**cdc37 is essential for JNK pathway activation and wound closure in Drosophila**

Chan-wool Lee, Young-Chang Kwon, Youngbin Lee, Min-Yoon Park, and Kwang-Min Choe*

Department of Systems Biology, Yonsei University, Seodaemun-gu, Seoul 03722, South Korea

**ABSTRACT** Wound closure in the Drosophila larval epidermis mainly involves nonproliferative, endocytosing epithelial cells. Consequently, it is largely mediated by cell growth and migration. We discovered that both cell growth and migration in Drosophila require the cochaperone-encoding gene cdc37. Larvae lacking cdc37 in the epidermis failed to close wounds, and the cells of the epidermis failed to change cell shape and polarize. Likewise, wound-induced cell growth was significantly reduced, and correlated with a reduction in the size of the cell nucleus. The c-Jun N-terminal kinase (JNK) pathway, which is essential for wound closure, was not typically activated in injured cdc37 knockdown larvae. In addition, JNK, Hep, Mkk4, and Tak1 protein levels were reduced, consistent with previous reports showing that Cdc37 is important for the stability of various client kinases. Protein levels of the integrin β subunit and its wound-induced protein expression were also reduced, reflecting the disruption of JNK activation, which is crucial for expression of integrin β during wound closure. These results are consistent with a role of Cdc37 in maintaining the stability of the JNK pathway kinases, thus mediating cell growth and migration during Drosophila wound healing.

**INTRODUCTION**

The healing of a mammalian skin wound is complex and involves various cellular processes, including blood clotting, inflammation, epithelial cell proliferation and migration, and matrix synthesis and remodeling, which span multiple tissues (Martin, 1997; Gurtner et al., 2008; Shaw and Martin, 2009). In contrast, wound healing in the Drosophila larval epidermis is simple: the epidermis consists of a single, nonproliferative cell layer that underlies the protective cuticle. Thus, wound closure involves primarily cuticle regeneration and cell growth and migration, but not proliferation.

Many signaling pathways are required for wound closure in the Drosophila epidermis (reviewed in Tsai et al., 2018). Of these, c-Jun N-terminal kinase (JNK), which is required for a broad range of wound healing processes, is the most crucial. Without JNK, cells cannot properly polarize, change shape, orient toward the wound center, or migrate to close the wound (Ramet et al., 2002; Galko and Krasnow, 2004; Kwon et al., 2010; Park et al., 2018). Conversely, some proteins acting upstream of JNK appear to be redundant in a pathway that includes both canonical and noncanonical factors in regard to the embryonic dorsal closure process (Lesch et al., 2010; Rios-Barrera and Riesgo-Escovar, 2013). Specifically, both JNK and the AP-1 transcription factors DJun (Ura) and Dfos (Kay) are absolutely required for wound closure, and larvae that are lacking any one of these factors cannot repair open wounds. In contrast, the Jun/stress-activated protein (SAP) 2 kinases Hep and Mkk4 are partially redundant, as are the Jun/SAP3 kinases Slpr and Tak1 (Lesch et al., 2010). Although the involvement of the JNK/SAPK pathway in wound healing is well known both in insects and mammals (Angel et al., 2001; Li et al., 2003), the mechanisms underlying the regulation of this pathway are not well understood.

Protein kinases are often associated with the molecular chaperone Hsp90, which helps these client proteins take on their active conformation (Workman et al., 2007; Taipale et al., 2010). Hsp90 interacts with at least 20 other factors, called cochaperones, which either modulate the activity of Hsp90 or affect the specificity of Hsp90 client proteins (Taipale et al., 2010). Cdc37 is one such cochaperone that is known to maintain the function and stability of client kinases (Pearl, 2005; Caplan et al., 2007; Karnitz and Felts, 2007; Taipale et al., 2010), and many kinases are regulated by...
FIGURE 1: cdc37-knockdown larvae display defects in wound closure. (A–E) The epidermis was examined in late third instar larvae of the indicated genotypes, either before wounding (A) or 24 h after injury to the dorsal epidermis (B–E). Cell boundaries were stained red with anti-FasIII antibody, and the nuclei were stained blue with DAPI. The dotted line indicates the wound hole. (A, B) A58-GAL4-only control. (C) A58-GAL4, UAS-cdc37-RNAi12019, UAS-GFP.nls (A58-cdc37-i12019, GFP.nls, hereafter). The strong blue staining patterns show hemocytes attached to the wound site. (D) A58-cdc37-i12019, cdc3770-moi, (E) A58-bskDN, (F) Quantification of the wound closure phenotype. For each genotype, 13 or more larvae were examined. Scale bar: 100 μm (A–E). (G, H) RNAi knockdown of cdc37 was confirmed in the larval epidermis by immunohistochemistry using anti-Cdc37 antibody, as shown in the heat map. cdc37-RNAi was driven using e16E-GAL4 in the posterior half of each segment, leaving the anterior half as an internal control. The dotted line indicates the anterior–posterior compartment boundary. Anterior is up. (G) e16E-only control. (H) e16E-cdc37-i12019. Scale bar: 25 μm (G, H).

Cdc37, but the relationship between Cdc37 and the JNK signaling pathway is not clear.

Cdc37 was originally identified as a yeast cell-cycle regulator that was later found to interact with Hsp90 and v-Src (reviewed in Karnitz and Felts, 2007). Hsp90 and Cdc37 are both structurally and functionally conserved in metazoans (Caplan et al., 2007). In Drosophila, cdc37 was initially isolated from a mutagenesis screen based on its involvement in eye development (Simon et al., 1991), and was later found to be essential for Sevenless receptor tyrosine kinase signaling (Cutforth and Rubin, 1994). Null mutations in cdc37 are recessively lethal, indicating it is required for cell viability (Cutforth and Rubin, 1994; Lange et al., 2002). Cdc37 inhibits Hh and Wnt signaling pathways in both flies and mammalian cells (Swarup et al., 2015) and mediates chromosome segregation and cytokinesis by modulating the function of Aurora B kinase (Lange et al., 2002).

In this study, we isolated cdc37 based on its RNA interference (RNAi) knockdown phenotype in larval epidermal wound closure in Drosophila, and found that cdc37 is required not only for reepithelialization but also for cells to change shape, polarize, and grow during epidermal wound closure, and all of these phenotypes are shared by larvae lacking JNK. Molecularly, cdc37 is required for maintaining the protein levels of JNK pathway components.

RESULTS
cdc37 knockdown disrupts epidermal wound closure

We isolated cdc37 from an RNAi-based genetic screen of essential genes using an epidermal wound closure assay and the larval epidermis-specific A58-GAL4 driver. Whereas wild-type third instar larvae closed a large wound generated by the abrasion of ~30 epidermal cells within 12–24 h (Figure 1, A and B, and Supplemental Figure S1, A–E; Galko and Krasnow, 2004; Kwon et al., 2010), cdc37-knockdown larvae were unable to close an introduced wound even after 30 h (Figure 1, C and F, and Supplemental Figure S1, F–J). In wild-type larvae, cells located one to three rows distal to the wound margin generally undergo the most dramatic change in cell shape due to cell migration (Supplemental Figure S1, B and C; Kwon et al., 2010), but in the A58-GAL4 (UAS-cdc37 RNAi (hereafter, A58-cdc37-i) larvae, these cells displayed a very strong open-wound phenotype. They often retained a pentagonal or hexagonal cell shape even 24–30 h after injury (Figure 1C and Supplemental Figure S1, G–J), which is similar to unwounded epidermis or wounded epidermis expressing a dominant negative Drosophila JNK bsk (bskDN) construct (Figure 1, A and E).

We confirmed the open-wound phenotype using three different approaches. First, we tested five different cdc37 RNAi strains. Although phenotypic strength varied, four of the five strains with the A58-GAL4 driver displayed open wounds (Figure 1F and Supplemental Figure S2, A–D). Second, we generated a UAS transgenic line for Drosophila mojavensis cdc37 (UAS-cdc3770-moi) and overexpressed it in the UAS-cdc37-i background, which displayed the strongest phenotype, for a phenotypic rescue without interference from Drosophila melanogaster cdc37 RNAi (Kondo et al., 2009). This experiment assumed the functional conservation of cdc37 between the two species. The sequence identity and similarity of the Cdc37 protein between the two species were 80.6% and 87.9%, respectively. Interestingly, A58-cdc37-i; cdc3770-moi larvae exhibited...
**cdc37 is required for cell polarization during wound healing**

During *Drosophila* wound healing, cells located near the wound margin polarize toward the wound center, a process that can be monitored by the localization of a GFP-Zip fusion protein (Franke et al., 2005; Baek et al., 2010; Kwon et al., 2010). In wild-type larvae, GFP-Zip proteins localize primarily to the perinuclear region but translocate to the rear side (opposite to the wound) of the cells upon wounding, as was clearly visible 4–10 h after injury in many of the cells in the first to third rows distal to the wound margin (Figure 2A; Kwon et al., 2010). In *cdc37*-knockdown larvae, GFP-Zip proteins were detected mainly around the perinuclear region and the localization was similar to that of unwounded tissues, as if the cells had not received wound signals (Figure 2B).

**cdc37 is required for JNK pathway activation**

The strong open-wound phenotype observed in the *cdc37*-knockdown larvae prompted us to evaluate whether the JNK pathway was properly activated in these larvae using the JNK pathway reporter *msn-lacZ* (Galko and Krasnow, 2004). In wild-type larvae, wound-induced *msn-lacZ* expression was visible up to five to six cell diameters away from the wound margin (Figure 3A), but in *cdc37*-knockdown larvae, *msn-lacZ* expression disappeared almost completely, similar to *bsk^DN*-expressing larvae (Figure 3, B and D). We wanted to confirm these results using another JNK pathway reporter *puc-lacZ*, and obtained essentially the same result (Supplemental Figure S3; Galko and Krasnow, 2004).
induction were rescued to near wild-type levels by the simultaneous overexpression of cdc37-D.moj (Figure 3C). We noticed, however, that msn-lacZ reporter induction was no stronger or broader in the wounded, rescued cdc37 knockdown larvae than in wounded wild-type controls, and that msn-lacZ expression was not induced in unwounded larvae (Figure 3E). These results indicate that cdc37-D.moj expression does not generate a gain-of-function phenotype. To investi-gate this finding further, we generated a UAS transgenic line of D. melanogaster cdc37 (UAS-cdc37-D.moj) and expressed cdc37-D.moj in the epidermis. Overexpression of cdc37-D.moj alone neither induced msn-lacZ expression in unwounded larvae nor increased the induction of msn-lacZ expression in wounded larvae (Figure 3F; unpublished data). Together, these results indicate that cdc37 is necessary, but not sufficient, for activation of the JNK pathway during wound healing, suggesting that cdc37 functions as a permissive factor in JNK signaling.

We next examined whether forced activation of the JNK pathway could rescue the wound healing defects of cdc37 knockdown larvae. First of all, overexpression of the constitutively active form of Hep (HepCA) degenerated epidermal tissues, and thus could not be used for wound analysis, while overexpression of Hep or Jra did not interfere with normal wound closure, examined at 24 h after injury. Overexpression of Jra enhanced msn-lacZ induction upon injury, whereas overexpression of Hep did not (Figure 4, A–C), indicating
cdc37 knockdown disrupts the wound-induced growth of cells and nuclei

Wounding induces endoreplication, and consequently cell growth, in the Drosophila adult epidermis (Losick et al., 2013; Zielke et al., 2013). We noted that the epidermal cells in cdc37 knockdown larvae remained small even 24 h after injury. Thus, we wanted to determine whether cdc37 is required for endoreplication. In unwounded epidermis, cdc37 knockdown via A58-GAL4 significantly reduced nuclear width (p < 0.01; similar to that observed following knockdown of cycE or cdk2, the genes that encode the cyclin and cyclin-dependent kinase required for S phase entry, respectively), consistent with the argument that cdc37 is required for the developmental endocycle of epidermal cells (Figure 5A). Knockdown of cycE or cdk2 did not noticeably interfere with wound closure or wound-induced msn-lacZ expression (Supplemental Figure S4, A–D), indicating that other factors may compensate for retarded cell growth, including cell migration, cell–cell fusion (Losick et al., 2013; Lee et al., 2017), and possibly the thinning of epidermal tissues, which would increase the two-dimensional width.

Next, we attempted to measure the growth of the cells and nuclei in the wounded epidermis. It is generally true that cells proximal to the wound tend to undergo the most prominent changes. However, we also noticed occasionally that cells away from the wound were enlarged greatly, while some cells of the wound margin shrank. These factors interfered with simple size measurement of wound-proximal cells. Therefore, we first measured the width of all cell nuclei in a defined region and then chose those with the largest nuclear areas (top 15%) for further analysis, by marking the cell boundaries for those cells. First, although the average size of the cell nuclei (top 15%) in wounded versus unwounded wild-type larvae increased significantly (p > 0.01), this increase was disrupted in both cdc37-knockdown larvae and bskDN-expressing larvae (Figure 5B). Second, the average size of the cells containing the largest nucleus (top 15%) also increased after wounding in wild-type larvae, but not in cdc37-knockdown or bskDN-expressing larvae (Figure 5C). These results suggest that cdc37 is required for endoreplication and cell growth during wound healing, which also depends on Cdk2, and is consistent with a previous report showing that Cdk2 interacts with Cdc37 (Prince et al., 2005).

JNK and integrin β subunit protein levels are reduced in cdc37-knockdown larvae

As a way to examine whether Cdc37 might influence protein stability, we measured global protein levels of the JNK pathway signaling components in the epidermis of cdc37-knockdown larvae compared with wild-type larvae by Western blotting. JNK protein levels were reduced in the epidermis of knockdown larvae, even before wound incubation (Figure 6, A and B; unpublished data). Activated JNK, assessed using an anti-phospho-JNK antibody, was also reduced, potentially reflecting a reduction in total protein levels (Figure 6, A and C), as were the levels of Hep, Mkk4, and Tak1, kinases acting upstream of JNK (Figure 6, A and D–F). These results are consistent with previous reports showing that Cdc37 increases the stability of client kinases (Caplan et al., 2007; Karnitz and Felts, 2007), although our results cannot exclude the potential involvement of transcriptional or translational regulation.

Because JNK up-regulates protein levels of the integrin β subunit βPS and integrins are essential for wound closure in Drosophila (Lee et al., 2017; Park et al., 2018), we also measured βPS and talin protein levels. βPS levels were greatly reduced in the epidermis of cdc37-knockdown larvae compared with wild-type controls, whereas talin levels did not differ between the two groups of larvae (Figure 6, A, G, and H). These results were confirmed by immunostaining the wounded larval epidermis with anti-βPS antibody (Figure 6, I and J).

**DISCUSSION**

Our results indicate that cdc37 is an essential factor in the activation of the JNK pathway during Drosophila wound healing. Without cdc37, the levels of many proteins involved in JNK pathway signaling, including JNK itself, were severely reduced, suggesting that the stability of these proteins is compromised in the absence of cdc37.
In Drosophila, JNK mediates diverse wound healing responses, including gene expression, cell shape change and polarization, reepithelialization, and cell fusion (Ramet et al., 2002; Galko and Krasnow, 2004; Bosch et al., 2005; Mattila et al., 2005; Campos et al., 2010; Kwon et al., 2010; Lesch et al., 2010; Brock et al., 2012; Losick et al., 2016; Lee et al., 2017; Park et al., 2018). The present study suggests that the JNK pathway also mediates wound-induced endoreplication and cell growth. However, prior reports have indicated that JNK suppresses wound-induced endoreplication in adult stages (Losick et al., 2016), which is a discrepancy that requires further investigation.

Larvae lacking cdc37 displayed disrupted activation of the JNK pathway and displayed phenotypes similar to those of larvae lacking active JNK. Thus, we conclude that most of the cdc37-knockdown phenotypes we analyzed were likely caused by the disruption of JNK activation during wound healing. It should be noted, however, that cell nucleus size and JNK protein levels were also reduced in the unwounded epidermis of cdc37 knockdown larvae, indicating that loss of cdc37 expression also causes developmental defects. This was not unexpected, given that cdc37 null mutations are cell lethal (Lange et al., 2002). Considering that A58-GAL4 is only active after early larval stages (Galko and Krasnow, 2004), and that endoreplicating cells are resistant to apoptosis (Mehrotra et al., 2008; Hassel et al., 2014; Zhang et al., 2014), the wound healing defects in cdc37-knockdown larvae may have been uncovered luckily due to cell stress caused by wound- ing in the apoptosis-resistant epidermal cells.

Cdc37 is best known as a cochaperone that confers client kinase specificity to Hsp90 (Karnitz and Felts, 2007; Taipale et al., 2010). The client kinases requiring the Hsp90-Cdc37 complex for activity and stability are diverse and include Cdk2, Cdk4, Src, Aurora B, Raf1, and RIP3 (Stepanova et al., 1996; Lange et al., 2002; Prince et al., 2005; Li et al., 2015; and reviewed in Hunter and Poon, 1997). However, Cdc37 may also function as an independent molecular chaperone alone, similar to Hsp90 (Kimura et al., 1997). Our investigation into the possible involvement of Hsp90 (also known as Hsp83 in Drosophila) in wound healing using multiple Hsp90 RNAi lines did not yield any definitive answer. We also assessed whether aurora B, cdc2, or ckII were involved in wound healing, as these factors reportedly interact with cdc37 in various contexts (Cutforth and Rubin, 1994; Kimura et al., 1997; Lange et al., 2002; Miyata and Nishida, 2004). Larvae deficient of each of these factors closed wounds normally (Supplemental Figure S4, E–G). Finally, we did not observe any noticeable changes in the protein level or localization of Cdc37 during wound healing (Supplemental Figure S5). Thus, the requirement of cdc37 for JNK activation is a novel finding. Nonetheless, defining the detailed molecular mechanisms underlying Cdc37 functions requires further investigation.

MATERIALS AND METHODS

Fly stocks

The following stocks were obtained from the Bloomington Stock Center: Oregon R, w1118, msn-lacZ (msn[2940]), puc-lacZ, UAS-Jra, UAS-hep, e16E-GAL4, hs-GAL4, UAS-cdc37-RNAi (JF03184, GD14633, HMS01401), UAS-bskRNAi, UAS-luciferase RNAi, UAS-GFP nlS, UAS-Dcr-2, UAS-cdk2-RNAi (HMS00174; Sopko et al., 2014), UAS-cycE-RNAi (HMS00060), and UAS-ckIIΔ256 (Lin et al., 2002). The following stocks were obtained from the Vienna Drosophila RNAi Center: UAS-hep, UAS-cdk2-RNAi (HMS00060), and UAS-ckIIΔ256 (Lin et al., 2002). The client kinases requiring the Hsp90-Cdc37 complex for activity and stability are diverse and include Cdk2, Cdk4, Src, Aurora B, Raf1, and RIP3 (Stepanova et al., 1996; Lange et al., 2002; Prince et al., 2005; Li et al., 2015; and reviewed in Hunter and Poon, 1997). However, Cdc37 may also function as an independent molecular chaperone alone, similar to Hsp90 (Kimura et al., 1997). Our investigation into the possible involvement of Hsp90 (also known as Hsp83 in Drosophila) in wound healing using multiple Hsp90 RNAi lines did not yield any definitive answer. We also assessed whether aurora B, cdc2, or ckII were involved in wound healing, as these factors reportedly interact with cdc37 in various contexts (Cutforth and Rubin, 1994; Kimura et al., 1997; Lange et al., 2002; Miyata and Nishida, 2004). Larvae deficient of each of these factors closed wounds normally (Supplemental Figure S4, E–G). Finally, we did not observe any noticeable changes in the protein level or localization of Cdc37 during wound healing (Supplemental Figure S5). Thus, the requirement of cdc37 for JNK activation is a novel finding. Nonetheless, defining the detailed molecular mechanisms underlying Cdc37 functions requires further investigation.
Generating cdc37D.mel and cdc37D.moj transgenic flies
Total RNA was isolated from mid- to late-third instar larvae of D. melanogaster or D. mojavensis. The corresponding cDNAs were synthesized and the cdc37 open reading frame (ORF) was amplified from the resulting cDNA pools using the following primer sets: 5′-AGCGGCGCATGCTGAGAAGAGA-3′ (forward) and 5′-AGACCTGAGTCTAGTTGGTGCGCAT-3′ (reverse) for D. melanogaster and 5′-AGCGGCGCATGCTGAGAAGAGA-3′ (forward) and 5′-AGACCTGAGTCTAGTTGGTGCGCAT-3′ (reverse) for D. mojavensis. These ORFs were subcloned into a pUAST vector at the NotI-XhoI sites and injected into w1118 embryos to produce transgenic flies.

Generating anti-Cdc37 antibody
Total RNA was isolated from third instar larval and D. mojavensis. The cDNA was amplified from the cDNA pool by PCR using the following primer sets: 5′-AACGATCCCTCCGCGGCTGCGCCACCAAGGGC-3′ (forward) and 5′-AAGCCTGAGTCTAGTTGGTGCGCAT-3′ (reverse) for N-terminal His tagging and 5′-AACCATGTTTCCGCGGCTGCGCCACCAAGGGC-3′ (forward) and 5′-AAGCCTGAGTCTAGTTGGTGCGCAT-3′ (reverse) for C-terminal His-tagging. The resulting fragments were cloned into the vector pET28a for expression in the Escherichia coli (BL21). Histagged proteins were purified using a Ni- bead column. Polyclonal antibodies against the purified Cdc37 peptide were raised in rabbits by Young in Young Prague (Seoul, Korea). In this study, we primarily used antisera obtained using the C-terminal His-tagged fusion (Supplemental Figure S5, A and B).

Wounding and dissection
Mid- to late-third instar larvae were wounded using a pair of forceps (Fine Science Tools; Cat. No. 11295-00) to pinch the outer integument to abrade ~30 epidermal cells on the dorsal side of segment A2 or A3. The larvae were then placed on cornmeal–agar media for recovery. Epidermal tissues were dissected in phosphate-buffered saline (PBS), as described in Park et al. (2018). Samples were fixed in 4% paraformaldehyde for 15 min. For Western blotting and coimmunoprecipitation, five different dorsal segments were wounded to maximize wound healing responses. Unless otherwise specified, at least six larvae were examined for each experiment.

Immunohistochemistry
Fixed samples were washed three times in PBS and incubated with primary antibodies diluted in PBS plus 0.5% Triton X-100 (PBST) supplemented with 1% normal goat serum for 2 h at room temperature. The following primary antibodies were used: mouse anti-Fasciclin III (1:50 dilution; Developmental Studies Hybridoma Bank [DSHB]; Cat. No. 7G10) and rabbit anti-Cdc37 (1:200 dilution; obtained from M. Therrien at the University of Montreal in Canada; Cutforth and Rubin, 1994). Samples were washed in PBST plus 5% normal goat serum three times for 10 min each and incubated with secondary antibodies diluted in PBST plus 1% normal goat serum overnight at 4°C. The following secondary antibodies were used: Cy3-conjugated goat anti-mouse immunoglobulin G (IgG) (1:100; Jackson ImmunoResearch; Cat. No. 75512), Alexa Fluor 488–conjugated goat anti-mouse IgG (1:200; Molecular Probes; Cat. No. A11001), and Alexa Fluor 546–conjugated goat anti-rat IgG (1:200; Molecular Probes; Cat. No. A11081). After washing in PBST three times for 10 min each, the samples were mounted in VECTASHIELD (Vector Laboratories; Cat. No. H-1000). Cell nuclei were counterstained in a 1:500 dilution of 4′,6-diamidino-2-phenylindole (DAPI; Molecular Probes; Cat. No. D1306).

β-Galactosidase staining
Mid- to late-third instar larvae were dissected in PBS and fixed in 2% glutaraldehyde for 15 min at room temperature. The samples were washed three times in PBS and incubated in 10 mM NaPO4, 150 mM NaCl, 1 mM MgCl2, 3.1 mM K3[FeII(CN)6], 3.1 mM K3[FeIII(CN)6], 0.3% Triton X-100, and 0.2% X-Gal for 30 min at 37°C.

Measurement of the cell and nucleus
Micrographs of DAPI-stained epidermal tissues were analyzed using ImageJ. Background signals were eliminated using the Threshold tool, and the widths of all of the cell nuclei in the trapezoidal area of a dorsal segment (Kwon et al., 2010) were measured. Cells containing the widest nuclei (widest 15%) were selected for further analysis. The cell boundaries of these cells were marked using the Hand tool and the width of the cell was measured. At least eight animals per genotype were analyzed and a Wilcoxon rank-sum test was used to perform the statistical analysis in Figure 5.

Western blot analysis and coimmunoprecipitation
For Western blotting, epidermal filets were boiled in SDS sample buffer (250 mM Tris-HCl, pH 6.8, 0.5M dithiothreitol, 10% SDS, 0.25% bromophenol blue, and 50% glycerol) at 100°C for 5 min, subjected to 8% or 10% SDS–PAGE, and transferred to nitrocellulose membranes. The membranes were then blocked with 5% skim milk in TBST (10 mM Tris, pH 8.0, 150 mM NaCl, and 0.05% Tween 20) for 2 h and probed with primary antibodies at 4°C overnight. The following antibodies were used: rabbit anti-JNK (1:1000 dilution; Santa Cruz Biotechnology; Cat. No. sc-571), rabbit anti-p-JNK (1:1000 dilution; Promega; Cat. No. V9793A), rat anti-Cdc37 (1:400 dilution; Figure 1; Supplemental Figures S2 and S5, C–F; Cutforth and Rubin, 1994), rabbit anti-Cdc37 (1:1000 dilution; Supplemental Figure S5, A and B), rabbit anti-Hep (1:1000 dilution; Rallis et al., 2010), guinea pig anti-Mkk4 (1:1000 dilution; Rallis et al., 2010), rabbit anti-Tak1 (1:1000 dilution; Paquette et al., 2010), rabbit anti-Slp (1:500 dilution; Polaski et al., 2006), mouse anti-β-JPS (1:1 dilution; DSHB; Cat. No. CF.6G11), mouse anti-talin (1:1 dilution; DSHB; Cat. No. A22A), and goat anti–β-tubulin (1:1000 dilution; Santa Cruz Biotechnology). The membranes were then washed three times with TBST and incubated with horseradish peroxidase–conjugated secondary antibodies (1:1000 dilution; anti-rabbit [Cat. No. sc-2004], anti-tar [Cat. No. sc-2006], anti-mouse [Cat. No. sc-2005], anti-β-galactosidase [Cat. No. sc-2065], and anti-guinea pig [Cat. No. sc-2438], all purchased from Santa Cruz Biotechnology) in TBST plus 1% skim milk for 1 h. After washing in TBST three times, the membranes were visualized using the WEST-ZOL Plus Western blot detection system (nNtRon; Cat. No. 16024).

For coimmunoprecipitation, epidermal filets were briefly lysed in NP-40 buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% NP-40) at 4°C and incubated with protein G–sepharose beads (Sigma Aldrich; Cat. No. P3296) plus antibodies for coupling at 4°C overnight. The beads were then washed with NP-40 buffer and used for Western blot analysis.
ACKNOWLEDGMENTS

We thank J. Ng, Y. Miyata, N. Silverman, B. Stronach, M. Therrien, The Bloomington Stock Center, The National Institute of Genetics in Japan, and The Vienna Drosophila Resource Center for fly stocks and antibodies. We are also very grateful to our colleagues in the laboratory for helpful discussions. This work was supported by a National Research Foundation of Korea grant funded by the Korean Government, Ministry of Science and ICT (Grant no. 2015R1A2A2A01006660) to K.-M.C.

REFERENCES

Angel P, Szabowski A, Schorpp-Kistner M (2001). Function and regulation of ATP-1 subunits in skin physiology and pathology. Oncogene 20, 2413–2423.

Baek SH, Kwon YC, Lee H, Choe KM (2010). Rho-family small GTPases are required for cell polarization and directional sensing in Drosophila wound healing. Biochem Biophys Res Commun 394, 488–492.

Bell GP, Fletcher GC, Brain R, Thompson BJ (2015). Aurora kinases phosphorylate Lgl to induce mitotic spindle orientation in Drosophila epithelia. Curr Biol 25, 61–68.

Bosch M, Serras F, Martin-Blanco E, Baguna J (2005). JNK signaling pathway required for wound healing in regenerating Drosophila wing imaginal discs. Dev Biol 280, 73–86.

Brook AR, Wang Y, Berger S, Renkawitz-Pohl R, Han VC, Wu Y, Galko MJ (2012). Transcriptional regulation of Profilin during wound closure in Drosophila larvae. J Cell Sci 125, 5667–5676.

Camps J, Geiger JA, Santos AC, Carlos V, Jacinto A (2010). Genetic screen in Drosophila melanogaster uncovers a novel set of genes required for embryonic epithelial repair. Genetics 184, 129–140.

Caplan AJ, Mandal AK, Theodoraki MA (2007). Molecular chaperones and cell fusion by JNK and JAK/STAT signaling during wound healing. Biochem Biophys Res Commun 393, 656–661.

Carlo R, Baguran J, Falck A, Hunt P, Johnson RS (2003). c-Jun is essential for organization of the epidermal leading edge. Dev Cell 4, 865–877.

Lin JM, Kiliman VL, Keegan K, Paddock B, Emery-Le M, Rosbash M, Allada R (2002). A role for casein kinase 2α in the Drosophila circadian clock. Nature 420, 816–820.

Losick VP, Fox DT, Spradling AC (2013). Polyploidization and cell fusion contribute to wound healing in the adult Drosophila epithelium. Curr Biol 23, 2224–2232.

Losick VP, Jun AS, Spradling AC (2016). Wound-induced polyploidization: regulation by Hippo and JNK signaling and conservation in mammals. PLoS One 11, e0151251.

Martin P (1997). Wound healing—aiming for perfect skin regeneration. Science 276, 75–81.

Mattila J, Omyelanchuk L, Kytälä S, Turunen H, Nokkala S (2005). Role of Hsp90 and Cdc37—a chaperone cancer conspiracy. Curr Opin Cell Biol 17, 347–353.

Mehrotra S, Maqbool SB, Kolpakas A, Mumen K, Calvi BR (2008). Embryonic wound healing cells do not apoptose in response to DNA recombination. Science 320, 787–790.

Miyata Y, Nishida E (2004). CK2 controls multiple protein kinases by phosphorylating a kinase-targeting molecular chaperone, Cdc37. Mol Cell 24, 4065–4074.

Paquette N, Broemer A, Aggarwal K, Chen L, Husson M, Erturk-Hademir D, Reichhart JM, Meier P, Silverman N (2010). Caspase-mediated cleavage, IAP binding, and ubiquitination: linking three mechanisms crucial for Drosophila NF-κB signaling. Mol Cell 37, 172–182.

Park SH, Lee CW, Lee JH, Park JY, Roshandel M, Brenneman CA, Choe KM (2018). Requirement for and polarized localization of integrin proteins during Drosophila wound closure. Mol Cell 70, 2137–2147.

Pearl LH (2005). Hsp90 and Cdc37—a chaperone cancer conspiracy. Curr Opin Genet Dev 15, 55–61.

Polaski S, Whitney L, Barker BW, Stronach B (2006). Genetic analysis of the Wnt signaling pathway in Drosophila. Genetics 174, 719–733.

Prince T, Sun L, Matts RL (2005). Cdk2: a genuine protein kinase client of p53. Mol Biol Cell 16, 4795–4804.

Rallis A, Moore C, Ng J (2010). Signal strength and signal duration define two distinct aspects of JNK-regulated axon stability. Dev Biol 339, 65–77.

Ramet M, Lanot R, Zachary D, Manfreuelli P (2002). JNK signaling pathway is required for efficient wound healing in Drosophila. Dev Biol 241, 145–156.

Rios-Barrera LD, Riesgo-Escovar JR (2013). Regulating cell morphogenesis: the Drosophila Jun N-terminal kinase pathway. Genesis 51, 147–162.

Shaw TJ, Martin P (2009). Wound repair at a glance. J Cell Sci 122, 3209–3213.

Simon MA, Bowtell DD, Dodson GS, Laverty TR, Rubin GM (1991). Ras1 functions in signal transduction. Genes Dev 11, 1775–1785.

Taipale M, Jarosz DF, Lindquist S (2010). HSP90 at the hub of protein chaperone cancer network. Nature 467, 583–587.

Stepanova L, Pradhan-Sundd T, Verheyen EM (2015). Genome-wide identification of phospho-regulators of Wnt signaling in Drosophila. Development 142, 1502–1515.

Wang Y, Galko MJ (2018). Cdk5 and the Cdk-related kinase Sgk as a tumor suppressor in flies. Mol Biol Cell 29, 2137–2147.

Wisdom RM, Johnson RS (2003). c-Jun is essential for organization of the epidermal leading edge. Dev Cell 23, 5017–5022.

Lin JM, Kiliman VL, Keegan K, Paddock B, Emery-Le M, Rosbash M, Allada R (2002). A role for casein kinase 2α in the Drosophila circadian clock. Nature 420, 816–820.