DRhoGEF2 regulates actin organization and contractility in the Drosophila blastoderm embryo.

Padash, Mojgan; Rogers, Stephen; Häcker, Udo

Published in:
Journal of Cell Biology

DOI:
10.1083/jcb.200407124

2005

DRhoGEF2 regulates actin organization and contractility in the *Drosophila* blastoderm embryo

Mojgan Padash Barmchi,[1] Stephen Rogers,[2] and Udo Häcker[1]

[1]Department of Cell and Molecular Biology, Lund Strategic Research Center for Stem Cell Biology and Cell Therapy, Lund University, BMC B13, 22184 Lund, Sweden
[2]Howard Hughes Medical Institute and Department of Cellular and Molecular Pharmacology, University of California, San Francisco, San Francisco, CA 94143

Morphogenesis of the *Drosophila melanogaster* embryo is associated with a dynamic reorganization of the actin cytoskeleton that is mediated by small GTPases of the Rho family. Often, *Rho1* controls different aspects of cytoskeletal function in parallel, requiring a complex level of regulation. We show that the guanine triphosphate (GTP) exchange factor DRhoGEF2 is apically localized in epithelial cells throughout embryogenesis. We demonstrate that *DRhoGEF2*, which has previously been shown to regulate cell shape changes during gastrulation, recruits Rho1 to actin rings and regulates actin distribution and actomyosin contractility during nuclear divisions, pole cell formation, and cellularization of syncytial blastoderm embryos. We propose that *DRhoGEF2* activity coordinates contractile actomyosin forces throughout morphogenesis in *Drosophila* by regulating the association of myosin with actin to form contractile cables. Our results support the hypothesis that specific aspects of *Rho1* function are regulated by specific GTP exchange factors.

Introduction

Animal development is associated with extensive morphological changes of individual cells and entire tissues. Often, these changes require a dynamic rearrangement of the cortical actomyosin-based cytoskeleton. One example is the early phase of embryogenesis in *Drosophila melanogaster*. Development of the *Drosophila* zygote begins with 13 synchronous nuclear division cycles that create a syncytial embryo containing ~6,000 nuclei. During mitotic cycles, 10–13 shallow metaphase furrows form transiently between neighboring nuclei at the cell cortex, thus preventing mitotic spindles from colliding. Formation of the furrows is accompanied by relocalization of actin from apical caps into actin rings located at the base of the furrows. Blastoderm cellularization occurs during interphase of cycle 14 when simultaneous cytokinesis transforms the monolayer of syncytial nuclei into a columnar epithelium. This process can be subdivided into a slow and a fast phase, and both phases require distinct cytoskeletal rearrangements. During the early slow phase, cell membranes begin to invaginate radially between the nuclei and form stable infoldings called the furrow canal. The leading edge of the furrow canal is rich in actin and myosin, which form a hexagonal array surrounding the nuclei. As the actomyosin network moves inward, the nuclei, which are spherical at the onset of cellularization, begin to elongate. When the cellularization front has reached the base of the nuclei, the rate of membrane invagination increases by twofold. During this fast phase, actomyosin rings in the furrow canal constrict to close the blastoderm cells basally (for review see Mazumdar and Mazumdar, 2002).

Although some of the genes involved in cytoskeletal rearrangement during cellularization, such as the small GTPase *Rho1* (Crawford et al., 1998), the cytoskeletal regulator *diaphanous* (*dia*; Afshar et al., 2000) and the zygotic genes *nullo*, *serendipity-α*, and *bottleneck* (*bnk*; Schejter and Wieschaus, 1993; Postner and Wieschaus, 1994) have been identified, the genetic circuitry regulating the actomyosin contractile apparatus is not well understood.

The small GTPase *Rho1* has been identified as a powerful regulator of cytoskeletal reorganization in many contexts. In *Drosophila*, *Rho1* has been demonstrated to play an essential role during oogenesis (Magie et al., 1999), cellularization (Crawford et al., 1998), gastrulation (Barrett et al., 1997; Häcker and Perrimon, 1998), segmentation, dorsal closure (Magie et al., 1999), planar polarity determination (Strutt et al., 1997) and cytokinesis (Prokopenko et al., 1999). In many of these processes, *Rho1* controls the spatial and temporal coordination of different aspects of cytoskeletal function such as actin polymerization or contraction of actomyosin fibers in parallel, implying a complex level of regulation of *Rho1* activity. Like

Correspondence to Udo Häcker: udo.haecker@medkem.lu.se

The present address of S. Rogers is Department of Biology and Carolina Center for Genome Sciences, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599.

The online version of this article contains supplemental material.

The Journal of Cell Biology, Vol. 168, No. 4, February 14, 2005 575–585
http://www.jcb.org/cgi/doi/10.1083/jcb.200407124

Supplemental Material can be found at:
http://www.jcb.org/cgi/content/full/jcb.200407124/DC1

other members of the small GTPase family, *Rho1* acts as a molecular switch that is inactive when GDP is bound and activated upon exchange of GDP for GTP by GTP exchange factors (for review see Settleman, 2001).

During cytokinesis, which is mechanistically related to blastoderm cellularization, a linear pathway that includes Dia—the formin homology protein—and profilin—an actin-binding protein that regulates actin polymerization—has been proposed to link Rho1 to the actin cytoskeleton. The RhoGEF *pebble* has been shown to activate Rho1 in this context. Pebble is localized to contractile actin rings during cytokinesis in a cell cycle–regulated fashion. The function of *pebble* is considered essential for the assembly of actin rings during cytokinesis in all cells during *Drosophila* embryogenesis subsequent to cell cycle 14 (Prokopenko et al., 1999). Recently, *pebble* has also been shown to regulate the lateral migration of mesodermal cells (Schumacher et al., 2004; Smallhorn et al., 2004). Interestingly, *pebble* is not required during syncytial nuclear divisions or during blastoderm cellularization, suggesting that a different RhoGEF may activate Rho1 during this phase of development (Lehner, 1992).

A likely candidate is *DRhoGEF2*, which is maternally contributed to the embryo and ubiquitously expressed throughout embryogenesis. *DRhoGEF2* has previously been shown to regulate the apical constriction of cells during invagination of the mesodermal and endodermal germ layers immediately after cellularization (Barrett et al., 1997; Häcker and Perrimon, 1998). We have used α-DRhoGEF2 antiserum to investigate the role of *DRhoGEF2* during early embryogenesis in more detail. Here, we show that DRhoGEF2 colocalizes with actin, myosin II, Rho1, Dia, and Bnk in the furrow canal during cellularization and is required for the recruitment of Rho1 to the cellularization front. *DRhoGEF2* regulates actin localization and actin ring constriction during pole cell formation, metaphase furrow formation, and cellularization. Based on our results, we propose that *DRhoGEF2* regulates a specific aspect of Rho1-mediated cytoskeletal reorganization throughout *Drosophila* morphogenesis.

Results

DRhoGEF2 is apically enriched in epithelia throughout embryogenesis

DRhoGEF2 transcripts are maternally contributed to the embryo and ubiquitously expressed throughout embryogenesis. However, we observed *DRhoGEF2*-dependent cytoskeletal reorganization in a tissue-specific fashion during development and suggest that *DRhoGEF2* activity may be regulated at the protein level. To investigate the subcellular localization of DRhoGEF2, we stained wild-type embryos with α-DRhoGEF2 antibodies (Rogers et al., 2004). DRhoGEF2 was detected predominantly in epithelia throughout embryogenesis and was enriched at the apical end of cells. In agreement with its previously reported role during gastrulation, DRhoGEF2 levels were elevated at the apical and apico-lateral membrane of cells in the ventral furrow (Fig. 1 A), in the posterior midgut primordium (Fig. 1 B), and in the cephalic furrow (Fig. 1 C). After germ band elongation, DRhoGEF2 was detectable at the apical end of cells in the epidermis (Fig. 1 D). After germ band retraction, DRhoGEF2 levels were elevated in a periodically repeated pattern in the ventral epidermal cells of thoracic and abdominal segments (Fig. 1 E). In the central nervous system, DRhoGEF2 was concentrated in longitudinal and commissural axon fascicles (Fig. 1, E and F). In the lateral epidermis, DRhoGEF2 was highly concentrated at the apical cortex of leading edge cells during dorsal closure (Fig. 1 G). These data suggest that *DRhoGEF2* may play a role in reorganizing the cortical actin cytoskeleton throughout embryonic development.

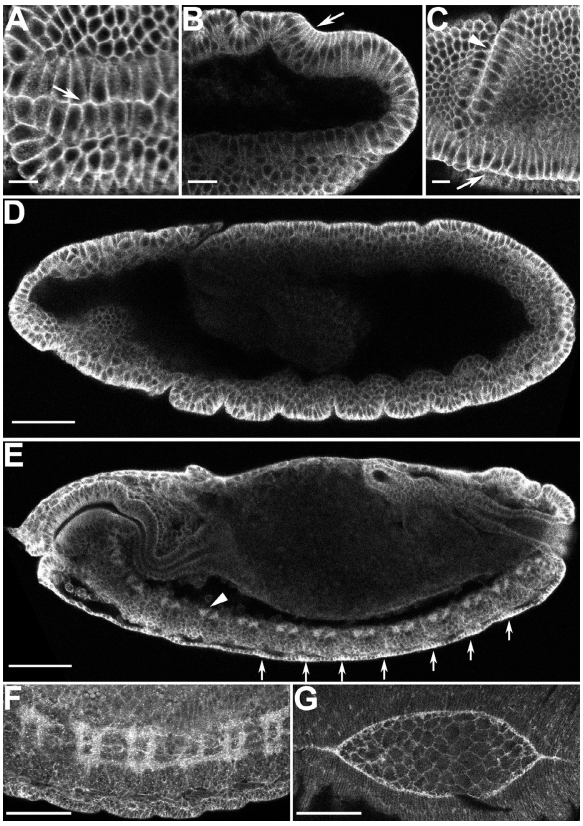

Figure 1. **Expression of *DRhoGEF2* during embryonic development.** (A–C) *DRhoGEF2* protein accumulates apically in cells of the invaginating ventral furrow (A and C, arrows), in cells of the posterior midgut primordium (B, arrow), and in cells in the cephalic fold (C, arrowhead). (D) At late stage 11, DRhoGEF2 is apically localized in all epidermal cells. (E) At late stage 14, DRhoGEF2 is enriched in a periodic pattern in the epidermis (arrows) and in commissural axon tracts (arrowhead). (F) DRhoGEF2 is enriched in longitudinal and commissural axon tracts in the central nervous system. (G) During dorsal closure of the epidermis, DRhoGEF2 is highly enriched at the apical cortex of leading edge cells. Anterior is to the left. B–E show lateral views; A and F show ventral views; and G shows dorsal view. Bars: (A–C) 10 μm; (D–G) 50 μm.

DRhoGEF2 is localized in actin- and myosin-rich regions during cellularization

During the interphase of syncytial nuclear cycles, DRhoGEF2 was found concentrated in the apical actin caps (Fig. 2 A). At prophase, DRhoGEF2 relocalized to the metaphase furrows (Fig. 2 B), where its levels were highest at the center of the broader actin domain (Fig. 2 B, arrow). At the onset of cellularization, the majority of DRhoGEF2

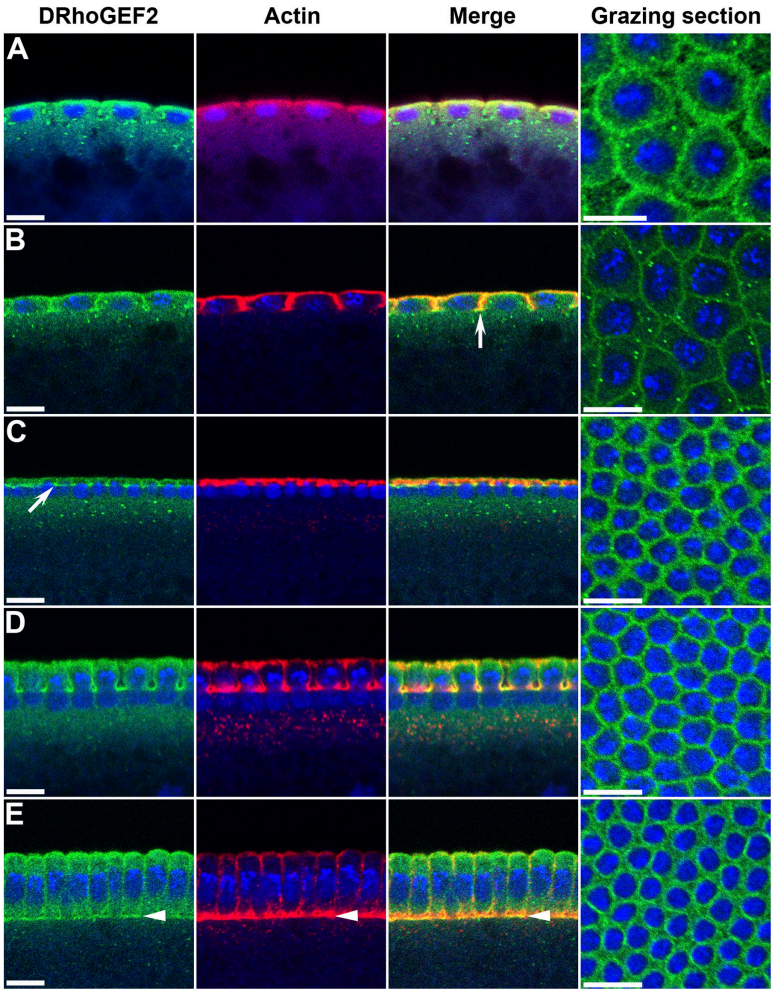

Figure 2. **Distribution of DRhoGEF2 during blastoderm cellularization.** DRhoGEF2 is shown in green; actin is shown in red, and DNA is shown in blue. (A) At the interphase of syncytial divisions DRhoGEF2 is concentrated in apical actin caps. (B) During syncytial nuclear cycles DRhoGEF2 redistributes to the metaphase furrows. The DRhoGEF2 domain is narrower than the actin domain (arrow). DRhoGEF2 is also observed in large cytoplasmic vesicles. (C) At the onset of cellularization, DRhoGEF2 redistributes to the furrow canal (arrow). Apical DRhoGEF2 levels are low. (D) DRhoGEF2 remains concentrated at the base of the furrow canal throughout membrane invagination. Apical DRhoGEF2 levels increase during the slow phase. (E) During basal closure, DRhoGEF2 is enriched at the basal-most edge of blastoderm cells in the region where actin rings are located (arrowheads). Left three panels show sagittal views; right panels show grazing view. Bars, 10 μm.

redistributed to the base of the furrow canal (Fig. 2 C). Apical levels were low at this time, but increased again gradually during the slow phase (Fig. 2 D), suggesting that DRhoGEF2 might play a role in the retraction of apical microvilli that occurs during cellularization (Grevengoed et al., 2003). Double labeling experiments showed that DRhoGEF2 colocalized with actin and myosin II at the base of the furrow canal (Fig. 2 and Fig. S1, available at http://www.jcb.org/cgi/content/full/jcb.200407124/DC1]). Moderate levels of DRhoGEF2 also remained associated with lateral membranes (Fig. 2, D and E). During basal closure of blastoderm cells, DRhoGEF2 was detectable in a thin stripe at the basal-most boundary of the actin and myosin II domains (Fig. 2 E and Fig. S1 C). The localization of DRhoGEF2 at sites of actin rings suggests that it might play a role in the regulation of actomyosin-based forces during cellularization.

It is noteworthy that DRhoGEF2 was also seen in large particles that are most abundant during the early phase of membrane invagination (Fig. 2, A–C). Similar particles that are transported to the cellularization front in a microtubule-dependent fashion have been reported for myosin II (Royou et al., 2004). Although we were unable to detect myosin II particles, it is possible that the DRhoGEF2 particles might have a similar function in transport of DRhoGEF2 to the apical cortex.

DRhoGEF2 regulates actomyosin contractility during early embryogenesis

It has previously been shown that *DRhoGEF2* is required for apical constriction of cells in the mesodermal and endodermal primordia during gastrulation (Barrett et al., 1997; Häcker and Perrimon, 1998). *DRhoGEF2* protein distribution suggests that *DRhoGEF2* may also have an earlier function. To investigate the role of *DRhoGEF2* during the syncytial phase of embryogenesis and cellularization, we generated germline clones from a *DRhoGEF2* null allele (embryos derived from *DRhoGEF2* mutant germline clones are hereafter referred to as *DRhoGEF2* mutants; see Materials and methods).

In wild-type embryos, actin cycles between apical actin caps and metaphase furrows during nuclear divisions 10–13 (Fig. 3, A and A′). At the onset of cellularization, actin relocalizes from the actin caps to the furrow canal where it forms a network of interlinked actin hexagons that surround the nuclei (Fig. 3, C and C′). In the course of the slow phase of membrane invagination, apical actin is almost completely redistributed to the base of the furrow canal (Fig. 3 E). When the cellularization front reaches the base of the nuclei, the actin hexagons detach from each other and begin to contract, thereby expanding the furrow canal (Fig. 3 E′). Contraction of actin rings eventually leads to basal closure of the newly formed blastoderm cells (Fig. 3, G and G′).

In *DRhoGEF2* mutants, the depth of metaphase furrows is more variable than in the wild type, and actin is more unevenly distributed (Fig. 3, B and B′). In some areas, metaphase furrows fail to invaginate or break down; this failure can cause adjacent mitotic spindles to collide (Fig. 3 B′, arrow) and leads to the elimination of nuclei from the cortex, which results in empty "pseudocells" (Fig. 3 B′ and Video 1 [available at http://www.jcb.org/cgi/content/full/jcb.200407124/DC1]; Grevengoed et al., 2003).

At the onset of cellularization, less actin is localized to the furrow canal and apical actin levels remain higher in *DRhoGEF2* mutants (Fig. 3 D). Actin rings appear rounded instead of hexagonal, as if they are not under tension. In the wild type, actin hexagons tightly surround the nuclei and slightly squeeze them. In contrast, nuclei in *DRhoGEF2* mutants are wider and abnormal, and multinucleated actin rings are frequently observed (Fig. 3 D′). Because abnormal nuclei are removed from the cell surface, we believe that they arise during cellularization rather than being the results of earlier defects during nuclear divisions. When the cellularization front reaches the base of the nuclei, actin rings stay in proximity to each other, and no significant constriction is observed (Fig. 3, F and F′). During the subsequent fast phase, constriction is irregular, and many multinucleated actin rings remain very large (Fig. 3, H and H′). Significant amounts of actin fail to localize to the cellularization front and remain apical (Fig. 3, F and H). We conclude from these results that *DRhoGEF2* activity is required for the organization and stabilization of actin rings at invaginating furrows and for the regulation of contractile actomyosin forces during cellularization.

The generation of contractile force requires actin to associate with myosin II, which is encoded by the *spaghetti squash* (*sqh*) gene. To assess whether the defects in actomyosin contraction may be caused by mislocalization of myosin II, we expressed an *sqh*-GFP fusion protein (Royou et al., 2004) in *DRhoGEF2* mutants, but no significant changes in localization or levels of myosin II were observed (Fig. S2, available at http://www.jcb.org/cgi/content/full/jcb.200407124/DC1). Next, we followed the time course of cellularization by time-lapse confocal microscopy (Video 2). Interestingly, a decrease in the rate of membrane invagination was not observed in *DRhoGEF2* mutants. Often, it seemed that the rate was slightly increased instead; this observation suggests that actomyosin contractility might not play a role or play only a very minor role in membrane invagination. In addition, these experiments revealed that the radial movement of nuclei was irregular in *DRhoGEF2* mutants. In the wild type, nuclei move inward in parallel to each other during the slow phase. In *DRhoGEF2* mutants, coordination of this movement is disrupted and some nuclei remain apical, suggesting that actomyosin-based forces may play a role in nuclear alignment.

DRhoGEF2 is required for Rho1 localization

DRhoGEF2 has previously been implicated in the regulation of *Rho1* activity (Barrett et al., 1997; Häcker and Perrimon, 1998). To investigate whether *Rho1* may be a target for *DRhoGEF2* during cellularization, we determined the localization of Rho1 with respect to DRhoGEF2 and found that Rho1 colocalizes precisely with DRhoGEF2 at the base of the furrow canal (Fig. 4, A–C). To define the domain of DRhoGEF2 and Rho1 localization more precisely, we double-labeled embryos with β-Heavy spectrin and Rho1. It has been shown that β-Heavy spectrin is present in the furrow canal in a domain apically adjacent to myosin II (Thomas and Kiehart, 1994). We found that Rho1 and, by inference, DRhoGEF2 are localized basal to β-Heavy spectrin at the basal-most boundary of the myosin II domain (Fig. 4, D and E).

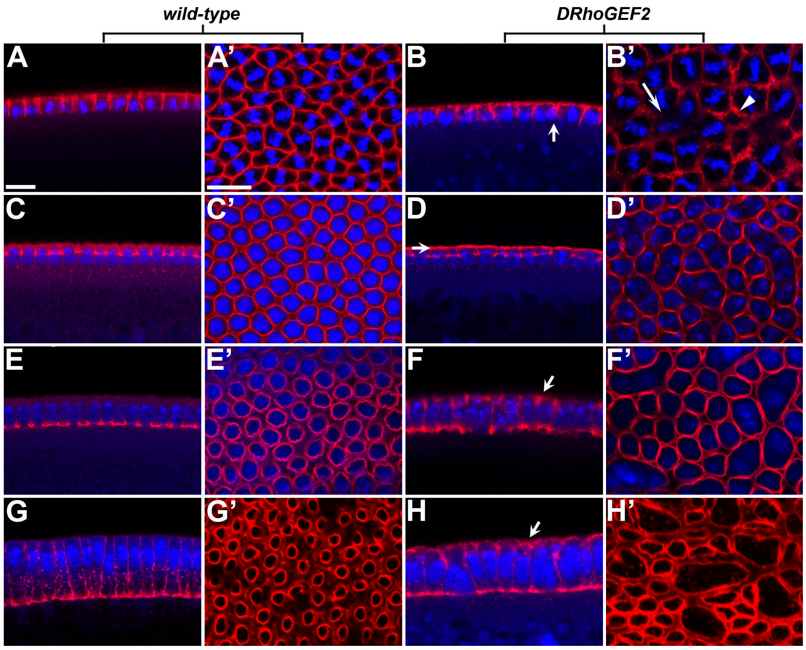

Figure 3. **Phenotype of *DRhoGEF2* mutants during blastoderm cellularization.** Actin is shown in red; and DNA is shown in blue. (A and A′) Metaphase furrows at cycle 13 in the wild type are shown. (B and B′) Same stage in *DRhoGEF2*$^{l(2)04291}$ is shown. Metaphase furrows invaginate to variable depths (B, arrow) and actin is irregularly distributed. Sometimes furrow formation fails (B′, arrow). Abnormal nuclei are eliminated from the cell surface (B′, arrowhead; and Video 1). (C and C′) Slow phase in the wild type is shown. (D and D′) Same stage in *DRhoGEF2*$^{l(2)04291}$ is shown. Significant amounts of actin fail to redistribute to the furrow canal, but remain apical (D, F, and H, arrows). Nuclei are not squeezed by the furrow and are wider than in the wild type. Actin rings have a rounded rather than a hexagonal shape, are irregular in size, and are frequently multinucleated (D′). (E and E′) Onset of the fast phase in the wild type is shown. (F and F′) Same stage in *DRhoGEF2*$^{l(2)04291}$ is shown. The furrow canal does not expand and no constriction of actin rings is observed. (G and G′) Shown is the basal closure in the wild type. (H and H′) Same stage in *DRhoGEF2*$^{l(2)04291}$. Actin rings are irregular in size and some remain very large. A–H show sagittal views at the same magnification; A′–H′ show grazing views at the same magnification. Bars, 10 μm.

Rho1 is essential for cytoskeletal regulation during oogenesis in *Drosophila*, and female germline clones mutant for *Rho1* null alleles do not survive (Magie et al., 1999), precluding a genetic analysis of *Rho1* function in the embryo. To overcome this problem, we have generated a *piggyBac* insertion in the 5′-untranslated region of *Rho1*. Embryos, derived from germline clones mutant for this allele, $Rho1^{L3}$, develop with variable defects that range from failure to establish organized actin structures in the most severe cases (Fig. 5 A and Fig. S3 A [available at http://www.jcb.org/cgi/content/full/jcb.200407124/DC1]) to irregular and often only partial membrane invagination in the least severe cases (Fig. 5 B and Fig. S3 B). Although low levels of Rho1 must be present in these embryos, the Rho1 that localized to the furrow canal was consistently below the detection threshold of α-Rho1 antibodies (Fig. 5 B). If Rho1 were required to recruit DRhoGEF2, one might expect that this reduction in Rho1 levels would result in a reduction of DRhoGEF2 at the furrow canal. However, this was not observed (Fig. 5 B). Although we cannot exclude that the reduced amount of Rho1 might be able to recruit similar levels of DRhoGEF2 to the furrow canal as in the wild type, we believe it is more likely that DRhoGEF2 is localized independent of Rho1. Conversely, when *DRhoGEF2* mutants were stained with α-Rho1 antibodies, localization of Rho1 was disrupted (Fig. 5 C), suggesting that DRhoGEF2 may recruit Rho1 to the furrow canal.

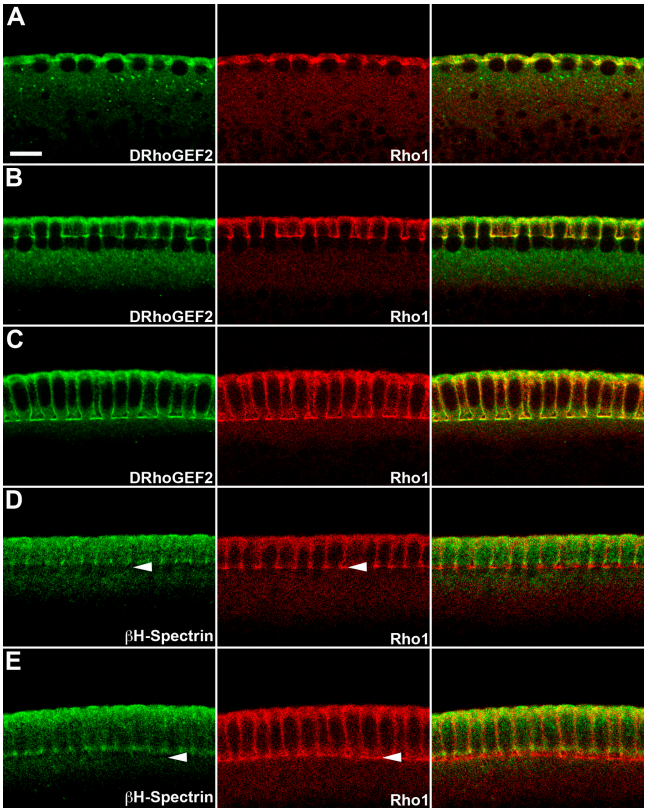

Figure 4. **Localization of DRhoGEF2 with respect to Rho1 and β-Heavy spectrin.** (A–C) DRhoGEF2 (green) and Rho1 (red) colocalize precisely at the furrow canal during membrane invagination. (D and E) β-Heavy spectrin (green) and Rho1 (red) are localized in adjacent nonoverlapping membrane subdomains (compare arrowheads). (right) Merge of the left two panels. All panels have the same magnification. Bar, 10 μm.

It has been proposed that the formin homology protein diaphanous (Dia) is a Rho1 effector during the assembly of actin rings. Dia is required for the localization of several actin-binding proteins such as anillin (Field and Alberts, 1995) and peanut (Pnut; Cooper and Kiehart, 1996) at the furrow canal (Afshar et al., 2000). To determine whether Dia may be involved in DRhoGEF2 localization, we generated germline clones of the null allele dia^5. Although maternally mutant dia^5 embryos have severe cellularization defects, DRhoGEF2 concentration at the furrow canal was unaffected (Fig. 5 D). Conversely, localization of Dia (Fig. 5 C) and the *Drosophila* septin family protein Pnut (not depicted) was unaffected in *DRhoGEF2* mutants. We conclude that DRhoGEF2 may be localized to the cellularization front independently of Dia and Rho1.

DRhoGEF2 acts in concert with *bnk* during cellularization

The actin-binding protein Bnk plays an important role during cellularization (Schejter and Wieschaus, 1993). It has been pro-

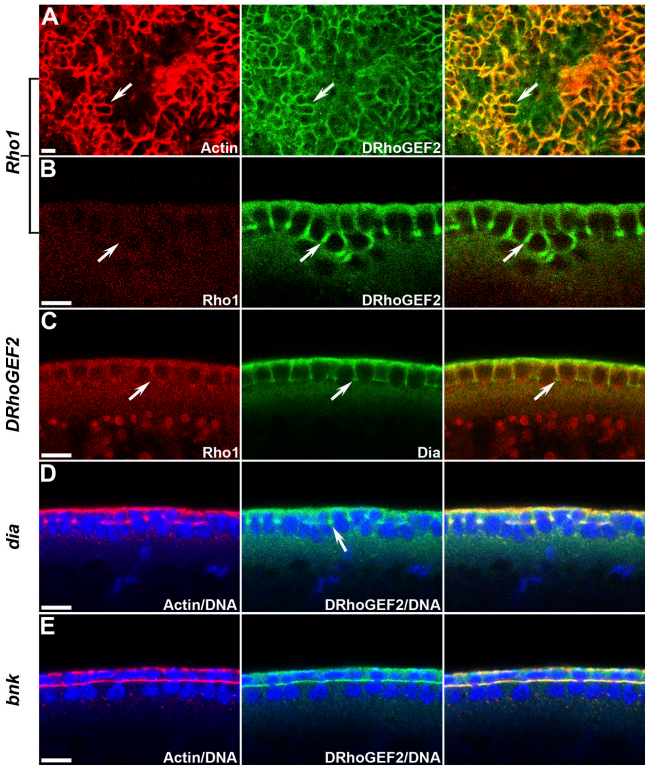

Figure 5. **Localization of DRhoGEF2 in $Rho1^{L3}$, dia^5 and, *bnk* mutants.** (A) $Rho1^{L3}$ embryo stained for actin (red) and DRhoGEF2 (green). Example of severe phenotype. Although actin structures are severely disrupted, DRhoGEF2 is concentrated at actin-rich structures (compare arrows). (B) $Rho1^{L3}$ embryo stained for Rho1 (red) and DRhoGEF2 (green). Example of less severe phenotype (possibly caused by paternal rescue; Fig. S3). DRhoGEF2 localization is unaffected although Rho1 is reduced to background levels (compare arrows). (C) $DRhoGEF2^{l(2)04291}$ embryo stained for Rho1 (red) and Dia (green). Concentration of Rho1 at the furrow canal is abrogated (compare arrows). Localization of Dia at the furrow canal is not affected. (D) dia^5 embryo stained for actin (red) and DRhoGEF2 (green). DRhoGEF2 concentration at the furrow canal (arrow) is unaffected. (E) *Df(3R)tll-e* embryo (*bnk* mutant) stained for actin (red) and DRhoGEF2 (green). Actin rings contract prematurely pinching the nuclei. DRhoGEF2 distribution is not affected by the absence of *bnk* function. DNA is stained in blue. (right) Merge of left two panels. Bars, 10 μm.

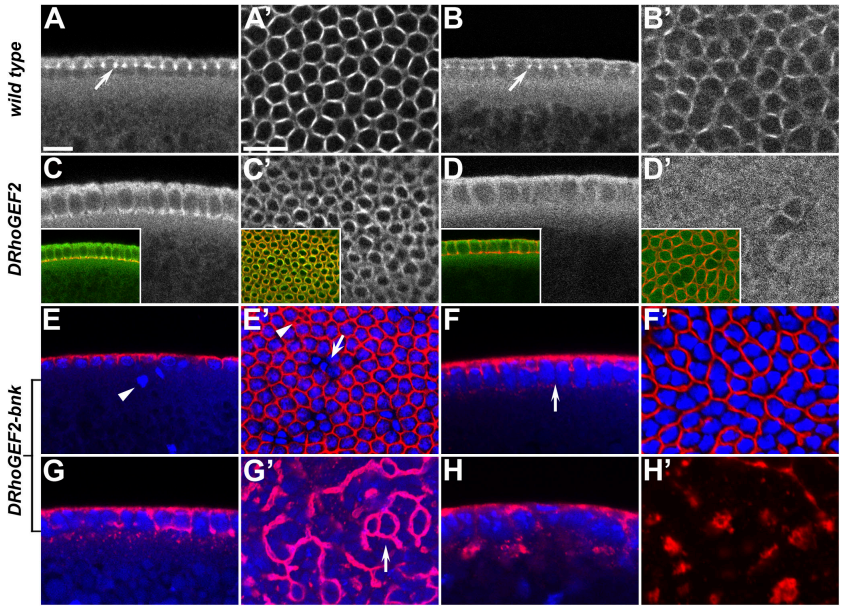

Figure 6. **Localization of Bnk in *DRhoGEF2* mutants and phenotype of *DRhoGEF2-bnk* double mutants.** (A–D) Bnk. Insets depict the same image with Bnk in green and actin in red. (E–H) Actin is shown in red; and DNA is shown in blue. (A′–H′) Corresponding grazing views of A–H. (A) Wild type at the slow phase is shown. (B) Same stage as A in *DRhoGEF2*$^{l(2)04291}$ is shown. Bnk concentration at the furrow canal is reduced (compare arrows in A and B). (C) Wild type at the end of the slow phase is shown. (D) Same stage as C in *DRhoGEF2*$^{l(2)04291}$ is shown. Bnk is not detectable at the furrow canal. (E–H) *DRhoGEF2-bnk* double mutants. (E) Metaphase furrows invaginate nonuniformly and occasionally fail to form (E′, arrow). Nuclei drop out of the cortical layer (E, arrowhead) and leave behind empty pseudocells (E′, arrowhead). (F) Early slow phase. Between some nuclei the furrow canal fails to invaginate (F, arrow) leading to multinucleated actin rings (F′). (G) Late slow phase. The actin network progressively disintegrates. Remaining actin rings do not constrict as in *bnk* mutants (arrow). (H) At later stages (as judged by the increased depth at which actin is found), actin forms randomly distributed aggregates. A–H show sagittal views at same magnification; A′–H′ show grazing views at same magnification. Bars, 10 μm.

posed that Bnk links the actomyosin hexagons surrounding individual nuclei and thereby creates a network that is kept under tension by the contractile force of actomyosin fibers (Theurkauf, 1994). Bnk is tightly associated with the lateral edges of adjacent actin hexagons, but is absent from the vertices (Fig. 6 A′). To investigate the functional relationship between *bnk* and *DRhoGEF2*, we stained wild-type and *DRhoGEF2* mutant embryos for Bnk.

bnk is a zygotically expressed gene and *bnk* protein is first detectable shortly after the onset of zygotic transcription when it is present at the apical surface during interphase and at metaphase furrows during syncytial divisions (Schejter and Wieschaus, 1993). At the onset of cellularization, Bnk redistributes to the base of the furrow canal (Fig. 6, A and A′), where it is detectable throughout the slow phase of membrane invagination (Fig. 6, C and C′). Bnk is rapidly degraded during the fast phase.

In *DRhoGEF2* mutants, a significant change in the overall levels of Bnk was not observed. However, levels of Bnk concentrated at the base of the furrow canal were decreased, as seen from the less intense staining and the more restricted distribution in the grazing confocal sections (Fig. 6, B and B′). At the time when the furrow canal had reached the base of the nuclei, Bnk was still present in the wild type (Fig. 6, C and C′), whereas no localized Bnk was detectable in *DRhoGEF2* mutants, (Fig. 6, D and D′). Conversely, *DRhoGEF2* protein levels or localization was unaffected in *bnk* mutants (Fig. 5 E). The premature disappearance of Bnk in *DRhoGEF2* mutants is not caused by a decreased rate of membrane invagination (see *DRhoGEF2* regulates actomyosin…).

This led us to speculate that DRhoGEF2 might play a role in Bnk stabilization. To test this hypothesis, we generated the *DRhoGEF2* expression construct *UAS-DRhoGEF2-RE*. When *UAS-DRhoGEF2-RE* was expressed ubiquitously using the *matα4Gal-VP16* driver (Häcker and Perrimon, 1998) in *DRhoGEF2* mutants, the ventral open cuticle defect, was rescued (Fig. 7 B), suggesting that *UAS-DRhoGEF2-RE* can provide *DRhoGEF2* function. When we expressed *UAS-DRhoGEF2-RE* using *prd-Gal4*, Bnk accumulated in a pair rule pattern in the epidermis (Fig. 7 C). This accumulation was not caused by *DRhoGEF2*-mediated transcriptional activation, because no increase in *bnk* mRNAs was observed (unpublished data). We conclude from these results that DRhoGEF2 and Bnk may be recruited to actin-rich regions independent of each other, and that DRhoGEF2 may play a role in the stabilization of Bnk.

The development of *bnk* mutants during syncytial nuclear divisions is indistinguishable from the wild type. However, shortly after the onset of membrane invagination, actin hexagons transform into rings, which constrict prematurely and cause the "bottleneck" phenotype (Fig. 5 E; Schejter and Wieschaus, 1993). To determine whether the premature constriction of actin rings requires *DRhoGEF2* function, we generated *DRhoGEF2-bnk* double mutants. During syncytial divisions,

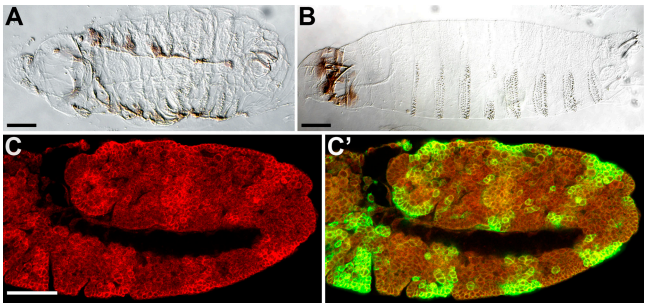

Figure 7. **Rescue of *DRhoGEF2* mutants and ectopic *DRhoGEF2* expression.** (A) *DRhoGEF2*$^{l(2)04291}$ cuticle showing ventral hole. (B) Cuticle of *DRhoGEF2*$^{l(2)04291}$ larva ubiquitously expressing *UAS-DRhoGEF2-RE*. The ventral open phenotype is rescued. (C) *prd-Gal4, UAS-DRhoGEF2-RE* embryo at stage 10 is shown. Bnk (red) accumulates in *prd*-domains in the epidermis. (C′) Same as in C showing DRhoGEF2 in green and Bnk in red. Bars, 50 μm. Anterior is to the left. Lateral views are shown, except for A, which is a ventro-lateral view.

the development of these embryos resembled *DRhoGEF2* single mutants. Metaphase furrow formation was variable and failed in some areas (Fig. 6, E and E′), which led to the loss of nuclei (Fig. 6 E) and the formation of empty pseudocells (Fig. 6 E′). During cellularization, the actin network of *DRhoGEF2-bnk* double mutants deteriorated progressively. Breaks in actin rings were observed more frequently than in the single mutants (Fig. 6, F and F′), and toward the end of the slow phase, the actin network was severely fragmented. Only isolated rings were found, but these did not constrict prematurely as observed in *bnk* single mutants (Fig. 6, G and G′). At later stages, as determined by the increased depth at which actin is found, the actin network was completely disintegrated. The majority of actin did not associate with the invagination front but remained localized at the cortex. We conclude that *bnk* and *DRhoGEF2* may act together to stabilize and organize actin filaments during cellularization and that the premature constriction of actin rings in *bnk* mutants requires *DRhoGEF2* function.

DRhoGEF2 is required for pole cell formation

The first individual cells that form in the *Drosophila* embryo are the germline precursors. After cortical migration of syncytial nuclei, actin caps form dome-like protrusions called cytoplasmic buds around interphase nuclei. During nuclear cycle 10, cytoplasmic buds at the posterior pole grow extensively, and constriction of an actin ring at the base of each bud results in the formation of a set of pole cells (Swanson and Poodry, 1980; Foe and Alberts, 1983). DRhoGEF2 is detectable at the membrane furrow between polar cytoplasmic buds and at the base of the forming pole cells (Fig. 8 A), in an area where actin rings are located. DRhoGEF2 colocalizes in these areas with the contractile ring marker Pnut (Fig. 8 A; Neufeld and Rubin, 1994), and we suggest that *DRhoGEF2* may play a role in pole cell formation.

In *DRhoGEF2* mutant embryos, polar cytoplasmic buds form in a manner very similar to that of the wild type. Although actin is more unevenly distributed, a set of pole cells forms at the posterior pole (Fig. 9 A). The concentration of Pnut and myosin II at the base between the forming pole cells suggests that contractile rings have been assembled (Fig. 8, B and C; and Video 1). In the wild type, the pole cells pinch off from the somatic syncytium immediately after nuclear division cycle 10. The pole cells of *DRhoGEF2* mutants show severe defects in actin and myosin II organization at this stage and fail to form independent cortical actin structures that are separated from the somatic nuclear layer (Fig. 9 B and Video 1). The defects are particularly severe during the metaphase of nuclear divisions (Fig. 9 C) and lead to the elimination of nuclei from the cortex, which accumulate in the yolk at the posterior pole (Fig. 9 D and Video 1). In contrast to wild-type pole cells, which sit on top of the syncytium, the pole cells of *DRhoGEF2* mutants remain embedded in the somatic nuclear layer. Cellularization occurs independent of these defects, and the inward movement of the cellularization front obliterates the majority of pole cells (Fig. 9, E–G). We conclude from these observations that *DRhoGEF2* function is required for actin distribution and for reorganization of the actin cytoskeleton of the pole cells during somatic syncytial divisions and cellularization.

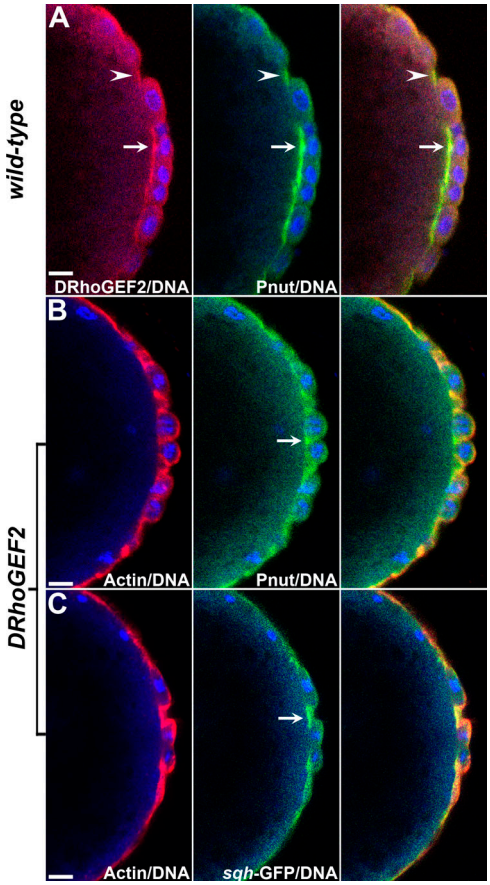

Figure 8. **Localization of DRhoGEF2 during pole cell formation.** (A) Wild type. DRhoGEF2 (red) colocalizes with Pnut (green) at the base of polar buds (arrowhead) and at contractile rings at the base of pole cells (arrow). (B) *DRhoGEF2*$^{l(2)04291}$. Localization of Pnut (green) at the contractile ring is unaffected (arrow). (C) *DRhoGEF2*$^{l(2)04291}$. Localization of myosin II (*sqh*-GFP, green) at the contractile ring is unaffected (arrow). (B and C) Actin is shown in red. (right) Merge of left two panels. Bars, 10 μm.

Discussion

The role of *DRhoGEF2* during development

Guanine nucleotide exchange factors regulate the activity of the small GTPase Rho1, which is thought to act as a molecular switch in a broad spectrum of morphogenetic processes that require a complex reorganization of the actin cytoskeleton. However, the manner in which different aspects of Rho1 function are regulated by RhoGEFs is not well understood. We found that *DRhoGEF2* protein is broadly distributed in epithelia during oogenesis (unpublished data) and embryonic development and concentrated at the apical surface of cells, suggesting that it may regulate *Rho1* throughout morphogenesis. The defects of *DRhoGEF2* mutants are less severe than those of *Rho1* mutants, suggesting that *DRhoGEF2* regulates specific aspects of *Rho1* function.

DRhoGEF2 has previously been shown to regulate cell shape changes during gastrulation. A recent paper implicates

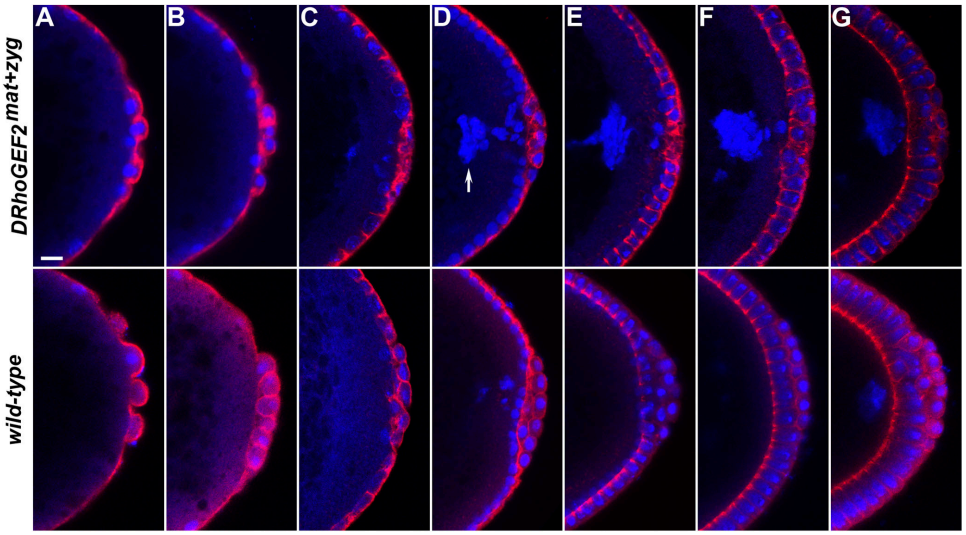

Figure 9. **Role of *DRhoGEF2* during pole cell formation.** Actin is shown in red; and DNA is shown in blue. (A) Actin is irregularly distributed in polar buds. (B) Cortical actin of pole cells fails to separate from the syncytium during interphase. (C) At metaphase, actin is disorganized and furrow formation fails in the pole cell region. (D) At the onset of cellularization, actin surrounding the pole cells is disorganized and nuclei accumulate in the yolk at the posterior pole (arrow). (E) During the slow phase, pole cells remain embedded in the somatic nuclear layer. (F) The invaginating cellularization front obliterates the pole cells. (G) At the fast phase, most pole cells have been lost. All panels same magnification. Bar, 10 μm.

DRhoGEF2 in epithelial folding during imaginal disc development, a process that depends on cell shape changes that are similar to those driving invagination of the germ layers (Nikolaidou and Barrett, 2004). In this paper, we show that *DRhoGEF2* regulates cytoskeletal reorganization and function during pole cell formation and blastoderm cellularization. All of these processes require the contraction of actomyosin rings. We propose that *DRhoGEF2* regulates *Rho1* activity during cell shape changes requiring actomyosin contractility. Our results support the hypothesis that individual RhoGEFs may regulate specific aspects of *Rho1* function during development (Van Aelst and D'Souza-Schorey, 1997).

Interestingly, *DRhoGEF2* has been found to be nonessential during cytokinesis (Nikolaidou and Barrett, 2004; (unpublished data), which also involves the function of contractile actin rings. The function of *Rho1* during cytokinesis is regulated by the RhoGEF *pebble* that initiates actin ring assembly (Prokopenko et al., 1999). In *pebble* mutants, cytokinesis is blocked at mitotic cycle 14 and subsequent mitoses occur without cytokinesis, creating polyploid, multinucleated cells. Although large multinucleated cells are also observed in *DRhoGEF2* mutants at the extended germ band stage (not depicted) it is not clear whether these cells are caused by a block in cytokinesis or are caused by earlier defects during cellularization. In contrast to *pebble*, *DRhoGEF2* may not be required for the assembly of actin rings, but may play a nonessential role in the separation of daughter cells. This is reminiscent of our observations during cellularization. Although the function of actin rings appears compromised throughout cellularization, our data suggest that some contractile activity remains that leads to the basal closure of blastoderm cells and is responsible for the cellularized appearance of *DRhoGEF2* mutants at the onset of gastrulation.

At the retracted germ band stage, DRhoGEF2 is enriched at the apical cortex of cells in the leading edge of the lateral epidermis, which is consistent with the view that it may regulate Rho1 during dorsal closure. Rho1 function is essential for dorsal closure, and the cuticles of zygotic Rho1 mutants show dorsal holes (Harden et al., 1999; Magie et al., 1999). In *DRhoGEF2* mutants, we observed that the lateral epithelial sheets closed the embryo dorsally. This does not exclude the possibility that constriction of actin cables may contribute to dorsal closure and that *DRhoGEF2* may play a role in this process. Overall, our data suggest that DRhoGEF2 function may not be essential for the generation of contractile force, but rather regulate the temporal and spatial coordination of actomyosin contractility.

The role of DRhoGEF2 during early embryogenesis

During syncytial nuclear divisions and cellularization, DRhoGEF2 is localized specifically at the invaginating furrows. In *DRhoGEF2* mutants, actin is irregularly distributed and metaphase furrow formation is less uniform than in the wild type. The defects in furrow formation lead to mitotic defects and the subsequent elimination of abnormal nuclei from the cortex so that, at the onset of cellularization, ~20% of the nuclei have been lost. These phenotypes are reminiscent of the defects seen in mutants of the nonreceptor tyrosine kinase Abelson (Abl; Grevengoed et al., 2003). The abnormalities in actin distribution observed in *abl* mutants are likely caused by the mislocalization of Dia, which leads to ectopic actin polymerization at the apical end of cells. Changes in Dia distribution were not observed in *DRhoGEF2* mutants, suggesting that *DRhoGEF2* may regulate actin distribution by a different mechanism (see next section). Perturbations in actin distribution are observed throughout early development in *DRhoGEF2*

mutants. During cellularization, significant amounts of actin fail to redistribute to the base of the furrow canal. These observations show that one of the roles of *DRhoGEF2* is to regulate furrow assembly. The defects in actin distribution also affect the pole cells, which fail to reorganize their cortical actin cytoskeleton and remain embedded in the somatic nuclear layer rather than sitting on top of it. Consequently, they are obliterated during invagination of the cellularization front.

We speculate that *DRhoGEF2* may have a function in the assembly of actin cables by regulating the association of actin with other proteins such as myosin II. The mislocalization of actin observed in *DRhoGEF2* mutants may be caused by failure of actin to associate with myosin. Interestingly, although myosin II is present at the metaphase furrows, it plays no essential role in their formation (Royou et al., 2004), and this suggests that the function of DRhoGEF2 in furrow assembly may be independent of actomyosin contractility.

Our phenotypic analysis suggests that *DRhoGEF2* regulates actomyosin contractility during cellularization. Previously, the actin-binding protein Bnk has been implicated in the regulation of contractile forces (Schejter and Wieschaus, 1993). In *bnk* mutants, actin hexagons detach from each other and constrict prematurely. Based on this phenotype, Schejter and Wieschaus (1993) proposed a model and suggested that, during the slow phase, cortical actin hexagons are linked to each other through Bnk, and that actomyosin constriction causes the network to contract as a whole, thereby pulling the membrane front inwards. Once the cellularization front has reached the base of the nuclei and Bnk is degraded, actin hexagons detach from each other and contract as individual rings, thereby closing the blastoderm cells basally. We propose that *DRhoGEF2*-mediated activation of *Rho1* may regulate the force that keeps actin hexagons under tension. Bnk counteracts contraction during the slow phase by linking individual actin rings to each other. Degradation of Bnk during the fast phase releases individual actin rings, and the *DRhoGEF2*-mediated contractile force now contributes to basal closure. Therefore, *DRhoGEF2* and *bnk* act in concert to coordinate actin ring contraction during cellularization. In *DRhoGEF2-bnk* double mutants, the actin network disintegrates progressively, suggesting that *DRhoGEF2* and *bnk* may play an additional role in the assembly or stabilization of actomyosin filaments.

It has been proposed that actin network contraction contributes to the inward movement of the furrow canal. Although our data suggest that network tension is severely reduced in *DRhoGEF2* mutants, we find that the rate of membrane invagination is unaffected. This is consistent with reports on the role of myosin II during cellularization, suggesting that network tension may not contribute to membrane invagination (Royou et al., 2004). In the wild type, actin rings squeeze the nuclei slightly and push them basal-wards as the actin network moves over them. This may contribute to the parallel alignment of astral microtubules surrounding the nuclei and to nuclear elongation (Bate and Martinez Arias, 1993). In *DRhoGEF2* mutants, nuclei are wider than in the wild type and irregularly aligned. We propose that network tension may create an ordered hexagonal array of actin rings that contributes to a parallel alignment of nuclei during cellularization. The force moving the actin network inward may be created by plus end–directed tracking of actin on astral microtubules and by membrane insertion as previously suggested (Bate and Martinez Arias, 1993). Our observations suggest that actomyosin contractility plays a role in the spatial coordination of cytoskeletal function during cellularization.

The molecular pathway transducing *DRhoGEF2* activity during blastoderm cellularization

Two effector pathways have been implicated in the transduction of Rho1 activation to the actin cytoskeleton. During cytokinesis, which is mechanistically related to cellularization, a linear pathway including profilin and Dia have been proposed to link *Rho1* to the contractile actomyosin ring (Prokopenko et al., 1999). The maternally supplied Dia plays a role in a spectrum of cytoskeletal functions during early embryogenesis that also require *DRhoGEF2* function, such as metaphase furrow formation, pole cell formation, and cellularization. Dia is localized at the cellularization front and is necessary for the recruitment of cytoskeletal components such as the actin-binding protein anillin and the septin homologue Pnut. The phenotypes of *dia* mutants suggest that *dia* is necessary for the assembly of contractile actin rings at sites of membrane invagination (Afshar et al., 2000).

The similarities between *dia* and *DRhoGEF2* mutants might suggest *dia* as a downstream effector of *DRhoGEF2*. However, the defects of *dia* mutants are morphologically different from those of *DRhoGEF2* mutants. In *dia* mutants, metaphase furrows do not form and contractile rings at the base of polar cytoplasmic buds fail to assemble. During cellularization, actin fails to condense into individual rings, and the network disintegrates during the second phase of cellularization. In *DRhoGEF2* mutants actin rings form and remain largely intact but fail to constrict. In addition, the temporal and spatial localization of Dia and Pnut to the cellularization front was unaffected in *DRhoGEF2* mutants and *dia* was not required for the localization of DRhoGEF2. These findings do not exclude that *DRhoGEF2* activity may in part be mediated by *dia*, however, they suggest that some *dia*-dependent aspects of *Rho1* function are still active in *DRhoGEF2* mutants and that another pathway may be involved in transduction of the *DRhoGEF2* signal.

A well-characterized pathway regulating actomyosin contractility in mammalian cells (Fukata et al., 2001) and in *Caenorhabditis elegans* (Wissmann et al., 1997) links Rho1 to actin via Rho kinase (Winter et al., 2001), the regulatory subunit of myosin light chain phosphatase (MBS; Mizuno et al., 2002; Tan et al., 2003) and myosin II. Rho kinase-mediated phosphorylation inhibits the activity of MBS and induces a conformational change in myosin II allowing it to form filaments that promote sliding of antiparallel actin filaments. Our data are consistent with a model in which *DRhoGEF2* regulates the association of actin with myosin II, thereby stabilizing actomyosin cables. We propose that failure to activate the Rho kinase pathway may compromise the recruitment of actin into

contractile cables. This may destabilize actin cables and lead to the mislocalization of actin and to the defects in actomyosin contractility observed in *DRhoGEF2* mutants. The *Drosophila* homologue of *Rho kinase*, *Drok*, and myosin II have recently been identified as downstream effectors of *DRhoGEF2* during the regulation of actomyosin contractility in Schneider (S2) cells (Rogers et al., 2004). In addition, myosin II is required for basal closure of blastoderm cells (Royou et al., 2004) and the myosin II heavy chain encoded by *zipper* (*zip*) interacts genetically with *DRhoGEF2* (Halsell et al., 2000). These data support the model that *DRhoGEF2* may regulate actomyosin contractility through the Rho kinase pathway. Mutants in *Drok* and *Drosophila myosin light chain phosphatase* have been identified, however, their role during early embryogenesis has not been reported. Interestingly, inhibition of *Drok* activity by injection of the specific Rho kinase inhibitor Y-27632 into embryos before cellularization disrupts the localization of myosin II (Royou et al., 2004). Similar observations have been made in *Drok* mutant cell clones in imaginal discs (Winter et al., 2001). By contrast, *DRhoGEF2* mutants reveal no significant changes in the localization of myosin II during cellularization. It is possible that the differences in myosin II localization between *DRhoGEF2* and *Drok* mutants are due to different mechanisms of action at the molecular level. In mammalian cells myosin II phosphorylation is required for the generation of contractile force but not for its localization (Fumoto et al., 2003). Further investigations will be necessary to resolve how the DRhoGEF2 signal is transduced to the cytoskeleton.

The localization of DRhoGEF2 during cellularization

Little is known about the events that regulate the specific subcellular localization and activation of DRhoGEF2. It has recently been shown that DRhoGEF2 particles are transported from the cytoplasm to the cell periphery by tracking microtubule plus ends in *Drosophila* S2 cells (Rogers et al., 2004). We have observed DRhoGEF2 particles during syncytial development that may be involved in a similar process in the embryo. We speculate that DRhoGEF2 may be delivered to specific membrane subdomains at the cellularization front by microtubules. The G-protein α-subunit encoding gene *concertina* (*cta*) has been shown to regulate the dissociation of DRhoGEF2 from microtubules. *cta* has previously been implicated in the activation of DRhoGEF2 during gastrulation (Barrett et al., 1997), but is not required during cellularization (Parks and Wieschaus, 1991). It has been suggested that the force moving the actin network inward may be generated by plus end-directed crawling of actin on astral microtubules (Bate and Martinez Arias, 1993). We speculate that DRhoGEF2 may regulate actin ring constriction during cellularization while associated with the tip of astral microtubules by recruiting Rho1 to the site of actin rings.

DRhoGEF2 is concentrated in actin-rich regions throughout development and the human orthologue of *DRhoGEF2*, *PDZ-RhoGEF*, has been shown to bind to actin directly (Banerjee and Wedegaertner, 2004). Although the domain structure of *DRhoGEF2* and *PDZ-RhoGEF* is very similar, the actin-binding region of *PDZ-RhoGEF* is not conserved in *DRhoGEF2*. Nevertheless, the localization of DRhoGEF2 is consistent with the view that it may associate with actin, however, further experiments are needed to corroborate this theory.

Materials and methods

Fly strains and genetics

Germline clones of *DRhoGEF2$^{l(2)04291}$*, *DRhoGEF2$^{4.1}$*, *DRhoGEF2^{3w18}*, *dia^{5}*, and *Rho1^{L3}* were generated using the autosomal FLP-DFS technique (Chou and Perrimon, 1996). *DRhoGEF2$^{l(2)04291}$* and *DRhoGEF2$^{4.1}$* are considered null alleles (Barrett et al., 1997; Häcker and Perrimon, 1998) and showed very similar phenotypes. All data shown regarding *DRhoGEF2* mutants were obtained from the analysis of embryos derived from females carrying *DRhoGEF2$^{l(2)04291}$* germline clones crossed with heterozygous males. Paternal rescue was not observed. In *DRhoGEF2$^{l(2)04291}$* mutants, no DRhoGEF2 was detectable. In embryos derived from *DRhoGEF2^{3w18}* germline clones wild-type DRhoGEF2 levels and distribution were observed and phenotypes were less severe, suggesting that *DRhoGEF2^{3w18}* is a hypomorphic allele.

Rescue of *DRhoGEF2$^{l(2)04291}$* mutants was performed by crossing *DRhoGEF2$^{l(2)04291}$/P{FRT(w[hs])}G13 P{ovoD1-18}2R; matα4-Gal-VP16/TM3, Sb* females carrying germline clones to UAS-DRhoGEF2-RE males. *DRhoGEF2$^{l(2)04291}$*-bnk double mutants were made by crossing *DRhoGEF2$^{l(2)04291}$*; Df(3R)tll-e females carrying germline clones with *Df(3R)tll-e/TM6b, Tb, ca* males. Double mutant embryos were identified by α-Bnk antibody staining. *DRhoGEF2* mutants expressing *sqh-GFP* (Royou et al., 2004) were made by crossing *DRhoGEF2$^{l(2)04291}$*; *sqh-GFP* females carrying germline clones with *sqh-GFP* males. The wild-type controls used were *sqh-GFP/TM3, Sb* females crossed with *sqh-GFP* males.

Rho1^{L3} was made by mobilizing *pBac{3xP3-EYFP, p*-GAL4-K10}, using *P{neoFRT}40A, P{FRT(w[hs])}G13* as a target chromosome and the *pBac* insertion site was determined as described previously (Häcker et al., 2003). The *Rho1^{L3}* insertion is in the 5′ untranslated region of *Rho1* at nt160164 in GenBank scaffold AE003808.3. The *bnk* mutant used was *Df(3R)tll-e* (Schejter and Wieschaus, 1993). Gal4 drivers used were matα4Gal-VP16 (gift of D. St. Johnston, University of Cambridge, Cambridge, UK) and prd-Gal4 (Yoffe et al., 1995). UAS-DRhoGEF2-RE was generated by *P* element–mediated germline transformation of pUAST-DRhoGEF2-RE (performed at the EMBL *Drosophila* injection service).

Molecular biology

We isolated several partial cDNAs, representing different parts of *DRhoGEF2* transcripts, by screening a random primed *Drosophila* embryonic cDNA library (CLONTECH Laboratories, Inc.). pUAST-DRhoGEF2-RE was generated by joining a partial cDNA encompassing the region from a unique central EcoRI site to the 3′-end of *DRhoGEF2* with a cDNA representing the transcript DRhoGEF2-RE (FlyBase) from the same EcoRI site to the 5′-end and cloning of the resulting DNA fragment into the XbaI site of pUAST (Brand and Perrimon, 1993).

Immunohistochemistry

For phalloidin stainings, embryos were dechorionated, fixed by shaking in 4 ml HEM (100 mM Hepes, 20 mM $MgSO_4$, and 1 mM EGTA), 4% formaldehyde for 20 min, devitellinized by hand using a needle, washed in PBT (PBS containing 0.1% Tween 20), incubated in rhodamine-conjugated phalloidin (Molecular Probes) for 1 h, washed three times in PBT, and mounted on a microscope slide. To visualize DRhoGEF2, Rho1, Dia, Pnut, β-Heavy spectrin, or Bnk embryos were fixed as just described, devitellinized by adding 10 ml methanol and stained using rabbit α-DRhoGEF2 (Rogers et al., 2004), mouse α-Rho1 (Magie et al., 2002), mouse α-Pnut, (obtained from the Developmental Studies Hybridoma Bank and developed under the auspices of the National Institute of Child Health and Human Development and maintained by the University of Iowa), rabbit α-Dia (Afshar et al., 2000) (gift of S. Wasserman, University of California San Diego, CA), rabbit α-β-Heavy spectrin (gift of G. Thomas, Pennsylvania State University, University Park, PA; Thomas and Kiehart, 1994) or rat α-Bnk (Schejter and Wieschaus, 1993) (gift of E. Wieschaus, Princeton University, Princeton, NJ) antibodies, respectively. DNA was stained using TO-PRO (Molecular Probes). Primary antibodies were detected with Cy2-, rhodamine RedX- or FITC-conjugated goat antisera (all obtained from Jackson ImmunoResearch Laboratories). Images were collected on a laser scanning confocal microscope (model TCS SP2; Leica) and imported directly into Adobe Photoshop software or assembled into movies using NIH image, ImageJ 1.62 software.

Online supplemental material
Time-lapse videos supplement Fig. 3 (Videos 1 and 2) and Fig. 8 and Fig. 9 (Videos 1 and 3). Videos play at 10 frames/s. Fig. S1 shows the distribution of DRhoGEF2 and myosin II during cellularization. Fig. S2 shows the distribution of myosin II in DRhoGEF2 mutants. Fig. S3 provides an overview of the phenotype of embryos derived from Rho1L3 germline clones. Online supplemental material is available http://www.jcb.org/cgi/content/full/jcb.200407124/DC1.

We express our thanks to Norbert Perrimon for his support and to Beth Stronach for help with the generation of full-length DRhoGEF2 cDNAs.

This work was supported by the Swedish Foundation for Strategic Research (SSF) Developmental Biology Programme, the Swedish Research Council VR grant 621-2001-2013, the Swedish Cancer Foundation grant 4554-B03-03XAC, the Crafoord Foundation, the Nilsson-Ehle Foundation (all to U. Häcker), and the National Institutes of Health number 5F32GM064966 (to S. Rogers). The Lund Stem Cell Center is supported by a Center of Excellence grant in life sciences from the SSF.

Submitted: 19 July 2004
Accepted: 7 January 2005

References

Afshar, K., B. Stuart, and S.A. Wasserman. 2000. Functional analysis of the *Drosophila* diaphanous FH protein in early embryonic development. *Development.* 127:1887–1897.

Banerjee, J., and P.B. Wedegaertner. 2004. Identification of a novel sequence in PDZ-RhoGEF that mediates interaction with the actin cytoskeleton. *Mol. Biol. Cell.* 15:1760–1775.

Barrett, K., M. Leptin, and J. Settleman. 1997. The Rho GTPase and a putative RhoGEF mediate a signaling pathway for the cell shape changes in *Drosophila* gastrulation. *Cell.* 91:905–915.

Bate, M., and A. Martinez Arias. 1993. The Development of *Drosophila melanogaster*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 1558.

Brand, A.H., and N. Perrimon. 1993. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development.* 118: 401–415.

Chou, T.B., and N. Perrimon. 1996. The autosomal FLP-DFS technique for generating germline mosaics in *Drosophila melanogaster*. *Genetics.* 144: 1673–1679.

Cooper, J.A., and D.P. Kiehart. 1996. Septins may form a ubiquitous family of cytoskeletal filaments. *J. Cell Biol.* 134:1345–1348.

Crawford, J.M., N. Harden, T. Leung, L. Lim, and D.P. Kiehart. 1998. Cellularization in *Drosophila melanogaster* is disrupted by the inhibition of rho activity and the activation of Cdc42 function. *Dev. Biol.* 204:151–164.

Field, C.M., and B.M. Alberts. 1995. Anillin, a contractile ring protein that cycles from the nucleus to the cell cortex. *J. Cell Biol.* 131:165–178.

Foe, V.E., and B.M. Alberts. 1983. Studies of nuclear and cytoplasmic behaviour during the five mitotic cycles that precede gastrulation in *Drosophila* embryogenesis. *J. Cell Sci.* 61:31–70.

Fukata, Y., M. Amano, and K. Kaibuchi. 2001. Rho-Rho-kinase pathway in smooth muscle contraction and cytoskeletal reorganization of non-muscle cells. *Trends Pharmacol. Sci.* 22:32–39.

Fumoto, K., T. Uchimura, T. Iwasaki, K. Ueda, and H. Hosoya. 2003. Phosphorylation of myosin II regulatory light chain is necessary for migration of HeLa cells but not for localization of myosin II at the leading edge. *Biochem. J.* 370:551–556.

Grevengoed, E.E., D.T. Fox, J. Gates, and M. Peifer. 2003. Balancing different types of actin polymerization at distinct sites: roles for Abelson kinase and Enabled. *J. Cell Biol.* 163:1267–1279.

Häcker, U., S. Nystedt, M.P. Barmchi, C. Horn, and E.A. Wimmer. 2003. piggyBac-based insertional mutagenesis in the presence of stably integrated *P elements* in *Drosophila*. *Proc. Natl. Acad. Sci. USA.* 100:7720–7725.

Häcker, U., and N. Perrimon. 1998. DRhoGEF2 encodes a member of the Dbl family of oncogenes and controls cell shape changes during gastrulation in *Drosophila*. *Genes Dev.* 12:274–284.

Halsell, S.R., B.I. Chu, and D.P. Kiehart. 2000. Genetic analysis demonstrates a direct link between rho signaling and nonmuscle myosin function during *Drosophila* morphogenesis. *Genetics.* 155:1253–1265.

Harden, N., M. Ricos, Y.M. Ong, W. Chia, and L. Lim. 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.

Lehner, C.F. 1992. The pebble gene is required for cytokinesis in *Drosophila*. *J. Cell Sci.* 103:1021–1030.

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

Magie, C.R., D. Pinto-Santini, and S.M. Parkhurst. 2002. Rho1 interacts with p120ctn and alpha-catenin, and regulates cadherin-based adherens junction components in *Drosophila*. *Development.* 129:3771–3782.

Mazumdar, A., and M. Mazumdar. 2002. How one becomes many: blastoderm cellularization in *Drosophila melanogaster*. *Bioessays.* 24:1012–1022.

Mizuno, T., K. Tsutsui, and Y. Nishida. 2002. *Drosophila* myosin phosphatase and its role in dorsal closure. *Development.* 129:1215–1223.

Neufeld, T.P., and G.M. Rubin. 1994. The *Drosophila* peanut gene is required for cytokinesis and encodes a protein similar to yeast putative bud neck filament proteins. *Cell.* 77:371–379.

Nikolaidou, K.K., and K. Barrett. 2004. A Rho GTPase signaling pathway is used reiteratively in epithelial folding and potentially selects the outcome of Rho activation. *Curr. Biol.* 14:1822–1826.

Parks, S., and E. Wieschaus. 1991. The *Drosophila* gastrulation gene concertina encodes a G alpha-like protein. *Cell.* 64:447–458.

Postner, M.A., and E.F. Wieschaus. 1994. The nullo protein is a component of the actin-myosin network that mediates cellularization in *Drosophila melanogaster* embryos. *J. Cell Sci.* 107:1863–1873.

Prokopenko, S.N., A. Brumby, L. O'Keefe, L. Prior, Y. He, R. Saint, and H.J. Bellen. 1999. A putative exchange factor for Rho1 GTPase is required for initiation of cytokinesis in *Drosophila*. *Genes Dev.* 13:2301–2314.

Rogers, S.L., U. Wiedemann, U. Häcker, C. Turck, and R.D. Vale. 2004. *Drosophila* RhoGEF2 associates with microtubule plus ends in an EB1-dependent manner. *Curr. Biol.* 14:1827–1833.

Royou, A., C. Field, J.C. Sisson, W. Sullivan, and R. Karess. 2004. Reassessing the role and dynamics of nonmuscle myosin II during furrow formation in early *Drosophila* embryos. *Mol. Biol. Cell.* 15:838–850.

Schejter, E.D., and E. Wieschaus. 1993. bottleneck acts as a regulator of the microfilament network governing cellularization of the *Drosophila* embryo. *Cell.* 75:373–385.

Schumacher, S., T. Gryzik, S. Tannebaum, and H.A. Muller. 2004. The RhoGEF Pebble is required for cell shape changes during cell migration triggered by the *Drosophila* FGF receptor Heartless. *Development.* 131: 2631–2640.

Settleman, J. 2001. Rac 'n Rho: the music that shapes a developing embryo. *Dev. Cell.* 1:321–331.

Smallhorn, M., M.J. Murray, and R. Saint. 2004. The epithelial-mesenchymal transition of the *Drosophila* mesoderm requires the Rho GTP exchange factor Pebble. *Development.* 131:2641–2651.

Strutt, D.I., U. Weber, and M. Mlodzik. 1997. The role of RhoA in tissue polarity and Frizzled signalling. *Nature.* 387:292–295.

Swanson, M.M., and C.A. Poodry. 1980. Pole cell formation in *Drosophila melanogaster*. *Dev. Biol.* 75:419–430.

Tan, C., B. Stronach, and N. Perrimon. 2003. Roles of myosin phosphatase during *Drosophila* development. *Development.* 130:671–681.

Theurkauf, W.E. 1994. Actin cytoskeleton. Through the bottleneck. *Curr. Biol.* 4:76–78.

Thomas, G.H., and D.P. Kiehart. 1994. β-heavy-spectrin has a restricted tissue and subcellular distribution during *Drosophila* embryogenesis. *Development.* 120:2039–2050.

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

Winter, C.G., B. Wang, A. Ballew, A. Royou, R. Karess, J.D. Axelrod, and L. Luo. 2001. *Drosophila* Rho-associated kinase (Drok) links Frizzled-mediated planar cell polarity signaling to the actin cytoskeleton. *Cell.* 105:81–91.

Wissmann, A., J. Ingles, J.D. McGhee, and P.E. Mains. 1997. *Caenorhabditis elegans* LET-502 is related to Rho-binding kinases and human myotonic dystrophy kinase and interacts genetically with a homolog of the regulatory subunit of smooth muscle myosin phosphatase to affect cell shape. *Genes Dev.* 11:409–422.

Yoffe, K.B., A.S. Manoukian, E.L. Wilder, A.H. Brand, and N. Perrimon. 1995. Evidence for engrailed-independent wingless autoregulation in *Drosophila*. *Dev. Biol.* 170:636–650.