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Functional Analysis of Cdc42 in Actin Filament Assembly, Epithelial Morphogenesis, and Cell Signaling during *Drosophila* Development

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Cdc42, a member of the Rho family of GTP binding proteins, functions in the formation of polarized actin structures, in elongation of cell shape, and in cell signaling. Although genetic mutations previously have not been available in multicellular organisms, studies have attempted to discern Cdc42 functions in organisms, including *Drosophila*, using dominant active or interfering alleles. Here, for the first time, we examine the functions of Cdc42 in developing tissues using loss-of-function mutations in the *Drosophila Cdc42* gene. We find that *Cdc42*$^{-}$ epithelial cells fail to elongate into a columnar cell shape and cannot maintain a monolayered epithelial structure. In contrast to previous studies, we find no requirement for Cdc42 in cell division or in activation of the Jun N-terminal kinase pathway. In addition, Cdc42 function is not required for cytoplasmic actin filament assembly in the nurse cells during oogenesis, although it may facilitate this process. Furthermore, our results indicate that Cdc42 plays a role in intercellular interactions between the germ line and the somatic follicle cells. These results confirm the role of Cdc42 in actin filament assembly and provide new insights into its functions in epithelial morphogenesis and regulating intercellular signaling events.

Key Words: **Rho family; cell polarity; JNK pathway; dorsal closure; cell proliferation.**

INTRODUCTION

Members of the Ras superfamily of small GTPase proteins have been shown to act as molecular switches that regulate cell proliferation, cell fate, and a range of other cellular and developmental processes. Within this superfamily, the Rho family, including Rho, Rac, and Cdc42, has been particularly well studied because of its role in regulating cytoskeletal assembly and related cellular events such as membrane trafficking, cell polarity, cell adhesion, and cell elongation (Van Aelst and D'Souza-Schorey, 1997). More recently, these proteins have also been suggested to function as switches in signal transduction pathways, such as the Jun N-terminal kinase (JNK) pathway, with functions that extend beyond cytoskeletal regulation. In addition, numerous studies implicate Rho family members in processes associated with oncogenesis, either directly or as effectors of oncogenic pathways. Thus it appears that Rho family proteins play crucial roles in regulating cytoskeletal processes and a range of related cellular and developmental functions.

Within the Rho family, the *Cdc42* gene is unique in that it was originally identified as a cell cycle mutation in the yeast *Saccharomyces cerevisiae* (Adams *et al.*, 1990). In the yeast, *CDC42* function is necessary during the assembly of the bud site, an actin-rich structure that forms at one end of the budding yeast cell. Mutations in the yeast *CDC42* gene disrupt the assembly of a ring of actin microfilaments that normally forms in the neck of the bud, thereby blocking bud formation and cell division. This phenotype has been interpreted to indicate that *CDC42* mutations disrupt the ability of cells to construct localized or "polarized" assemblies of actin microfilaments.

Experiments using cultured cells have implicated human *Cdc42* in the formation of filopodia in growth factor-stimulated cells, presumably via the assembly of membrane-associated microfilaments in these structures (Nobes and Hall, 1995). Interestingly, other Rho family members appear to regulate the assembly of morphologically distinct cellular processes, such as lamellipodia and stress fibers. In addition, several lines of evidence implicate

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Cdc42 or another Rho family member as functioning upstream of the JNK pathway. Activated forms of both Rac and Cdc42 stimulate the JNK pathway in HeLa, NIH-3T3, and Cos cells (Coso *et al.*, 1995; Minden *et al.*, 1995). However, constitutively activated forms of Cdc42 and Rho appear to activate the JNK pathway in human kidney 293T cells (Teramoto *et al.*, 1996). These seemingly contradictory results could indicate that the Rho family regulation of the JNK pathway occurs in a tissue-specific manner. Alternatively, it is possible that the mutationally activated forms of these highly related proteins result in nonspecific interactions between the structurally similar members of the Rho family.

The developmental functions of Cdc42 and the Rho family members have been investigated in *Drosophila* using dominant interfering or activating alleles (Luo *et al.*, 1994). Dominant active and negative mutations in *Rac* disrupt axon outgrowth in the embryonic nervous system and affect myoblast fusion. Similar forms of Cdc42 affect neurite outgrowth in the peripheral nervous system but do not affect myoblast fusion. Dominant negative Cdc42 has also been shown to disrupt the process of dorsal closure late in embryonic development (Riesgo-Escovar *et al.*, 1996). Studies of *Drosophila* oogenesis using ectopically expressed dominant negative Cdc42 suggest that Cdc42 may function to regulate the assembly of actin microfilaments that are necessary for proper transfer of cytoplasm from the nurse cells to the oocyte (Murphy and Montell, 1996). Dominant alleles of *Cdc42* also affect the ability of imaginal epithelial cells to elongate into a columnar cell shape and disrupt planar polarity (rather than apical–basal polarity) of these epithelial sheets (Eaton *et al.*, 1995, 1996).

We have used genetic mutations in *Drosophila Cdc42* (Fehon *et al.*, 1997) to examine the effects of loss of Cdc42 function in developing tissues. Reduction of Cdc42 function in embryonic, imaginal, and follicular epithelial cells results in a loss of epithelial character—mutant cells are unable to maintain a columnar cell shape, lose cohesion necessary to maintain cell sheets, and form multiple cell layers. Reduction of Cdc42 function in the embryonic epidermis leads to a failure in germ-band retraction and a degeneration of the ventral epidermis prior to the process of dorsal closure, yet axon outgrowth in the central and peripheral nervous systems still occurs. Functional Cdc42 is also required for proper differentiation of imaginal epithelial cells and in both the germ-line and the follicle cells for proper specification of the follicular stalk cells. In contrast, actin filament assembly in the developing nurse cells of the ovary is not dependent on Cdc42 function. In addition, contrary to predictions from previous studies (Agnès *et al.*, 1999; Riesgo-Escovar *et al.*, 1996), loss of Cdc42 function does not appear to affect activation of the JNK pathway during dorsal closure or cytokinesis in proliferating epithelial cells. Thus, these studies, which are the first to use loss-of-function *Cdc42* alleles in a higher eukaryote, provide new insights into the functions of this ubiquitous cytoskeletal regulatory switch.

MATERIALS AND METHODS

Identification of the Cdc42 Mutations

The molecular lesions associated with *Cdc42* alleles were determined as previously described (Fehon *et al.*, 1997).

Clonal Analysis

To generate clones in the imaginal discs and the follicular epithelium, *y w Cdc42 P{neoFRT}19A/FM6* virgin females were mated to *y w P{w*$^{+mC}$ *= piM}5A P{w*$^{+mC}$ *= piM}10D P{neoFRT}19A; P{ry*$^{+7.2}$ *= hsFLP},Sb/TM6, Tb* males. Mitotic clones were generated in the imaginal discs at 36 and 72 h after egg laying (AEL) using two 60-min heat shocks at 37°C, separated by 60 min at 25°C. Follicle cell clones were induced using a similar protocol in late third-instar larvae. Expression of the clonal marker, piMyc, was induced by heat shocking larvae for 60 min at 37°C 1 h prior to dissection.

Germ-line clones were generated by crossing *ovo*^{D1} *P{FRT(w*^{hs}*)}101* males to *y w Cdc42*$^{-}$ *P{neoFRT}19A/FM6* virgin females. Clones were induced using 1057 rads from a ^{137}Cs source at 36 ± 6 h as described previously (Wieschaus *et al.*, 1981), and all ovaries were removed for staining 48 h after eclosion.

To generate clones in a *Minute* background, *M(1)18C*^{1}*/FM6* virgin females were crossed to *y w Cdc42*^{3}*P{neoFRT}19A; P{cosMer*$^{+}$*}/+* males. *P{cosMer*$^{+}$*}* is a 36-kb piece of genomic DNA that encodes both the *Merlin* and the *Cdc42* genes (Fehon *et al.*, 1997). Clones were induced by gamma irradiation (1057 rads) at 72 h AEL and were examined in adult eyes.

Immunofluorescence

All tissues were dissected, fixed, and stained as previously described (McCartney and Fehon, 1996) with the exception of PS1 staining. PS1 staining was performed on living tissue for 30 min in Shields and Sang M3 insect medium (Sigma) prior to fixation. Primary antibodies were used at the following concentrations: anti-Coracle (mAb C566.9C and 615.16B) 1:500, mAb 22C10 (S. Benzer, CalTech) 1:1000, anti-Myc (mAb 1-9E10.2) 1:5, anti-α Spectrin (Ab 905, D. Kiehart, Duke University) 1:1000, anti-Armadillo (M. Peifer, University of North Carolina) 1:500, anti-Vasa (H. Lin, Duke University) 1:1000, and mouse anti-PS1 (D. Brower, University of Arizona) 1:500. Secondary antibodies (Jackson ImmunoResearch) were used at 1:1000. FITC-conjugated anti-horseradish peroxidase (1:750; Cappel) and rhodamine-conjugated phalloidin (1:1000; Molecular Probes) were added with the secondary antibodies. SPIF and propidium iodide nuclear staining were performed as previously described (Lundell and Hirsh, 1994; Orsulic and Peifer, 1994). Confocal microscopy was performed using a Zeiss LSM410 laser scanning confocal microscope with krypton/argon and helium/neon lasers.

Follicle Cell Counts

The average number of propidium iodide-stained follicle cells per confocal optical section was determined as described previously (Lee *et al.*, 1997). Egg chambers that were composed of more than 75% mutant follicle cells and had not fused with adjacent egg chambers were used for this analysis. For egg chambers prior to stage 7, staging was determined using standard criteria (Mahowald and Kambysellis, 1980). Later stages, which were grouped for this analysis, were determined using the size of the nurse cell nuclei,

TABLE 1
Cdc42 Mutations

Allele	Phenotype	Site[a]	Mutation	Effect
Cdc42^{1}	Lethal	50	G → A	Gly^{10} → Asp
Cdc42^{2}	Visible	311	T → A	Splice donor consensus
Cdc42^{3}	Lethal	423	G → A	Gly^{114} → Asp
Cdc42^{4}	Lethal	370	G → A	Splice acceptor consensus
Cdc42^{5}	Visible	214	G → A	Asp^{65} → Asn
Cdc42^{6}	Visible	269	C → T	Ser^{83} → Leu

[a] According to Sequence Accession Nos. AF153423–AF153429.

because yolk deposition did not occur normally in these egg chambers. Six to ten egg chambers of each developmental time point were used to generate the average number of follicle cells per optical section.

Construction of a Myc-Tagged Cdc42 Transgene

The complete coding sequence for *Drosophila Cdc42* (bp 159–771) was amplified by PCR from cDNA and cloned into pBSSK-myc (LaJeunesse *et al.*, 1998), 3′ to sequences encoding the Myc epitope. The resulting *Myc–Cdc42* fusion coding sequence was then excised with *Eco*RI and *Xba*I restriction enzymes and cloned into the *Ubiquitin*-promoter-containing plasmid pUp2-RHX (Fehon *et al.*, 1997). The polyadenylation signal from the *Alcohol dehydrogenase* gene (taken from pRmHa-3; Bunch *et al.*, 1988) was then cloned 3′ to the *Cdc42* coding sequence using a *Xba*I site. The *Ubiquitin* promoter, *Myc*-tagged *Cdc42* coding sequence, and polyadenylation signal were then excised using flanking *Not*I sites and cloned into the pCaSpeR-3 transformation vector.

In Situ Hybridization

The *Decapentaplegic* (*dpp*) probe was made from a cDNA clone in pNB40. *In situ* hybridization was performed as described (Ashburner, 1989) with the following modifications: Embryos were fixed in a 1:1 mixture of 4% paraformaldehyde:heptane, and when necessary, the staining process was allowed to progress overnight.

RESULTS

Mutations in the Drosophila Cdc42 Gene

To determine the functions of Cdc42 during *Drosophila* development, we have examined the phenotypes of six previously isolated mutations in the *Drosophila Cdc42* gene (Fehon *et al.*, 1997). The molecular lesions associated with two of these alleles, *Cdc42*^{1} and *Cdc42*^{2}, were previously characterized (Fehon *et al.*, 1997) and we identified the remainder for this study (Table 1). Of the lethal alleles, two (*Cdc42*^{1} and *Cdc42*^{3}) are missense mutations while the other (*Cdc42*^{4}) is a mutation in the splice acceptor site. *Cdc42*^{3} and *Cdc42*^{4} displayed a similar range of phenotypes and produced similar degrees of maternal-effect lethality (see below), suggesting that they reduce Cdc42 function to similar levels. Rescue experiments using a *Ubiquitin* promoter–*Cdc42*$^{+}$ transgene indicate that *Cdc42*^{1} is only partially rescuable, while all of the other alleles can be rescued completely, consistent with the notion that *Cdc42*^{1} has dominant negative functions (data not shown).

Cdc42 Is Required for Development of the Embryonic Epithelium

Previous studies of *Cdc42* in yeast and mammalian cells have indicated that it plays a fundamental role in cellular morphogenesis. It is therefore surprising that lethal *Cdc42* mutations in *Drosophila* display no embryonic phenotype (Fehon *et al.*, 1997). One possible explanation for this apparent paradox is that *Cdc42* is maternally expressed and loaded into the egg, thereby providing sufficient Cdc42 function for embryogenesis. *Cdc42*$^{-}$ germ-line clones could not be used to address this possibility because Cdc42 is required during oogenesis for the production of fertile eggs (see below). Instead we used heteroallelic combinations of weak and strong alleles to produce females that, while viable, had reduced Cdc42 function. Examination of two such combinations, *Cdc42*^{3}/*Cdc42*^{6} and *Cdc42*^{4}/*Cdc42*^{6}, demonstrated that both combinations produce over 70% embryonic lethality, even when outcrossed to wild-type males [71.5% (n = 1069) and 72.2% (n = 816), respectively]. Given the Mendelian expectation that 25% of the progeny will be hemizygous for the lethal allele, the high embryonic lethality indicates that Cdc42 is maternally contributed to the embryo and is essential.

To examine the role of *Cdc42* in embryogenesis, we examined the phenotypes of embryos with reduced maternal Cdc42 function. Cuticle preparations and staining with anti-Coracle, which specifically stains ectodermally derived epithelial cells (Fehon *et al.*, 1994), revealed epidermal defects in these embryos, prior to the process of dorsal closure, that first appeared at the onset of germ-band retraction. In the embryos that failed to hatch, germ-band retraction failed to proceed to completion, leaving embryos open on the dorsal side (Figs. 1C–1F). In addition, holes frequently appeared in the embryonic epidermis along the ventral midline (Figs. 1D and 1F). In extreme cases, possibly corresponding to embryos that also lacked zygotic Cdc42 function, the ventral epidermis was observed to tear apart along almost the entire anterior–posterior axis of the retracting embryo (Fig. 1F).

A previous study using a dominant activated *Cdc42* allele has proposed that Cdc42 functions in neuronal cells to regulate neurite outgrowth (Luo *et al.*, 1994). To determine if Cdc42 is required for normal neurite formation, embryos with reduced maternal *Cdc42* contribution were examined for defects in the central and peripheral nervous systems. Because the embryonic defects we observed were not completely penetrant and some embryos (<30%) completed embryogenesis normally, we selected embryos with obvious germ-band retraction and

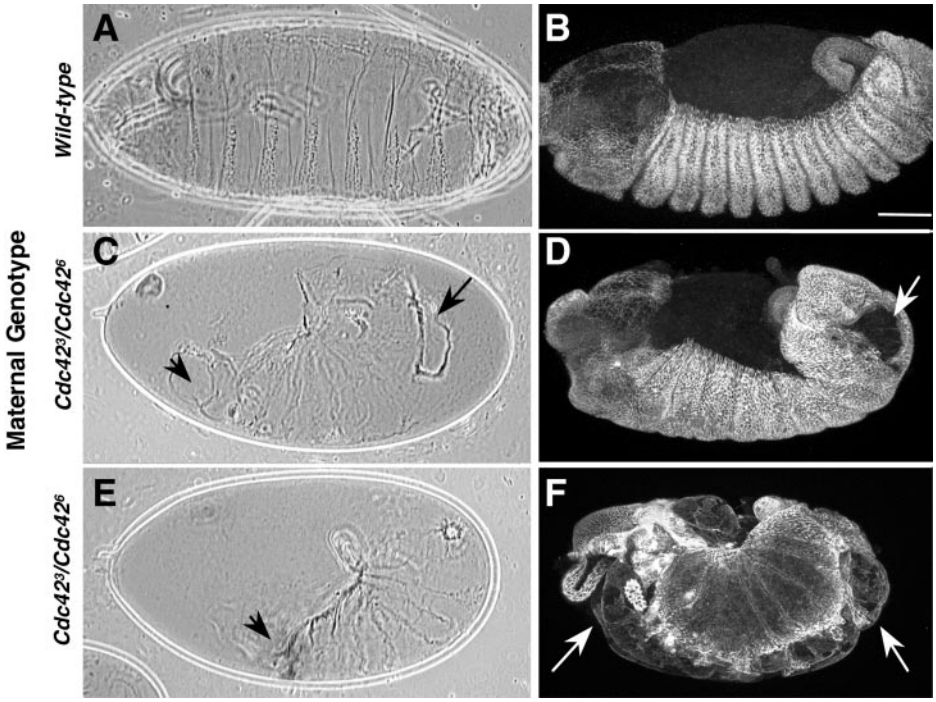


FIG. 1. Cdc42 function is required for development of the embryonic epidermis. Cuticle preparations (A, C, E) and staining with anti-Coracle (B, D, F) of representative embryos produced by females of the indicated genotypes. (A, B) Embryos derived from wild-type females display normal cuticular differentiation (A) and epidermal development (B). In contrast, most embryos produced by $Cdc42^{3}/Cdc42^{6}$ females display disruptions in epidermal development (shown by absence of Coracle staining), including incomplete germ-band retraction (D, F), ventral holes in the epidermis (arrows, D and F), ventral holes in cuticle produced by epidermal cells (long arrows, C; not shown in E), and anterior open phenotypes (short arrows, C and E). Ventral holes range in size from isolated disruptions (D) to openings in the entire ventral surface of the embryo (F). Scale bar represents 50 μm.

epidermal defects as an indication of reduced Cdc42 function. Patterns of neuronal differentiation, as determined by mAb 22C10 and anti-horseradish peroxidase (anti-HRP), were largely normal, even in areas where the integrity of the overlying epidermis was disrupted (Figs. 2A–2D). In certain embryos defects in the central nervous system (CNS) such as incomplete formation and midline fusion of the longitudinal commissures were observed (Fig. 2B). However, in all embryos the axons of the peripheral nervous system as detected by antibody 22C10 appeared to extend properly (Fig. 2C). The defects observed in the CNS could represent a requirement for Cdc42 function in this tissue or they could be secondary to the other embryonic defects, such as failure in germband retraction and epidermal holes. In either case, the formation of axon commissures in the CNS and the apparently correct projection of neurites in the peripheral nervous system suggest that Cdc42 function is not essential for neurite outgrowth. Alternatively, it is possible that the requirement for Cdc42 function is higher in the epidermis than in other tissues or that tissues of the nervous system contain greater amounts of the maternal Cdc42 product.

Cdc42 Is Required for Patterning and Morphogenesis in Postembryonic Development

We examined the function of Cdc42 in postembryonic development using analysis of phenotypes caused by partial loss-of-function mutations and somatic mosaic analysis of lethal mutations. Flies homozygous for weak *Cdc42* alleles or carrying combinations of weak and strong alleles displayed distinct visible phenotypes (Fig. 3). Scanning electron micrographs of *Cdc42* mutant eyes revealed minor flaws in the ommatidial array, primarily in the dorsal posterior quadrant (Fig. 3B). In this region, we observed occasional fusions of the ommatidia and frequent loss or duplication of bristles. Although these phenotypes suggest possible defects in the structure of ommatidial units, examination of histological sections from *Cdc42* mutant eyes revealed that all of the photoreceptor cells are present and in the proper orientation (data not shown).

Cdc42 mutant adults also possessed extra crossveins on the wing between veins II and III and veins III and IV (Fig. 3D). Some allelic combinations displayed blisters in the wings where the dorsal and ventral surfaces were not fully apposed. Extra crossveins were also observed in 12% of flies

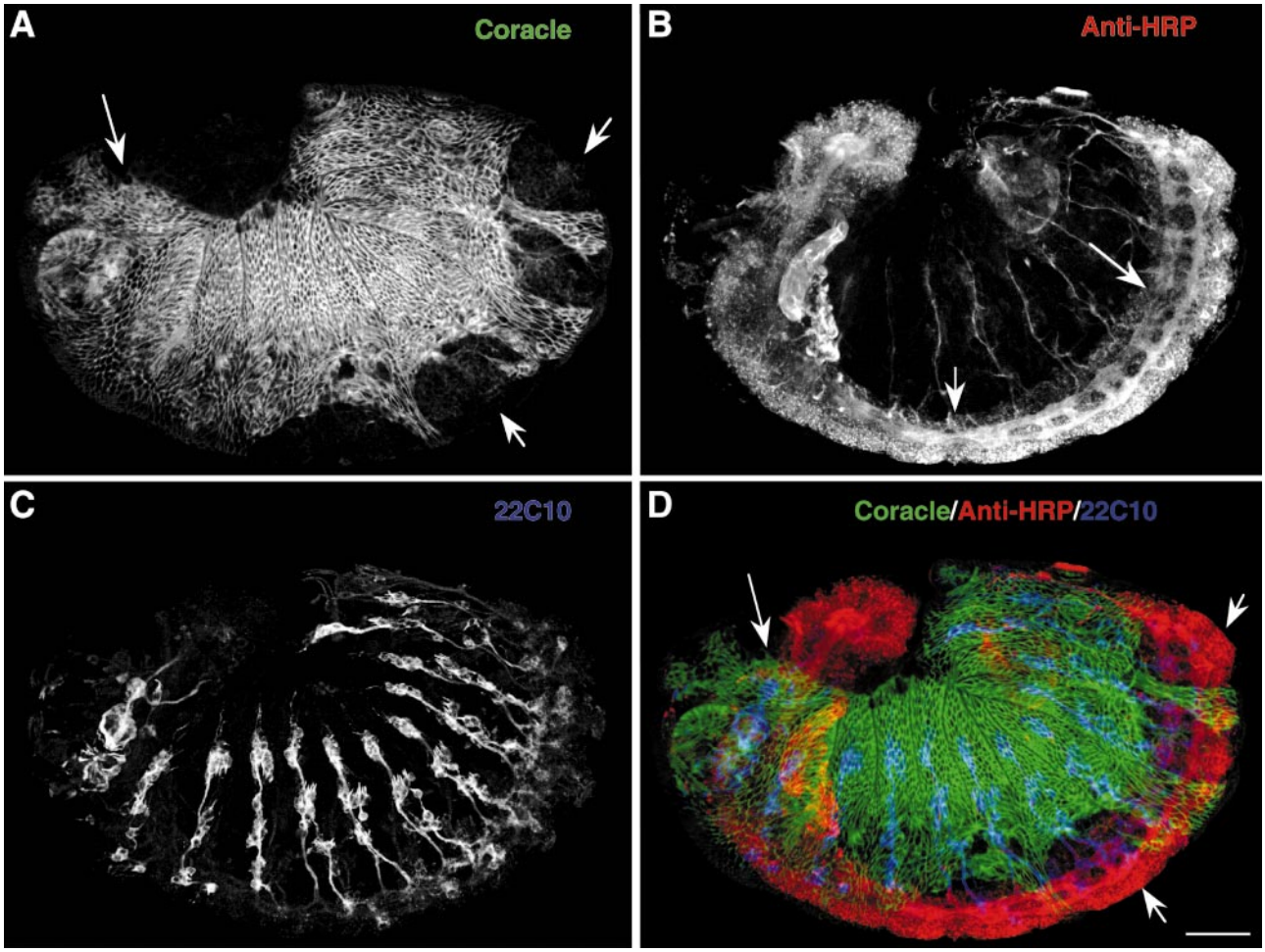


FIG. 2. Axon extension occurs in *Cdc42* mutant embryos. An embryo produced by a $Cdc42^{3}/Cdc42^{6}$ female, stained with anti-Coracle (A; green in D), was examined for neuronal defects as determined by anti-HRP staining (B; red in D) and mAb 22C10 (C; blue in D). Anti-Coracle staining reveals holes along most of the ventral epithelium (short arrows, A and D) and defects in the head region (long arrow, A and D) along with a failure in germ-band retraction. The central nervous system (B), although mostly normal, displays some disruption of the longitudinal commissures (long arrow, B) and a region where the longitudinal commissures appear to fuse at the ventral midline (short arrow, B). However, the overall organization and axonal projections of the peripheral nervous system appear normal (C). Scale bar represents 50 μm.

heterozygous for $Cdc42^{1}$, consistent with the assertion that this allele has dominant negative properties.

To examine the function of *Cdc42* at the cellular level, somatic mosaic clones were generated to produce cells homozygous for *Cdc42* lethal alleles in the wing imaginal discs. Clones generated early in larval development, at 36 h AEL, did not survive to the wandering third-instar stage (Fig. 4B), leaving only sister clones that mark where a recombination event occurred. Mutant clones that were generated later in larval development, at 72 h AEL, were smaller than their sister clones at the end of larval development (Fig. 4C). In these discs, mutant clones that occurred in the notum were only slightly reduced in size relative to their sister clones, whereas the mutant clones generated in the blade were either absent or much smaller than their sisters (Fig. 4C). This clonal pattern is characteristic of the cell competition effect that has been described previously (Burke and Basler, 1996; Simpson, 1979) and suggests that *Cdc42* mutant cells are at a proliferation disadvantage relative to their wild-type neighbors.

As in the wing discs, $Cdc42^{-}$ clones generated in the eye at 36 h AEL were not observed in wandering third-instar larvae. Clones generated throughout the eye later in development, at 72 or 96 h AEL, did survive to the wandering third-instar stage (Fig. 4D), but did not survive to the adult stage. In histological sections of such eyes, mild pattern disruptions were observed around small scars near the sister clones, but no mutant cells had differentiated into photo-

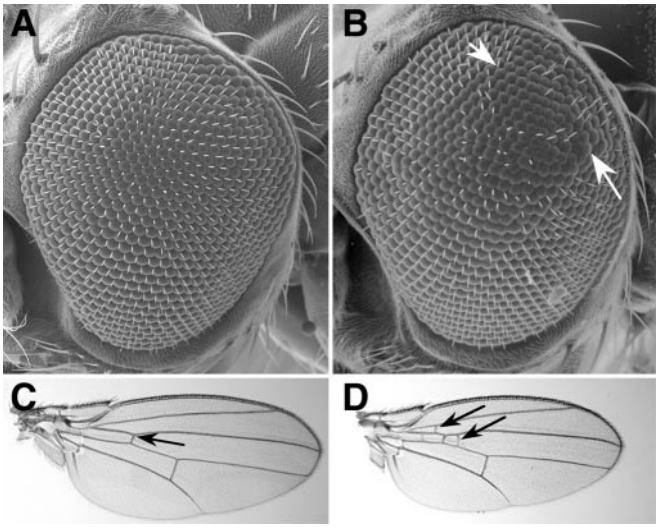


FIG. 3. Viable *Cdc42* alleles display phenotypes in the eye and wing. (A, B) Scanning electron micrographs of wild-type (A) and $Cdc42^{1}/Cdc42^{5}$ (B) eyes. *Cdc42* mutant eyes display disruptions in the normal ommatidial pattern, ommatidial fusion (long arrow, B), and loss of bristles in the dorsal posterior quadrant (short arrow, B). In wild-type flies (C) the wing has a single anterior crossvein (arrow), while the wings of $Cdc42^{1}/Cdc42^{5}$ flies (D) often have extra anterior crossveins (arrows).

receptors or other recognizable cell types (data not shown). To overcome the implied competition disadvantage of *Cdc42* mutant cells and to examine their ability to differentiate adult structures, eye clones were generated in a *Minute* background. Control clones generated in the developing eye at 72 or 96 h AEL in a *Minute* background grew to encompass the majority of the eye (Fig. 4H). In contrast, clones of *Cdc42* mutant cells failed to produce adult ommatidia and instead left scars on the eye (Fig. 4I). This result suggests that, in this case, the competition effect is due to a cause other than impaired proliferation.

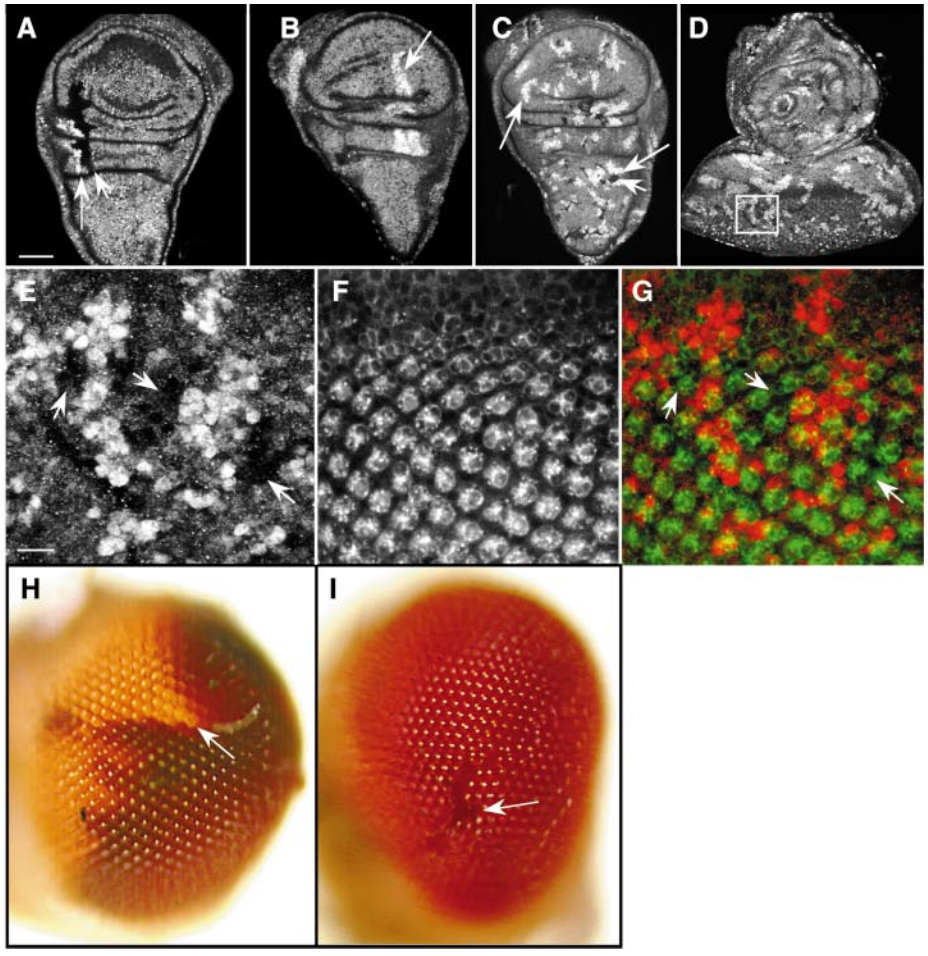
To examine the development of these clones, we asked if, in a wild type background, *Cdc42* mutant cells could initiate neuronal differentiation. As determined by anti-HRP staining for developing neurons, mutant clones generated in eye discs 72 h AEL initiated differentiation as photoreceptor cells in the larval eye imaginal disc (Figs. 4D–4G). These results indicate that although *Cdc42* function is not required to initiate neuronal cell fate in the eye, it is necessary to complete photoreceptor differentiation.

Cdc42 Function Is Required in the Follicle Cells and the Germ Line during Oogenesis

Previous studies using ectopically expressed dominant alleles of yeast *CDC42* and *Drosophila Cdc42* in flies have suggested that *Cdc42* may have important functions during *Drosophila* oogenesis (Murphy and Montell, 1996). To examine the effects of loss of *Cdc42* function in the somatic and germ-line cells of the developing egg chamber, we generated $Cdc42^{-}$ clones via mitotic recombination. As described in detail below, follicle cell clones had three highly penetrant phenotypes: loss of columnar cell shape, formation of multiple layers of cells at the posterior end of the egg chamber, and apparent fusion of adjacent egg chambers. Germ-line clones displayed minor defects in

FIG. 4. Cdc42 function is required for differentiation of imaginal tissues. (A–C) Late third-instar wing imaginal discs stained with anti-Myc antibody to reveal the presence (wild-type cells) or absence (mutant cells) of the pi-Myc cell marker. Wild-type control clones induced 36 h AEL (short arrow, A) are roughly equal in size to their sister clones, which show increased Myc staining relative to other cells in the disc (long arrow, A). $Cdc42^{3}$ clones induced at the same developmental stage fail to survive to the late third instar (B), though their wild-type sister clones do survive (arrow, B). Clones induced later in development, at 72 h AEL, can survive to late third-instar stage (C, D). In the wing disc, clones in the notum (short arrow, C) are smaller than their wild-type sister clones (long arrow, C). Although sister clones are found in the blade (long arrow, C) the $Cdc42^{3}$ mutant cells are frequently absent. $Cdc42^{3}$ mutant clones induced at 72 h throughout the eye–antennal disc also survive to the wandering third-instar stage (D). (E–G) A higher magnification view of the area bracketed in (D) shows that mutant cells (arrows, E and G; Myc staining is red) can initiate differentiation as photoreceptor cells, as indicated by anti-HRP staining (F and G; anti-HRP staining is green). (H, I) Superficial illumination of adult eyes in which $Cdc42^{3}$ clones had been generated at 72 h AEL in a *Minute* background. In (H), control clones were induced in a $y\ w\ Cdc42^{3}/w^{+}\ M(1)18C^{1}; P\{cosMer^{+}\}/+$ eye disc. The large yellow patch of ommatidia (arrow, H) indicates homozygous $Cdc42^{3}$ cells that are rescued by the $P\{cosMer^{+}\}$ duplication (which carries $Cdc42^{+}$). Flies lacking the transgene have obvious scars (arrow, I) but do not contain *Cdc42* mutant ommatidia. Scale bars represent 50 μm in A–D and 10 μm in E–G.

FIG. 5. Cdc42 function is required for columnar epithelial cell shape. (A–D) An egg chamber carrying a large clone induced at late third instar that comprises all of the anterior follicle cells (anterior is to the left in all images). Arrows indicate the boundary between mutant and wild-type cells. Anti-Myc (A) stains only wild-type cells while the nuclear stain SPIF shows all nuclei (B). There is noticeable reduction of follicle cell density in the mutant clone, as indicated by decreased nuclear density (B). Anti-α Spectrin staining (C) demonstrates that wild-type follicle cells have a columnar cell shape while homozygous $Cdc42^{1}$ cells are cuboidal in shape. The merged image (D) shows that loss of columnar cell shape and the change in cell density coincide at the clone margin. (E and F) Optical section of a different egg chamber stained with anti-Myc (E) to indicate the clone boundaries (arrows) and anti-Armadillo (F). Although epithelial structure is disrupted, Armadillo is still apically localized in the $Cdc42^{3}$ mutant cells (outside arrows, F). Scale bars represent 10 μm.



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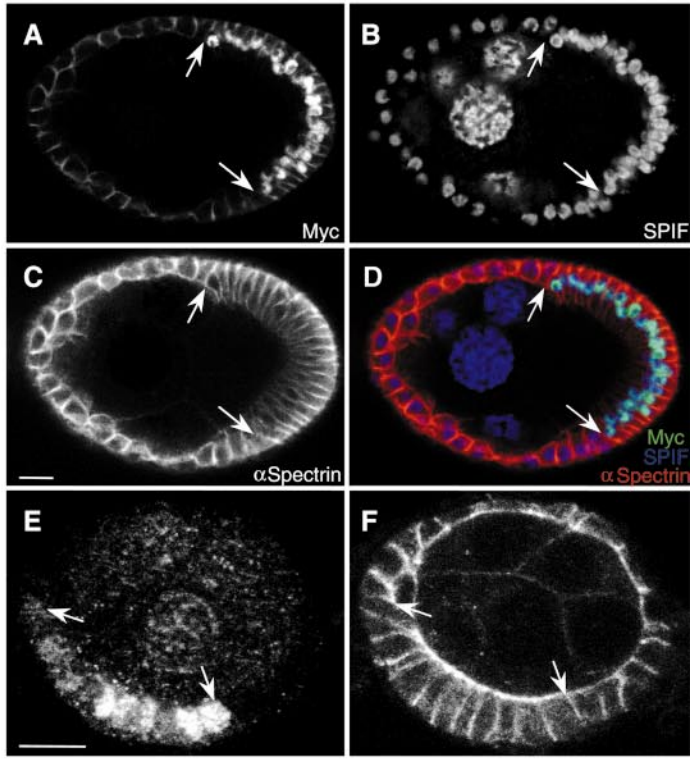

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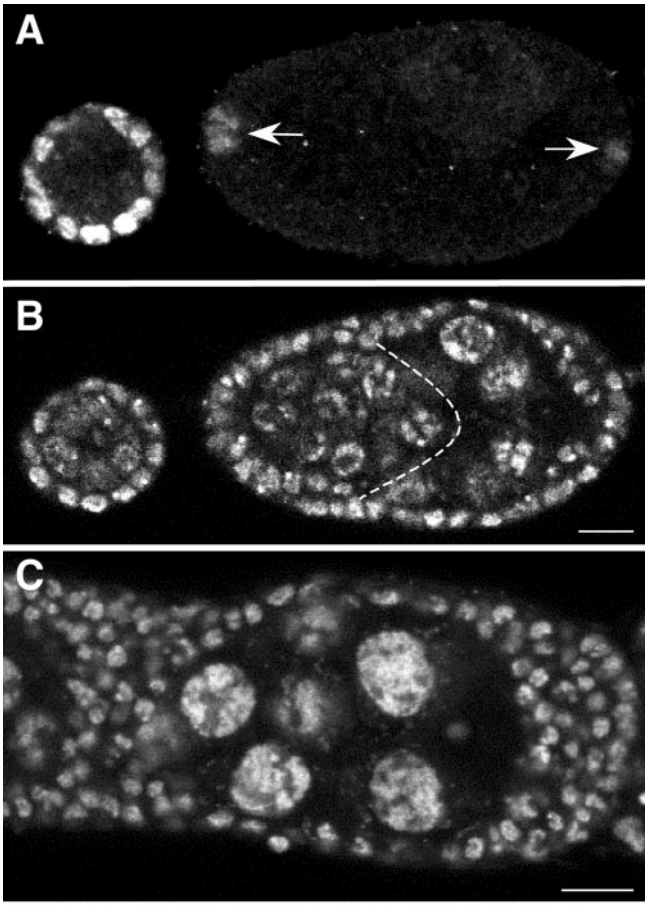

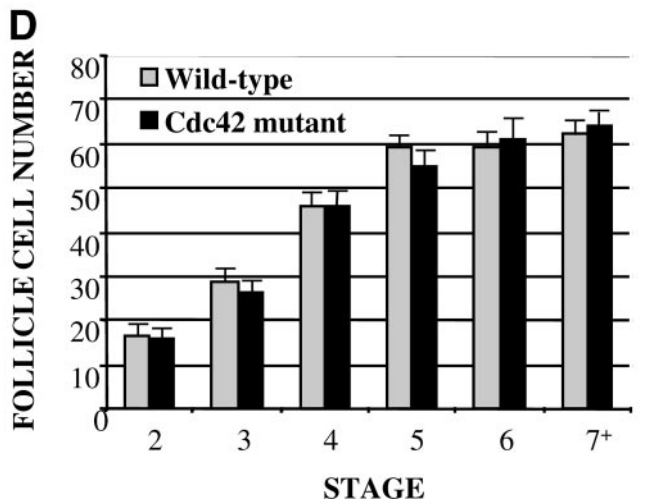

FIG. 6. Loss of Cdc42 function in the follicle cells results in egg chamber fusion and disruption of the monolayered epithelium, but does not affect cell proliferation. (A, B) Two egg chambers stained with anti-Myc (A) and the nuclear marker SPIF (B). The smaller chamber has wild-type follicle cells, while the larger is almost entirely mutant (except for polar follicle cells, arrows) and appears to be the result of the fusion of two adjacent egg chambers. Egg chamber fusion is indicated by the presence of two size classes of nurse cell nuclei (dashed line in B indicates approximate division between the two size classes). (C) An egg chamber stained with SPIF in which all follicle cells are *Cdc42*^{3} (Myc staining not shown) and which displays multiple layers of follicle cells at both poles, indicating that mutant follicle cells lose the ability to maintain an epithelial monolayer. (D) To ascertain if *Cdc42* mutant follicle cells overproliferate, the average number of follicle cells per egg chamber section was determined as described under Materials and Methods. The average number of follicle cells in egg chambers composed of >75% mutant follicle cells does not increase compared to wild type. Scale bars represent 25 μm.

actin filament assembly and, unexpectedly, defects in a subset of the follicle cells.

At stage 9 of egg chamber development, wild-type follicle cells change from a cuboidal cell shape to a more columnar shape. However, in mosaic egg chambers *Cdc42* mutant cells remain cuboidal or irregular in cell shape while the wild-type cells abutting the mutant clone become columnar (Figs. 5A–5D). Anti-Armadillo staining indicated that although the cells are abnormally shaped, the adherens junctions remain intact and cell polarity is maintained (Figs. 5E and 5F). Other apical markers, such as filamentous actin and Notch, also indicated that the apical–basal polarity is normal (data not shown).

Clones of *Cdc42* mutant follicle cells appear to lose the characteristics of a monolayered epithelium and form multiple layers of cells, primarily at the poles of the egg chamber (Figs. 6A–6C). Mutations in *α Spectrin*, which produce a similar multilayered phenotype, have been shown to cause overproliferation in the follicle cells (Lee *et al.*, 1997). To determine if the *Cdc42* phenotype is due to overproliferation or alternatively is caused by redistribution of mutant cells to the posterior end of the egg chamber, we measured follicle cell number in egg chambers containing over 75% mutant follicle cells. These egg chambers did not appear to accumulate yolk in the oocyte and they degenerated by stage 9. The average follicle cell number in egg chambers containing *Cdc42* mutant cells was not significantly different from that of wild-type egg chambers (Fig. 6D). In addition, cell division, as determined using anti-phosphohistone H3, an antibody marker for mitotic cells, ceases at stage 6 in mutant clones as it does in wild-type cells (data not shown). These results indicate that *Cdc42* mutant follicle cells do not overproliferate, but rather display an inability to form a normal, single-layered epithelium.

A third phenotype observed in follicle cell clones that comprised the entire egg chamber was that egg chambers often appeared abnormally large, containing germ-line cells of two distinct size classes (Fig. 6B). These characteristics indicate that adjacent cysts may have fused or failed to separate during oogenesis. To examine this phenotype further, we stained egg chambers with anti-PS1 integrin, a stalk-cell-specific marker (Goode *et al.*, 1996). Fused egg chambers appeared to lack stalk cells (data not shown),

suggesting that *Cdc42* function is necessary for stalk cell differentiation or placement at the poles of egg chambers.

In the female germ line, highly organized bundles of cytoplasmic filaments are rapidly polymerized at stage 10b in the nurse cells. To examine Cdc42 localization during the polymerization of these actin filaments, we constructed a *Myc*-tagged $Cdc42^{+}$ transgene whose expression was regulated by the *Drosophila Ubiquitin* promoter. This transgene rescued lethal *Cdc42* alleles (data not shown), indicating that the addition of the Myc epitope tag did not affect Cdc42 function. In nurse cells prior to and during stage 10b, Myc-tagged Cdc42 was observed throughout the cytoplasm and was concentrated at the plasma membrane (Figs. 7B and 7E). In addition, we observed that during stage 10b Cdc42 accumulated in regions where the cytoplasmic actin filaments intersected the plasma membrane (Figs. 7D–7F). This localization pattern suggests that Cdc42 is recruited to sites on the plasma membrane where actin assembly is occurring.

Given the localization of Myc-tagged Cdc42 to the base of cytoplasmic actin filaments, it is reasonable to propose that Cdc42 function is required for the formation of these filaments. To test this hypothesis, ovaries were examined from mutant germ-line clones, produced using the ovo^{D1} dominant, female-sterile mutation and in females carrying hypomorphic combinations of alleles. In egg chambers containing *Cdc42* mutant germ-line clones, the characteristic cytoplasmic actin filaments still formed and had essentially normal morphology. However, compared to wild-type nurse cells, *Cdc42* mutant nurse cells appeared to have fewer cytoplasmic actin filaments (Figs. 7G–7J). In addition, we often observed that those actin filaments directly associated with the ring canals appeared longer than normal (Figs. 7I and 7J). Dumping of nurse cell contents into the oocyte was delayed and did not appear to proceed to completion. Although these egg chambers failed to produce functional oocytes, staining with anti-Vasa antibody indicated that their overall anterior–posterior polarity was properly established (Figs. 7K and 7L).

Recent studies have shown that numerous interactions occur between the germ-line cells and surrounding somatic follicle cells during oogenesis. To determine if any of these interactions depend on *Cdc42* function in the germ line, we examined egg chambers containing mutant germ-line cells and wild-type follicle cells (as determined by the structure of the follicular epithelium). Staining with anti-PS1 showed a greater number of stalk cells adjacent to *Cdc42* mutant germ-line clones than in wild-type controls (Table 2; Fig. 8). Interestingly, a previous study of the *Drosophila toucan* gene, which encodes a novel protein, has shown that this gene also functions in the germ line to regulate stalk cell number (Grammont *et al.*, 1997). This result indicates that interactions between the germ line and the soma are important for the differentiation of the stalk cells and that Cdc42 function is required in the germ line for this process.

The Role of Cdc42 in the JNK Pathway

Various lines of evidence from mammalian tissue culture suggest that Cdc42 functions in regulating the JNK signaling cascade (Coso *et al.*, 1995). In *Drosophila*, the JNK pathway plays an integral role in dorsal closure, a morphogenetic process involving cell shape changes and local signaling events that occurs late in embryogenesis. One demonstrated function of the JNK pathway is to promote expression of the morphogen Decapentaplegic in cells at the leading edge of the lateral epidermis during dorsal closure. Consistent with this notion, previous studies have shown that *dpp* expression in the leading-edge epidermal cells is disrupted in embryos carrying mutations in members of the JNK signaling pathway (Glise and Noselli, 1997; Riesgo-Escovar and Hafen, 1997; Sluss and Davis, 1997). Reduction but not complete loss of maternally contributed *hemipterous* results in complete loss of *dpp* expression while loss of a negative regulator, *puckered*, results in increased *dpp* expression (Agnès *et al.*, 1999; Martin-Blanco *et al.*, 1998). Thus *dpp* expression at the leading edge is sensitive to the level of JNK pathway function.

Previous studies using ectopic expression of dominant *Cdc42* alleles have suggested that Cdc42 is necessary for dorsal closure and functions upstream of the JNK pathway at the leading edge (Agnès *et al.*, 1999; Harden *et al.*, 1999; Riesgo-Escovar *et al.*, 1996). If this is so, then loss of Cdc42 function should disrupt JNK signaling and therefore *dpp* expression in these cells. To test this hypothesis, *in situ* hybridization to *dpp* mRNA was performed on embryos derived from $Cdc42^{4}/Cdc42^{6}$ mothers. Although ~70% of embryos produced by these females displayed epithelial defects and lethality (see previous section), normal levels of *dpp* expression were observed in all embryos that developed to the onset of dorsal closure [Fig. 9; wild type 92% (n = 370), $Cdc42^{4}/Cdc42^{6}$ 89% (n = 478)], including those that had arrested development due to insufficient levels of Cdc42 function. Thus, unlike known upstream components of the JNK pathway, reduction in Cdc42 function has no apparent effect on *dpp* expression by leading-edge cells at the time of dorsal closure.

DISCUSSION

Previous studies of *Cdc42* have implicated this small GTPase in a variety of processes from regulation of the actin cytoskeleton to signal transduction. To critically test these putative functions, we have examined the effects of loss-of-function *Cdc42* mutations during *Drosophila* development. This approach is important because it allows the specific reduction or elimination of Cdc42 function, without affecting the highly related *Rac* and *Rho* genes. Thus, this is the first study using loss-of-function *Cdc42* alleles in a multicellular organism. Our results demonstrate that *Cdc42* has essential functions during the morphogenesis of embryonic, imaginal, and follicular epithelia.

Cdc42 Is Not Essential for Cellular Proliferation

Cdc42 was initially isolated as a cell division mutation that affects the process of budding in the yeast *S. cerevisiae* (Adams *et al.,* 1990). It has also been implicated in the proper formation of the actin and myosin contractile ring during cytokinesis (Drechsel *et al.,* 1997). Taken together, these results suggest that Cdc42 is essential for cell division in all organisms. However, the results presented here indicate that this hypothesis is incorrect: Cdc42 function is not required for proliferation of the follicular epithelium. In the ovary we frequently observed *Cdc42* mutant follicle cell clones induced 6 days prior to dissection that were large enough to comprise almost an entire ovariole (Fig. 6). The size of the follicle cell clones and the extent of the ovariole that they cover indicate that the recombination was induced in stem cells that subsequently underwent many rounds of cell division. Given the length of time that these clones persisted and their size, it is unlikely that perdurance played a role in the survival of the mutant cells. Thus, Cdc42 does not appear to be required directly for cytokinesis or other aspects of cell proliferation.

The Role of Cdc42 in Epithelial Morphogenesis

Cdc42 mutant epithelial cells displayed phenotypes indicating an inability to undergo changes in cell shape that normally occur during embryogenesis, oogenesis, and imaginal development. At stage 9 during oogenesis, follicle cells undergo a transition from cuboidal to columnar cell shape. Simultaneously, most of the follicle cells migrate toward the posterior end of the egg chamber, leaving only a few specialized squamous cells covering the nurse cells at the anterior end of the egg chamber. In contrast to wild-type cells, $Cdc42^{-}$ cells fail to undergo this change of cell shape and instead remain more cuboidal or irregular in shape (Fig. 5). Defects in cell elongation were also observed when dominant negative Cdc42 was expressed during *Drosophila* development (Eaton *et al.,* 1995, 1996; Luo *et al.,* 1994). Although improperly shaped, *Cdc42* mutant follicle cells still migrate, which results in multiple layers of cuboidal cells at the posterior end of the egg chamber (Fig. 6). This phenotype indicates that while still competent to migrate, mutant follicle cells are unable to undergo the changes in cell shape necessary for proper epithelial organization.

Results using somatic mosaic analysis in the imaginal epithelium differ in some respects from those in the follicular epithelium. Although we have observed very large clones of mutant cells in the follicular epithelium, we never find such clones in the imaginal discs. Instead, *Cdc42* mutant clones are lost rapidly from the imaginal epithelium in a manner resembling cell competition. The inability of cells to compete in the imaginal epithelium has been shown to be characteristic of cells that proliferate more slowly than their wild-type neighbors. However, our observation that $Cdc42^{-}$ cells in the follicular epithelium proliferate normally suggests that an inability to differentiate properly was the cause of clone loss. Consistent with this hypothesis, we have shown that *Cdc42* mutant clones induced in the background of a *Minute* mutation, which slows the growth of neighboring cells thereby rescuing clones with a proliferative disadvantage, still fail to survive to the adult stage. A previous study using ectopic expression of a dominant negative *Cdc42* allele in wing imaginal disc cells has shown that these cells are unable to elongate properly to a columnar shape (Eaton *et al.,* 1995), a phenotype similar to that we have observed for $Cdc42^{-}$ follicle cells. Thus it is possible that cells that are unable to elongate properly to form the columnar shape that is typical of imaginal disc cells are eliminated from the epithelium by cell competition. If so, cell competition may represent a general response to eliminate cells that do not function normally, rather than a specific response to proliferation defects.

In the developing embryonic epithelium, we also observe phenotypes indicating a role for *Cdc42* in epithelial morphogenesis. In embryos derived from females that are hypomorphic for *Cdc42* function, early embryogenesis through germ-band extension occurs normally, presumably by using maternally encoded *Cdc42*. However, holes appear in the embryonic epithelium at the onset of germ-band retraction, particularly along the ventral midline (Fig. 1). Epidermal cells along the ventral midline undergo extensive cell shape changes and rearrangements during germ-band retraction. Taken together, the phenotypes observed

FIG. 7. Cdc42 localization and function during actin filament assembly in the germ line. (A–F) Subcellular localization of filamentous actin revealed by rhodamine phalloidin staining (A, D) and Myc-tagged Cdc42 protein (B, E) in stage 10b germ-line cells. Merged images (C, F) show filamentous actin in red and Cdc42 in green. Myc–Cdc42 accumulates at the membrane and diffusely throughout the cytoplasm in nurse cells, but does not accumulate in the oocyte (B). At higher magnification (D–F), it is apparent that Myc–Cdc42 accumulates at higher levels in regions where the cytoplasmic actin filaments that assemble at stage 10b intersect with the plasma membrane (arrows). (G–J) Effect of loss of Cdc42 on cytoplasmic actin filament formation in the germ line. In wild-type nurse cells (G, I), cytoplasmic actin filaments fill the cytoplasm at stage 10b leaving a hole at the position of the nucleus. In $Cdc42^{3}$ nurse cells (H), the cytoplasmic filaments still form, but are reduced in number relative to wild-type cells. In contrast, the specialized filaments that project outward from the ring canals (arrows in I and J) appear to be enhanced in $Cdc42^{4}/Cdc42^{6}$ nurse cells (J). (K, L) Staining of wild-type (K) and $Cdc42^{4}$ mutant (L) oocytes with anti-Vasa, which normally localizes to the posterior pole of the developing oocyte, indicates that loss of *Cdc42* function in the germ line does not affect overall anterior–posterior polarity of the oocyte. Scale bar represents 10 μm in D–F; all other bars indicate 25 μm.

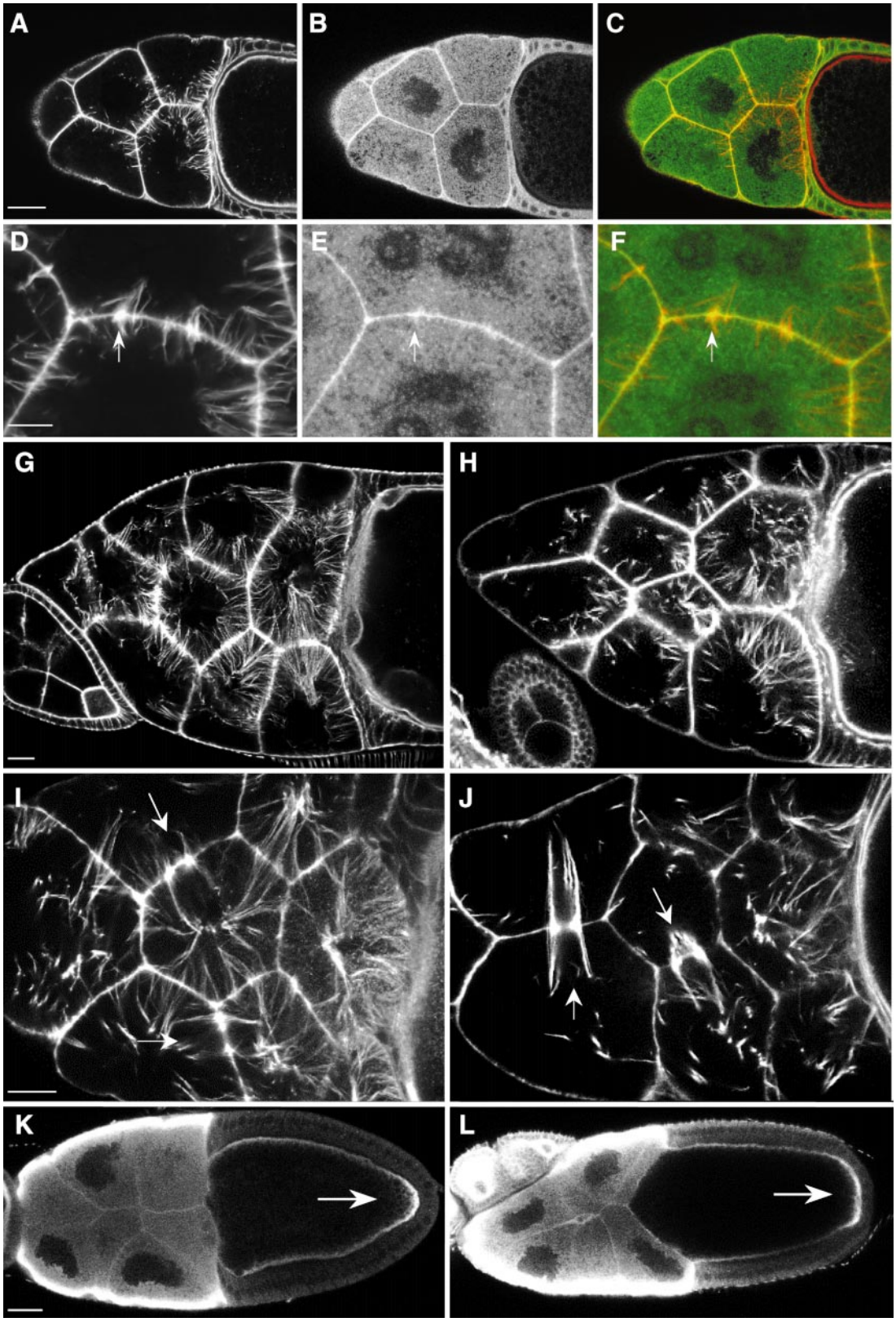

TABLE 2
Effect of Cdc42 Germ-Line Clones on Stalk Cell Number

Germ-line genotype	Stalk cell number[a]	*n*
w^{1118}	5.6 ± 0.2	10
$Cdc42^{1}/Cdc42^{1}$	9.1 ± 0.5	13
$Cdc42^{4}/Cdc42^{4}$	9.5 ± 0.8	10

[a] Mean ± standard error.

in developing *Drosophila* epithelial cells demonstrate a role for Cdc42 in cell elongation and other dynamic cell shape changes. In addition, the presence of epithelial holes may indicate that Cdc42 mutant cells are unable to either form or maintain normal adhesive contacts.

How Does Cdc42 Function in Cell Shape and Morphogenesis?

In epithelial cells, *Cdc42* is essential for elongation into a columnar cell shape, a process that is likely to be dependent on actin filament assembly. Although the small size of *Drosophila* epithelial cells did not allow a direct analysis of cytoskeletal structure in *Cdc42* mutant cells, the cortical actin filaments that form in the nurse cells during stage 10b of oogenesis are much larger and are readily observable. In $Cdc42^{-}$ nurse cells we find that the number of cortical actin filaments is typically reduced relative to wild-type cells, but those that form appear morphologically normal (Fig. 7). Myc-tagged Cdc42 protein localizes to the plasma membrane in these cells and is concentrated at the base of the clusters of cortical actin filaments (Fig. 7). These results suggest that *Cdc42* functions at the plasma membrane to facilitate the nucleation of actin filaments, but is not absolutely required for their formation.

This view of *Cdc42* function *in vivo* agrees well with evidence from studies *in vitro*. Recent studies indicate that *Cdc42* plays a primary role in the nucleation of new actin filaments by regulating the activity of an actin filament nucleating complex composed of the Wiskott–Aldrich syndrome protein (WASP) and the Arp2/3 complex (Rohatgi *et al.*, 1999). Although Arp2/3 and WASP can nucleate new actin filaments in the absence of *Cdc42*, this activity increases dramatically in the presence of activated *Cdc42* and phospholipids. Thus the reduction (rather than complete loss) of assembly of cortical actin filaments that we have observed is consistent with the hypothesis that *Cdc42* functions by regulating the rate of actin filament assembly, presumably in response to activation of an as yet unknown signaling pathway.

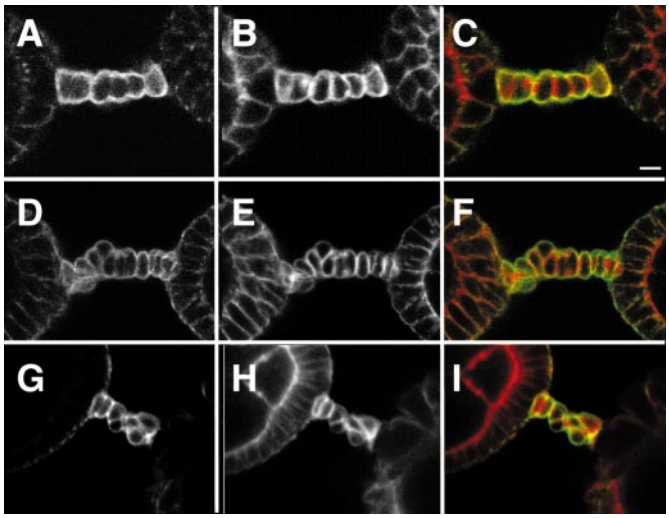

FIG. 8. Loss of Cdc42 function in the germ line affects stalk cell differentiation. Stalk cells separating egg chambers with wild-type (A–C), $Cdc42^{1}$ (D–F), or $Cdc42^{4}$ (G–I) germ-line cells stained with the stalk-cell-specific marker anti-PS1 (A, D, G) and rhodamine phalloidin (B, E, H). The merged images (C, F, I) display PS1 in green and filamentous actin in red. In all cases germ-line clones were induced in ovo^{D1} heterozygous females. As indicated in Table 2 and in D–I, the number of stalk cells, which are wild type, increases when the adjacent germ-line cells are mutant for *Cdc42*. Scale bar represents 5 μm.

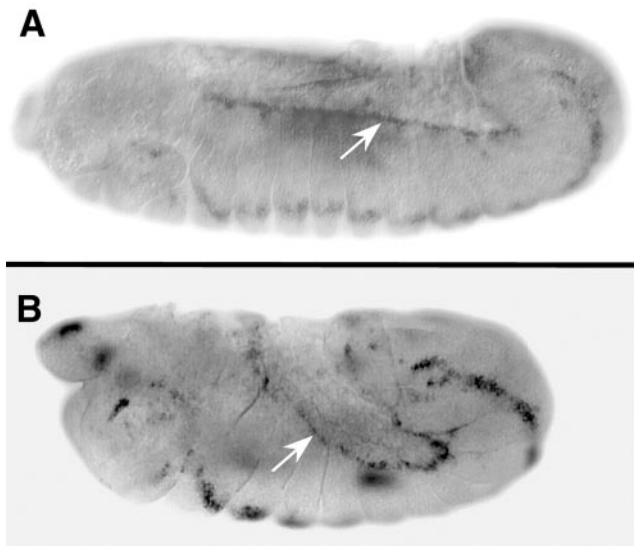

FIG. 9. Loss of *Cdc42* function does not affect *dpp* expression at the leading edge during dorsal closure. *dpp* expression as revealed by *in situ* hybridization in a w^{1118} embryo (A) or an embryo derived from a $Cdc42^{4}/Cdc42^{6}$ female (B). *dpp* expression at the leading edge (arrows) appears normal in both classes of embryo.

Cdc42 in Cell Signaling

Given the structural similarity between Cdc42, Rho, and related proteins, including Ras, it seems likely that *Cdc42*

functions as a component of a signaling cascade triggered by cues that are external to the cell. Although many components of this pathway have not been identified, previous studies have suggested that *Cdc42* functions upstream of the JNK signaling cascade in mediating cytoskeletal rearrangements (Agnès *et al.,* 1999; Riesgo-Escovar *et al.,* 1996). In *Drosophila,* the JNK pathway appears to regulate the process of embryonic dorsal closure, in part by regulating the expression of DPP, a diffusible ligand that is expressed by the dorsalmost row of epidermal cells. If this model is correct, we would expect that reduction of *Cdc42* function would reduce or eliminate *dpp* expression in the dorsal epidermis. However, the evidence presented here indicates that *dpp* expression is independent of *Cdc42* function in the leading-edge cells (Fig. 9). In addition, the JNK pathway may be activated independent of the Rho family of proteins (Liu *et al.,* 1999; Su *et al.,* 1998). We therefore conclude that Cdc42 function is not essential for activation of the JNK pathway and *dpp* expression at the leading edge during the process of dorsal closure.

Our finding that *dpp* expression is independent of Cdc42 function seems at odds with a previous study using ectopic expression of a dominant negative *Cdc42* allele (Riesgo-Escovar *et al.,* 1996). Similarly, a more recent study indicates that ectopic expression of constitutively active Cdc42 in the wing imaginal epithelium results in ectopic expression of *puckered* via the JNK pathway (Agnès *et al.,* 1999). Other examples of discrepancies between the results in this study, which used loss-of-function mutations, and previous studies using dominant interfering or activating mutations have been mentioned previously. The cause of these discrepancies is not certain, but one likely possibility is that dominant negative or active Cdc42 may "cross-talk" nonspecifically with other members of the Rho family of small GTPases. Thus, it is possible that expression of dominant negative Cdc42 results in partial or complete suppression of Rho and/or Rac function in addition to Cdc42 function. Consistent with this notion, several studies indicate that Rac rather than Cdc42 functions upstream of JNK pathway activation (Coso *et al.,* 1995; Minden *et al.,* 1995; Teramoto *et al.,* 1996). In addition, phenotypes from ectopic expression of dominant negative Cdc42 in the germ line (Murphy and Montell, 1996), which differ from the loss-of-function phenotypes reported here (Fig. 7), closely mimic those recently reported for loss-of-function Rho mutations (Magie *et al.,* 1999). On the other hand, because of maternal contribution and the possibility that our alleles partially rather then completely eliminate Cdc42 function, it is difficult to be certain that the phenotypes we report represent the null *Cdc42* phenotype. Thus the absence of an obvious axon outgrowth phenotype in *Cdc42* mutant embryos that display epidermal defects could indicate that neurons have a lower threshold requirement for Cdc42 function than do epidermal cells. Resolution of these questions will require further study into loss of function *Cdc42* phenotypes and the mode of action of dominant negative and active *Cdc42* alleles. In the meantime, it seems clear that caution needs to be taken in interpreting the results of either type of experiment and that both are necessary to fully elucidate the role of Cdc42 and the related Rho family members during development and cellular morphogenesis.

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REFERENCES

Adams, A. E., Johnson, D. I., Longnecker, R. M., Sloat, B. F., and Pringle, J. R. (1990). *CDC42* and *CDC43,* two additional genes involved in budding and the establishment of cell polarity in the yeast *Saccharomyces cerevisiae. J. Cell Biol.* **111,** 131–142.

Agnès, F., Suzanne, M., and Noselli, S. (1999). The Drosophila JNK pathway controls the morphogenesis of imaginal discs during metamorphosis. *Development* **126,** 5453–5462.

Ashburner, M. (1989). "*Drosophila:* A Laboratory Handbook." Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Bunch, T. A., Grinblat, Y., and Goldstein, L. S. B. (1988). Characterization and use of the *Drosophila* metallothionein promoter in cultured *Drosophila melanogaster* cells. *Nucleic Acids Res.* **16,** 1043–1061.

Burke, R., and Basler, K. (1996). Dpp receptors are autonomously required for cell proliferation in the entire developing *Drosophila* wing. *Development* **122,** 2261–2269.

Coso, O. A., Chiariello, M., Yu, J. C., Teramoto, H., Crespo, P., Xu, N., Miki, T., and Gutkind, J. S. (1995). The small GTP-binding proteins Rac1 and Cdc42 regulate the activity of the JNK/SAPK signaling pathway. *Cell* **81,** 1137–1146.

Drechsel, D. N., Hyman, A. A., Hall, A., and Glotzer, M. (1997). A requirement for Rho and Cdc42 during cytokinesis in Xenopus embryos. *Curr. Biol.* **7,** 12–23.

Eaton, S., Auvinen, P., Luo, L., Jan, Y. N., and Simons, K. (1995). CDC42 and Rac1 control different actin-dependent processes in the *Drosophila* wing disc epithelium. *J. Cell Biol.* **131,** 151–164.

Eaton, S., Wepf, R., and Simons, K. (1996). Roles for Rac1 and Cdc42 in planar polarization and hair outgrowth in the wing of Drosophila. *J. Cell Biol.* **135,** 1277–1289.

Fehon, R. G., Dawson, I. A., and Artavanis-Tsakonas, S. (1994). A *Drosophila* homologue of membrane-skeleton protein 4.1 is associated with septate junctions and is encoded by the *coracle* gene. *Development* **120,** 545–557.

Fehon, R. G., Oren, T., LaJeunesse, D. R., Melby, T. E., and McCartney, B. M. (1997). Isolation of mutations in the Drosophila homologues of the human *Neurofibromatosis 2* and yeast *CDC42* genes using a simple and efficient reverse-genetic method. *Genetics* **146,** 245–252.

Glise, B., and Noselli, S. (1997). Coupling of Jun amino-terminal kinase and Decapentaplegic signaling pathways in Drosophila morphogenesis. *Genes Dev.* **11,** 1738–1747.

Goode, S., Melnick, M., Chou, T. B., and Perrimon, N. (1996). The neurogenic genes egghead and brainiac define a novel signaling pathway essential for epithelial morphogenesis during *Drosophila* oogenesis. *Development* **122,** 3863–3879.

Grammont, M., Dastugue, B., and Couderc, J. L. (1997). The Drosophila toucan (toc) gene is required in germline cells for the somatic cell patterning during oogenesis. *Development* **124,** 4917–4926.

Harden, N., Ricos, M., Ong, Y. M., Chia, W., and Lim, L. (1999). Participation of small GTPases in dorsal closure of the Drosophila embryo: Distinct roles for Rho subfamily proteins in epithelial morphogenesis. *J. Cell Sci.* **112,** 273–284.

LaJeunesse, D. R., McCartney, B. M., and Fehon, R. G. (1998). Structural analysis of Drosophila Merlin reveals functional domains important for growth control and subcellular localization. *J. Cell Biol.* **141,** 1589–1599.

Lee, J. K., Brandin, E., Branton, D., and Goldstein, L. S. (1997). alpha-Spectrin is required for ovarian follicle monolayer integrity in Drosophila melanogaster. *Development* **124,** 353–362.

Liu, H., Su, Y. C., Becker, E., Treisman, J., and Skolnik, E. Y. (1999). A Drosophila TNF-receptor-associated factor (TRAF) binds the ste20 kinase Misshapen and activates Jun kinase. *Curr. Biol.* **9,** 101–104.

Lundell, M. J., and Hirsh, J. (1994). A new visible light DNA fluorochrome for confocal microscopy. *Biotechniques* **16,** 434–440.

Luo, L., Liao, Y. J., Jan, L. Y., and Jan, Y. N. (1994). Distinct morphogenetic functions of similar small GTPases: *Drosophila* Drac1 is involved in axonal outgrowth and myoblast fusion. *Genes Dev.* **8,** 1787–1802.

Magie, C. R., Meyer, M. R., Gorsuch, M. S., and Parkhurst, S. M. (1999). Mutations in the Rho1 small GTPase disrupt morphogenesis and segmentation during early Drosophila development. *Development* **126,** 5353–5364.

Mahowald, A. P., and Kambysellis, M. P. (1980). Oogenesis. *In* "Genetics and Biology of *Drosophila*" (M. Ashburner and T. R. F. Wright, Eds.), Vol. 2, pp. 141–224. Academic Press, London.

Martin-Blanco, E., Gampel, A., Ring, J., Virdee, K., Kirov, N., Tolkovsky, A. M., and Martinez-Arias, A. (1998). puckered encodes a phosphatase that mediates a feedback loop regulating JNK activity during dorsal closure in Drosophila. *Genes Dev* **12,** 557–570.

McCartney, B. M., and Fehon, R. G. (1996). Distinct cellular and subcellular patterns of expression imply distinct functions for the *Drosophila* homologues of moesin and the neurofibromatosis 2 tumor suppressor, merlin. *J. Cell Biol.* **133,** 843–852.

Minden, A., Lin, A., Claret, F. X., Abo, A., and Karin, M. (1995). Selective activation of the JNK signaling cascade and c-Jun transcriptional activity by the small GTPases Rac and Cdc42Hs. *Cell* **81,** 1147–1157.

Murphy, A. M., and Montell, D. J. (1996). Cell type-specific roles for Cdc42, Rac, and RhoL in *Drosophila* oogenesis. *J. Cell Biol.* **133,** 617–630.

Nobes, C. D., and Hall, A. (1995). Rho, rac and cdc42 GTPases: Regulators of actin structures, cell adhesion and motility. *Biochem. Soc. Trans.* **23,** 456–459.

Orsulic, S., and Peifer, M. (1994). A method to stain nuclei of Drosophila for confocal microscopy. *Biotechniques* **16,** 441–447.

Riesgo-Escovar, J. R., and Hafen, E. (1997). Common and distinct roles of DFos and DJun during Drosophila development. *Science* **278,** 669–672.

Riesgo-Escovar, J. R., Jenni, M., Fritz, A., and Hafen, E. (1996). The Drosophila Jun-N-terminal kinase is required for cell morphogenesis but not for DJun-dependent cell fate specification in the eye. *Genes Dev.* **10,** 2759–2768.

Rohatgi, R., Ma, L., Miki, H., Lopez, M., Kirchhausen, T., Takenawa, T., and Kirschner, M. W. (1999). The interaction between N-WASP and the Arp2/3 complex links Cdc42- dependent signals to actin assembly. *Cell* **97,** 221–231.

Simpson, P. (1979). Parameters of cell competition in the compartments of the wing disc of Drosophila. *Dev. Biol.* **69,** 182–193.

Sluss, H. K., and Davis, R. J. (1997). Embryonic morphogenesis signaling pathway mediated by JNK targets the transcription factor JUN and the TGF-beta homologue decapentaplegic. *J. Cell. Biochem.* **67,** 1–12.

Su, Y. C., Treisman, J. E., and Skolnik, E. Y. (1998). The Drosophila Ste20-related kinase misshapen is required for embryonic dorsal closure and acts through a JNK MAPK module on an evolutionarily conserved signaling pathway. *Genes Dev.* **12,** 2371–2380.

Teramoto, H., Crespo, P., Coso, O. A., Igishi, T., Xu, N., and Gutkind, J. S. (1996). The small GTP-binding protein rho activates c-Jun N-terminal kinases/stress-activated protein kinases in human kidney 293T cells. Evidence for a Pak-independent signaling pathway. *J. Biol. Chem.* **271,** 25731–25734.

Van Aelst, L., and D'Souza-Schorey, C. (1997). Rho GTPases and signaling networks. *Genes Dev.* **11,** 2295–2322.

Wieschaus, E., Audit, C., and Masson, M. (1981). A clonal analysis of the roles of somatic cells and germ line during oogenesis in *Drosophila. Dev. Biol.* **88,** 92–103.

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