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Binding site for p120/δ-catenin is not required for *Drosophila* E-cadherin function in vivo

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Homophilic cell adhesion mediated by classical cadherins is important for many developmental processes. Proteins that interact with the cytoplasmic domain of cadherin, in particular the catenins, are thought to regulate the strength and possibly the dynamics of adhesion. β-catenin links cadherin to the actin cytoskeleton via α-catenin. The role of p120/δ-catenin proteins in regulating cadherin function is less clear. Both β-catenin and p120/δ-catenin are conserved in *Drosophila*. Here, we address the importance of cadherin–catenin interactions in vivo, using mutant variants of *Drosophila* epithelial cadherin (DE-cadherin) that are selectively defective in p120ctn (DE-cadherin-AAA) or β-catenin–armadillo (DE-cadherin-Δβ) interactions. We have analyzed the ability of these proteins to substitute for endogenous DE-cadherin activity in multiple cadherin-dependent processes during *Drosophila* development and oogenesis; epithelial integrity, follicle cell sorting, oocyte positioning, as well as the dynamic adhesion required for border cell migration. As expected, DE-cadherin-Δβ did not substitute for DE-cadherin in these processes, although it retained some residual activity. Surprisingly, DE-cadherin-AAA was able to substitute for the wild-type protein in all contexts with no detectable perturbations. Thus, interaction with p120/δ-catenin does not appear to be required for DE-cadherin function in vivo.

Introduction

Classic cadherins are major mediators of homophilic cell–cell adhesion during animal development (Tepass, 1999). In many developmental processes, cadherin-dependent adhesive interactions between cells need to be regulated. The question of how such regulation is achieved is of considerable interest. Cell culture studies have shown that both clustering of cadherin molecules and linkage to the actin cytoskeleton dramatically affect the strength of adhesion. The link between the intracellular domain of cadherin and the actin cytoskeleton is provided by proteins of the catenin family; β-catenin binds to the COOH-terminal part of the cadherin intracellular domain and to α-catenin, which in turn links to the actin cytoskeleton by interacting with multiple proteins (Fig. 1 A). In addition, members of the p120/δ-catenin family of proteins can bind to the juxtamembrane domain of cadherin. In vitro studies have suggested that p120/δ-catenin proteins are regulators of cadherin-dependent adhesion, although there is some controversy on whether they are positive or negative regulators (Anastasiadis and Reynolds, 2000).

Drosophila is a useful model for studying the function and regulation of cadherin in the animal. Three classical cadherins have been identified in the *Drosophila* genome; *Drosophila* epithelial cadherin (DE-cadherin)* and two N-cadherins. DE-cadherin, encoded by the *shotgun* (*shg*) locus, is associated with junctional complexes in epithelia (Oda et al., 1994). Genetic analyses have revealed a role for DE-cadherin in different types of adhesion (Tepass, 1999). DE-cadherin is required for epithelial integrity; in particular, continued synthesis of DE-cadherin is required in morphogenetically active epithelia of the embryo (Tepass et al., 1996; Uemura et al., 1996). DE-cadherin also mediates differential cell affinities, including correct positioning of the oocyte in egg chambers (Godt and Tepass, 1998; González-Reyes and St. Johnston, 1998). Finally, DE-cadherin is absolutely required for the invasive migration of border cells during oogenesis (Niewiadomska et al., 1999). It appears to be the key mediator of dynamic adhesion of migrating border cells to their substratum, the germ line cells.

The intracellular domain of DE-cadherin is highly related to that of mammalian classical cadherins and is likely to mediate the same protein interactions (Tepass et al., 2001). The importance of interaction with β-catenin is supported by

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*Abbreviations used in this paper: *arm*, *armadillo*; DE-cadherin, *Drosophila* epithelial cadherin; RNAi, RNA-mediated interference; *shg*, *shotgun*.

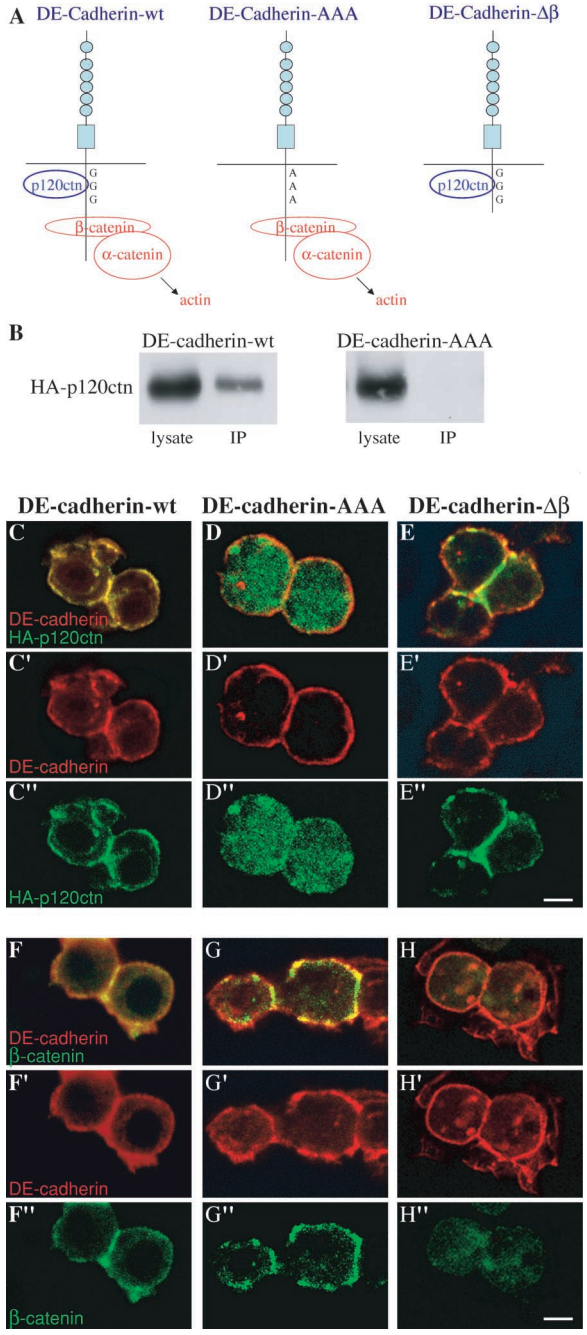


Figure 1. **Cortical recruitment of p120ctn and β-catenin by DE-cadherin variants in Schneider cells.** (A) Schematic representation of DE-cadherin variants and catenins associated. (B) Immunoprecipitation from Schneider cells transfected with pRm-HA-p120ctn and pRm-DE-cadherin-wt or pUAST-DE-cadherin-AAA+pRmGal4. 4% of lysates and all immunoprecipitates (IP) were loaded. (C–H) Schneider cells transfected with pRm-HA-p120ctn (C–E, green, and C′′–E′′) or pRm-β-catenin (F–H, green, and F′′–H′′), and pRm-DE-cadherin-wt (C and F), pRm-DE-cadherin-AAA (D and G), or pRm-DE-cadherin-Δβ (E and H, red, and C′–H′). HA-p120ctn was often enriched at the cortex between two cadherin overexpressing cells, suggesting that recruitment to the membrane is affected by cadherin interactions. Bars, ~5 μm.

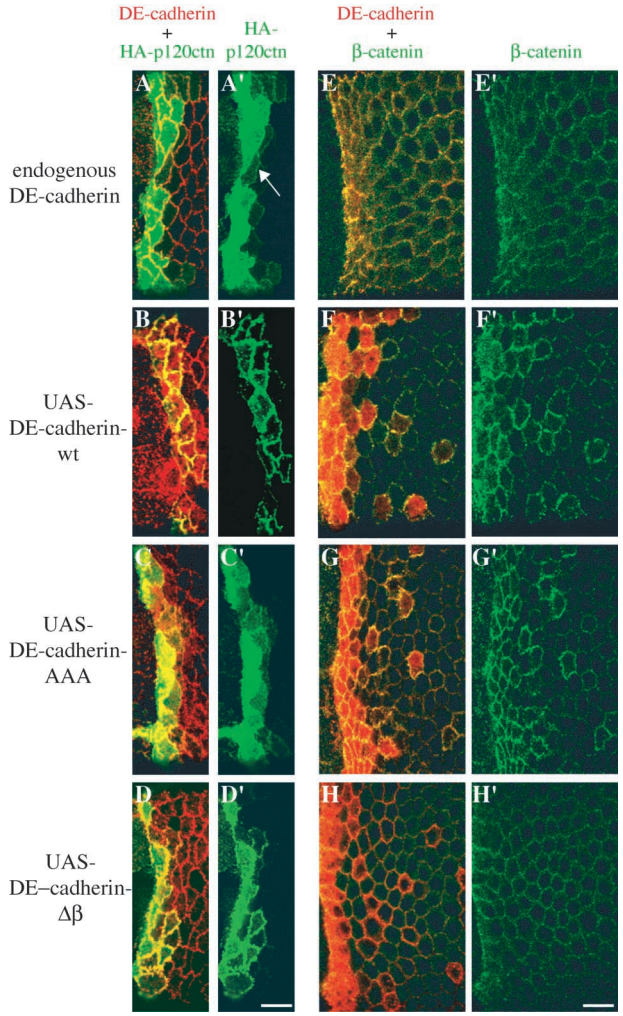


Figure 2. **Cortical recruitment of p120ctn and β-catenin by DE-cadherin variants in follicular cells.** Localization of overexpressed HA-p120ctn (A–D, green, and A′–D′) or endogenous β-catenin (E–H, green, and E′–H′) in follicular cells expressing only endogenous DE-cadherin (A and E), or overexpressing DE-cadherin-wt (B and F), DE-cadherin-AAA (C and G) or DE-cadherin-Δβ (D and H; red). Levels of endogenous DE-cadherin are too low to recruit all overexpressed p120ctn to the cortex, except in cells where overexpression is milder (arrow). (A–D) Basal sections of the follicular epithelium are shown. UAS-HA-p120ctn and UAS-DE-cadherin transgenes were overexpressed with slboGal4 (Rørth et al., 1998). Bars, ~20 μm.

genetic analysis of mutations in *armadillo* (*arm*), the single *Drosophila* β-catenin. In addition to the established effects on Wg/Wnt signaling, *arm* mutants display phenotypes consistent with a requirement in cadherin-dependent adhesion (Peifer et al., 1993). A single member of the p120/δ-catenin family (p120ctn) is encoded in the *Drosophila* genome, but no mutants are yet available. The conserved interactions with the intracellular domain of DE-cadherin have allowed us to investigate the requirements for catenin interactions in different cadherin-dependent processes in vivo.

Results and discussion

To specifically disrupt the interaction between DE-cadherin and p120ctn, we mutated a conserved triplet of glycine in the juxtamembrane region of DE-cadherin to alanine (Fig. 1 A; DE-cadherin-AAA). The corresponding mutation in human E-cadherin disrupts the interaction with p120 with-

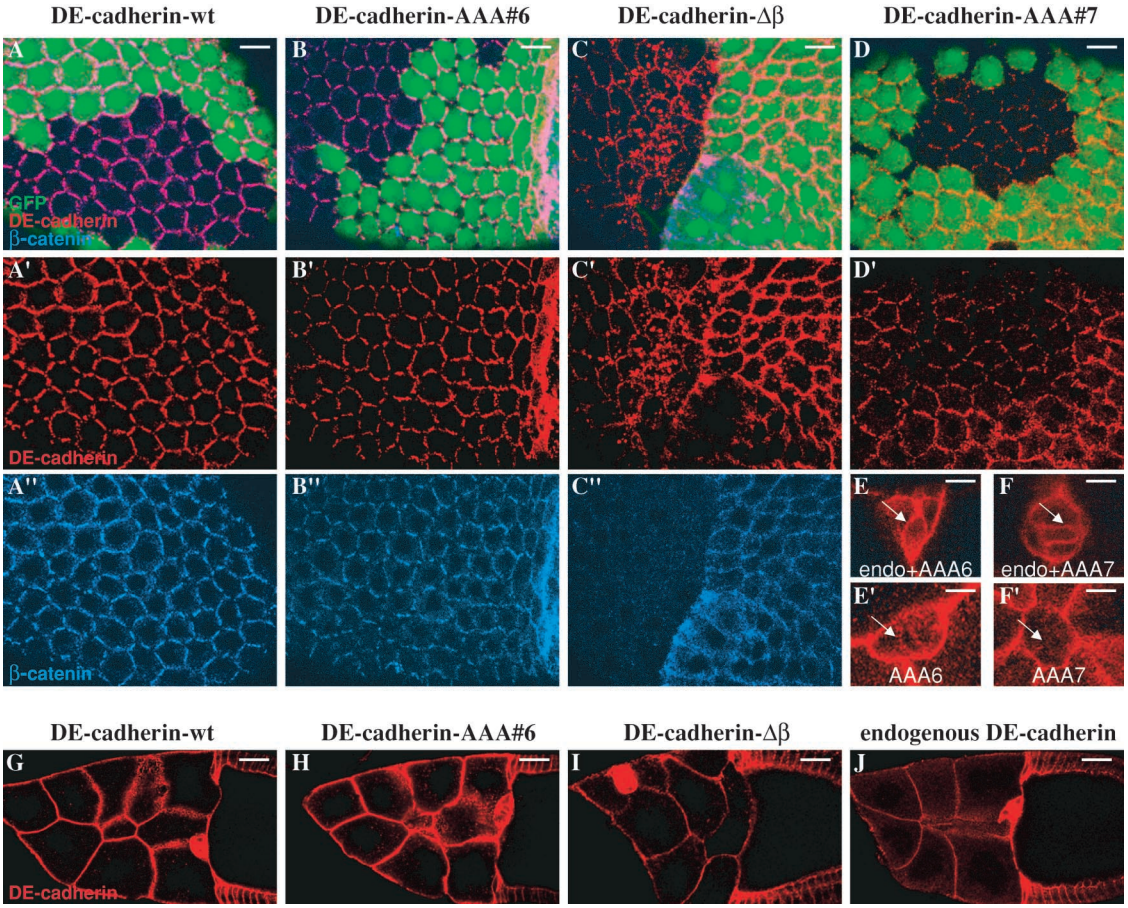


Figure 3. **Expression of tubulin-DE-cadherin transgenes in the ovary.** (A–D) Follicular epithelium from females carrying tubulin-DE-cadherin-wt (A), tubulin-DE-cadherin-AAA-#6 (B), tubulin-DE-cadherin-Δβ (C), or tubulin-DE-cadherin-AAA-#7 (D). Absence of GFP (green) marks *shg* mutant cells. Cadherin is in red and endogenous β-catenin in blue. A''–C'' show that DE-cadherin-wt and DE-cadherin-AAA recruit endogenous β-catenin even in the absence of endogenous DE-cadherin. Bars, ~10 μm. (E–F) Expression levels of DE-cadherin-AAA-#6 (E and E′) and #7 (F and F′) in border cells were estimated by comparison of DE-cadherin levels at the inner membranes of border cells (arrow) in *shg* mutant cells (E′ and F′) and non mutant cells (E and F). Bars, ~10 μm. (G–J) DE-cadherin in nurse cells expressing only the indicated tubulin-DE-cadherin transgene (G–I, germ line cells are *shg* mutant clones) or only endogenous DE-cadherin (J). Bars, ~20 μm.

out affecting the interaction with β-catenin (Thoreson et al., 2000). We also made a version of DE-cadherin that should be unable to interact with β-catenin by deleting the COOH-terminal β-catenin interaction domain (Fig. 1 A; DE-cadherin-Δβ). The mutant DE-cadherin molecules were tested by transfection in Schneider cells (Fig. 1) as well as by overexpression with the UAS-Gal4 system in the follicular epithelium of egg chambers (Fig. 2).

Wild-type (DE-cadherin-wt) as well as mutant cadherin proteins were enriched at the cell cortex, presumably the plasma membrane, in Schneider cells (Fig. 1, C–H) as well as in follicular cells (Fig. 2, B–D). As a measure of the ability of the different forms of DE-cadherin to bind β-catenin and p120ctn, we looked at their ability to recruit p120ctn and β-catenin to the cortex. Schneider cells were cotransfected with tagged versions of p120ctn and β-catenin. Without coexpressed cadherin, HA-p120ctn and β-catenin were cytoplasmic (unpublished data); β-catenin was present only at a low level in the cytoplasm due to its instability. Coexpression of wild-type DE-cadherin resulted in recruitment of HA-p120ctn (Fig. 1 C) and in recruitment and/or stabilization of β-catenin at the cortex (Fig. 1 F). In follicular cells, we looked at overexpressed HA-p120ctn and endogenous β-catenin. A small amount of HA-p120ctn (Fig. 2 A, arrow) and endogenous β-catenin was present at the cell cortex (Fig. 2 E), presumably due to association with endogenous DE-cadherin. Overexpression of DE-cadherin-wt resulted in efficient recruitment of HA-p120ctn (Fig. 2 B) and of more β-catenin (Fig. 2 F). In both Schneider cells and follicular cells, DE-cadherin-AAA recruited β-catenin (Fig. 1 G and Fig. 2 G), but not HA-p120ctn (Fig. 1 D and Fig. 2 C), whereas DE-cadherin-Δβ recruited HA-p120ctn (Fig. 1 E and Fig. 2 D), but not β-catenin (Fig. 1 H and Fig. 2 H). Immunoprecipitation from Schneider cell extracts confirmed an interaction between HA-p120ctn and DE-cadherin-wt, but not DE-cadherin-AAA (Fig. 1 B). Thus, DE-cadherin-AAA and DE-cadherin-Δβ appear to be good tools to selectively affect DE-cadherin interaction with p120ctn or with β-catenin.

Cell aggregation is commonly used to measure cadherin-dependent adhesion and importance of cytoplasmic interactions in mammalian cells (Thoreson et al., 2000). As a first functional analysis of the DE-cadherin mutants, we thus compared the ability of DE-cadherin-wt, DE-cadherin-

AAA, and DE-cadherin-Δβ to induce aggregation of Schneider cells. A form of DE-cadherin lacking the full cytoplasmic tail (DE-cadherin-Δcyt) was also tested. All four constructs mediated DE-cadherin–dependent aggregation, and the degree of aggregation was indistinguishable. Thus, DE-cadherin–mediated aggregation of Schneider cells is independent of its cytoplasmic tail. This does not appear to be due to lack of p120ctn; Schneider cells express some p120ctn as detected by Northern blot. Also, co-overexpression of HA-p120ctn or β-catenin had no effect. We attempted to perform aggregation assays in CHO cells, but did not obtain proper expression of DE-cadherin in this heterologous system.

To analyze the biological activity of the mutant DE-cadherin proteins during development, the following strategy was used to replace endogenous DE-cadherin with mutant forms: We generated transgenic flies that express DE-cadherin-wt, DE-cadherin-AAA, or DE-cadherin-Δβ ubiquitously, under the control of the tubulin-α1 promoter. Endogenous DE-cadherin was removed from specific cells by generating homozygous *shg* mutant clones or zygotic expression was removed by analyzing *shg* homozygous mutant embryos. Both *shg* alleles used in this study are strong alleles, one (shg^{R69}) is a molecular null allele. Next, we determined whether the transgene-encoded DE-cadherin could substitute for endogenous DE-cadherin, that is, rescue the *shg* mutant phenotype. Membrane localization and expression levels were checked by immunostaining of egg chambers containing patches of *shg* mutant cells. These cells expressed only the transgene-encoded DE-cadherin, allowing comparison with endogenous levels (Fig. 3). In follicle cells, DE-cadherin-wt transgenes and one of the DE-cadherin-AAA transgenes (#6) were expressed at somewhat higher levels than endogenous DE-cadherin (Fig. 3, A and B). Another DE-cadherin-AAA transgene (#7) and DE-cadherin-Δβ transgenes gave similar levels of expression, slightly lower than endogenous DE-cadherin (Fig. 3, C and D). In nurse cells, DE-cadherin-Δβ transgenes gave expression levels similar to endogenous DE-cadherin, whereas DE-cadherin-wt and both DE-cadherin-AAA transgenes were expressed at much higher levels (Fig. 3, G–J)

We were particularly interested in how DE-cadherin might be regulated during border cell migration, which occurs during stage 9 of oogenesis. Border cells are a group of 6 to 10 somatic follicle cells that delaminate from the anterior tip of the egg chamber, invade the germ line cluster, and migrate to the oocyte. Border cells migrate in between the nurse cells and apparently use these cells as substratum. Absence of DE-cadherin from border cells or from nurse cells results in total lack of invasive migration, suggesting that border cells adhere to the nurse cell substratum through homophilic DE-cadherin interaction (Fig. 4, A and B; Niewiadomska et al., 1999). Adhesion to a substratum must in some way be dynamic for cells to move productively across it. Relative to the well-characterized function of cadherin in epithelial cell adhesion, DE-cadherin function in border cell migration might therefore require additional regulatory mechanisms. In addition, border cells normally express higher levels of DE-cadherin than other follicle cells, and migration is quite sensitive to reduction of DE-cadherin level; even weak *shg* alleles cause detectable delays in migration (Niewiadomska et al., 1999). For these reasons, it seemed likely that border cell migration would be sensitive to perturbations of cadherin–catenin interactions. To test this, we analyzed the activity of mutant DE-cadherin proteins in border cells as well as in the substratum, the nurse cells. In both border cells and nurse cells, expression of DE-cadherin-wt as well as DE-cadherin-AAA-#6 completely rescued the migration (Fig. 4, A′′ and B′′). Thus, DE-cadherin–p120ctn interaction does not appear to be required for the migration. DE-cadherin-AAA-#7 gave full rescue in nurse cells (Fig. 4 B′′), but incomplete rescue in border cells (Fig. 4 A′′). In border cells, DE-cadherin-AAA-#6 was expressed at levels similar to endogenous DE-cadherin, whereas DE-cadherin-AAA-#7 was expressed at lower levels (Fig. 3, E and F). Given that border cell migration is sensitive to cadherin expression level, this can explain the incomplete rescue by cadherin-AAA-#7. DE-cadherin-Δβ expression in border cells did not rescue the *shg* phenotype at all, suggesting that DE-cadherin must be anchored to the actin cytoskeleton via β-catenin to function in the migrating cells. DE-cadherin-Δβ could partially compensate for endogenous DE-cadherin in nurse cells (Fig. 4 B′′). Strong linkage to the cytoskeleton via β-catenin may be less critical for cadherin to function as substratum than in the actively migrating cell itself.

Next, we addressed whether the DE-cadherin–p120ctn interaction was required for other types of adhesion. Early in oogenesis, DE-cadherin–mediated adhesion is necessary to correctly position the oocyte at the posterior pole of the egg chamber (Fig. 4, C and D; Godt and Tepass, 1998; González-Reyes and St. Johnston, 1998). If either the germ line or the somatic cells are mutant for *shg*, the oocyte is often mispositioned. Both DE-cadherin-wt and DE-cadherin-AAA-#6 rescued the *shg* defects in both germ line and follicular cell clones (Fig. 4, C′′ and D′′). Thus, adhesion between the oocyte and the follicular cells does not require the DE-cadherin–p120ctn interaction. DE-cadherin-AAA-#7 failed to rescue the lack of endogenous DE-cadherin in follicular cells, suggesting that levels of expression are important. DE-cadherin-Δβ was completely unable to rescue the mispositioning phenotype (Fig. 4, C′′ and D′′).

Within the follicular epithelium, *shg* mutant cells sort away from wild-type cells generating a smooth boundary at the interface (Fig. 4 E; González-Reyes and St. Johnston, 1998). This is thought to reflect preferential adhesion between DE-cadherin expressing cells. If DE-cadherin–p120ctn interaction alters adhesion, cells expressing only DE-cadherin-AAA might sort away from cells also expressing wild-type DE-cadherin. However, this was not observed (Fig. 4 E′′). In contrast, cells expressing only DE-cadherin-Δβ did sort from cells also expressing wild-type DE-cadherin. Thus, the lack of interaction between DE-cadherin and β-catenin resulted in a decrease of adhesion strong enough to obtain sorting, whereas the lack of interaction between DE-cadherin and p120ctn did not. At early to mid-oogenesis, *shg* mutant follicle cells maintained correct cell shape within the epithelium. This may reflect functional overlap between DE-cadherin and N-cadherin at these stages, as *arm* mutant clones have stronger defects (Tanentzapf et al., 2000). Late in oogenesis, *shg* mutant follicle cells

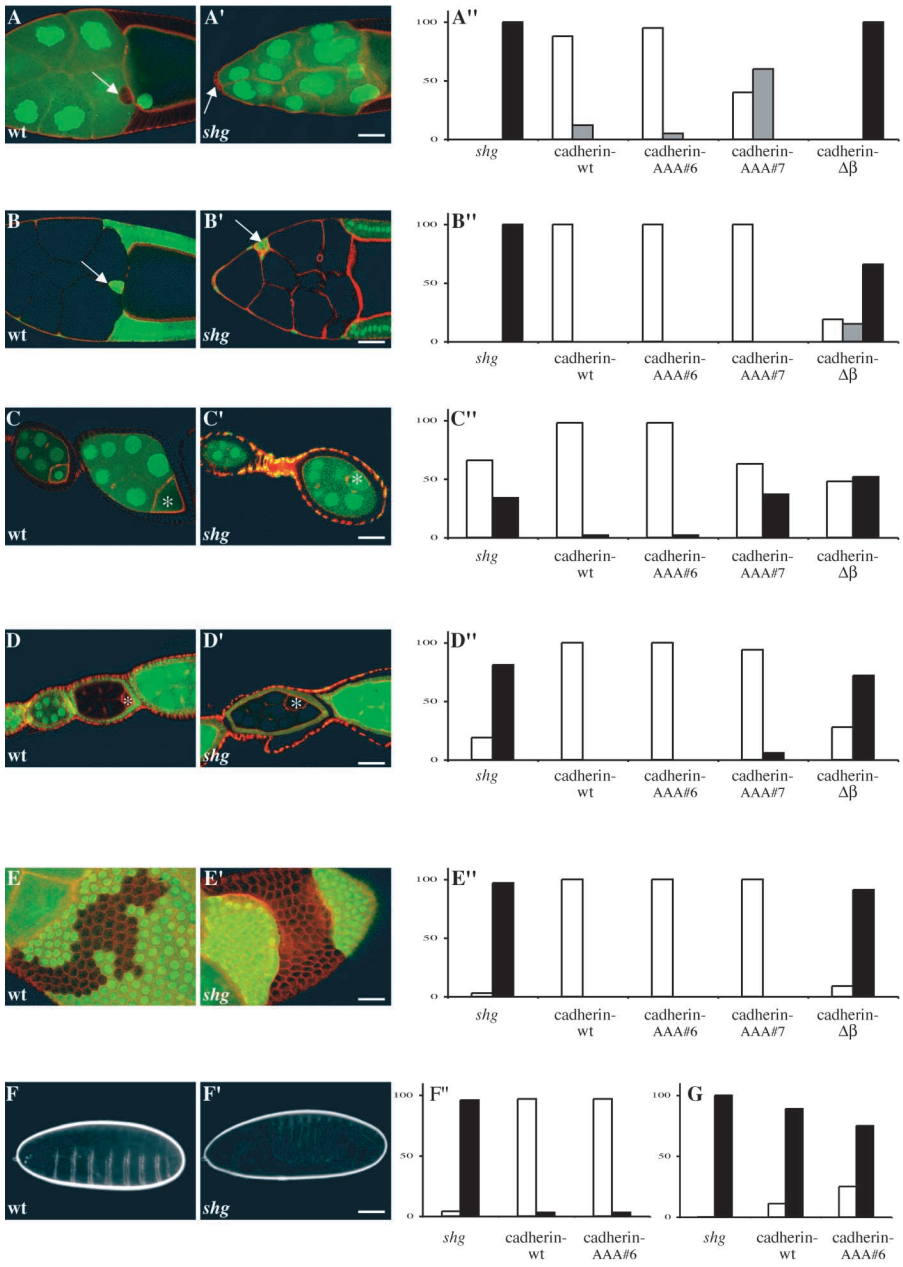
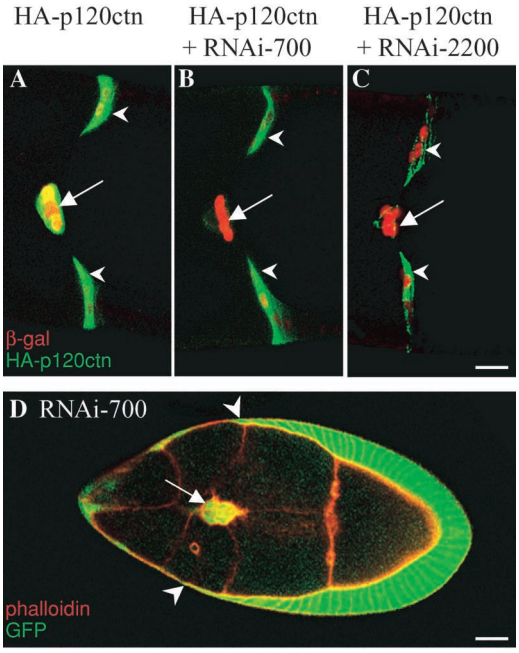


Figure 4. **DE-cadherin-wt and DE-cadherin-AAA but not DE-cadherin-Δβ transgenes can rescue *shg* phenotypes.** (A–E) Phalloidin (red) stains the actin cytoskeleton of egg chambers and non-GFP (non-green) cells are wild type (A–E) or *shg* mutant (A′–E′) clones. (A and B) Border cell migration. In stage 10 wild type egg chambers, border cells (arrow) have reached the oocyte (A and B). Lack of DE-cadherin in border cells (A′) or in nurse cells (B′) prevent border cells from penetrating between the nurse cells. (A″ and B″) Migration defects in *shg* mutant border cells (A″) and germ line (B″) clones and in the presence of the indicated transgenes ($15 \leq n \leq 73$). White, full migration; gray, incomplete migration; black, no migration. Bars, ~20 μm. (C and D) Oocyte mispositioning. The oocyte (asterisk) is located at the posterior of a wild type egg chamber (C and D). *shg* mutant clones often causes a mislocalization of the oocyte (C′ and D′, follicular cells and germ line clones, respectively). All ovarioles are represented anterior (younger egg chambers) to the left. (C″ and D″) Oocyte mispositioning in *shg* mutant follicular cells (C″) and germ line (D″) clones and in the presence of the indicated transgenes ($17 \leq n \leq 57$). White, oocyte posteriorly located; black, mispositioned oocyte. Bars, ~15 μm. (E) Follicular cell sorting. Wild type follicular clones intermingle (E), whereas *shg* mutant clones sort (E′). (E″) Sorting in *shg* mutant clones and in the presence of the indicated transgenes ($27 \leq n \leq 68$). White, no sorting; black, sorting. Bar, ~20 μm. (F) Cuticle phenotype. F is a wild-type cuticle and F′ a cuticle of a homozygous *shg* mutant embryo. (F″) Cuticle defects in homozygous *shg* mutant embryos and in the presence of the indicated transgenes ($32 \leq n \leq 232$). White, wild type cuticle; black, cuticle with *shg* phenotype. Bar, ~50 μm. (G) Hatching of homozygous *shg* mutant embryos and in the presence of the indicated transgenes ($n \geq 278$). White, hatching; black, no hatching.

showed cell shape defects in the epithelium. This defect was also rescued by DE-cadherin-AAA, but not DE-cadherin-Δβ (unpublished data).

To analyze additional types of DE-cadherin mediated adhesion in other tissues, we turned to the embryo. DE-cadherin is maternally supplied, but zygotic expression is required for embryonic development, for example, in the embryonic epidermis. Homozygous shg^{1} mutant embryos lack the head and ventral epidermis, which results in the lack of head and ventral cuticle (Fig. 4 F). Both DE-cadherin-wt and DE-cadherin-AAA transgenes fully rescued this phenotype (Fig. 4 F″). We also tested rescue of the *shg* zygotic lethality. Despite the use of a heterologous promoter to express DE-cadherin, 10–25% of shg^{1}/shg^{1} embryos carrying a DE-cadherin-wt or DE-cadherin-AAA-#6 transgene hatched, and the resulting larvae looked normal (Fig. 4 G). A few percent of shg^{R69}/shg^{R69} mutants were even rescued to adulthood. The rescued females allowed us to test the ability of DE-cadherin-AAA to substitute for maternally provided DE-cadherin. Females expressing only DE-cadherin-AAA-#6 gave rise to viable progeny also carrying only DE-cadherin-AAA. Thus, DE-cadherin-AAA rescued both maternal and zygotic DE-cadherin functions. p120ctn protein is present in the embryo (Magie et al., 2002) and is expressed in the ovary, in a pattern identical to that of DE-cadherin (Magie, C., and S. Parkhurst, personal communication). However, our results



E	normal border cell migration	oocyte correctly positioned	follicular cell clones non sorted	hatching rate (zyg)	hatching rate (mat+zyg)
UAS-RNAi-700	100% (n>240)	100% (n>200)	100% (n>120)	91% (n>2500)	86% (n>2000)
UAS-RNAi-2200	100% (n>120)	100% (n>130)	100% (n>70)	74% (n>2500)	98% (n>2500)
wildtype	nd	nd	nd	84% (n>1200)	90% (n>2500)

Figure 5. **Effect of p120ctn-RNAi transgenes.** (A–C) Levels of HA-p120ctn in border cells (arrow) and centripetal cells (arrowheads) overexpressing HA-p120ctn (A) and different RNAi constructs (B and C); stage 10b egg chambers. All constructs are expressed using slboGal4, which starts expressing in border cells at stage 9 and in centripetal cells at stage 10. HA-p120ctn levels are severely reduced in border cells, estimated to have been expressing RNAi constructs for 12–24 h. Bar, ~10 μm. (D) Stage 9 egg chamber from a female of the genotype *hsFLP/actin-Flipout-Gal4; UAS-RNAi/+; UAS-GFP/+*. Larval heat shock was used to initiate constitutive expression of the RNAi in somatic cells (marked by GFP) one week before analysis. Border cell migration is normal. Bar, ~15 μm. (E) Quantification of phenotypes after expression of p120ctn-RNAi transgenes in the ovary (as in D) and in embryos (maternal [mat] and zygotic [zyg] expression).

indicate that the DE-cadherin–p120ctn interaction is not essential for any critical process during development.

In an attempt to independently compromise p120ctn function, we used double-stranded RNA-mediated interference (RNAi). Two p120ctn-RNAi transgenes (RNAi-700 and RNAi-2200) were able to severely reduce the levels of co-overexpressed HA-p120ctn (Fig. 5, A–C), suggesting that levels of endogenous p120ctn would also be strongly reduced. Expression of p120-RNAi in somatic cells did not cause any detectable phenotype in the ovary (Fig. 5, D and E). This is consistent with p120ctn not being important for DE-cadherin function during oogenesis. However, the lack of phenotype may also reflect incomplete removal of p120ctn. Maternal and zygotic expression of p120ctn-RNAi transgenes did not affect embryonic viability (Fig. 5 E). Severe defects have been observed on injection of p120ctn double-stranded RNA in embryos (Magie et al., 2002). Resolving the discrepancy between these results awaits analysis of a p120ctn mutant.

The general inability of DE-cadherin-Δβ to replace endogenous DE-cadherin probably reflects that β-catenin is an important, often essential, interaction partner for cadherin. This supports the view that linkage to the actin cytoskeleton through β-catenin and α-catenin is critical for cadherin function. We cannot rule out that other proteins bind to the deleted COOH terminus of DE-cadherin, but the requirement for β-catenin interaction is supported by the cellular defects in *arm* (β-catenin) mutants (Peifer et al., 1993).

In contrast to DE-cadherin-Δβ, DE-cadherin-AAA was able to replace wild-type DE-cadherin in all assays. To investigate different modes of cadherin function, different types of DE-cadherin function were tested; stable interactions within an epithelium and between cell layers, as well as dynamic adhesion required for migration. Given that the AAA mutation disrupts or strongly attenuates the interaction with p120ctn, our results indicate that interaction with p120ctn is not required for DE-cadherin function. It remains possible that the interaction has a subtle modulatory effect on DE-cadherin function or a role in a nonessential process that we did not test directly. In chick embryos, experiments based on N-cadherin overexpression suggested a regulatory role of the juxtamembrane domain, but not the p120 binding site (Horikawa and Takeichi, 2001). Thus, a limited role of the p120ctn–cadherin interaction may not be restricted to *Drosophila* E-cadherin.

Materials and methods

Cloning

For DE-cadherin-AAA, three glycines (aa 1376–1378) were mutated to alanines by replacing GGTGGCGGC with GCGGCCGCC (NotI site). For DE-cadherin-Δβ and DE-cadherin-Δcyt, DE-cadherin cDNA was truncated at bp 4954 and 4746. DE-cadherin-AAA and -Δβ were subcloned in pUAST (Asp718/XbaI; Brand and Perrimon, 1993). DE-cadherin-wt, -AAA, and -Δβ were subcloned in pCaSpeR-tubulin (EcoRI/KpnI fragment of tubulin-α1 promoter in pCaSpeR-4; Asp718/XbaI). DE-cadherin-wt and -AAA were cloned into pRmHa3 (Asp718/BamHI; Bunch et al., 1988) and DE-cadherin-Δβ and -Δcyt into pRmHa3b (EcoRI/NotI). DE-cadherin-wt, -AAA, -Δβ, and -Δcyt were cloned into pcDNA-3 (Asp718/XbaI). p120ctn cDNA (obtained from EST LD02621) was cloned in frame with the HA tag of pRmHa-Nle-Ha (Royet et al., 1998), in place of the Nle insert (AscI/SalI). HA-p120ctn (BamHI/SalI) was then subcloned into pUAST. β-Catenin cDNA was cloned from pMK33-myc (Yanagawa et al., 1997) to pRmHa3b (BamHI). For p120ctn-RNAi, 1000 bp inverted repeats from the cDNA (bp 700 to1700 for RNAi-700 or bp 1200–2200 for RNAi-2200) were inserted with 500 bp spacers into pUAST.

Cell culture

Schneider S2 and CHO cells were transfected with CellFECTIN® (Invitrogen), with pRm and pcDNA3 constructs, respectively. pRm plasmids were induced with 0.7 mM $CuSO_4$ for 24 h. Immunoprecipitations were done in 1% NP-40 lysis buffer with DCAD2 antibody (Oda et al., 1994). Western blot was done with mouse anti-HA antibody (1:10,000; BAbCo). For aggregation assays, S2 and CHO cells were cotransfected with pRm-eGFP and pcDNA3-eGFP, respectively. S2 cells were transferred in Petri dishes and shaken at 50 rpm for 2 h before quantification. Percentage of GFP cells in aggregates and average size of aggregates were quantified in three independent experiments. There were some variations from experiment to experiment, but in each experiment, all four DE-cadherin constructs induced similar aggregation.

Flies

Tubulin-DE-cadherin-wt and AAA stocks were verified by PCR on genomic DNA followed by a NotI digest. For clonal analysis, flies of the genotype *hsFLP/+; FRTG13shg*^{1}/FRTG13GFP; tubulin-cadherin/+ were heat shocked as larvae or adults, at least 4 d before analysis.

For cuticles analysis and quantification of hatching, *shg*^{1}/*shg*^{1}; tubulin-cadherin/+ embryos (18–24 h) were obtained by sorting non-GFP embryos from *shg*^{1}/CyO,krGFP; tubulin-cadherin stocks (COPAS SELECT embryo sorter; Union Biometrica, Inc.). Rescue of lethality was tested using *shg*^{R69}/*CyO,krGFP;* tubulin-cadherin stocks. To test maternal rescue, *shg*^{R69}/*shg*^{R69};*tub-cad-AAA-#6/+* females were crossed to *shg*^{R69}/CyO; *tubulin-cad-AAA-#6/+* males. For embryo zygotic RNAi, *tubGal4/TM3Ser* females were crossed to *UAS-p120ctn-RNAi/UAS-p120ctn-RNAi* males. For maternal and zygotic RNAi, UAS-p120ctn-RNAi/+; nosGal4 females were crossed to *tubGal4/TM3Ser*.

Immunostainings

Cells were fixed with 3% PFA and ovaries with 4% PFA. Primary antibodies were rat DCAD2 (1:100), mouse anti-HA (1:1,000), mouse anti–armadillo N2–7A1 (1:10; Peifer, 1993). Fluorescent secondary antibodies (Jackson ImmunoResearch Laboratories) were used 1:200 and rhodamine-phalloidin (Molecular Probes, Inc.) 1:500. All images were captured using confocal microscopy (Leica).

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Note added in proof. The article in this issue by Myster et al. (Myster, S.H., R. Cavallo, C.T. Anderson, D.T. Fox, and M. Peifer. 2003. *J. Cell Biol.* 160: 443–449) describes the isolation and analysis of a p120ctn lack-of-function mutant in *Drosophila*. Their finding that p120ctn is not essential for cadherin-mediated adhesion during development is in agreement with our analysis.

References

Anastasiadis, P.Z., and A.B. Reynolds. 2000. The p120 catenin family: complex roles in adhesion, signaling and cancer. *J. Cell Sci.* 113:1319–1334.

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

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

Godt, D., and U. Tepass. 1998. *Drosophila* oocyte localization is mediated by differential cadherin-based adhesion. *Nature.* 395:387–391.

González-Reyes, A., and D. St. Johnston. 1998. The *Drosophila* AP axis is polarised by the cadherin-mediated positioning of the oocyte. *Development.* 125: 3635–3644.

Horikawa, K., and M. Takeichi. 2001. Requirement of the juxtamembrane domain of the cadherin cytoplasmic tail for morphogenetic cell rearrangement during myotome development. *J. Cell Biol.* 155:1297–1306.

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

Niewiadomska, P., D. Godt, and U. Tepass. 1999. DE-Cadherin is required for intercellular motility during *Drosophila* oogenesis. *J. Cell Biol.* 144:533–547.

Oda, H., T. Uemura, Y. Harada, Y. Iwai, and M. Takeichi. 1994. A *Drosophila* homolog of cadherin associated with armadillo and essential for embryonic cell-cell adhesion. *Dev. Biol.* 165:716–726.

Peifer, M. 1993. The product of the *Drosophila* segment polarity gene armadillo is part of a multi-protein complex resembling the vertebrate adherens junction. *J. Cell Sci.* 105:993–1000.

Peifer, M., S. Orsulic, D. Sweeton, and E. Wieschaus. 1993. A role for the *Drosophila* segment polarity gene armadillo in cell adhesion and cytoskeletal integrity during oogenesis. *Development.* 118:1191–1207.

Rørth, P., K. Szabo, A. Bailey, T. Laverty, J. Rehm, G.M. Rubin, K. Weigmann, M. Milán, V. Benes, W. Ansorge, and S.M. Cohen. 1998. Systematic gain-of-function genetics in *Drosophila. Development.* 125:1049–1057.

Royet, J., T. Bouwmeester, and S.M. Cohen. 1998. Notchless encodes a novel WD40-repeat-containing protein that modulates Notch signaling activity. *EMBO J.* 17:7351–7360.

Tanentzapf, G., C. Smith, J. McGlade, and U. Tepass. 2000. Apical, lateral, and basal polarization cues contribute to the development of the follicular epithelium during *Drosophila* oogenesis. *J. Cell Biol.* 151:891–904.

Tepass, U. 1999. Genetic analysis of cadherin function in animal morphogenesis. *Curr. Opin. Cell Biol.* 11:540–548.

Tepass, U., E. Gruszynski-DeFeo, T.A. Haag, L. Omatyar, T. Torok, and V. Hartenstein. 1996. shotgun encodes *Drosophila* E-cadherin and is preferentially required during cell rearrangement in the neurectoderm and other morphogenetically active epithelia. *Genes Dev.* 10:672–685.

Tepass, U., G. Tanentzapf, R. Ward, and R. Fehon. 2001. Epithelial cell polarity and cell junctions in *Drosophila. Annu. Rev. Genet.* 35:747–784.

Thoreson, M.A., P.Z. Anastasiadis, J.M. Daniel, R.C. Ireton, M.J. Wheelock, K.R. Johnson, D.K. Hummingbird, and A.B. Reynolds. 2000. Selective uncoupling of p120(ctn) from E-cadherin disrupts strong adhesion. *J. Cell Biol.* 148:189–202.

Uemura, T., H. Oda, R. Kraut, S. Hayashi, Y. Kotaoka, and M. Takeichi. 1996. Zygotic *Drosophila* E-cadherin expression is required for processes of dynamic epithelial cell rearrangement in the *Drosophila* embryo. *Genes Dev.* 10: 659–671.

Yanagawa, S., J.S. Lee, T. Haruna, H. Oda, T. Uemura, M. Takeichi, and A. Ishimoto. 1997. Accumulation of Armadillo induced by Wingless, Dishevelled, and dominant-negative Zeste-White 3 leads to elevated DE-cadherin in *Drosophila* clone 8 wing disc cells. *J. Biol. Chem.* 272:25243–25251.