Rho1 regulates *Drosophila* adherens junctions independently of p120ctn

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Summary

During animal development, adherens junctions (AJs) maintain epithelial cell adhesion and coordinate changes in cell shape by linking the actin cytoskeletons of adjacent cells. Identifying AJ regulators and their mechanisms of action are key to understanding the cellular basis of morphogenesis. Previous studies linked both p120catenin and the small GTPase Rho to AJ regulation and revealed that p120 may negatively regulate Rho. Here we examine the roles of these candidate AJ regulators during *Drosophila* development. We found that although p120 is not essential for development, it contributes to morphogenesis efficiency, clarifying its role as a redundant AJ regulator. Rho has a dynamic localization pattern throughout ovarian and embryonic development. It preferentially accumulates basally or basolaterally in several tissues, but does not preferentially accumulate in AJs. Further, Rho1 localization is not obviously altered by loss of p120 or by reduction of core AJ proteins. Genetic and cell biological tests suggest that p120 is not a major dose-sensitive regulator of Rho1. However, Rho1 itself appears to be a regulator of AJs. Loss of Rho1 results in ectopic accumulation of cytoplasmic DE-cadherin, but ectopic cadherin does not accumulate with its partner Armadillo. These data suggest Rho1 regulates AJs during morphogenesis, but this regulation is p120 independent.

Key words: Cadherin, Morphogenesis, RhoGEF2, Shotgun

Introduction

During animal development, coordinated changes in cell shape and position build the body plan and drive morphogenesis. These changes are executed in part by the actin cytoskeleton, and neighboring cells act in concert by linking their cytoskeletons to cell-cell and cell-matrix junctions (reviewed by Perez-Moreno et al., 2003). In epithelial cells, adherens junctions (AJs) mediate cell-cell adhesion, via interactions between cadherins on neighboring cells. Within the cell, the cadherin cytoplasmic tail indirectly interacts with apical actin via β- and α-catenin (reviewed by Tepass et al., 2001).

In addition to these core AJ components, regulatory proteins modulate both AJ stability and connections to the cytoskeleton (reviewed by Gumbiner, 2000). Identifying how these regulators modify AJs during development is crucial to understanding morphogenesis. Studies in cultured mammalian cells and other systems identified many candidate AJ regulators, including the catenin p120 and the small GTPase Rho (mammalian RhoA or *Drosophila* Rho1).

p120 binds the juxtamembrane region of cadherins (reviewed by Anastasiadis and Reynolds, 2000). Initially, the regulatory relationship between p120 and AJs was unclear. Overexpression of mutant E-cadherins lacking the juxtamembrane domain in different mammalian cell types gave opposing results suggesting that p120 either promotes (Yap et al., 1998) or downregulates adhesion (Ozawa and Kemler, 1998). siRNA knockdown of p120 in mammalian cells clarified this, showing that p120 promotes AJ stability by blocking E-cadherin endocytosis (Davis et al., 2003; Xiao et al., 2003).

In invertebrates, p120 also promotes adhesion but may be dispensable for viability. In *Caenorhabditis elegans*, *p120/jac-1* RNAi enhances the *hmp-1*/α-catenin phenotype, but *jac-1* RNAi alone does not disrupt morphogenesis (Pettitt et al., 2003). Similarly, loss of p120 enhances the phenotype of *Drosophila* E-Cadherin (DE-Cad; *shotgun* – FlyBase) mutants but loss of p120 alone (Myster et al., 2003) or expression of *p120* RNAi transgenes (Pacquelet et al., 2003) do not affect viability or cell adhesion. However, injection of *p120* double-stranded RNA (dsRNA) in embryos was reported to disrupt morphogenesis (Magie et al., 2002). This suggested that rapid depletion of p120 might have more severe consequences than chronic depletion.

In mammalian cells p120 also may function outside of AJs (Anastasiadis and Reynolds, 2000). In the cytoplasm, p120 can negatively regulate RhoA. Rho regulates many cellular processes, including actin organization, cell migration and cell polarity (reviewed by Etienne-Manneville and Hall, 2002). siRNA knockdown of mammalian p120 increases RhoA activity and promotes stress fiber formation (Shibata et al., 2004). Conversely, p120 overexpression causes fibroblasts to lose stress fibers (Anastasiadis et al., 2000; Noren et al., 2000) and contractility (Grosheva et al., 2001), both RhoA-dependent processes (Ridley and Hall, 1992; Chrzanowska-Wodnicka and Burridge, 1996).

Recently, the relationship between p120 and Rho has begun to be tested in vivo, but the results do not yield a consistent mechanistic picture. Embryonic defects caused by knockdown of p120 family members in *Xenopus* can be partially rescued by both dominant-negative (Fang et al., 2004) and wild-type RhoA (Ciesiolka et al., 2004). These contrasting results are consistent with p120 activating or repressing RhoA, respectively. In *Drosophila,* p120 preferentially binds Rho1-GDP and regulates Rho1 localization. Overexpression of p120 enhances the *Rho1* phenotype (Magie et al., 2002), suggesting that fly p120 negatively regulates Rho1. Thus p120 may regulate morphogenesis by regulating AJs and/or Rho.

Additionally, Rho itself regulates AJ stability. Blocking RhoA function in keratinocytes prevents the formation of stable AJs (Braga et al., 1997). In *Drosophila*, both *Rho1* loss of function and expression of dominant-negative *Rho1* during embryogenesis alter DE-Cad localization (Magie et al., 2002; Bloor and Kiehart, 2002). However, the regulation of AJs by Rho is probably complex, as manipulation of different RhoA effectors can promote or decrease AJ stability in mammalian cells (Sahai and Marshall, 2002; Vaezi et al., 2002). Additionally, AJs may regulate Rho activity, as cadherin-cadherin engagement can either activate (Charrasse et al., 2002; Nelson et al., 2004) or inhibit (Noren et al., 2001; Noren et al., 2003) RhoA activity in vitro.

Work in cultured cells supports roles for both p120 and Rho as AJ regulators, but the interactions among p120, Rho and AJs are complex. Ultimately, we want to understand how Rho and p120 regulate AJs during the intricate events of embryonic morphogenesis. One key question is whether Rho and p120 act together in this process. Here we use *Drosophila* to investigate this.

Materials and methods

RNAi

Around 0.7 kb of *p120*, *ftz* or *gfp* coding sequence were amplified from cDNA using primers introducing T7 promoters (see Table 1) and used to synthesize dsRNAs using the MEGAscript kit (Ambion). For *p120::GFP* injections, the control dsRNA was against *dysfusion* (Jiang and Crews, 2003). Syncytial blastoderm embryos were bleach-dechorionated and injected under halocarbon oil at the posterior end with dsRNA at 5 μmol/l. For hatch rate estimates, injected *yellow white* embryos were left 3 days at 18°C, and unhatched embryos collected for cuticle preparations. Injected *p120::GFP* embryos were aged 24 hours at 18°C and transferred to petriPERM plates (Sartorius Corp.) for imaging.

Primer sequences used were: *p120*, 5′-ATGGAAGCGC-GATCTCTC-3′ (forward) and 5′-TACGTAAAGCACCACAGGCA-3′ (reverse); *ftz*, 5′-GCCAGAGCCACTACAGCTA-3′ (forward) and 5′-TGATGCCAAAGTCTCCTCG-3′ (reverse); and *gfp*, 5′-ATGGT-GAGCAAGGGCGAGGAG-3′ (forward) and 5′-GTACAGCTCGTC-CATGCCGAG-3′ (reverse).

Fly stocks

Mutations are described at http://flybase.bio.indiana.edu/. $Rho1^{rev220}$ and $Rho1^{1B}$ (Magie et al., 2002; Magie et al., 2005) were from S. Parkhurst (Fred Hutchison). Homozygous *Rho1* mutants were identified using a *Kr::gfp* balancer (Casso et al., 1999); controls were *Kr::gfp* positive siblings. For Rho1 localization in *p120*, *histone::gfp* was the control. Otherwise it was *yellow white*. Recombinant genotypes were confirmed by failure to complement an independently derived allele; $p120^{308}$ was confirmed by PCR. Cuticle preparations were as in Wieschaus and Nüsslein-Volhard (Wieschaus and Nüsslein-Volhard, 1986). Unless noted, experiments were done at 25°C. Live imaging utilized wild-type or $p120^{308}$ mutants expressing moesin::GFP. Follicle cell clones: *heat-shock-FLP/+; FRT42Dshg*^{R69}*/FRT42D gfp* females were heat-shocked for 1 hour at 37°C for 2 consecutive days before dissection.

Table 1. *p120 RNAi* is not lethal

	dsRNA		
	p120	*gfp*	*ftz*
% embryos hatched (*n*)	68.8% (655)	63.5% (388)	4.1% (393)
Unhatched embryos-cuticle phenotypes			
Wild-type	35.7%	29.5%	0%
Head defects	30.9%	34.6%	2.2%
Segmental fusion	8.3%	10.3%	0.7%
Tail up	7.1%	9.0%	0.7%
Disrupted	17.8%	16.7%	6.0%
ftz phenotype	0%	0%	90.2%
n	84	78	134

Table 2. *p120 RNAi* significantly depletes p120::GFP

	dsRNA	
Phenotype	*p120*	Control (*dys*)
Normal p120::GFP signal	10.0%	57.1%
Dim but junctional signal	3.3%	25.0%
Dim, cytoplasmic signal	3.3%	7.1%
No signal	83.3%	10.7%
n	30	28

Immunofluorescence

Ovaries were treated as in Magie et al. (Magie et al., 2002). Embryos were fixed in 1:1 PBS+3.7% formaldehyde:heptane for 20 minutes, except for Rho1 staining, which was as in Padash-Barmchi et al. (Padash-Barmchi et al., 2005). Embryos were methanol-devitellinized (or hand-devitellinized for phalloidin), blocked and stained in PBS/1% goat serum/0.1%Triton-X-100. Antibodies: anti-Rho1p1D9 (1:50), anti-βPS1 integrin (1:3), anti DE-Cad2 (1:200), anti-ArmN2 (1:200; all Developmental Studies Hybridoma Bank), anti-DRhoGEF2 (1:500) (Rogers et al., 2004), anti-phosphotyrosine (1:1000, Upstate Biotechnology). Alexa-phalloidin was used at 1:1000; secondary antibodies were Alexas 488, 568 and 647 (Molecular Probes). Embryos were mounted in Aqua-Polymount (Polysciences). Fixed samples were imaged using a Zeiss LSM510 confocal microscope and LSM software. Live imaging used a Perkin-Elmer Ultraview spinning-disc confocal, an ORCA-ER digital camera (Hamamatsu), and Metamorph software. To analyze dorsal closure timing, we began analysis of all movies when the maximum separation between the leading edges was 52 μm (as measured in Metamorph), and ended analysis when the leading edges met along their entire length. All images were acquired at 40×. Adobe Photoshop 7.0 was used to adjust brightness and contrast. When comparing wild-type and mutants, images were adjusted identically.

Results

p120 RNAi is not lethal

Our previous genetic analysis suggested that p120 is not essential for *Drosophila* development but plays an important positive-modulatory role in cell adhesion that is revealed by reducing levels of DE-Cad (Myster et al., 2003). However, Magie et al. (Magie et al., 2002) suggested that embryos suddenly deprived of p120 have developmental defects. We tested this by injecting blastoderm-stage embryos with dsRNA directed against the *p120* coding region. *p120* RNAi did not

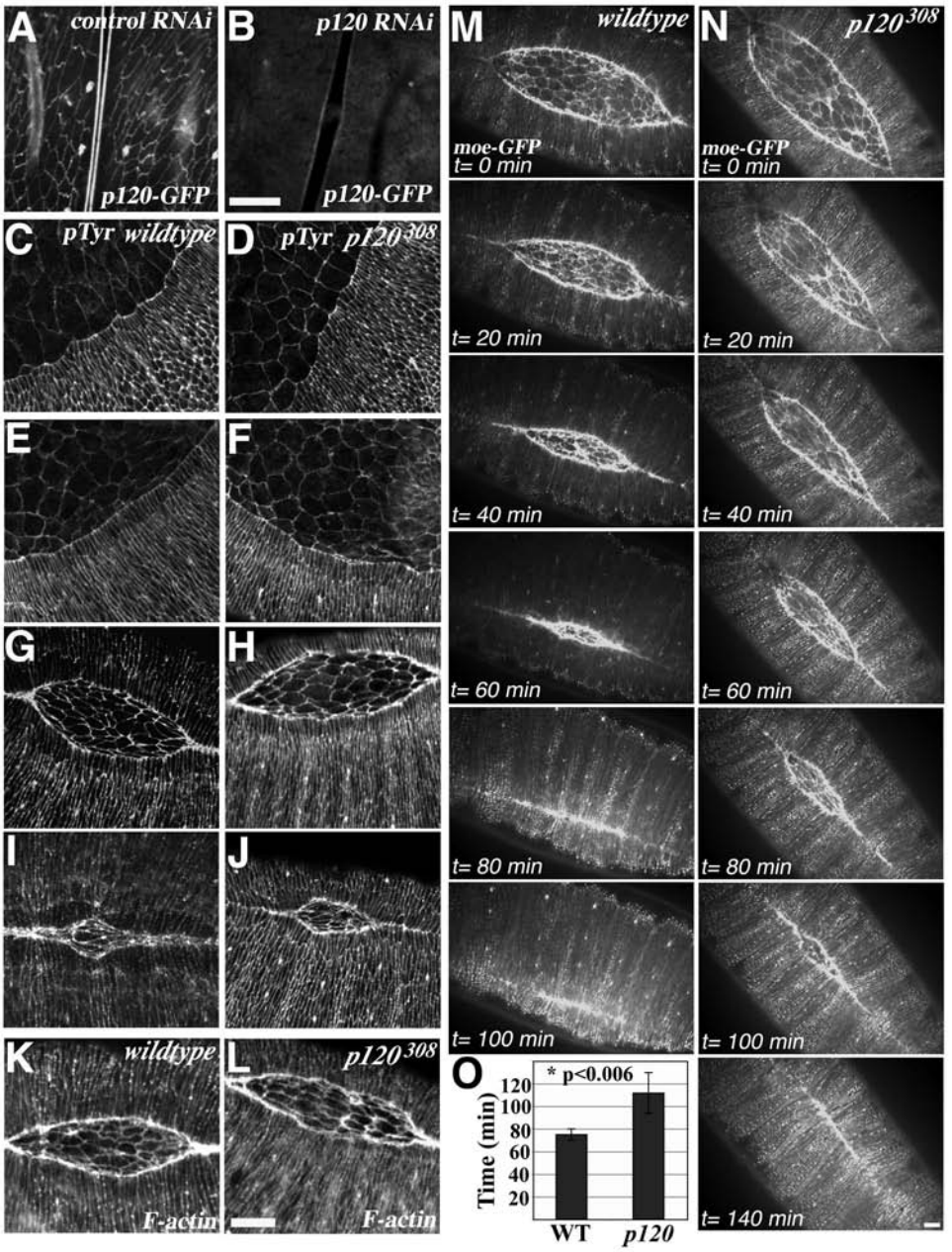

Fig. 1. Loss of *p120* slows but does not disrupt dorsal closure. Embryos. Unless noted, in all figures anterior is left. (A,B) *p120* RNAi depletes p120:GFP. GFP fluorescence, p120::GFP embryos injected with control dsRNA (A) or *p120* dsRNA (B). (C-J) Anti-phosphotyrosine. (K,L) Phalloidin. Wild type (C,E,G,I,K). *p120* null mutants (D,F,H,J,L). (M-N) Stills, movies of representative wild-type (M) and *p120* null mutants (N), both expressing moesin-GFP. Times, lower left. (O) Mean time to complete dorsal closure ±s.d. Scale bars: 25 μm.

result in embryonic lethality (68.8% of embryos hatched versus 63.5% of embryos injected with a negative control (*gfp*) dsRNA; Table 1), while RNAi of the essential gene *ftz* resulted in embryonic lethality (Table 1). As about one-third of both *p120*-dsRNA and *gfp*-dsRNA injected embryos died, we suspected this residual lethality resulted from injection-associated defects. Consistent with this, *p120* and *gfp* RNAi gave similarly variable distributions of cuticle defects (Table 1), while 90% of *ftz* RNAi embryos displayed the characteristic loss of every other body segment (Wakimoto et al., 1984). To rule out the possibility that we failed to knock down p120 protein, we injected embryos expressing GFP-tagged p120 (Myster et al., 2003), using the same preparation of *p120* dsRNA. More than 80% of *p120* dsRNA-injected *p120::GFP* embryos lost detectable junctional p120::GFP (Table 2; Fig. 1B; Fig. S1 in the supplementary material), whereas >80% of *p120::GFP* embryos injected with control dsRNA retained junctional p120::GFP (Table 2; Fig. 1A; Fig. S1 in the supplementary material). Thus RNAi of *Drosophila p120*, like *p120* loss-of-function mutations, is not lethal.

Loss of p120 slows but does not disrupt dorsal closure

p120 mutants are viable and fertile, and AJ proteins and actin accumulate normally in *p120* mutant embryos (Myster et al., 2003). We noted, in passing, that a fraction of *p120* mutants exhibit slight defects in cell shape during dorsal closure, a process during which lateral sheets of epithelial cells elongate and migrate dorsally, enclosing the embryo in epidermis. To examine whether p120 plays a subtle role in morphogenesis, we looked in detail at dorsal closure, examining cell shape changes and cell behavior in fixed and live *p120* mutants. We examined each stage in dorsal closure and observed no consistent differences between *p120* mutants and wild type (Fig. 1C-J). F-actin localization was also similar (compare Fig. 1K and Fig. 1L).

We next examined whether more subtle changes in cell behavior were revealed by imaging dorsal closure in living embryos expressing a GFP-tagged F-actin-binding fragment of Moesin that highlights the cytoskeleton (Moesin::GFP) (Edwards et al., 1997). Once again, we saw no gross defects in cell shape in *p120* mutants. However, loss of p120 slowed the rate of dorsal closure. On average, wild-type embryos completed dorsal closure within 75 minutes (Fig. 1O). *p120* mutants were significantly slower, taking 112 minutes (Fig. 1O). Despite this, *p120* mutants completed dorsal closure without apparent defects. Thus, loss of p120, while not lethal, alters the efficiency of morphogenesis.

Rho1 exhibits dynamic localization

In mammalian cells, p120 regulates Rho activity. One

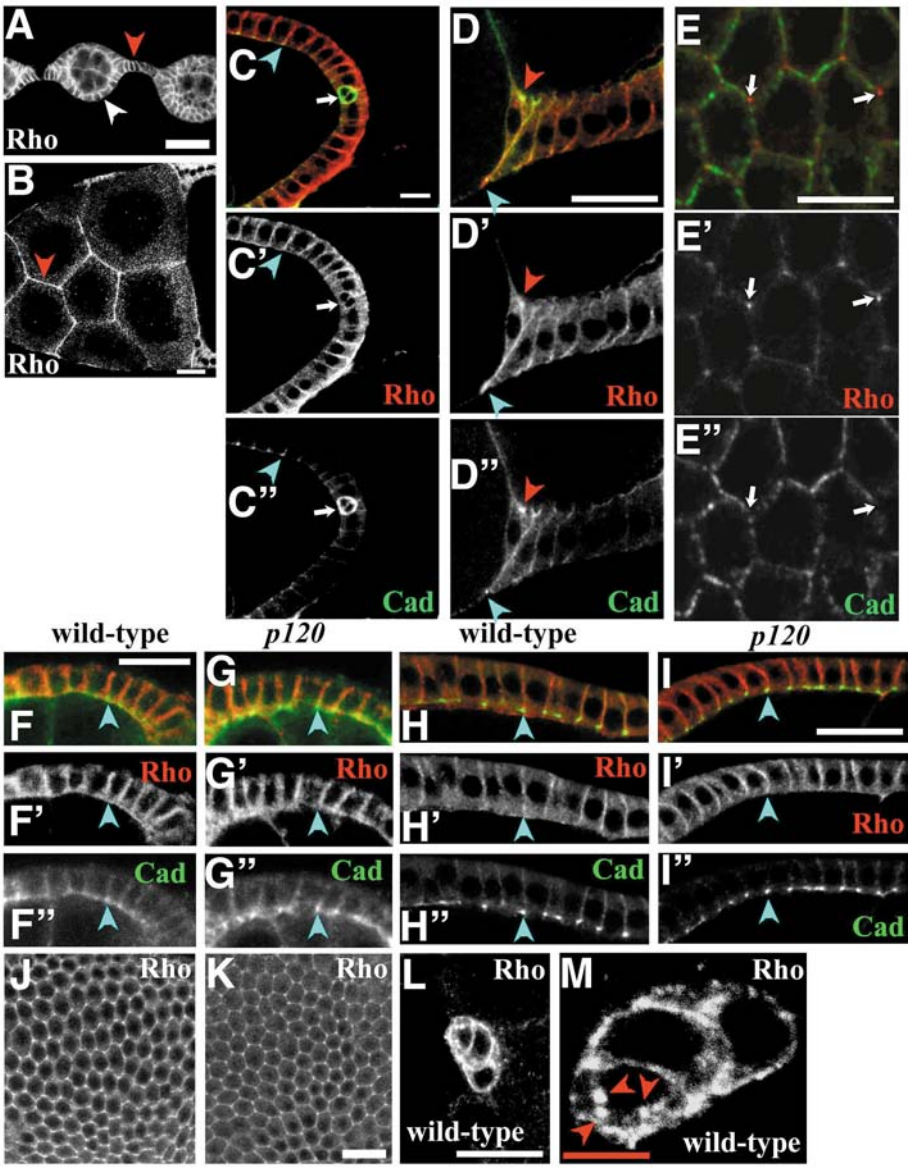

Fig. 2. Rho1 localization during oogenesis. Egg chambers. Rho1 (red). DE-Cad (green). (A) Cross-section, stage 2-3. Rho1 accumulates laterally in follicle cells (white arrowhead) and stalk cells (red arrowhead). (B) Stage 10. Rho1 at germ cell boundaries (arrowhead). (C) DE-Cad at apical AJs of follicle cells (blue arrowhead) and in polar follicle cells (white arrow). Rho1 does not preferentially accumulate in either place. (D) Cross-section, stage 10B. DE-Cad and Rho1 elevated apically in centripetal follicle cells (red arrowhead). Rho1 accumulates basolaterally (blue arrowhead). (E) Grazing section basal to AJs, stage 10B follicle cells. DE-Cad is absent from tricellular junctions, where Rho1 accumulates (arrows). (F-I′′) Cross-sections, stage 5 (F,G) and 10B (H,I). Wild type (F,H). *p120* mutants (G,I). In both Rho1 is enriched along lateral membranes. DE-Cad is enriched at apical AJs (arrowheads). (J,K) Grazing sections, stage 10B. Rho1 at tricellular junctions. Wild-type (J). *p120* (K). (L,M) Migrating border cells. Rho1 enriched at plasma membrane and in intracellular vesicles (M, arrowhead). Scale bars: 20 μm in A-F,I,K,L; 5 μm in M.

mechanism to regulate Rho is by controlling its subcellular localization. Previous workers examined Rho1 localization in both ovaries and embryos (Magie et al., 2002; Padash-Barmchi et al., 2005). *Drosophila* Rho1 was reported to localize to AJs (Magie et al., 2002), suggesting that this might be a mechanism by which it both regulates and is regulated by AJs. We re-examined Rho1 localization compared to that of AJs. This revealed new information about Rho1 dynamic localization, sometimes confirming previous work and in other cases contradicting it.

We began with oogenesis (utilizing the same protocol and anti-Rho1 monoclonal antibody used in Magie et al., 2002). Ovarian follicle cells form an epithelium with its apical surface inward, providing an excellent place to examine AJs. After egg chambers formed, Rho1 localized to follicle cell apical and lateral membranes (Fig. 2A, white arrowhead), and along lateral membranes of stalk cells (Fig. 2A, red arrowhead). Rho1 remained enriched at follicle cell lateral membranes (Fig. 2F,H), but apical enrichment decreased at later stages (Fig. 2H). DE-Cad was strongly enriched in apical AJs (Fig. 2C,F,H blue arrowheads). Rho1 localization sometimes overlapped DE-Cad at AJs, but it was not enriched there (Fig. 2C,F,H). In grazing sections at stage 10, Rho1 accumulated in puncta, where three follicle cells meet (Fig. 2E, arrows; Fig. 2J); these puncta were basal to the strongest DE-Cad staining (data not shown) and did not co-localize with DE-Cad (Fig. 2E). Thus Rho1 was not enriched in AJs of most follicle cells. However, like AJ proteins (Peifer et al., 1993; Oda et al., 1997), Rho1 did accumulate at germ cell boundaries (Magie et al., 2002) (Fig. 2B, arrowhead).

Posterior polar follicle cells preferentially accumulate AJ proteins (Peifer et al., 1993; Niewiadomska et al., 1999) (Fig. 2C, arrow). By contrast, Rho1 levels in these cells resembled those of their neighbors (Fig. 2C, arrow). Border cells also accumulate elevated levels of AJ proteins (Oda et al., 1997; Niewiadomska et al., 1999) and require DE-Cad to migrate to the anterior end of the oocyte. During migration, border cells accumulate Rho1 (Magie et al., 2002) (Fig. 2L) at both the plasma membrane and in cytoplasmic puncta (Fig. 2M, arrowheads). These may represent multivesicular bodies enriched in border cells (Peifer et al., 1993). DE-Cad was enriched in apical AJs of centripetal follicle cells that migrate between nurse cells and the oocyte (Fig. 2D, red arrowhead). Rho1 accumulated with DE-Cad in these cells apically (Fig. 2D, red arrowhead); Rho1 was also enriched in more basal puncta (Fig. 2D, blue arrowhead). Thus in the ovary, while Rho1 and DE-Cad overlapped in some places, Rho1 did not preferentially localize to AJs.

We also compared Rho1 and DE-Cad localization in

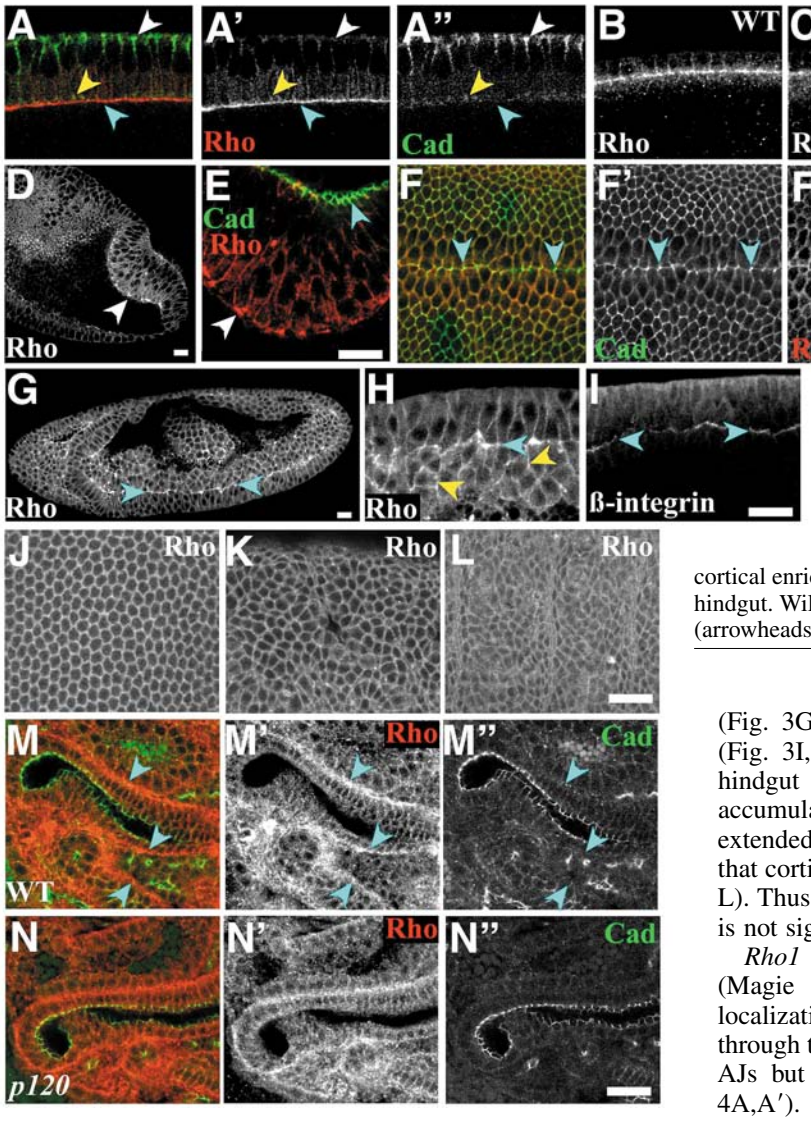

Fig. 3. Rho1 localization during embryogenesis. Rho1 (red). DE-Cad (green). (A-C) Cross-sections, cellularization. Wild type (A,B). *p120* (C). (A) Rho1 enriched at furrow canals (A, blue arrowhead). DE-Cad accumulates at basal junctions (A, yellow arrowhead) and apical AJs (A, white arrowhead). (B,C) Loss of p120 (C) does not alter Rho1 localization. (D-E) Cross-section, posterior midgut (E, close-up). Rho1 in basal puncta in invaginating midgut cells (arrowhead). DE-Cad (green, E) at sites of apical constriction. (F) Ventral furrow. DE-Cad accumulates at AJs of apically constricting cells (arrowheads) while Rho1 does not. (G-I) Cross sections, stage 8. Rho1 accumulates where ectodermal and mesodermal cells meet (G,H, arrowheads). H, close-up of G. (I) βPS1 integrin. (J-L) Basolateral sections. Stages 5 (J), 9 (K), 15 (L). Rho1 cortical enrichment decreases. (M-N′′) Cross-sections, stage 15 hindgut. Wild type (M). *p120* mutant (N). Rho1 accumulates basally (arrowheads). DE-Cad accumulates in apical AJs. Scale bars: 20 μm.

embryos, extending earlier work [(Magie et al., 2002; Padash-Barmchi et al., 2005); the pictures presented use the protocol of Padash-Barmchi et al. (Padash-Barmchi et al., 2005), but similar results were also seen with the protocol of Magie et al. (Magie et al., 2002)]. Rho1 localization was very dynamic. During cellularization, Rho1 was enriched at invaginating furrow canals (Fig. 3A, blue arrowhead), as previously observed (Padash-Barmchi et al., 2005), while DE-Cad localized both to basal (Fig. 3A, yellow arrowhead) and developing apical AJs (Fig. 3A, white arrowhead). At gastrulation, DE-Cad was enriched in AJs of posterior midgut cells (Fig. 3E, blue arrowhead), while Rho1 accumulated in basal puncta (Fig. 3D,E white arrowheads) that may be furrow canal remnants. DE-Cad was also enriched in apical AJs of invaginating cells in the ventral furrow, while Rho1 was only weakly enriched in the ventral furrow (Fig. 3F, arrowheads). After mesodermal cells invaginated, they accumulated cortical Rho1 (Fig. 3H, yellow arrowheads). In epithelial cells, we observed two general features of Rho1 localization. In several cell types, Rho1 localized basally. After germband extension, Rho1 accumulated basally where ectoderm meets mesoderm (Fig. 3G,H arrowheads). βPS1-integrin also localized there (Fig. 3I, arrowheads). Later, Rho1 localized basally in the hindgut epithelium (Fig. 3M, blue arrowheads; DE-Cad accumulated apically). Second, comparison of blastoderm, extended germband, and dorsal closure-stage embryos showed that cortical enrichment of Rho1 decreases over time (Fig. 3J-L). Thus Rho1 localization varies in different cell types, but it is not significantly enriched in AJs of most cells.

Rho1 zygotic mutants have defects during dorsal closure (Magie et al., 1999). We thus closely examined Rho1 localization at that stage, collecting sections in the *z*-axis through the lateral epithelia. Apically, Rho1 did not localize to AJs but accumulated at low levels in the cytoplasm (Fig. 4A,A′). Basal to AJs (where DE-Cad was seen at AJs of invaginating segmental groove cells; Fig. 4B,B′), Rho1 levels increased and were more cortical. Thus in these cells Rho1 was enriched basal to AJs. Another important input in Rho1 regulation is localized activation by RhoGEFs. While a comprehensive study is beyond the scope of our work, we examined RhoGEF2 during dorsal closure, which co-localizes with Rho1 during cellularization (Padash Barmchi et al., 2005). During dorsal closure, RhoGEF2 accumulated basal to AJs (Fig. 4H,H′), like Rho1. However, RhoGEF2 was more cortical, poising it to activate a cortical pool of Rho1. Thus, during oogenesis and embryogenesis Rho1 localization varies among different cell types, with basal or basolateral accumulation in many epithelia. Importantly, we found no evidence for preferential Rho1 accumulation at AJs, although the localizations do sometimes overlap.

Rho1 localization is independent of p120 and core AJ protein function

Both p120 and α-catenin can bind Rho1 (Magie et al., 2002). These authors reported that RNAi of either *p120* or *α-catenin* altered Rho1 localization. While we did not observe strong enrichment of Rho1 at AJs, it remained possible that p120 or core AJ proteins could regulate Rho1 localization. To examine

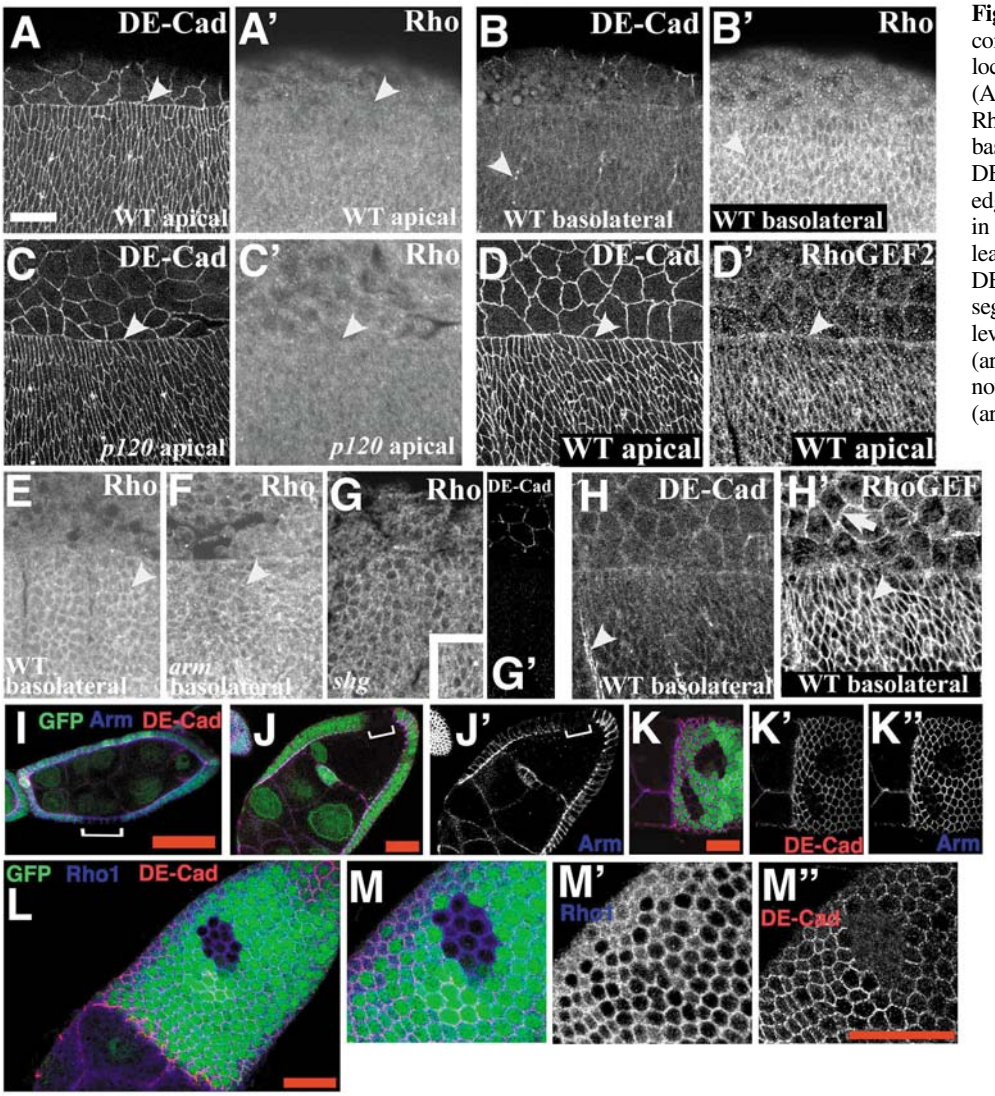

Fig. 4. Neither p120 nor core AJ components are essential for Rho1 localization during dorsal closure. (A,B) Wild type. DE-Cad (A,B) versus Rho1 (A′,B′) in apical (A,A′) and basolateral (B,B′) sections. (A,A′) Apically, DE-Cad (A) accumulates at AJs. Leading edge (arrowhead). Rho1 (A′) is at low levels in the cytoplasm and is not enriched at the leading edge (arrowhead). (B,B′) Basally, DE-Cad (B) accumulates at AJs in segmental grooves (arrowhead). Rho1 (B′) levels are higher and it is cortically enriched (arrowhead). (C,C′) *p120* mutant. Rho1 is not elevated at the leading edge (arrowheads, C′ versus A′). (D,D′) Apical RhoGEF2. Arrowheads, leading edge. (E-G) Basolateral Rho1 (arrowhead) is similar in wild type (E), arm^{YD35} (F), or shg^{R69} zygotic mutants (G). (G′) DE-Cad in a portion of G. (H,H′) RhoGEF2 is enriched basolaterally and is more cortical than Rho1 (H′ versus B′) at both amnioserosal (arrow) and epidermal cell (arrowhead) boundaries. (I-M′′) Rho1 in shg^{R69} mutant follicle cell clones. (I-K′) GFP, green; Arm, blue; DE-Cad, red. (I) Cross-section, early egg chamber. shg^{R69} mutant clone (bracket) indicated by lack of GFP. Arm accumulates at AJs at this stage despite loss of DE-Cad. (J-K′) Cross-section (J,J′), grazing section (K,K′), later egg chamber. shg^{R69} mutant clone (bracket). Arm and DE-Cad are severely reduced. (L-M′′) Grazing section, similar stage egg chamber as J,K. GFP, green; Rho1, blue; DE-Cad, red. (L) Rho1 accumulates normally in shg^{R69} clone. (M,M′′) Close-up of L. Scale bars: 20 μm in A; 50 μm in I-L,M′′.

this, we first examined *p120* null mutant ovaries and embryos. As previously observed in embryos (Myster et al., 2003), *p120* mutant follicle cells exhibited normal DE-Cad localization to AJs (Fig. 2G,I). In *p120* mutant follicle cells, Rho1 localization to the cytoplasm, the lateral membrane (compare Fig. 2F to Fig. 2G, and Fig. 2H to Fig. 2I) and its accumulation in puncta in late-stage follicle cells (Fig. 2J versus Fig. 2K) were indistinguishable from wild type. We also saw no change in Rho1 localization in *p120* mutant embryos; for example, Rho1 localization to furrow canals (Fig. 3B versus Fig. 3C) and its basal localization in the hindgut (Fig. 3M versus Fig. 3N) were unchanged. Further, there were no changes in apical (Fig. 4C′ versus Fig. 4A′) or basolateral (data not shown) Rho1 localization during dorsal closure in *p120* mutants. In particular, we did not observe elevated Rho1 accumulation at the leading edge, as was previously reported for *p120* RNAi (Magie et al., 2002). Thus p120 is not required for normal Rho1 localization in ovarian or embryonic epithelia.

We next examined whether core AJ proteins are crucial for Rho1 localization. We addressed this in the follicle cell epithelium by generating homozygous null DE-Cad (*shotgun;*shg^{R69}) mutant clones (marked by loss of GFP; Fig. 4I-M′′). DN-Cadherin can act in place of DE-Cad (Tanentzapf et al., 2000) during early oogenesis; thus in early-stage egg chambers Arm remains at AJs despite loss of DE-Cad (Fig. 4I). However, by stage 10 this redundancy is less apparent; loss of DE-Cad substantially reduces Arm accumulation in AJs (Fig. 4J-K′′). We thus confined our analysis to late stages. Rho1 localization was unchanged in *shg* null mutant follicle cells (Fig. 4L,M). We also examined Rho1 localization in embryos zygotically mutant for core AJ proteins. In zygotic *arm* null mutants, DE-Cad was no longer detectable by dorsal closure (data not shown). Zygotic *shg* null mutants lost DE-Cad from the epidermis, although some maternal DE-Cad remained in the aminoserosa (Fig. 4G′). In both *arm* (Fig. 4E versus Fig. 4F) and *shg* (Fig. 4G) zygotic mutants, Rho1 localization was unchanged. Thus core AJ components do not play a major role in Rho1 localization in *Drosophila* epithelia.

Drosophila p120 is not a key regulator of Rho1 function

Although p120 is not essential for Rho1 localization, it might

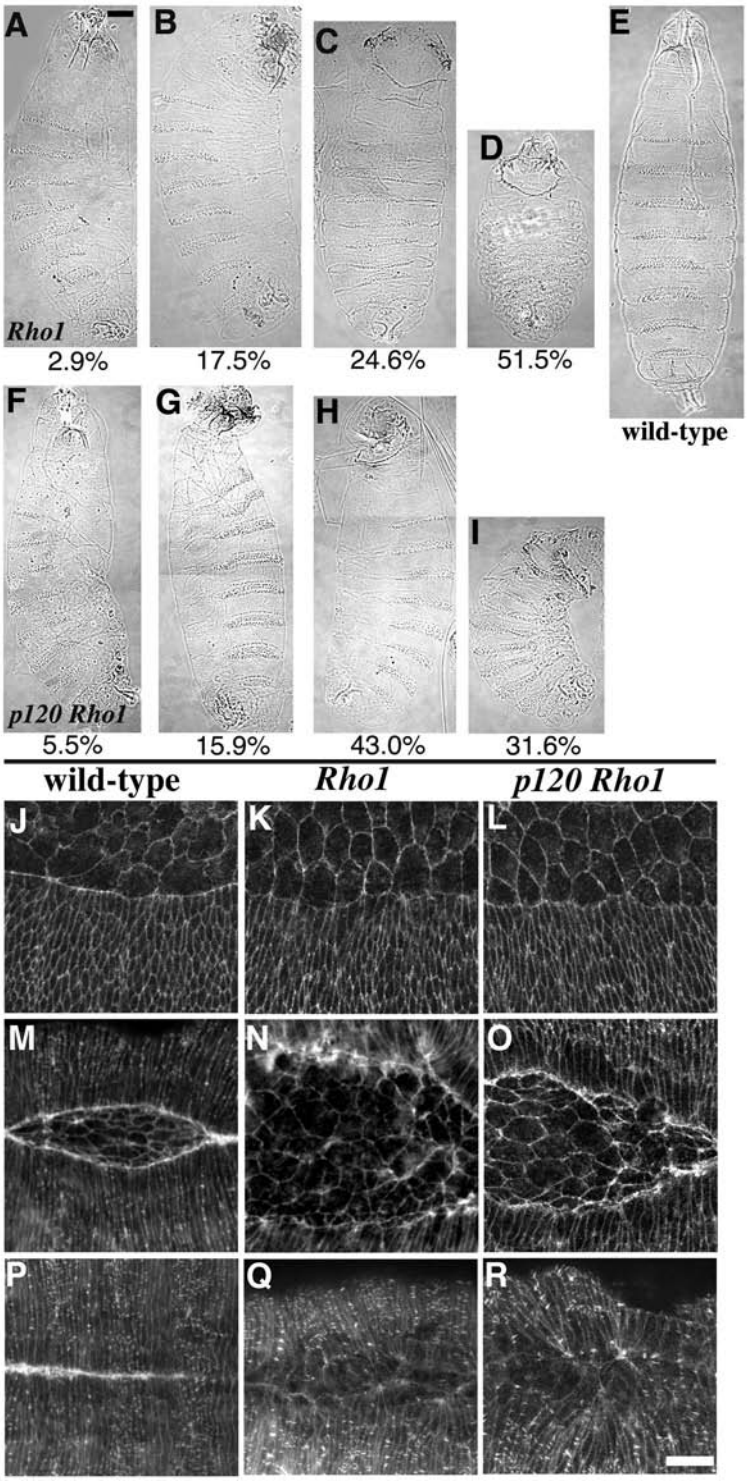

Fig. 5. *p120* loss of function does not substantially enhance or suppress *Rho1*. (A-I) Cuticle preparations, anterior up. *Rho1* (A-D), wild type (E), or *p120 Rho1* (F-I). Embryos were divided into phenotypic classes of increasing severity (Table 3). The percentage of embryos in each class is listed below a representative cuticle. (J-R) F-actin (Phalloidin). Wild type (J,M,P). *Rho1* (K,N,Q). *p120 Rho1* (L,O,R). (J-L) Lateral view. Early dorsal closure. (M-R) Dorsal views. (M-O) Late dorsal closure. (P-R) After closure. Scale bars: 25 μm in A-I; 20 μm in J-R.

regulate Rho1 function by other mechanisms. Often regulators, even those that are partially redundant, can be identified by looking for phenotypic effects in a sensitized genetic background. For example, the supporting role of p120 in AJs was revealed by the fact that loss of p120 strongly enhances DE-Cad mutants (Myster et al., 2003). In zygotic *Rho1* mutants, maternal Rho1 is gradually depleted, and thus we reasoned that altering a Rho1 regulator might modify the consequences of reduced levels of Rho1.

To test whether *p120* and *Rho1* genetically interact, we generated *p120 Rho1* double mutants and compared their phenotypes to those of *Rho1*. *Rho1* mutant embryos had the defects described previously (Magie et al., 1999) (unless noted, we used the strong allele $Rho1^{rev220}$). Most embryos lack head cuticle; the remaining cuticle differs significantly in size among different embryos (Fig. 5; Table 3), perhaps due to the varying degrees of 'bowing' of the dorsal surface previously observed (Magie et al., 1999). *p120* null mutants ($p120^{308}$, used for all experiments) have normal cuticles and are embryonically viable.

Complete removal of *p120* did not substantially modify the *Rho1* phenotype, nor was a novel double-mutant phenotype seen. The percentage of embryos with head holes was similar, for example (76.1% of *Rho1* mutants versus 74.6% of *p120 Rho1* double mutants; Table 3). We did observe an effect on one aspect of the phenotype: *p120 Rho1* double mutants had twice as many long cuticles as *Rho1* single mutants (Table 3; Fig. 5; this was seen in two separate experiments). This phenotypic shift may reflect subtle suppression of *Rho1* by *p120*. However, this is much more subtle than the effect of p120 loss on the *shg* phenotype (Myster et al., 2003) and may simply reflect differences in genetic background or sample preparation – we observed comparable variation between different cuticle preparations of the same genotype (data not shown).

As a second test for genetic interactions, we examined whether p120 overexpression modifies the *Rho1* phenotype, ubiquitously expressing a p120 transgene under the control of the GAL4-UAS system (Myster et al., 2003) (using actin-GAL4). Magie et al. (Magie et al., 2002) previously reported that *p120* overexpression using actin-GAL4 enhanced the *Rho1* phenotype. However, using an independently derived UAS-p120 transgene, we did not observe phenotypic enhancement. Instead, our results suggested a slight suppression of the *Rho1* phenotype. The overall range of phenotypes was similar, with a small shift toward the less severe categories (Table 3; data not shown). However, this effect was fairly small, and may reflect differences in genetic background.

We also compared the effect of loss of Rho1 with the loss of both Rho1 and p120 on F-actin during dorsal closure. As previously observed by Magie et al. (Magie et al., 1999), *Rho1* mutants were nearly normal during early dorsal closure (Fig. 5J versus Fig. 5K), with defects becoming apparent during late dorsal closure. The *Rho1* phenotype was variable – in more severely affected *Rho1* mutants, both the leading-edge actin

Table 3. Neither loss nor overexpression of p120 substantially modifies the *Rho1* phenotype

	Phenotype	*Rho/+×Rho/+*	*p120Rho/p120+× p120Rho/p120+*	*Rho/+×Rho/+*	*Rho/+; UASp120× Rho/+; actin-GAL4*
Least severe	Wild-type or mild head defects	2.9%	5.5%	2.2%	9.8%
↓	Severe head defects	17.5%	15.9%	8.6%	12.3%
↓	Head hole with remnant head skeleton	3.4%	3.9%	5.3%	11.8%
↓	Head hole, body >0.6, field at 20× magnification	24.6%	43.0%	33.3%	17.1%
Most severe	Head hole, body <0.6 field at 20× magnification	51.5%	31.6%	50.6%	48.9%
	n	1165	634	324	519

cable and cell shape changes were less uniform (Fig. 5M versus Fig. 5N). In less severely affected mutants, when leading edges met at the dorsal midline the two epithelial sheets did not line up or intercalate normally (Magie et al., 1999) (Fig. 5P versus Fig. 5Q). *p120 Rho1* double mutants exhibited the same range of phenotypes as *Rho1* single mutants during early (Fig. 5K versus Fig. 5L) and later stages of dorsal closure (Fig. 5N versus Fig. 5O; Fig. 5Q versus Fig. 5R). Thus, *p120* does not behave genetically as a key regulator of Rho1 function, contrasting with its strong genetic interactions with DE-Cad (Myster et al., 2003).

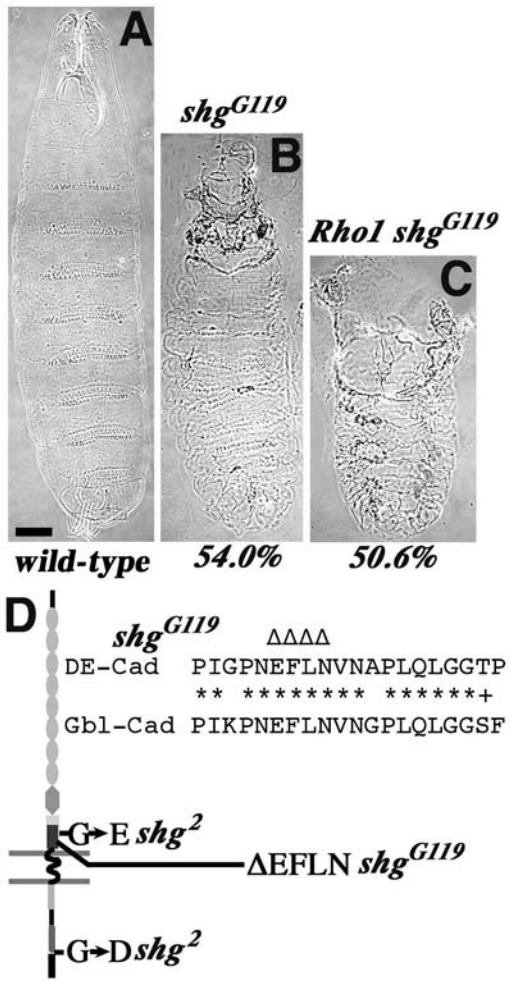

Fig. 6. *Rho1* enhances a weak *shg* allele. Cuticle preparations, anterior up. (A) Wild type. (B) shg^{G119}. Note head hole and small ventral scar. (C) *Rho1* shg^{G119}. Note head and ventral holes. (D) Schematic of shg^{G119} and shg^{2} lesions, and sequence alignment of relevant region in shg^{G119}. Gbl-cad, *Gryllus bimaculatus* (cricket), DE-cad homolog. Scale bar: 25 μm.

Rho1 and *shg* genetically interact

These data suggest that p120 is not a required regulator of Rho function in *Drosophila*. However, there are other strong links between Rho and AJs: evidence from mammalian cell culture and *Drosophila* strongly suggest that Rho regulates AJ protein localization and function. To probe the relationship between Rho and AJs during embryonic morphogenesis, we asked whether *Rho1* genetically interacts with DE-Cad (*shg*) by constructing double mutants for *Rho1* and each of three *shg* alleles: a weak allele, shg^{G119} (Tepass et al., 1996); a strong allele, shg^{2} [(Uemura et al., 1996) (shg^{IH81}); (Nüsslein-Volhard et al., 1984)]; and a protein-null allele, shg^{R69} (Godt and Tepass, 1998). Embryonic epithelia have differential sensitivity to DE-Cad loss (Tepass et al., 1996). The head epidermis, which undergoes extensive rearrangements, is most sensitive. Thus, weak alleles such as shg^{G119} mainly disrupt head cuticle (Fig. 6; Table 4). The next most sensitive tissue is the ventral epidermis, site of neuroblast delamination. Strong *shg* alleles such as shg^{2} disrupt head and, to varying degrees, ventral epidermis (Fig. 7; Table 5). Finally, null *shg* alleles such as shg^{R69} lack head, ventral cuticle and, to some extent, dorsal cuticle (Fig. 7; Table 5). We reasoned that novel phenotypes in double mutants or suppression of one mutation by another might indicate a genetic interaction.

Loss of *Rho1* enhances the phenotype of the weak allele shg^{G119} (Fig. 6; Table 4). Most double mutant embryos have holes in their ventral epidermis that are not present in most shg^{G119} single mutants. As both *Rho1* and shg^{G119} affect head involution, the enhancement of this phenotype may simply reflect additive effects. However, because loss of Rho1 does not affect integrity of the ventral epidermis, we believe enhancement of this aspect of the phenotype is likely to reflect a genetic interaction. We also assessed whether reduction of the dose of Rho1 had an effect on the shg^{G119} phenotype: there was no apparent effect of reducing the maternal and zygotic Rho1 dose by half. Surprisingly, however, *Rho1* did not enhance, but instead partially suppressed, the phenotype of stronger *shg* alleles (Fig. 7; Table 5). For both alleles, a larger fraction of double mutant progeny fell into less severe phenotypic categories. Thus, *Rho1* behaves genetically as a regulator of AJ function, but the nature of this genetic interaction is complex.

We also sequenced the two non-null *shg* alleles. shg^{G119} has an in-frame deletion of four conserved amino acids in the membrane-proximal lamininG domain in the extracellular domain (Fig. 6D). shg^{2} has two mutations: mis-sense changes

Table 4. *Rho1* enhances a weak *shg* allele

	Phenotype	*Rho/Cy×Rho/+*	*shg^{G119}/Cy×shg^{G119}/+*	*Rhoshg^{G119}/Cy×Rhoshg^{G119}/+*
Least severe	Wild type	0.8%	7.6%	0.5%
↓	Head defect/head hole	97.6%	16.8%	17.4%
↓	Scar in ventral cuticle	0%	50.6%	8.7%
↓	Ventral holes	0.4%	21.7%	54.0%
↓	Fragmentary ventral cuticle	0%	1.4%	17.4%
↓	Dorsal cuticle only	0.4%	1.8%	1.5%
↓	Dorsal cuticle with holes	0.4%	0%	0%
↓	U-shaped dorsal cuticle	0%	0%	0%
Most severe	Fragmented cuticle	0.4%	0%	0.3%
	n	255	498	378

in a conserved amino acid in the lamininG domain and in a conserved residue in the cytoplasmic tail (Fig. 6D; Fig. 7G) at the C-terminal end of the Arm-binding site (Pai et al., 1996; Huber and Weis, 2001; Pokutta and Weis, 2000).

Rho1 regulates DE-Cad but not Arm localization

Rho1 was reported to be required for correct localization of AJ proteins (Magie et al., 2002; Bloor and Kiehart, 2002). We examined embryos lacking zygotic Rho1. During dorsal closure, DE-Cad accumulated ectopically, as previously reported (Magie et al., 2002). Ectopic DE-Cad accumulated in the cytoplasm of epithelial or amnioserosal cells near the leading edge (Fig. 8A versus Fig. 8B). Ectopic DE-Cad also accumulated in *Rho1* embryos prior to (Fig. 8C versus Fig. 8D) and following (Fig. 8E versus Fig. 8F) dorsal closure. Importantly, however, ectopic DE-Cad did not co-localize with its binding partner Arm (Fig. 8B). Finally, we used the ectopic DE-Cad phenotype of *Rho1* mutants to further test whether p120 regulates Rho1. *p120 Rho1* double mutants accumulated ectopic DE-Cad during dorsal closure in a fashion identical to *Rho1* single mutants (Fig. 8G versus Fig. 8B). Thus, Rho1 regulates DE-Cad but not Arm localization and the effect of loss of Rho1 is not enhanced or suppressed by removing p120.

Discussion

The regulation of adhesion and its coupling to the actin cytoskeleton are crucial for morphogenesis. Rho and p120 both regulate adhesion and the cytoskeleton, but the precise nature of their roles and the interrelationship among them are less clear. We addressed these issues during development in *Drosophila*.

Drosophila p120: a redundant regulator of AJs

In mammalian cells, p120 is a key regulator of cadherin-based adhesion (Davis et al., 2003; Xiao et al., 2003). However, the

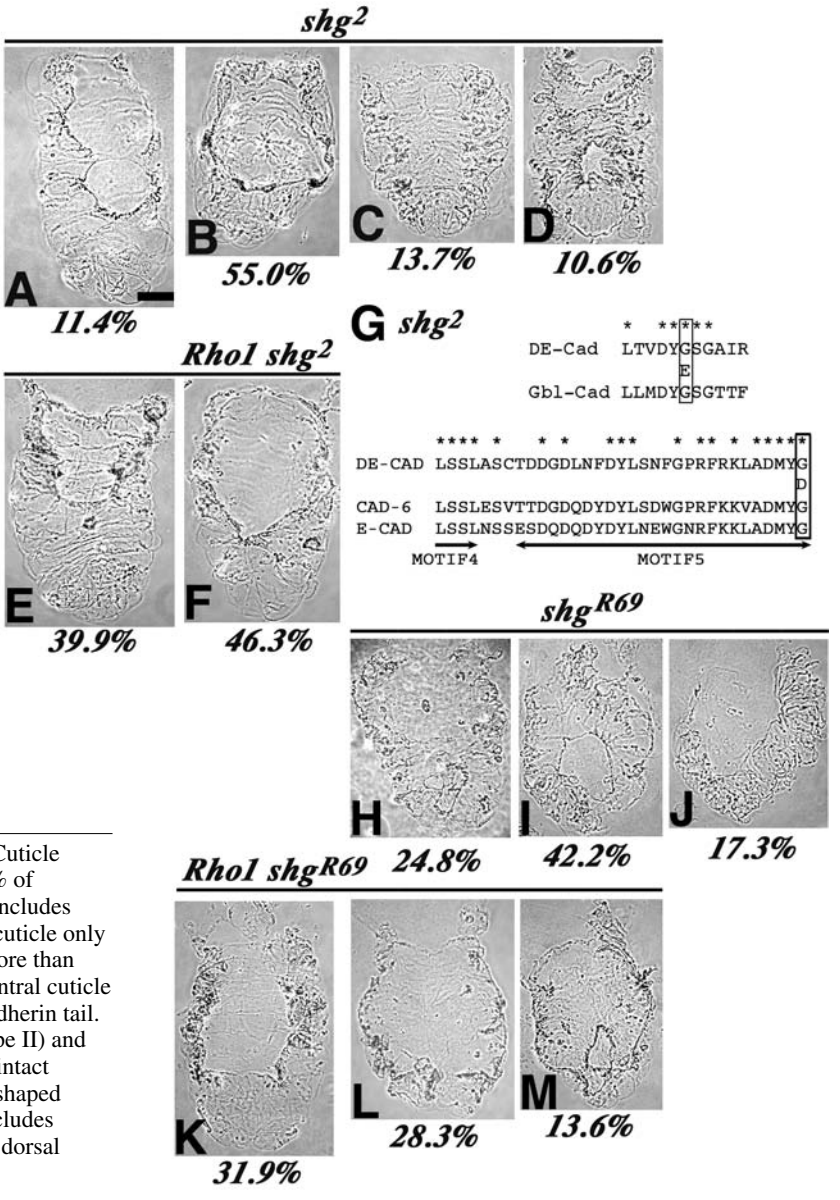

Fig. 7. *Rho1* suppresses both strong and null *shg* alleles. Cuticle preparations, anterior up. Major phenotypic classes (>10% of cuticles), *shg* or *Rho1 shg*. (A-D) *shg^2*. Range of defects includes ventral holes (A), fragmentary ventral cuticle (B), dorsal cuticle only (C) and dorsal cuticle with holes (D). (E,F) *Rho1 shg^2*. More than 80% of embryos have ventral holes (E) or fragmentary ventral cuticle (F). (G) *shg^2* lesions in lamininG repeat and motif 5 of cadherin tail. DE-Cad versus Gbl-Cad, or mouse cadherins CAD-6 (Type II) and E-CAD (Type I). (H-J) *shg^{R69}*. Range of defects includes intact dorsal cuticle only (H), dorsal cuticle with holes (I) or U-shaped dorsal cuticle (J). (J-M) *Rho1 shg^{R69}*. Range of defects includes fragmentary ventral cuticle (K), dorsal cuticle only (L) or dorsal cuticle with holes (M). Scale bar: 25 μm.

Table 5. *Rho1* partially suppresses both a strong and a null *shg* allele

	Phenotype	$shg^2/Cy\times shg^2/+$	$Rhoshg^2/Cy\times Rhoshg^2/+$	$shg^{R69}/Cy\times shg^{R69}/+$	$Rhoshg^{R69}/Cy\times Rhoshg^{R69}/+$
Least severe	Wild-type	4.5%	5.9%	6.6%	5.1%
	Head defect/head hole	1.5%	2.0%	0.8%	15.4%
	Scar in ventral cuticle	0%	0%	0.3%	0.7%
	Ventral holes	11.4%	39.9%	1.0%	4.0%
	Fragmentary ventral	55.0%	46.3%	6.1%	31.9%
	Dorsal cuticle only	13.7%	4.4%	24.8%	28.3%
	Dorsal cuticle with holes	10.6%	1.4%	42.2%	13.6%
	U-shaped dorsal cuticle	2.2%	0%	17.3%	0.7%
Most severe	Fragmented cuticle	0%	0%	0.5%	0%
	n	131	203	374	272

universality of this role was called into question by the viability of *p120* mutant flies (Myster et al., 2003). One caveat remained, however. Magie et al. (Magie et al., 2002) reported that rapidly depleting fly embryos of p120 by RNAi led to defects in morphogenesis and Rho1 localization. To resolve this discrepancy, we carried out *p120* RNAi. Our data confirm that knockdown of p120 does not result in lethality. Thus, *Drosophila* (these data) (Myster et al., 2003; Pacquelet et al., 2003) and *C. elegans* p120 (Pettitt et al., 2003) are dispensable for development. By contrast, p120 knockdown in *Xenopus* or *Mus musculus* is lethal (Fang et al., 2004) (Al Reynolds, personal communication), suggesting differences in the importance of p120 in vertebrates versus invertebrates.

As *Drosophila* has a single p120 family member, simple redundancy does not explain the difference between vertebrates and invertebrates. We imagine two possible explanations. First, p120 proteins may play fundamentally different roles in the two groups of animals. Alternatively, the role of p120 in both may be similar, but the relative importance of p120 and unrelated, partially redundant regulators of cadherin and/or Rho may differ. We favor the latter possibility, because p120 binds to and promotes the function of AJs in vertebrates and invertebrates (Anastasiadis and Reynolds, 2000; Myster et al., 2003; Pettitt et al., 2003), and p120 has a conserved role in regulating morphogenesis, contributing to dorsal closure efficiency (these data) and regulating dendrite morphology (Li et al., 2005) in *Drosophila* and regulating gastrulation and craniofacial morphogenesis in *Xenopus* (Fang et al., 2004; Ciesiolka et al., 2004). One role of p120 is to inhibit cadherin endocytosis. Perhaps in invertebrates other regulators of cadherin trafficking compensate for its absence.

Drosophila p120: a redundant regulator of Rho1?

The second postulated role for p120 is as a Rho regulator. The viability of *p120* mutants suggested that *Drosophila* p120 is not an essential Rho1 regulator. However, this did not rule out a role as one of several Rho1 regulators with overlapping functions. For example, Magie et al. (Magie et al., 2002) suggested overlapping roles for p120 and α-catenin, with p120 regulating Rho1 localization during dorsal closure. We thus looked for dose-sensitive genetic interactions between *p120* and *Rho1*. Loss of p120 did not substantially affect Rho1 function, as assessed by cuticle phenotype. Further, loss of p120 did not enhance or suppress the effect of *Rho1* on F-actin or DE-Cad localization during dorsal closure. p120 overexpression had only a slight effect on the *Rho1* phenotype, a result that may reflect variation in genetic background. Thus, although *Drosophila* p120 preferentially binds inactive Rho1 (Magie et al., 2002), it is not a major dose-sensitive regulator of Rho1.

We also tested the hypothesis that p120 regulates Rho1 localization, examining several places in which Rho1 exhibits striking subcellular localization, and examining the place where Rho1 exhibits its zygotic phenotype: the dorsal closure front.

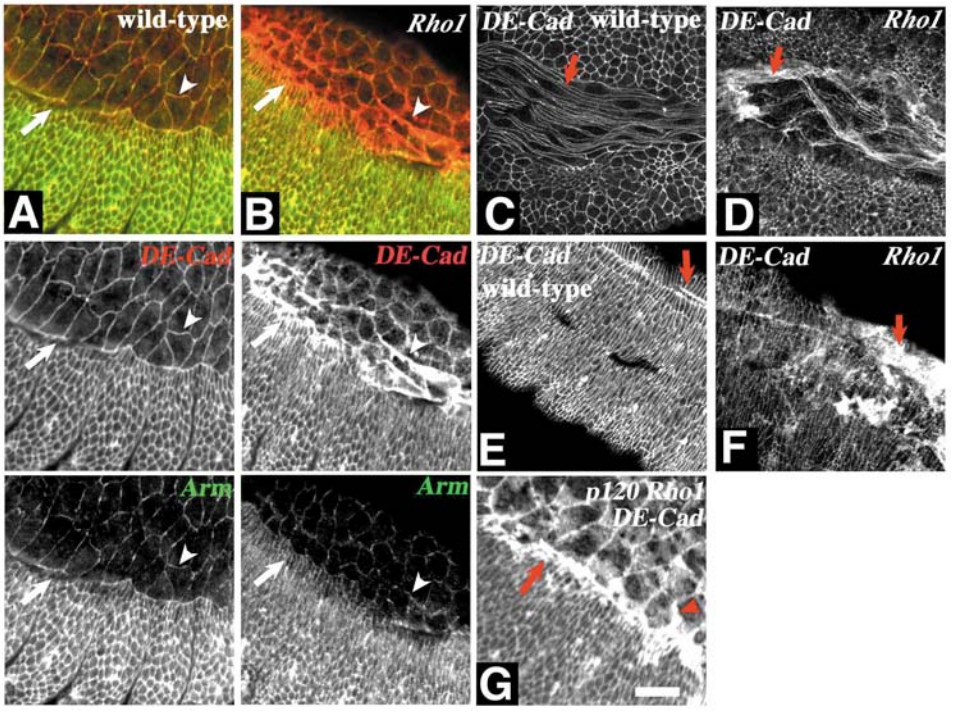

Fig. 8. Rho1 regulates DE-Cad but not Arm localization. (A,B) Wild-type (A) or *Rho1* (B) during dorsal closure. DE-Cad (red). Arm (green). Arrow, leading edge; arrowhead, amnioserosa. (C-F) DE-Cad in wild type (C,E) and $Rho1^{1B}$ (D,F). Stage 9 (C,D; arrows, amnioserosa). Stage 17 (E,F; arrows, dorsal midline). (G) *p120 Rho1* double mutant during dorsal closure. Scale bar: 20 μm.

We saw no change in Rho localization in *p120* mutants. Therefore, if p120 regulates Rho1 localization, it must do so redundantly with other putative Rho1 regulators, such as α-catenin (Magie et al., 2002). These data do not rule out the possibility that p120 recruits a pool of active Rho1, which may be only a small fraction of total cellular Rho1.

p120 appears to regulate RhoA during *Xenopus* development (Fang et al., 2004; Ciesiolka et al., 2004). Perhaps redundant Rho regulators act in parallel to p120 in flies. Alternatively, the role of p120 as a Rho regulator may not be conserved: the N-terminal domain of p120, which is implicated in regulating transitions between its adhesive and cytoplasmic roles, is not well conserved between mammalian and fly p120. As *p120* (Myster et al., 2003) and *Rho1* (Fig. 5) mutations modify *shg* mutant phenotypes differently, p120 and Rho1 may act in separate pathways to regulate AJs in *Drosophila*.

Rho1 localization and its regulation

We extended previous analyses of Rho1 localization (Magie et al., 2002; Padash-Barmchi et al., 2005). It is dynamic, with Rho1 accumulating at different subcellular sites in distinct cell types, some consistent with proposed Rho functions. For example, mammalian RhoA regulates integrin-based cell-matrix junctions (reviewed by Burridge and Wennerberg, 2004). The basal localization of Rho1 raises the possibility that it may regulate integrins in *Drosophila*. Rho1 accumulation in mesodermal cells is consistent with its role in regulating cell shape during mesoderm spreading (Wilson et al., 2005). Relative levels of cortical Rho1 decrease through development. Perhaps at later stages Rho1 is activated by localized RhoGEFs. Consistent with this, RhoGEF2 is more cortically enriched during dorsal closure than Rho1. Thus, future studies will need to examine the localization of Rho1 regulators and effectors. Recent advances also allow visualization of active Rho GTPases (e.g. Benink and Bement, 2005). As much of the Rho1 pool may be inactive, application of this approach to flies will advance our understanding of Rho1 function.

It was previously proposed that Rho1 is enriched at *Drosophila* AJs and that this is regulated by core AJ proteins (Magie et al., 2002). We examined this in follicle cells and embryos. In follicle cells, Rho1 localized to lateral and apical membranes in early egg chambers, and to lateral membranes later. In neither case did we observe enrichment in AJs, although Rho1 was not excluded from them. In embryonic epithelia, Rho1 sometimes localized uniformly to the basolateral membrane, while in other places it was enriched basally. During dorsal closure, when *Rho1* exhibits its zygotic phenotype, Rho1 accumulated basal to AJs. The lack of preferential Rho1 localization at AJs does not rule out accumulation of a pool of active Rho1 at AJs: this will require reagents to measure Rho1 activation in vivo. We also tested the hypothesis that AJs regulate Rho1 localization. In follicle cells mutant for DE-Cad and embryos mutant for *arm* or DE-Cad during dorsal closure, Rho1 localization was not obviously disturbed.

Rho is an important regulator of AJs during embryonic morphogenesis

In cultured cells, Rho and AJs have a complex relationship. Rho regulates AJ stability, and conversely AJs regulate Rho activity (reviewed by Yap and Kovacs, 2003). Further, different Rho effectors can promote or decrease AJ stability in cultured mammalian cells (Sahai and Marshall, 2002; Vaezi et al., 2002). We examined this complex relationship during morphogenesis, using genetic and cell biological assays. Our data support the hypothesis that Rho1 is an important regulator of cadherin-based adhesion during embryonic development.

Loss of Rho1 leads to DE-Cad mislocalization (Magie et al., 2002), while dominant-negative Rho1 reduces DE-Cad in AJs (Bloor and Kiehart, 2002), implicating Rho1 in regulating DE-Cad localization. Our results support this hypothesis. Cytoplasmic DE-Cad accumulation is consistent with a role for Rho1 in regulating either DE-Cad transport to or recycling from AJs. We observed that the ectopic DE-Cad in *Rho1* mutants accumulates independently of its binding partner Arm. In mammalian cells, newly-synthesized E-cadherin must bind β-catenin before it can be transported to AJs (Chen et al., 1999), while endocytosed E-cadherin accumulates with either no (Xiao et al., 2003) or reduced (Le et al., 1999) amounts of β-catenin. Thus our data are more consistent with ectopic DE-Cad accumulating after endocytosis. Consistent with this, mammalian RhoA regulates clathrin-mediated endocytosis (Lamaze et al., 1996), and *Drosophila* Rho1 regulates endocytosis of the ligand Wingless (Magie et al., 2005). Further, constitutively active Rac1, which can inhibit RhoA (Sander et al., 1999), triggers E-cadherin recruitment to intracellular vesicles in keratinocytes (Akhtar and Hotchin, 2001). As high levels of Rho1 do not accumulate at AJs, either a small pool of active Rho1 at AJs is sufficient to inhibit cadherin endocytosis or the effect is more indirect, with Rho1 acting on the actin cytoskeleton or regulators of endocytic trafficking. The mechanism by which Rho1 regulates DE-Cad trafficking is an interesting question for future studies.

Mammalian p120 also regulates cadherin endocytosis (Davis et al., 2003; Xiao et al., 2003). The viability of fly *p120* mutants suggests that in flies this role is not rate limiting, although the enhancement of mutants with reduced DE-Cad by *p120* is consistent with p120 playing a similar role (Myster et al., 2003). p120 and Rho could regulate DE-cad trafficking via the same or distinct pathways. The effect on DE-Cad trafficking in zygotic *Rho1* mutants, which should have limiting levels of maternal Rho1, is not enhanced by removing *p120*. This is more consistent with a model in which the two proteins work in different pathways, and in which p120 acts partially redundantly with another unknown regulator.

Our analysis of *Rho1* and *Rho1 shg* mutants is consistent with the hypothesis that Rho1 regulates AJs, but suggests that their interactions are complex. A weak *shg* allele was enhanced, but stronger alleles were suppressed. There are several possible explanations for these contrasting results. Weak alleles (e.g. shg^{G119}) make protein with reduced but residual function. If Rho1 negatively regulates cadherin endocytosis, more mutant DE-Cad protein might be endocytosed in Rho1's absence, further reducing functional DE-Cad and enhancing the phenotype. However, null or very strong *shg* alleles accumulate no functional DE-Cad at AJs (for shg^2 see Uemura et al., 1996), rendering regulation of cadherin endocytosis a moot point. The slight suppression by *Rho1* of strong *shg* alleles may result from a reduction of morphogenetic movements, reducing cuticle disruption (as in Tepass et al., 1996). Alternatively, some mutant DE-Cad proteins may be capable of coupling to Rho1 while others are not. Rho1 can bind α-catenin (Magie et al., 2002), and active

Rho1 may be recruited to AJs by that interaction. shg^{G119} has a wild-type cytoplasmic domain and could presumably couple to Rho1; reducing Rho1 might further impair its function. By contrast, the shg^{2} mutation may impair Arm and/or α-catenin binding and thus Rho1 recruitment; if so this mutant protein would not be further impaired by Rho1 removal. Finally, the complex genetic interactions might reflect different requirements for Rho1 during neuroblast delamination and head involution, which are affected by strong or weak reduction in DE-Cad function, respectively (Tepass et al., 1996). Future studies of Rho regulation of and by AJs will help distinguish between these possibilities.

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Supplementary material

Supplementary material for this article is available at
http://dev.biologists.org/cgi/content/full/132/21/4819/DC1

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