59       |
| CG14275   | 1800410    | 48       |
| CG14544   | 191091     | 42       |
| CG14598   | 201297     | 67       |
| CG14655   | 214488     | 35       |
| CG14830   | 220532     | 48       |
| CG14894   | 234037     | 43       |
| CG14957   | 245937     | 52       |
| CG14992   | 250653     | 31       |
| CG15170   | 260629     | 75       |
| CG15173   | 2705160    | 21       |
| CG15436   | 2807763    | 51       |
| Gene Symbol | Gene ID | p-value |
|-------------|---------|---------|
| CG15443     | 0.03556 | 83      |
| CG15443     | 0.03827 | 34      |
| CG15443     | 0.03116 | 101     |
| CG15625     | 0.03818 | 36      |
| CG15625     | 0.03581 | 56      |
| CG15625     | 0.08079 | 98      |
| CG15695     | 0.04218 | 55      |
| CG15696     | 0.04976 | 60      |
| CG15706     | 0.06092 | 48      |
| CG1600EP    | 0.0198  | 140     |
| CG1603      | 0.02443 | 62      |
| CG16075     | 0.06671 | 108     |
| CG16786     | 0.03819 | 60      |
| CG1681      | 0.06400 | 38      |
| CG16833     | 0.01119 | 84      |
| CG16865     | 0.04739 | 53      |
| CG16904     | 0.03307 | 33      |
| CG16904     | 0.03104 | 54      |
| CG16947     | 0.00837 | 50      |
| CG16977     | 0.02981 | 64      |
| CG17086     | 0.02395 | 28      |
| CG17141     | 0.05038 | 84      |
| CG17202     | 0.01979 | 116     |
| CG17211     | 0.03045 | 36      |
| CG17221     | 0.00569 | 61      |
| CG17350     | 0.03687 | 48      |
| CG17379     | 0.05380 | 40      |
| CG17562 | 68 | 13.2 |
| CG17597 | 110 | 0.9 |
| CG17612 | 91 | 18.7 |
| CG17658 | 57 | 7.0 |
| CG17712 | 63 | 0.0 |
| CG1776 | 75 | 8.0 |
| CG17838 | 105 | 21.0 |
| CG17912 | 52 | 5.8 |
| CG18131 | 51 | 2.0 |
| CG18158 | 60 | 0.0 |
| CG18522 | 62 | 19.4 |
| CG18539 | 48 | 8.3 |
| CG18606 | 55 | 12.7 |
| CG18619 | 51 | 7.8 |
| CG18877 | 65 | 0.0 |
| CG1894 | 49 | 4.1 |
| CG1957 | 25 | 8.0 |
| CG19620 | 26 | 3.8 |
| CG2107 | 50 | 0.0 |
| CG2121 | 53 | 5.7 |
| CG2493 | 118 | 21.2 |
| CG2747 | 40 | 12.5 |
| CG2767 | 103 | 15.5 |
| CG2819 | 142 | 79.6 |
| CG2921 | 36 | 2.8 |
| CG2950 | 55 | 9.1 |
| CG3001 | 104 | 42.3 |
| Gene       | Value1 | Value2 |
|------------|--------|--------|
| CG30158    | 65     | 3.1    |
| CG30299    | 55     | 1.8    |
| CG30431    | 58     | 8.6    |
| CG30437    | 42     | 14.3   |
| CG30497    | 106    | 0.0    |
| CG31004    | 59     | 10.2   |
| CG31005    | 137    | 22.6   |
| CG31120    | 61     | 27.9   |
| CG31121    | 36     | 16.7   |
| CG31211    | 55     | 5.5    |
| CG31316    | 51     | 5.9    |
| CG31337    | 65     | 10.8   |
| CG31360    | 157    | 29.3   |
| CG31374    | 48     | 6.3    |
| CG31600    | 20     | 0.0    |
| CG31710    | 47     | 0.0    |
| CG31720    | 53     | 5.7    |
| CG31730    | 47     | 14.9   |
| CG31736    | 58     | 17.2   |
| CG31805    | 114    | 33.3   |
| CG31851    | 42     | 11.9   |
| CG31855    | 140    | 27.1   |
| CG31871    | 34     | 20.6   |
| CG31877    | 73     | 1.4    |
| CG31899    | 46     | 0.0    |
| CG31901    | 64     | 3.1    |
| CG31902    | 52     | 0.0    |
| Gene       | Expression | Log2 Fold Change |
|------------|------------|------------------|
| CG3190     | 51         | 11.8             |
| CG3193      | 53         | 3.8              |
| CG3195      | 43         | 18.6             |
| CG3196      | 50         | 24.0             |
| CG3197      | 18         | 22.2             |
| CG3208      | 47         | 0.0              |
| CG3211      | 60         | 3.3              |
| CG3213      | 156        | 15.4             |
| CG3217      | 56         | 7.1              |
| CG3226      | 52         | 5.8              |
| CG3235      | 68         | 10.3             |
| CG3237      | 58         | 3.4              |
| CG3241      | 60         | 3.3              |
| CG3242      | 0          | *                |
| CG3249      | 149        | 18.1             |
| CG3250      | 63         | 3.2              |
| CG3267      | 62         | 17.7             |
| CG3269      | 56         | 7.1              |
| CG3285      | 65         | 1.5              |
| CG3294      | 221        | 32.6             |
| CG3297      | 41         | 14.6             |
| CG3316      | 48         | 2.1              |
| CG3317      | 163        | 13.5             |
| CG3319      | 280        | 47.9             |
| CG3322      | 61         | 13.1             |
| CG3335      | 27         | 11.1             |
| CG3393      | 51         | 5.9              |
| Code     | Value1 | Value2 |
|----------|--------|--------|
| CG3412   | 112    | 8.0    |
| CG3412   | 49     | 0.0    |
| CG3436   | 57     | 8.8    |
| CG3436   | 68     | 0.0    |
| CG3436   | 59     | 6.8    |
| CG3438   | 42     | 16.7   |
| CG3440   | 98     | 16.3   |
| CG3542   | 56     | 17.9   |
| CG3563   | 56     | 8.9    |
| CG3590   | 198    | 26.8   |
| CG3609   | 107    | 29.0   |
| CG3609   | 48     | 16.7   |
| CG3645   | 51     | 21.6   |
| CG3662   | 51     | 3.9    |
| CG3683   | 44     | 0.0    |
| CG3700   | 43     | 2.3    |
| CG3714   | 58     | 12.1   |
| CG3764   | 65     | 3.1    |
| CG3803   | 50     | 4.0    |
| CG3983   | 55     | 29.1   |
| CG4389   | 167    | 38.9   |
| CG4398   | 61     | 9.8    |
| CG4484   | 42     | 4.8    |
| CG4497   | 119    | 22.7   |
| CG4554   | 85     | 0.0    |
| CG4594   | 113    | 4.4    |
| CG4658   | 56     | 3.6    |
| Gene     | Expression |
|----------|------------|
| CG4674   | 27         |
| CG4738   | 50         |
| CG4757   | 43         |
| CG4774   | 116        |
| CG4774   | 76         |
| CG4836   | 75         |
| CG4848   | 51         |
| CG4851   | 58         |
| CG4933   | 50         |
| CG4942   | 26         |
| CG4959   | 24         |
| CG5003   | 48         |
| CG5091   | 59         |
| CG5126   | 53         |
| CG5147   | 48         |
| CG5148   | 48         |
| CG5149   | 61         |
| CG5149   | 70         |
| CG5156   | 53         |
| CG5168   | 38         |
| CG5181   | 46         |
| CG5189   | 41         |
| CG5198   | 147        |
| CG5276   | 68         |
| CG5290   | 91         |
| CG5342   | 64         |
| CG5384   | 55         |

CG4674: 086455
CG4738: 09322
CG4757: 07133
CG4774: 01874
CG4774: 01121
CG4836: 04238
CG4848: 03040
CG4851: 02813
CG4933: 01978
CG4942: 00071
CG4959: 02213
CG5003: 02566
CG5091: 04215
CG5126: 09916
CG5147: 03412
CG5148: 05646
CG5149: 06708
CG5149: 03983
CG5156: 05647
CG5168: 04080
CG5181: 01211
CG5189: 01140
CG5198: 07150
CG5276: 03385
CG5290: 002563
CG5342: 01976
CG5384: 06779
| Gene   | Feature | Value |
|--------|---------|-------|
| CG5451 | e03563  | 71    |
|        |         | 1.4   |
| CG5451 | e03690  | 64    |
|        |         | 9.4   |
| CG5508 | f04927  | 36    |
|        |         | 2.8   |
| CG5515 | e02644  | 93    |
|        |         | 5.4   |
| CG5525 | EP642   | 51    |
|        |         | 2.0   |
| CG5567 | f03921  | 88    |
|        |         | 10.2  |
| CG5589 | f06132  | 53    |
|        |         | 0.0   |
| CG5602 | f06992  | 46    |
|        |         | 6.5   |
| CG5626 | f02731  | 30    |
|        |         | 10.0  |
| CG5640 | f01321  | 76    |
|        |         | 47.4  |
| CG5645 | f03479  | 48    |
|        |         | 0.0   |
| CG5660 | f01783  | 93    |
|        |         | 6.5   |
| CG5758 | c01197  | 112   |
|        |         | 28.6  |
| CG5758 | f0197   | 59    |
|        |         | 13.6  |
| CG5780 | EP315   | 46    |
|        |         | 2.2   |
| CG5807 | f05043  | 41    |
|        |         | 2.4   |
| CG5850 | f03122  | 36    |
|        |         | 0.0   |
| CG5888 | f02257  | 61    |
|        |         | 6.6   |
| CG5931 | f01711  | 62    |
|        |         | 14.5  |
| CG5970 | f00883  | 41    |
|        |         | 22.0  |
| CG6005 | f07117  | 100   |
|        |         | 38.0  |
| CG6113 | f07009  | 67    |
|        |         | 10.4  |
| CG6126 | f09005  | 64    |
|        |         | 6.3   |
| CG6136 | f02814  | 36    |
|        |         | 2.8   |
| CG6171 | f07144  | 43    |
|        |         | 0.0   |
| CG6180 | f02988  | 233   |
|        |         | 9.0   |
| CG6196 | f09865  | 107   |
|        |         | 10.3  |
| ID     | Value | 13   | 0.0   |
|--------|-------|------|-------|
| CG6225 |       | 13   | 0.0   |
| CG6357 |       | 60   | 23.3  |
| CG6393 |       | 57   | 12.3  |
| CG6568 |       | 54   | 9.3   |
| CG6608 |       | 36   | 5.6   |
| CG6637 |       | 35   | 8.6   |
| CG6678 |       | 59   | 8.5   |
| CG6686 |       | 126  | 19.0  |
| CG6724 |       | 59   | 0.0   |
| CG6729 |       | 43   | 0.0   |
| CG6739 |       | 46   | 4.3   |
| CG6746 |       | 51   | 17.6  |
| CG6750 |       | 212  | 37.3  |
| CG6792 |       | 60   | 0.0   |
| CG6856 |       | 120  | 18.3  |
| CG6907 |       | 224  | 27.7  |
| CG6931 |       | 52   | 5.8   |
| CG6951 |       | 33   | 0.0   |
| CG7029 |       | 64   | 12.5  |
| CG7081 |       | 59   | 15.3  |
| CG7191 |       | 43   | 0.0   |
| CG7202 |       | 49   | 2.0   |
| CG7214 |       | 72   | 0.0   |
| CG7263 |       | 55   | 18.2  |
| CG7371 |       | 75   | 9.3   |
| CG7394 |       | 0    | *     |
| CG7532 |       | 70   | 5.7   |
| Gene  | Expression | Code   |
|-------|------------|--------|
| CG7549| 31         | 0.0    |
| CG7627| 282        | 30.9   |
| CG7638| 52         | 1.9    |
| CG7639| 16         | 6.3    |
| CG7759| 0          | **     |
| CG7806| 103        | 27.2   |
| CG7816| 66         | 1.5    |
| CG7816| 50         | 2.0    |
| CG7816| 82         | 24.4   |
| CG7844| 60         | 8.3    |
| CG7861| 61         | 13.1   |
| CG7870| 64         | 18.8   |
| CG7891| 40         | 5.0    |
| CG7911| 40         | 5.0    |
| CG8064| 75         | 6.7    |
| CG8083| 43         | 0.0    |
| CG8086| 46         | 0.0    |
| CG8233| 105        | 21.9   |
| CG8270| 30         | 13.3   |
| CG8412| 61         | 9.8    |
| CG8414| 59         | 11.9   |
| CG8419| 40         | 7.5    |
| CG8494| 83         | 27.7   |
| CG8516| 33         | 3.0    |
| CG8552| 59         | 25.4   |
| CG8552| 105        | 23.8   |
| CG8745| 58         | 3.4    |
| Gene ID  | Frequency | Clustering |
|----------|-----------|------------|
| CG8777   | 67        | 13.4       |
| CG8861   | 34        | 5.9        |
| CG9003   | 78        | 1.3        |
| CG9143   | 54        | 25.9       |
| CG9162   | 60        | 0.0        |
| CG9249   | 93        | 7.5        |
| CG9249   | 182       | 51.1       |
| CG9264   | 30        | 3.3        |
| CG9265   | 79        | 8.9        |
| CG9289   | 72        | 27.8       |
| CG9293   | 93        | 24.7       |
| CG9296   | 49        | 0.0        |
| CG9320   | 51        | 25.5       |
| CG9510   | 52        | 1.9        |
| CG9555   | 88        | 15.9       |
| CG9596   | 46        | 19.6       |
| CG9603   | 32        | 9.4        |
| CG9603   | 59        | 8.5        |
| CG9669   | 68        | 1.5        |
| CG9778   | 105       | 27.6       |
| CG9922   | 34        | 2.9        |
| CG9932   | 60        | 0.0        |
| CG9987   | 70        | 7.1        |
| CheA29a  | 130       | 5.4        |
| CHORD    | 50        | 12.0       |
| cir5     | 55        | 0.0        |
| CB1       | 61        | 0.0        |
| Gene/Protein | Accession | Value |
|-------------|-----------|-------|
| Cpr50Ch     | e02005    | 53    |
| Cpr51A      | e03998    | 56    |
| Cpr62Be     | e01009    | 65    |
| Cpr64Ad     | e02305    | 43    |
| CSN3        | e02855    | 52    |
| CSN7        | e02176    | 65    |
| Cyp4c3      | e02505    | 43    |
| CSN7        | e02176    | 65    |
| cv-004940   |           | 50    |
| cv-007633   |           | 69    |
| CycA        | e05304    | 50    |
| Cyp4c3      | e06288    | 34    |
| cype        | e03803    | 54    |
| d-00148     |           | 93    |
| Dcr-2       | e095544   | 50    |
| def-00109   |           | 52    |
| def-02039   |           | 80    |
| Dip-C       | e06706    | 49    |
| DXApol-α    | e02592    | 114   |
| DopErR      | e02142    | 52    |
| dream       | e00801    | 189   |
| dr-01777    |           | 36    |
| dys-00009    |           | 50    |
| dyn-p2.5-02174 |       | 53    |
| e02022      |           | 55    |
| Eaat2       | e03003    | 22    |
| Eaf6        | e06605    | 37    |
| Ect4        | e03349    | 136   |
| Ect4        | e03749    | 49    |
| Gene          | Value |
|--------------|-------|
| Edg84A        | 48    |
| egfP0093     | 55    |
| dIF-1A        | 46    |
| dIF2B-gamma   | 280   |
| elr80020     | 64    |
| Elongin-C     | 51    |
| emp01154     | 68    |
| Ena01613      | 58    |
| Ena02743      | 51    |
| EP1244        | 109   |
| EP2404        | 32    |
| EP2515        | 40    |
| EP2520        | 59    |
| EP3542        | 57    |
| EP732         | 57    |
| EP937         | 58    |
| EP995         | 63    |
| Eps-1         | 19    |
| esd01154      | 140   |
| emp00417      | 61    |
| Etv21C        | 58    |
| f04861        | 68    |
| fred02229     | 60    |
| gavz          | 70    |
| Gas185365     | 111   |
| gskhe         | 36    |
| gka03598      | 50    |
| Gene     | Strain    | Length | Activity |
|----------|-----------|--------|----------|
| glf02674 |           | 221    | 59.7     |
| Glw-R1B  | f01737    | 49     | 4.1      |
| gog02564 |           | 34     | 0.0      |
| Gp28h01184|          | 92     | 6.5      |
| Gop7.5g5403|          | 43     | 0.0      |
| gyp00087 |           | 171    | 43.9     |
| GpiH02488|           | 162    | 35.2     |
| GaiD606796|          | 62     | 17.7     |
| GaiDg00084|          | 44     | 9.1      |
| GaiEg00084|          | 167    | 18.6     |
| Ga49Bh0219|          | 47     | 21.3     |
| GY33A00834|          | 108    | 25.0     |
| Hand003901|          | 0      | **       |
| hag00028 |           | 55     | 0.0      |
| Hdl25E02545|          | 48     | 22.9     |
| hep00087 |           | 60     | 21.7     |
| HepEP2450|          | 63     | 4.8      |
| Hepg00656 |          | 34     | 0.0      |
| HGTX00083 |          | 0      | *        |
| Hepf00246 |           | 74     | 6.8      |
| HephSP410 |          | 41     | 0.0      |
| Hrb27C04375|          | 0      | **       |
| hsl007110 |           | 49     | 0.0      |
| HtaA2003785 |          | 50     | 17.2     |
| ima007155 |           | 108    | 4.6      |
| ire-1f00170 |          | 0      | *        |
| itpEP2287 |           | 66     | 13.6     |
| Gene      | Library | Scores |
|-----------|---------|--------|
| jhp004551| 69      | 5.8    |
| k05816b   | 60      | 0.0    |
| KaiRIAd   | 68      | 20.6   |
| Khc02141  | 67      | 3.0    |
| kdc03205  | 63      | 20.6   |
| Kk02512   | 167     | 18.0   |
| kaf01902  | 37      | 13.5   |
| krimp00583| 110     | 28.2   |
| krc02503  | 49      | 14.3   |
| ks011183  | 362     | 49.7   |
| l(2)34Fa  | 126     | 10.3   |
| l(2)k07433| 56      | 3.6    |
| leaEP2502 | 0       | ***    |
| lig04268  | 112     | 20.5   |
| Liprin-αEp2141 | 63 | 0.0 |
| lmg00301  | 92      | 19.6   |
| lolaEP2537 | 54 | 0.0 |
| loq00791  | 51      | 2.0    |
| Lr4700177  | 52      | 7.7    |
| max01209  | 40      | 5.0    |
| mats00377  | 62      | 14.5   |
| Mdh411968  | 98      | 6.1    |
| MED15004180 | 58 | 8.6 |
| MED20099555 | 32 | 6.3 |
| Mg2EP28002a | 38 | 0.0 |
| MESR3EP2221 | 57 | 5.3 |
| Met75Ca000116 | 44 | 2.3 |
| gene   | expression | fold change |
|--------|------------|-------------|
| mlp00910 | 83         | 16.9        |
| mlp4009474 | 50         | 0.0         |
| M1d001445 | 25         | 4.0         |
| mlp002679 | 60         | 18.3        |
| morgEP1184 | 91         | 17.6        |
| mRpLJ005962 | 45         | 0.0         |
| mRpL2406092 | 48         | 16.7        |
| mRpL300543 | 80         | 10.0        |
| mRpL5104701 | 66         | 3.0         |
| mRpLg00642 | 95         | 5.3         |
| mRpS2103199 | 57         | 3.5         |
| mRpS2802339 | 50         | 6.0         |
| mRpS2803336 | 52         | 0.0         |
| mRpS3301766 | 103        | 20.4        |
| Mt3GPh04049 | 116        | 24.1        |
| MTA1-like00146 | 39         | 5.1         |
| NaCP60EP148 or RpL41EP148 | 71         | 5.6         |
| nAcRα-34E00672 | 30         | 16.7        |
| Ncks30C00661 | 37         | 10.8        |
| Nep402841 | 41         | 2.4         |
| nep00249 | 53         | 1.9         |
| Neu3-01935 | 229        | 48.5        |
| NLag01602 | 110        | 29.1        |
| nocturnin005983 | 50         | 12.0        |
| nox65142 | 48         | 8.3         |
| nox006114 | 62         | 25.8        |
| Notum00039 | 47         | 14.9        |
| Gene        | Value1 | Value2 |
|------------|--------|--------|
| n-3yb      | 45     | 0.0    |
| nxf2      | 38     | 5.3    |
| Nat1F      | 57     | 10.5   |
| dlf41      | 82     | 22.0   |
| Optix      | 63     | 0.0    |
| Os35a      | 56     | 3.6    |
| otf6278    | 40     | 0.0    |
| Osit087607 | 0      | **     |
| olkEP2017  | 105    | 16.2   |
| Pcaf       | 45     | 4.4    |
| Pcaf      | 50     | 0.0    |
| P01890     | 311    | 54.7   |
| PDCD-5     | 52     | 15.4   |
| Pde1I      | 59     | 13.6   |
| Pde1c      | 40     | 0.0    |
| pdm39828   | 30     | 16.7   |
| pgant402186 | 63 | 12.7   |
| Pcoa92367  | 58     | 8.6    |
| pae03506   | 74     | 4.1    |
| Phe98E     | 81     | 23.5   |
| Pldp06925  | 56     | 3.6    |
| pld01145   | 0      | **     |
| Pp2A-29B   | 52     | 9.6    |
| frominin-lik | 37 | 2.7    |
| ProsQ      | 40     | 0.0    |
| Pp18G05974 | 74     | 0.0    |
| Pp61P065292 | 56 | 1.8    |
| Protein/Genome | Gene ID | Value 1 | Value 2 |
|----------------|---------|---------|---------|
| Ptpmeg90147 or meth11090147 |         | 47      | 0.0     |
| pag93481 |         | 44      | 4.5     |
| qkr54B02079 |         | 121     | 9.9     |
| Rab1-01287 |         | 62      | 11.3    |
| Rab36G01805 |         | 62      | 0.0     |
| Rab6EP2397 |         | 38      | 0.0     |
| Rad1002242 |         | 107     | 26.2    |
| RhoGapEP1773 |       | 53      | 11.3    |
| Repgap1-04534 |       | 47      | 4.3     |
| Rec1-02614 |         | 42      | 9.5     |
| Rab002994 |         | 93      | 25.8    |
| Rep01510 |         | 63      | 0.0     |
| rep011801 |         | 42      | 0.0     |
| rho-5101415 |       | 66      | 0.0     |
| RhoGAPI00F08128 | | 61      | 0.0     |
| RhoGEF2-01784 |       | 83      | 14.5    |
| RhaC-2EP2102 |       | 58      | 12.1    |
| Roc2EP2187 |         | 46      | 4.3     |
| Rph11-01628 |       | 47      | 17.0    |
| Rpm1EP05388 |       | 67      | 26.9    |
| RpS15-01611 |       | 60      | 15.0    |
| Rpt1EP2153 or CG17985EP2153 | | 47      | 6.4     |
| Rep1-00605 |         | 43      | 4.7     |
| SAA00622 |         | 45      | 15.6    |
| Samuelo2949 |       | 59      | 20.3    |
| Sh02563 |         | 0       | *       |
| Sep05078 |         | 52      | 9.6     |
| Gene  | Symbol | Product | P-value | q-value |
|-------|--------|---------|---------|---------|
| SdhB  | c00364 | 37      | 5.4     |         |
| sds2  | c00975 | 62      | 21.0    |         |
| sec10 | 000365 | 77      | 1.3     |         |
| sec31 | 02461  | 51      | 11.8    |         |
| sec45 | 00080  | 80      | 22.5    |         |
| sec12 | 00416  | 212     | 43.9    |         |
| sepp  | 00211  | 63      | 12.7    |         |
| Shab  | 00095  | 61      | 9.8     |         |
| sidb  | 000577 | 130     | 59.2    |         |
| SMC2  | 004642 | 46      | 8.7     |         |
| sidb  | 00174  | 49      | 14.3    |         |
| sur   | 00971  | 84      | 6.0     |         |
| spic1 | EP2282 | 36      | 0.0     |         |
| sp4   | EP2237 | 60      | 0.0     |         |
| Spn1  | 02145  | 113     | 23.0    |         |
| Spn5  | 01214  | 108     | 22.2    |         |
| srm   | EP2786 | 73      | 27.4    |         |
| SroG4 | 04709  | 65      | 3.1     |         |
| Ssb-c31a| 02272 | 63      | 4.8     |         |
| sta6  | 01639  | 62      | 21.0    |         |
| Stam  | 00677  | 183     | 43.2    |         |
| Strn  | Mlck   | 105     | 22.9    |         |
| su(Hgr)| 04961  | 0       | *       |         |
| Sur-G | 02803  | 81      | 12.3    |         |
| Surp  | 00074  | 69      | 14.5    |         |
| Surf  | 004274 | 64      | 1.6     |         |
| synp  | 02397  | 51      | 11.8    |         |
| Gene | Symbol | ID   | Value |
|------|--------|------|-------|
| SynX5 | SynX5  | P2313| 51    |
| Taf6 | Taf6   | 00090| 139   |
| Tbp6 | Tbp6   | 03198| 54    |
| T-cf | T-cf   | 05367| 0*    |
| Taf11 | Taf11 | 000976| 73    |
| Tex | Tex   | 000549| 101   |
| TEAM | TEAM   | 01714| 32    |
| TjIIIA-L | TjIIIA-L | 00087| 70    |
| TjHIIalpha | TjHIIalpha | 01362| 59    |
| TK | TK    | 000233 or Ect3 | 121 |
| TexFP1162 | TexFP1162 | 40   | 0.0   |
| Top3α | Top3α  | EP2372| 47    |
| Topfor | Topfor | 03115| 36    |
| TaoC | TaoC  | 01760| 228   |
| TafDEP578 | TafDEP578 | 138  | 0.0   |
| tex00572 | tex00572 | 55   | 9.1   |
| TscJ | TscJ  | 000910| 58    |
| tup | tup   | 000913| 0*    |
| Ubx | Ubx   | 000281| 66    |
| UGP | UGP   | 007256| 96    |
| UGP | UGP   | 005315| 56    |
| Ugt58Fa | Ugt58Fa | 05973| 64    |
| Ugt86De | Ugt86De | 00862| 58    |
| UK11 | UK11  | 05386| 45    |
| unc-104 | unc-104 | 11204| 51    |
| uncEP124 | uncEP124 | 46   | 4.3   |
| Usp | Usp   | 04888| 68    |
| Gene       | n | % Holes |
|------------|---|---------|
| wls06300   | 52| 19.2    |
| Wwox04545  | 82| 14.6    |
| yellow-e3e01012 | 112| 25.9  |
| epsilon0907 | 59| 22.0    |
| Zeta060020 | 0 | **      |

655 insertional mutant lines obtained from Bloomington Stock Centre and re-balanced with CyO-CTG or TM3-TTG, depending on insertion site, were screened for wound healing phenotypes as described in Material and Methods. Number of embryos analysed (n) and percentage of opened wounds approximately 16 hours post wounding (% holes) are depicted. * - lines impossible to balance with CyO-CTG or TM3-TTG, depending on insertion site; ** - lines impossible to wound or score due to strong morphological defects; *** - contaminated stocks.