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Drosophila p120catenin plays a supporting role in cell adhesion but is not an essential adherens junction component

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Cadherin–catenin complexes, localized to adherens junctions, are essential for cell–cell adhesion. One means of regulating adhesion is through the juxtamembrane domain of the cadherin cytoplasmic tail. This region is the binding site for p120, leading to the hypothesis that p120 is a key regulator of cell adhesion. p120 has also been suggested to regulate the GTPase Rho and to regulate transcription via its binding partner Kaiso. To test these hypothesized functions, we turned to *Drosophila,* which has only a single p120 family member. It localizes to adherens junctions and binds the juxtamembrane region of DE-cadherin (DE-cad). We generated null alleles of *p120* and found that mutants are viable and fertile and have no substantial changes in junction structure or function. However, *p120* mutations strongly enhance mutations in the genes encoding DE-cadherin or Armadillo, the β-catenin homologue. Finally, we examined the localization of p120 during embryogenesis. p120 localizes to adherens junctions, but its localization there is less universal than that of core adherens junction proteins. Together, these data suggest that p120 is an important positive modulator of adhesion but that it is not an essential core component of adherens junctions.

Introduction

The ability of cells to assemble into tissues, organs, and animals depends on cell–cell adhesion (for reviews see Yap et al., 1997; Tepass et al., 2001). The central mediators of cell adhesion are proteins of the cadherin–catenin complex, which localize to adherens junctions (AJs),* adhesive junctions near the apical end of the lateral cell interface of epithelial cells. Transmembrane cadherins mediate homophilic adhesion, whereas catenins anchor cadherins to actin at adhesion sites. β-catenin (β-cat) and its *Drosophila* ortholog Armadillo (Arm) bind directly to both the distal region of the cadherin cytoplasmic tail and to α-catenin (α-cat). α-Cat interacts with actin both directly and indirectly. Cadherins, β-cat, and α-cat play essential roles in adhesion-genetic experiments in animals and in cell culture reveal that adhesion is abolished in their absence. Consistent with this, the cadherin tail is important for strong cell–cell adhesion, at least in some cells.

However, cadherin–catenin adhesion is not simply glue (for reviews see Yap et al., 1997; Tepass et al., 2001). It must be modulated during development, tissue remodeling, and wound repair. A series of experiments suggest that regulation of adhesion occurs, at least in part, through a region of the cadherin cytoplasmic tail distinct from the β-cat–binding site. This region, referred to as the juxtamembrane (JM) region, is highly conserved in all classic cadherins (for review see Anastasiadis and Reynolds, 2000). Although many studies suggest that the JM domain regulates adhesion, the nature of its role differs depending on the cells or assays employed. Ozawa and Kemler (1998) transfected cells lacking endogenous cadherins with wild-type or mutant cadherins. Cadherins completely lacking a cytoplasmic tail conferred adhesion, though the adhesion was not as robust as that conferred by wild-type cadherin. In contrast, a cadherin carrying the JM domain but not the β-cat–binding domain lacked adhesive activity entirely. This suggested that the JM domain inhibits adhesion, and the authors provided data that it might do so by regulating cadherin-dimer formation. Yap et al. (1998) came to a quite different conclusion using a different assay. Cells expressing wild-type cadherins or mutant cadherins carrying the JM region but not the β-cat–binding region

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*Abbreviations used in this paper: AJ, adherens junction; Arm, Armadillo; BDGP, Berkeley *Drosophila* Genome Project; α-cat, α-catenin; β-cat, β-catenin; CNS, central nervous system; DE-cad, DE-cadherin, *shg*, *shotgun*; IP, immunoprecipitate; JM, juxtamembrane; RNAi, RNA interference; Wg, Wingless.

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adhered strongly to cadherin-coated substrates, and this promoted lateral clustering of cadherins, whereas a cadherin with the β-cat–binding site but not the JM domain failed to promote either strong adhesion or lateral clustering. These data contrast with those of Ozawa and Kemler (1998), suggesting that the JM domain promotes adhesion, perhaps by regulating cadherin clustering. In addition to these seemingly opposing effects on cell adhesion, other studies suggested that the JM domain influences cadherin-mediated cell migration and axon outgrowth (for review see Anastasiadis and Reynolds, 2000).

The JM region may modulate adhesion by serving as the binding site for regulatory proteins. One candidate, p120ctn (henceforth p120), was identified by scientists studying the effects on cell adhesion of the oncogenic kinase Src (for review see Anastasiadis and Reynolds, 2000). Src activation leads to profound changes in cell–cell adhesion and morphology, perhaps by altering AJs. p120 is phosphorylated by Src and was subsequently found to be part of the cadherin–catenin complex where it binds to the JM region of cadherins. These and other data led to a model that suggested that p120 was a target by which signal transduction pathways regulate AJs.

Given this, the seemingly contradictory effects of the JM region on adhesion might be explained if this region receives regulatory inputs that vary depending on cell type, possibly via posttranslational modification of p120. This possibility was supported by experiments using kinase inhibitors and other reagents to perturb signal transduction and potentially p120 phosphorylation: these modify the effect of the JM domain on adhesion (Aono et al., 1999; Ohkubo and Ozawa, 1999). A role for p120 in adhesion regulation was further supported by Thoreson et al. (2000), who generated point mutations in the p120 binding site on the cadherin tail and found that abolishing p120 binding reduces (although it does not eliminate) cell adhesion. Other data suggest that p120 may also have cadherin-independent roles in regulating Rho and in transcriptional regulation (for review see Anastasiadis and Reynolds, 2000; Magie et al. 2002). p120-independent functions for the JM region are also possible. The JM region binds presenilins, components of the γ-secretase, an unusual protease that cleaves various transmembrane proteins in or near the membrane (for review see Kopan and Goate, 2000) (presenilins also bind β-cat and p120 family members). Presenilins and p120 compete for cadherin binding (Baki et al., 2001).

One means to assess the roles of p120 or other potential adhesion regulators is by loss-of-function genetic analysis. However, mammals have four p120 subfamily members: p120 itself, ARVCF (Armadillo repeat gene deleted in Velo-Cardio-Facial Syndrome), p0071, and δ-catenin (for review see Anastasiadis and Reynolds, 2000). All share sequence similarity in the Arm repeats, NH_2, and COOH-terminal domains, and all bind classic cadherins. Mammals also have three more distant p120 relatives, plakophilins 1, 2, and 3, that are components of desmosomes, a different cell–cell adhesive junction (for review see Green and Gaudry, 2000). Finally, there are multiple splice forms of p120. This complexity makes genetic analysis in mammals challenging, with full loss of function likely requiring the generation of double, triple, or even quadruple mutants. Thus far, no mutations in p120 subfamily proteins have been reported. Mutations in human desmosomal *plakophilin1* are found in patients with ectodermal dysplasia, a fragile skin disorder (for review see Green and Gaudry, 2000). In contrast, fruit flies have only one p120 family member, simplifying genetic analysis. We examined *Drosophila* p120 localization and function. Our data demonstrate that p120 plays an important role in promoting cell adhesion, but it is not an essential AJ component.

Results

Identification of fly p120

We searched for p120 homologues in a fly cDNA library using degenerate PCR with primers to regions conserved in vertebrate p120s. One primer pair gave a product of the expected size (see Materials and methods) that encoded an ORF with similarity to human p120. This was used to probe a cDNA library. From 20 positive clones (all derived from a single gene) a full-length coding sequence was assembled (sequence data available from GenBank/EMBL/DDBJ under accession no. AF220496). This was subsequently confirmed by sequencing of two full-length cDNAs by the Berkeley *Drosophila* Genome Project (BDGP; information was obtained from http://www.fruitfly.org). A single conservative change (I357V) in the coding sequence was observed among the three sequences. We mapped the *p120* gene to band 41C near the heterochromatin of the right arm of chromosome 2 by hybridization to a genomic P1 blot and in situ hybridization to polytene chromosomes (unpublished data). This was subsequently confirmed by the BDGP/Celera sequencing project (http://www.fruitfly.org). *p120* includes four exons spanning ~14 kb (Fig. 1 A). BDGP's gene prediction programs predict slightly different splice junctions and include a fifth exon, but these predictions are not borne out by our or the BDGP cDNAs.

In contrast to mammals, there appears to be only a single p120 family member in *Drosophila*. Extensive searching of releases 2.5 and 3.0 of the genome and of the large collection of full-length and partial cDNAs failed to identify any additional genes or mRNAs similar to p120. Although it is formally possible that a p120 relative is present in the small unsequenced portion of the euchromatin or in the heterochromatin, we think this is unlikely for two reasons. First, whereas 61 EST clones from several developmental stages and tissues are derived from p120, no ESTs encoding proteins related to but distinct from this were found (unpublished data). Second, the recently completed *Anopheles gambiae* (mosquito) genome contains only a single p120 family member (sequence data available from GenBank/EMBL/DDBJ under accession no. EAA05214.1) (Fig. 1 B), as does the *Caenorhabditis elegans* genome (*jac-1*).

Drosophila p120 encodes a predicted 781 aa protein of 86.7 kD (Fig. 1 B). No alternate splicing was evident from BDGP EST clones or our RT-PCR experiments (see below). By structure and sequence, *Drosophila* p120 is a clear member of the p120 subfamily; they share 10 Arm repeats with conserved inserts within the repeat region (Fig. 1, B and C). In fly p120, the Arm repeats are flanked by 210 aa NH_2-ter-

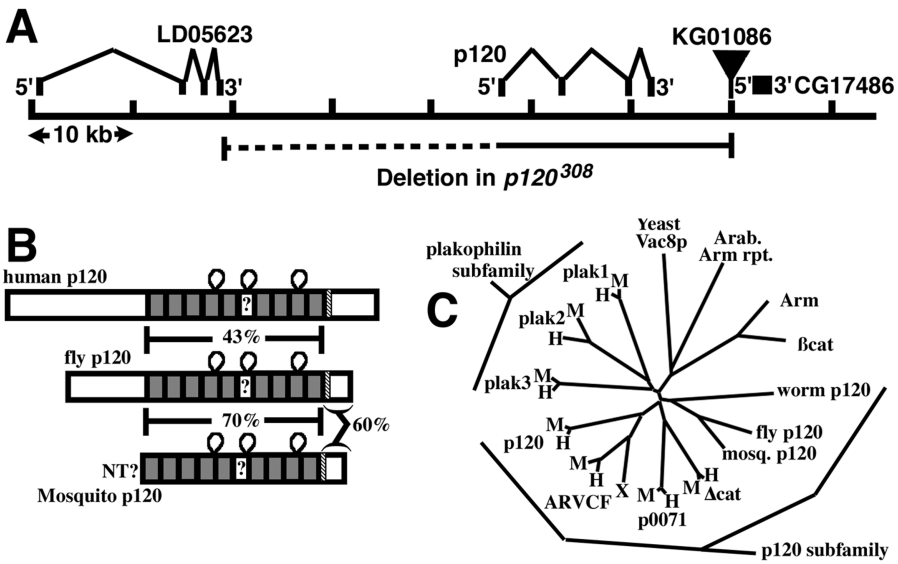

Figure 1. ***Drosophila* p120 is a member of the p120 subfamily.** (A) Gene structure of *p120* and the two adjacent genes, *LD05623* and *CG17486*. *KG01086*, the P element insertion used to generate *p120* mutants, is indicated, as is the region deleted in $p120^{308}$ (uncertainty in the left boundary is indicated as a dotted line). (B) Human, fly, and mosquito p120. Gray boxes represent Arm repeats. Repeat 6, which diverges from the consensus, is indicated by a "?". Loops represent conserved inserts in Arm repeats. Hatched box shows conserved region of similarity. Amino acid identities in pairwise comparisons of the regions bracketed are indicated. (C) Unrooted tree of the p120 subfamily, plakophilin subfamily, and selected other Arm repeat proteins. H, human; M, mouse; X, *Xenopus*.

minal and 91 aa COOH-terminal regions. We compared fly p120 to the seven mammalian p120 family members and the single p120 homologues in *C. elegans (jac-1)* and the mosquito (*A. gambiae*) using ClustalW and Treeview to create an unrooted tree (Fig. 1 C). Several other Arm repeat proteins were included for comparison. The p120 family is clearly distinct from other Arm repeat proteins. p120 family members form two distantly related groups (Fig. 1 C): one contains mammalian desmosomal plakophilins, whereas the other (the p120 subfamily) includes insect and nematode p120s, mammalian p120, ARVCF, p0071, and δ-catenin. The four mammalian p120 subfamily members are all more closely related to each other than any is to insect or nematode p120s. In the p120 subfamily, sequence similarity is highest in the Arm repeats, where fly p120 is 43–51% identical to its human relatives (although high, this similarity is lower than that between fly Arm and human β-cat, which are ~70% identical in their Arm repeats). There are clear blocks of sequence similarity among the NH_2-terminal domains of human p120 subfamily members, but these are not conserved in fly p120. The COOH termini are also divergent, with a short conserved region immediately after the Arm repeats. Fly and mosquito p120, which diverged ~250 million years ago, are much more similar than either is to mammalian p120, both in the Arm repeats (70% identity) and COOH-terminal domain (~60% identity) (Fig. 1 B).

p120 localizes to AJs and the cytoplasm

To further examine whether fly p120 was a p120 ortholog, we examined its subcellular localization. To do so, we generated rat and rabbit polyclonal antisera to its COOH-terminal 96 aa. The work described below, unless noted, uses affinity-purified rat anti-p120. In immunoblots of embryo extracts, this primarily recognizes a single protein of ~88 kD (Fig. 2 A; p120's predicted mol wt = 86.7 kD). This protein is absent in a null *p120* mutant (Fig. 2 A; see below), confirming that it is encoded by *p120*. This ~88 kD protein is also the major protein recognized by rabbit anti-p120 (Fig. 2 B). Both antisera variably cross-react with other proteins, but no other protein was consistently recognized. The rat antisera specifically recognize p120 in embryos. In null $p120^{308}$ mutants, cell junctional staining was lost and overall staining reduced (Fig. 2, F versus H). In parallel to generating antibodies, we generated fusions of p120 with six myc epitopes at the NH_2 terminus (myc-p120) or GFP at the COOH terminus (p120-GFP). These were expressed ubiquitously using the ubiquitin promoter or at specific times and places using the GAL4-UAS system. Since they were not expressed from the *p120* promoter, we primarily used them to confirm p120's subcellular localization.

In mammalian cells, p120 subfamily members localize to AJs and accumulate in the cytoplasm (for review see Anastasiadis and Reynolds, 2000). *Drosophila* p120 localizes in a similar fashion, accumulating both in cell–cell junctions and the cytoplasm in several epithelial tissues (Fig. 2, C and F). p120-GFP (Fig. 2 I) and myc-p120 (Fig. 2, J–L) localize in a similar fashion. This junctional accumulation matches that of Arm (Fig. 2 G) and DE-cadherin (DE-cad) (Fig. 2 K), though in some tissues p120 localization to junctions was more variable (Fig. 2 F; also see below). To determine whether p120 specifically accumulates in AJs, we examined its localization in optical cross-sections through polarized epithelia. Endogenous p120 localizes to the apical region of cells in the ectoderm (Fig. 2 D, arrows), consistent with AJ localization. In the developing gut, both endogenous p120 (Fig. 2, M and N) and p120-GFP (Fig. 2 O) colocalize with Arm and DE-cad at apical AJs. In ovaries, p120-GFP colocalized with Arm at AJs of the follicular epithelium (Fig. 2 P). Thus, *Drosophila* p120's localization is consistent with a role as the ortholog of the mammalian p120 subfamily.

p120 interacts with the juxtamembrane region of DE-cad

Mammalian p120 subfamily members interact with cadherins via conserved sequences in the JM region (for review see Anastasiadis and Reynolds, 2000). We thus examined whether *Drosophila* p120 interacts with DE-cad and Arm in AJ complexes and whether p120 binds the DE-cad JM region. We immunoprecipitated myc-p120 with anti-myc antibodies and looked for coimmunoprecipitation of DE-cad

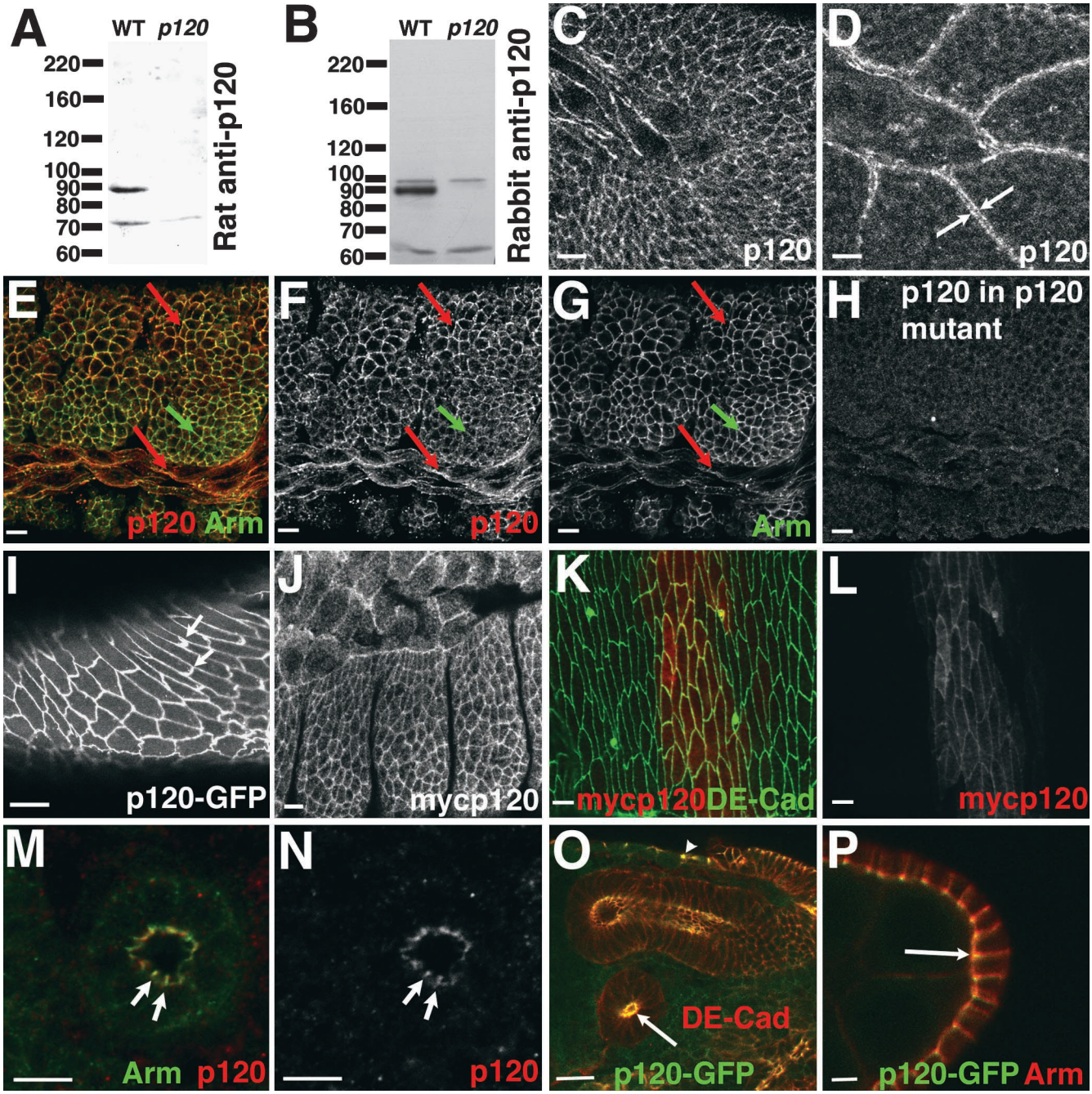

Figure 2. **p120 localizes to AJs and the cytoplasm.** (A and B) Wild-type (WT) and homozygous *p120*^{308} (*p120*) embryonic extracts immunoblotted with affinity-purified rat anti-p120 (A) or rabbit anti-p120 (B). (C–O) Embryos. Embryonic stages are as in Wieschaus and Nüsslein-Volhard (1986). (C and D) Stage 11. (C) Apical section through epidermis; more basal section, cutting across the folded epithelium (D). p120 localizes to the apical cell cortex (arrows). (E–G) Stage 11. p120 (red); Arm (green). Cells are indicated in which p120 accumulation is relatively high (red arrows) or low (green arrow). (H) Stage 11 *p120*^{380} mutant. (I) Live image; p120-GFP. Higher levels are seen at the ends of cells that are stretched (arrows). (J) Stage 12, ubiquitin-myc-p120. (K and L) Stage 12, expressing myc-p120 in *prd* stripes. Myc (red), DE-cad (green). DE-cad is uniform across the embryo; thus, myc-p120 overexpression does not affect its localization. (M–O) Stage 17, optical cross sections. (M and N) p120 (red) and Arm (green) colocalize to apical AJs of the gut (white arrows). (O) p120-GFP (green) and DE-cad (red) colocalize at AJs of the gut (arrow) and epidermis (arrowhead). (P) Egg chamber in ovary. p120-GFP (green) and Arm (red) colocalize at follicle cell AJs (arrow). Bars, 5 μm.

(Fig. 3 A). DE-cad was specifically detected in myc-immunoprecipitates (IPs) from embryos expressing myc-p120 and not from wild-type embryos. Interestingly, Arm was also detected in the IPs, although endogenous p120 was not. The absence of endogenous p120 suggests we did not immunoprecipitate large oligimeric cadherin complexes; the presence of Arm thus supports the idea that Arm and p120 may bind the same cadherin cis-dimer. IPs with a control antibody, anti-BicD, confirmed the specificity of this coimmunoprecipitation (Fig. 3 A). Next, we immunoprecipitated endogenous cadherin–catenin complexes using anti-Arm (Fig. 3 B). As expected, Arm antibodies coimmunoprecipitated DE-

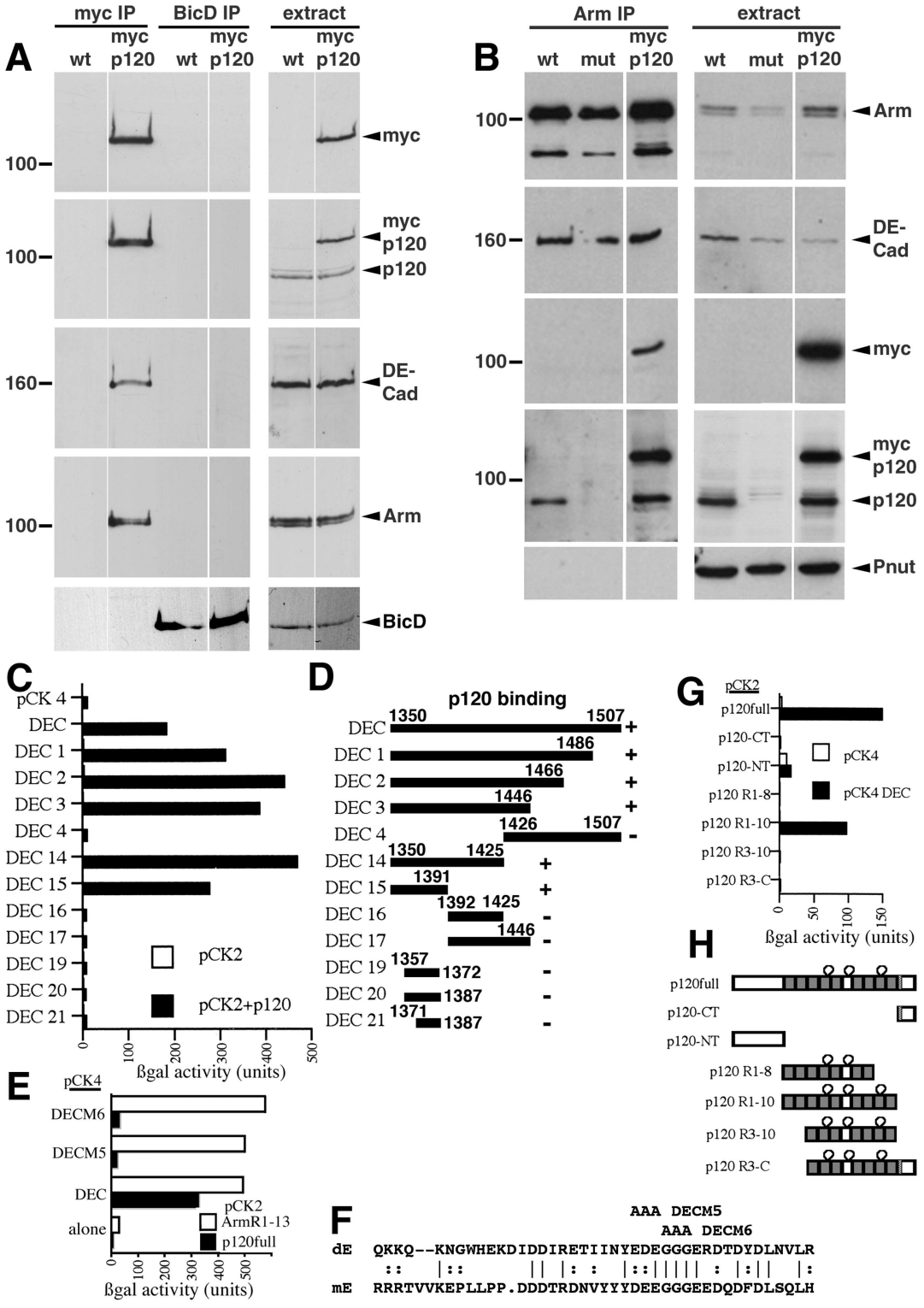

Figure 3. **p120 is in AJ complexes and binds the JM region of DE-cad.** (A) Cell extracts or IPs using anti-myc or anti-BicD (negative control) from wild-type or myc-p120 embryos were immunoblotted with antibodies against myc, p120, DE-cad, Arm, and BicD. Proteins identified are indicated to the right, and selected mol wt markers (kD) are on the left. Rat anti-p120 recognizes both endogenous and myc-p120. (B) Cell extracts or anti-Arm IPs from wild-type (wt), *p120* mutant (mut), and myc-p120 expressing embryos were immunoblotted with antibodies against Arm, DE-cad, myc, p120, and Pnut (a negative control). (A and B) ~1% of extract and ~50% of each IP was loaded. (C–H) Yeast two-hybrid interactions assessed by β-galactosidase activity. (C) Interaction between p120 and the DE-cad cytoplasmic tail (DEC), DE-cad deletion constructs (DECXX; black bars) or vector control (white bars). (D) Schematic illustrating DEC deletion constructs in C. (E and F) Clustered point mutations in the JM region of the DE-cad cytoplasmic tail (DECM5 and DECM6, diagram in F; dE, DE-cad; mE, mouse E-cad) abolished binding of full-length p120 (E, black bar), but had no effect on interaction of the Arm repeats of Arm with DE-cad (E, white bars). (G and H) Arm repeats 1–10 of p120 are required to confer strong binding to DE-cad (black bars). Vector control (white bars). (H) Diagram of constructs used.

cad. They also coimmunoprecipitated both endogenous p120 and myc-p120 (Fig. 3 B), suggesting that both are part of cadherin–catenin complexes. Controls with *p120* mutants or embryos not expressing myc-p120 confirmed the specificity of this interaction (Fig. 3 B).

To map the p120 binding site on the cadherin cytoplasmic tail, we used the yeast two-hybrid system (Fig. 3 C), fusing full-length p120 to the LexA DNA-binding domain (pCK2-p120) and fusing the cytoplasmic domain of DE-cad (aa 1,350–1,507; DEC) to the GAL4 transcriptional activation domain. p120 and DE-cad interacted strongly, as assessed by β-galactosidase assays (Fig. 3, C and D). We then used deletion analysis of the cytoplasmic tail to delineate the region that bound p120 (Fig. 3, C and D). The shortest portion that interacted included the first 41 aa (aa 1,350–1,391; DEC15), whereas removal of additional aa from either end (DEC 19, 20, or 21) abolished binding. We next tested clustered point mutations in the DE-cad cytoplasmic tail (Fig. 3 E). Two clustered point mutations in the Arm/β-cat–binding site did not affect binding (unpublished data), whereas two clustered point mutations in conserved aa in the JM region (Fig. 3 F) substantially reduced binding by p120 but not by Arm, as assessed in yeast (Fig. 3 E). Finally, we mapped the region of p120 that interacted with DE-cad (Fig. 3, G and H). The COOH and NH_2 termini are dispensable, since the Arm repeat domain alone interacted (p120 R1–10). However, removal of two NH_2-terminal (p120 R3–10) or two COOH-terminal Arm repeats (p120 R1–8) abrogated the interaction. All of these results closely match those obtained with mammalian p120 (Thoreson et al., 2000) and δ-catenin (Lu et al., 1999), supporting the idea that *Drosophila* p120 is their fly ortholog.

Genetic analysis revealed that p120 is nonessential

To examine p120 function, we set out to create *p120* mutations and characterize their effects on development. Because of its position near the heterochromatin, few genetic reagents were available. In particular, when we initiated this analysis there were no P elements in the immediate vicinity of *p120*. Thus, we began genetic analysis by selecting a Deficiency removing *p120*, *Df(2R)M41A8*, and carrying out a genetic screen for lethal mutations in the region. This was based on the premise that p120, like core AJ proteins in flies and mammals, would be encoded by an essential gene. We screened 6284 chromosomes and isolated 226 lethals. However, none of these lethals results from a mutation in *p120* (unpublished data).

We thus turned to an unbiased approach to obtaining *p120* mutants. The BDGP recently initiated a screen for P element insertions in new genomic regions (http://flypush.imgen.bcm.tmc.edu/pscreen/). One P element, *KG01086*, is ~7 kb 3′ to *p120*, and 2 kb 5′ to the adjacent gene, *CG17486* (Fig. 4 A). *KG01086* is homozygous viable. We removed secondary P element insertions and other background mutations by replacing the other chromosomes and recombining off the left arm of the 2nd chromosome. We then mobilized *KG01086* and screened for transposition to a nearby site or deletions beginning in the P element and extending into adjacent DNA (unpublished data). Three deletions affecting *p120* were isolated. All remove the entire

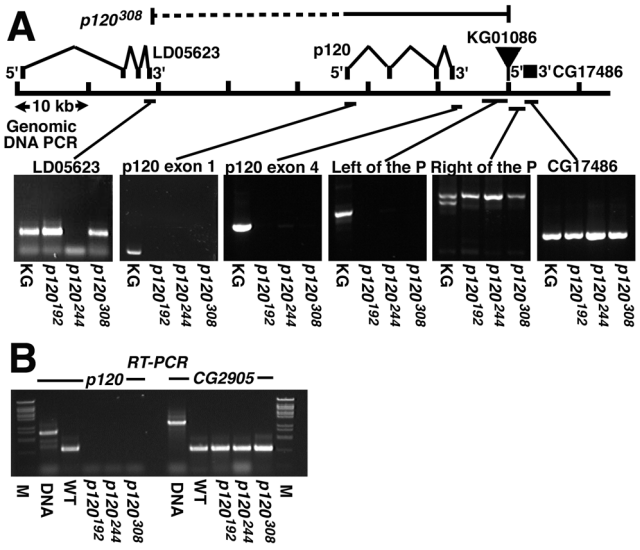

Figure 4. ***p120*^{308} is a null allele.** (A) *p120*^{308} deletes the entire *p120* coding region, but does not affect other genes. Schematic as in Fig. 1 A. Genomic DNA from single wild-type or homozygous mutant flies was PCR amplified using primer pairs from the indicated regions between *LD05623* and *CG17486*. (B) The *p120* mutants are mRNA nulls. cDNA generated from oligo-dT-primed total RNA from *p120* mutants and wild-type was amplified with primers spanning the *p120* third intron. An unrelated gene, *CG2905,* is a control. A DNA control confirmed we were examining mRNA.

p120 coding sequence as assessed by PCR of genomic DNA across the region (Fig. 4 A; unpublished data), and two of the three do not affect adjacent genes (Fig. 4 A). We focused on one of these alleles, *p120*^{308}. We verified that this allele is null, since it does not make *p120* mRNA as assessed by RT-PCR (Fig. 4 B) or stable p120 protein as assessed by immunoblotting with two different antisera (Fig. 2, A and B).

To our surprise, all three *p120* mutants are zygotically viable. Further, we could generate homozygous stocks of all of them, demonstrating that *p120* mutants are not male or female sterile, nor is a zygotic phenotype covered by maternal contribution of *p120*. We collected embryos from homozygous mutant mothers and fathers and saw no significant embryonic lethality above the normal background for a wild-type stock (93% viability for the homozygous mutant [n = 330] versus 96% for a wild-type strain [n = 318]). These data are in stark contrast to results with mutations in either *DE-cad* or *arm*. Both are zygotically embryonic lethal, and both are essential for adhesion at the onset of gastrulation (for review see Yap et al., 1997; Tepass et al., 2001). Thus, p120 is not an essential core component of the cadherin–catenin complex.

To determine the effect of loss of p120 on AJs in more detail, we examined the levels and localization of AJ proteins in *p120*^{308}mutants compared with wild-type. We immunoblotted cell extracts from wild-type or *p120*^{308}-null mutant embryos with antibodies to DE-cad, α-cat, and Arm (Fig. 5 A). No noticeable changes in levels of these proteins were seen, though we cannot rule out slight changes (less than twofold). We also examined the levels and localization during embryogenesis of Arm (Fig. 5, B–G), DE-cad (Fig. 5, H and I), and α-cat (Fig. 5, J and K), and

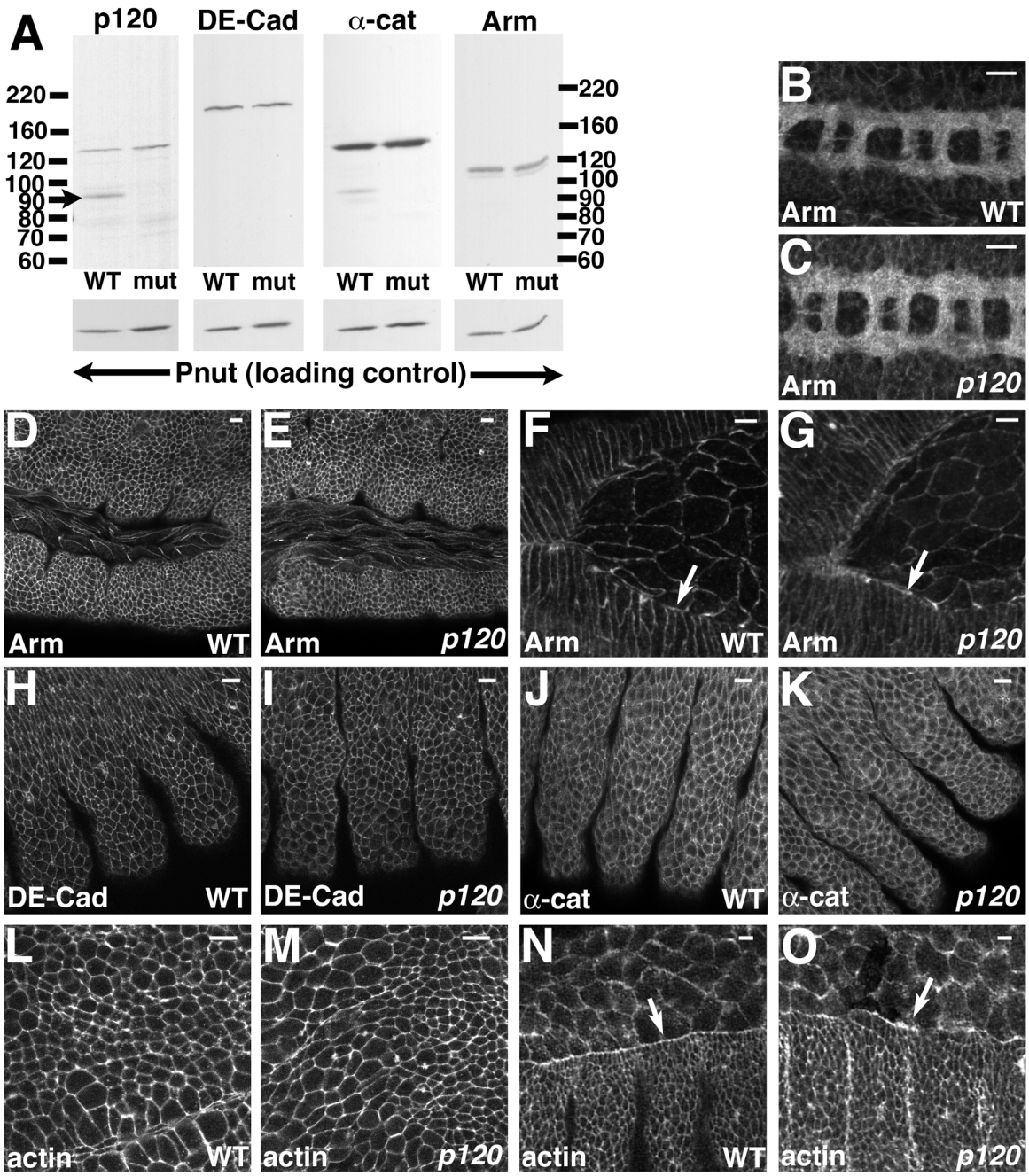

Figure 5. **AJ proteins are not significantly altered in levels or localization in *p120* mutants.** (A) Embryonic extracts from 3–8 h wild-type (WT) and $p120^{308}$ (mut) strains immunoblotted with antibodies to p120 (arrowhead), DE-cad, α-cat, and Arm. Anti-Pnut is a loading control. Mol wt standards (kD) are at left. (B–O) Wild-type and $p120^{308}$ mutant embryos labeled with Arm (B–G), DE-cad (H and I), α-cat (J and K), or phalloidin to show F-actin (L–O). (F, G, N, and O) arrows indicate the leading edge during dorsal closure. Bars, 5 μm.

the localization of actin to the cortex (Fig. 5, L–O) by immunofluorescence confocal microscopy. To control the experiment, we mixed homozygous $p120^{308}$mutants with wild-type embryos carrying a histone-GFP transgene, fixed and stained them together, and visualized them on the same slide with the same confocal settings. Images were scored blind by two observers, and no consistent difference in either the levels or localization of any AJ proteins or of cortical actin was observed. In each case, a slight preponderance of mutant embryos stained more weakly, but there were no systematic differences, and both wild-type and mutants fell across the spectrum of variation in staining intensity seen among embryos.

During this analysis, we noted a subtle but reproducible change in cell arrangement during dorsal closure in $p120^{308}$ mutants. In wild-type embryos cells of the leading edge form a discrete and relatively straight row, maintained by tension in the actin cable anchored at cell junctions at the leading edge (Fig. 5 N, arrow) (Kiehart et al., 2000). In many $p120^{308}$ mutants, the leading edge cell front was irregular, and at times the actin cable underlying it was less uniform (Fig. 5 O, arrow). Although this irregularity was observed in most mutants, some

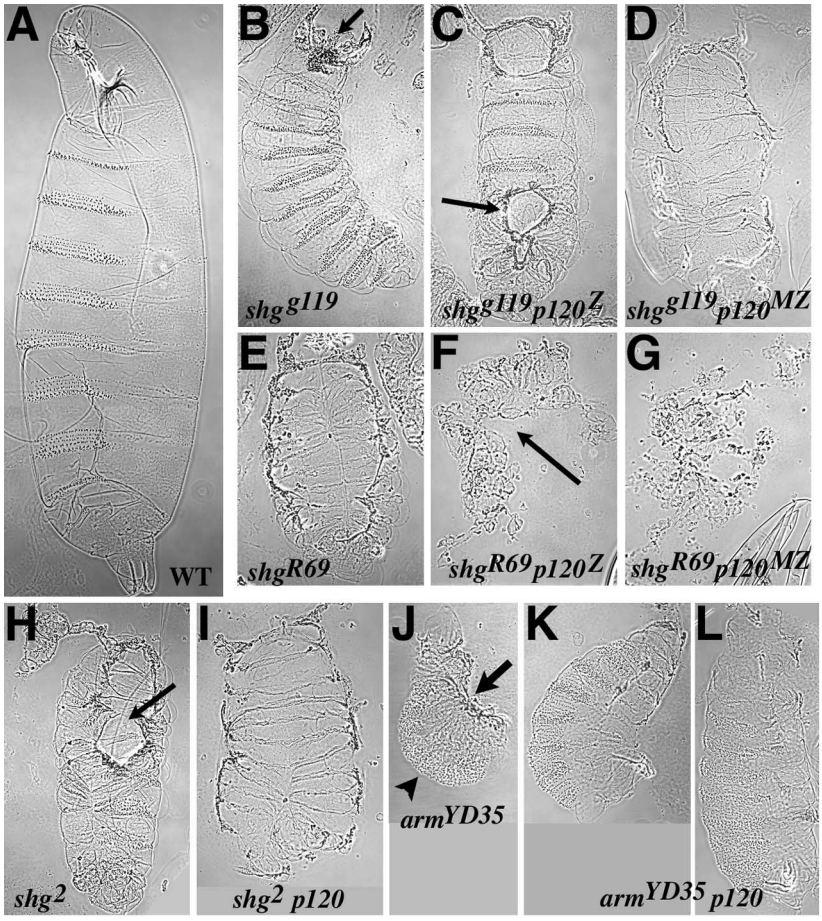

Figure 6. ***p120* mutations strongly enhance *shg* and *arm*.** Cuticle preps, anterior up. (A) Wild-type. Note alternating denticle bands and naked cuticle on the ventral epidermis and normal head exoskeleton (top). (B) shg^{g119}. Note head involution defects (arrow) but intact ventral epidermis. (C) Zygotic shg^{g119} $p120^{308}$. Note hole in ventral epidermis. (D) Zygotic shg^{g119} $p120^{308}$ mutant that is also maternally $p120^{308}$ mutant. The entire ventral epidermis is lost. (E) shg^{R69}. Ventral epidermis is lost, but dorsal and lateral epidermis remain. (F) Zygotic shg^{R69} $p120^{308}$. Dorsal epidermis is disrupted (arrow). (G) Zygotic shg^{R69} $p120^{308}$ mutant that was also maternally $p120^{308}$ mutant. The remaining cuticle is fragmented. (H) shg^{2}. Note holes in ventral epidermis. (I) Zygotic shg^{2} $p120^{308}$. Note complete loss of ventral epidermis. (J) arm^{YD35}. Note shortened body and lawn of denticles ventrally (arrowhead), and defects in dorsal closure (arrow). (K and L) Zygotic arm^{YD35} $p120^{308}$ mutant that was also maternally $p120^{308}$ mutant. Cuticles are longer, and in L dorsal closure defects are suppressed.

mutants were relatively wild type (Fig. 5, F compared with G). These data suggest there may be subtle defects in adhesion and/or cytoskeletal organization in *p120* mutants during morphogenesis. Dorsal closure is resistant to changes in the balance of forces driving leading edge progression (Kiehart et al., 2000), thus likely explaining why these minor defects do not disrupt development. Further, one can reduce the levels of DE-cadherin or Arm substantially before defects in adhesion occur (Cox et al., 1996; Tepass et al., 1996; Uemura et al., 1996), suggesting that AJ function might be diminished in *p120* mutants without obvious consequences.

p120 mutations strongly enhance mutations in the genes encoding DE-cad and Arm

One possible explanation for the lack of a strong phenotype for *p120* is that it is just one of several regulators of AJs and that its loss does not drop function below the threshold level needed for viability. To test this hypothesis, we examined whether reduction or loss of p120 enhanced or suppressed the phenotypes of mutations in two other AJ proteins: DE-cad (encoded by *shotgun* [*shg*]) and Arm. *shg* and *p120* both map to the 2nd chromosome, so to test for interactions we made recombinant chromosomes carrying the null allele $p120^{308}$ and various *shg* mutations. In the case of *arm*, we generated stocks heterozygous for the null allele arm^{YD35} and homozygous for $p120^{308}$.

In both situations, we saw genetic interactions consistent with p120 playing a positive role in modulating AJ function. Mutations in *p120* strongly enhanced the phenotypes of *shg* mutants. Wild-type larvae are entirely enclosed in cuticle (Fig. 6 A). To make a proper cuticle, epithelial tissues must retain their integrity and morphogenetic movements such as head involution and dorsal closure must occur properly. Zygotic loss-of-function mutations in *shg* disrupt morphogenesis and epithelial integrity (Tepass et al., 1996; Uemura et al., 1996), with different processes differentially sensitive to the level of remaining DE-cad function (in all cases, maternal wild-type DE-cad remains, since without it oogenesis is disrupted, and thus this is the phenotype of DE-cad depletion, not its total absence). A weak allele (shg^{g119}) primarily has defects in head involution such that the embryo secretes cuticle without head structures (Fig. 6 B). A stronger allele (shg^{2}) has holes in the ventral cuticle (Fig. 6 H): ventral neurectoderm is more sensitive to reductions in DE-cad function due to stresses imposed by the ingression of neural cells (Tepass et al., 1996). Most zygotic null mutants (shg^{R69}) secrete only a sheet of dorsal cuticle (Fig. 6 E) due to disruption of the integrity of the ventral epidermis.

Zygotic homozygosity for $p120^{308}$significantly enhanced the cuticle phenotype of all three *shg* alleles (Fig. 6; Tables I and II), making a given double mutant resemble in phenotype a stronger *shg* mutation. For example, shg^{g119} $p120^{308}$ double mutants exhibit large holes in their ventral cuticle (Fig. 6, B versus C). This enhancement is nearly completely rescued by p120GFP (Table I), confirming that it is due to p120 and suggesting that this transgene provides nearly wild-type function, at least in this context. shg^{2} $p120^{308}$ dou-

Table I. **Part I. *p120* mutations enhance the phenotype of mutations in *shg***

Cross	Phenotypic classes of lethal embryos (presumptive *shg* homozygotes) Increasing severity → Head defects but cuticle intact[a]	Scar in ventral cuticle	Ventral hole(s)[b]	Fragmentary ventral cuticle[c]	No ventral cuticle[d]	*n*
	%	%	%	%	%	
shg^{g119}/+ × shg^{g119}/+	48	41	10	1	0	145
shg^{g119} $p120^{308}$/+ × shg^{g119} $p120^{308}$/+	8	20	68	4	0	140
shg^{g119} $p120^{308}$/+; p120GFP × shg^{g119} $p120^{308}$/+; p120GFP	47	34	16	3	0	116
shg^{g119} $p120^{308}$/$p120^{308}$ × shg^{g119} $p120^{308}$/+	3	2	46	12	28	169[e]
shg^{2}/+ × shg^{2}/+	0	0	39	58	3	153
$shg^{2}p120^{308}$/+ × $shg^{2}p120^{308}$/+	0	0	1	34	65	91

In each case, we scored the lethal embryonic progeny of each cross. We presume they are the *shg* homozygotes, which are in some cases also homozygous mutant for $p120^{308}$. All crosses produced a small number of "wild-type" but dead embryos (<10%). Since these are observed in wild-type embryos, they were not included in the total. Examples of some of the phenotypic classes are shown in Fig. 6, as follows:
[a]Fig. 6 B.
[b]Fig. 6 C.
[c]Fig. 6 H.
[d]Fig. 6, D, E, and I.
[e]In the two crosses in which p120 was also maternally mutant, 10–15% of the progeny died with head defects but were otherwise normal. Our assessment of total embryonic lethality in these crosses suggests they are likely to be p120 homozygotes that are heterozygous for *shg*. They are also not included in the total.

ble mutants were more severe than shg^{2} alone, with the majority having no ventral cuticle remaining (Fig. 6, H versus I). In shg^{R69} $p120^{308}$ double mutants, the sheet of dorsal cuticle seen in shg^{R69} was disrupted (Fig. 6, E versus F). These genetic interactions were seen despite the fact that zygotic double mutants retained maternal p120. To further reduce p120, we generated *shg p120* zygotic double mutants whose mothers were homozygous *p120* mutant. These embryos were thus maternally and zygotically mutant for $p120^{308}$ ($p120^{MZ}$) and zygotically mutant for *shg*. In this case, we saw an even more dramatic enhancement of *shg*. For example, 40% of shg^{g119} $p120^{308}$ double mutants who were maternally *p120* mutant had only fragmentary ventral cuticle or no ventral cuticle at all (Fig. 6, B versus D). This also further enhanced shg^{R69} $p120^{308}$ double mutants, with the majority having only fragments of cuticle remaining (Fig. 6, E versus G). Together, these data suggest that p120 plays an important supporting role in adhesion that is revealed when DE-cad levels are reduced. During the course of these experiments, we observed an additional genetic interaction further supporting the idea that p120 is a critical modulator of adhesion when cadherin levels are reduced. In generating adults homozygous $p120^{308}$mutant and heterozygous for *shg*, we found that these animals are not recovered at Mendelian ratios. Reducing the dose of DE-cad reduced the viability of $p120^{308}$homozygotes that were *shg* heterozygous to 20–60% of that of their shg^{+} siblings (Table III). Thus, when DE-cad levels are reduced *p120* becomes virtually essential.

Table II. **Part II. *p120* mutations enhance the phenotype of mutations in *shg***

Cross	Phenotypic classes of lethal embryos (presumptive *shg* homozygotes) Increasing severity → Some ventral cuticle[a]	Dorsal cuticle intact[b]	Dorsal cuticle with hole	Dorsal cuticle U shaped[c]	Dorsal cuticle separated	Scraps of cuticle[d]	*n*
	%	%	%	%	%	%	
shg^{R69}/+ x shg^{R69}/+	13	24	45	18	0	0	128
shg^{R69} $p120^{308}$/+ × shg^{R69} $p120^{308}$/+	0	8	58	30	3	0	119
shg^{R69} $p120^{308}$/$p120^{308}$ × shg^{R69} $p120^{308}$/+	7	2	11	19	29	31	201[e]

In each case, we scored the lethal embryonic progeny of each cross. We presume they are the *shg* homozygotes, which are in some cases also homozygous mutant for $p120^{308}$. All crosses produced a small number of "wild-type" but dead embryos (<10%). Since these are observed in wild-type embryos, they were not included in the total. Examples of some of the phenotypic classes are shown in Fig. 6, as follows:
[a]Fig. 6 H.
[b]Fig. 6, D, E, and I.
[c]Fig. 6 F.
[d]Fig. 6 G.
[e]In the two crosses in which p120 was also maternally mutant, 10–15% of the progeny died with head defects but were otherwise normal. Our assessment of total embryonic lethality in these crosses suggests they are likely to be p120 homozygotes that are heterozygous for *shg*. They are also not included in the total.

Table III. **Reducing the dose of DE-cad reduces the adult viability of homozygous *p120* mutants**

	Progeny genotype		
	p120	*p120 shg*	
	Cy	*p120*	
Cross	**Adult progeny**		***n***
	%	%	
Predicted viability if no interaction	50	50	NA
$p120^{308}$, shg^{R69}/*Cy* × $p120^{308}$/$p120^{308}$	67	33	864
$p120^{308}$, shg^{g119}/*Cy* × $p120^{308}$/$p120^{308}$	80	20	886
$p120^{308}$, shg^{2}/*Cy* × $p120^{308}$/$p120^{308}$	91	9	345

	Progeny genotype		
	y w	*y w*	
	Cy	*p120 or p120 shg*	
Control cross	**Adult progeny**		***n***
	%	%	
$p120^{308}$/*Cy* × *y w*	42	58	139
$p120^{308}$, shg^{R69}/*Cy* × *y w*	48	52	124

In each case, we scored the adult progeny of the cross. The control crosses reveal that progeny carrying the *Cy* marker are less viable than their siblings, thus biasing our experiment somewhat against the conclusion we reached.

The dual roles of Arm in Wingless (Wg) signaling and cell adhesion complicate the analysis of *arm;* $p120^{308}$ double mutants. The zygotic null arm^{YD35} affects both ventral cell fate choices and dorsal closure, due to its dual roles in adhesion and Wg signaling. Mutant embryos thus have segment polarity defects, with all surviving cells taking on denticle fates (Fig. 6 J, arrowhead), and have defects in dorsal closure (Fig. 6 J, arrow). We found previously that reducing the dose of DE-cad suppresses the Wg signaling defect of a strong *arm* mutant, presumably by releasing maternal wild-type Arm from junctional complexes and allowing it to act in signaling (Cox et al., 1996). We saw a similar affect of loss of *p120* function: it suppressed arm^{YD35} such that double mutant embryos had longer cuticles and in some cases their dorsal closure defects were suppressed (Fig. 6, J versus K and L; Table IV). These data also support a positive modulatory role of p120 in cell–cell adhesion.

p120 accumulation during embryogenesis is consistent with a role as an adhesion regulator

Together these data suggest that p120 is not an essential core component of AJs but instead is a modulator that plays a critical role when cadherin–catenin function is compromised. Thus, one might hypothesize that although p120 would localize to AJs, its level there might vary depending on the situation. Embryogenesis provides a superb place to address this, since one can examine cells facing diverse cell biological challenges. We first examined the expression of *p120* mRNA (Fig. 7). It is maternally contributed, and maternal mRNA persists to late stages, since it can be detected in late stage embryos homozygous for a Deficiency removing *p120* (Fig. 7 F). When zygotic transcription begins, *p120* mRNA is found in all cells (Fig. 7 A). It remains on in all three germ layers during gastrulation (Fig. 7 B, arrows), and expression continues in all or virtually all tissues throughout embryogenesis (Fig. 7, C, D, and F). However, there is elevated expression in certain tissues, many of which are undergoing morphogenetic movements. These include the invaginating posterior midgut (Fig. 7 B, black arrow), the central nervous system (CNS) (Fig. 7, C and F, white arrows), the migrating anterior and posterior midguts (Fig. 7 C, black arrows), the foregut and hindgut, and cells forming the midgut constrictions (Fig. 7 D, arrow).

We next compared the localization of p120 at different developmental stages to that of the core AJ protein Arm. p120 accumulates at cell–cell borders through most of embryogenesis, largely paralleling Arm and DE-cad. However, some intriguing differences were observed. p120 is not as highly enriched in junctional structures during early stages of embryogenesis as are core AJ proteins. In early *Drosophila* development, 13 rounds of nuclear division occur without cytokinesis. The last three occur at the egg cortex, and during mitosis transient membrane invaginations called pseudocleavage furrows separate each spindle from the others. Arm, α-cat, and DE-cad localize to pseudocleavage furrows (McCartney et al., 2001) (Fig. 8 A). In contrast, p120 localization to pseudocleavage furrows was very weak (Fig. 8, A and B). During cellularization, which ends the syncytial phase, Arm and DE-cad localize to basal junctions just behind the advancing contractile apparatus and later localize to nascent AJs (for review see Tepass et al., 2001). Endogenous

Table IV. ***p120* mutations genetically interact with mutations in *arm***

	Phenotypic classes of lethal embryos (presumptive *arm* mutants)				
	Increasing severity →				
Cross	**Segment polarity and/or dorsal closure defects strongly suppressed[a]**	**Moderate segment polarity and/or dorsal closure defects[b]**	**Very strong segment polarity phenotype**	**Null segment polarity phenotype and dorsally open[c]**	***n***
	%	%	%	%	
arm^{YD35}/+ × +/*Y*	1	3	10	86	135
arm^{YD35}/+; $p120^{308}$ × +/*Y*; $p120^{308}$	16	25	12	47	89

In each case, we scored the lethal embryonic progeny of each cross. We presume they are the *arm/Y* mutants, which in the second cross are also homozygous mutant for $p120^{308}$. Examples of some of the phenotypic classes are in Fig. 6, as follows:
[a]Fig. 6 L.
[b]Fig. 6 K.
[c]Fig. 6 J.

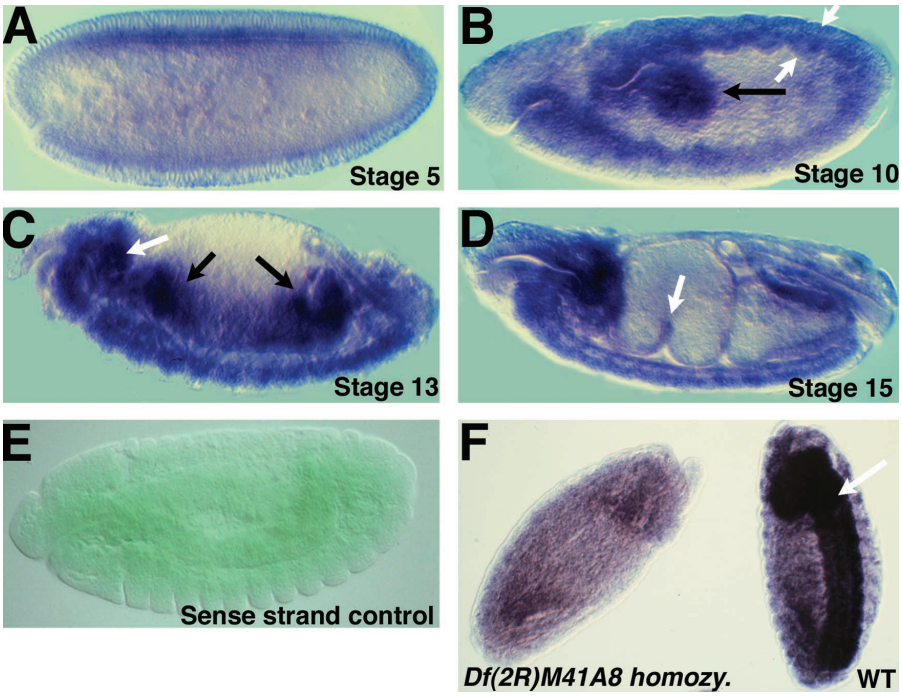

Figure 7. ***p120* mRNA is ubiquitously expressed but enriched in certain tissues.** Embryos at indicated stages, probed by situ hybridization for expression of *p120* mRNA (A–D and F) or with a sense strand *p120* control probe (E). Anterior is to the left and dorsal is up. In B, white arrows indicate the neurectoderm and mesoderm of the germband. Certain tissues accumulate elevated levels of *p120* mRNA, e.g., the posterior midgut (B, black arrow), brain and CNS (C and F, white arrows), migrating anterior and posterior midgut (C, black arrows), cells forming the midgut constriction (D, arrow). (F) Stage 14 wild-type and mutant homozygous for a deletion removing *p120* (*Df[2R]M41A8*), showing remaining maternal mRNA.

p120 colocalizes with Arm to basal junctions at early cellularization (Fig. 8, C–F) but at mid-late cellularization p120 is only weakly detectable at junctional structures (Fig. 8, G–H). Instead, p120 accumulates in the apical cytoplasm (Fig. 8, I and J, arrow). Thus, p120 is not as prominent a component of all early junctional structures as are core AJ proteins. Interestingly, when we expressed p120-GFP in early embryos (probably at levels exceeding endogenous p120), it localized to pseudocleavage furrows, basal junctions, and nascent AJs (Fig. 8, K–M; unpublished data).

During cellularization, we also observed p120 in a surprising location. Endogenous p120 accumulated in dot-like structures near the apical cell surface that were sometimes observed in pairs (Fig. 8, C and D, arrows). We also observed these with p120-GFP (Fig. 8, L and M, arrows) and the rabbit anti-p120 (Fig. 8 N). We suspected that these might be centrosomes. This was supported by the fact that p120-GFP colocalizes with the centrosomal proteins γ-tubulin (Fig. 8, O and P) and centrosomin (Fig. 8, Q and R). Centrosomal localization of p120-GFP persisted during germband extension (unpublished data), but in extended germband embryos it was no longer apparent (Fig. 8 S). p120-GFP also accumulated in the region of the spindle and DNA (Fig. 8, P and R).

During the extended germband stage, p120 begins to accumulate in epidermal cell junctions (Fig. 9), and it remains there through germband retraction. Although its localization roughly matches that of Arm, recruitment of p120 to junctions at several stages in development was less uniform. This was striking during germband elongation (Fig. 9, A–D). Arm outlined all ectodermal cells, regardless of the plane of focus (Fig. 9, A and C, green), reflecting the accumulation of Arm and DE-cad all along the lateral membrane (although they are enriched in AJs). In contrast, p120 was observed at cell junctions in a subset of cells (Fig. 9, B and D). This may reflect a more restricted localization of p120 along the lateral membrane, or it may suggest that strong recruitment of p120 to junctions lags behind that of Arm and DE-cad. As development proceeded, p120 localization to junctions became more uniform (Fig. 9, E and I, and Fig. 2, C and F). Interestingly, in mitotic cells the levels of cytoplasmic p120 drop sharply (Fig. 9, E–H). Differences in the localization of Arm and p120 reappeared during late dorsal closure when p120 was highly enriched in junctions of amnioserosa cells relative to those of the ectoderm, although Arm localized relatively uniformly (Fig. 9, J–N). p120-GFP, which is ubiquitously expressed from a heterologous promoter, does not show as striking a difference (Fig. 9 O).

p120 is also enriched in other tissues where AJ proteins are enriched. p120 accumulates at high levels in trachea (Fig. 10, A–D). Arm and DE-cad accumulate at especially high levels in fusion cells, where tracheal elements that invaginated from different segments join (Uemura et al., 1996) (Fig. 10, B–D, arrows). p120 is not strikingly enriched there (although p120-GFP is) (Fig. 10 E). In the nervous system, both p120 and p120GFP accumulate in axons (Fig. 10, I–L) and in sensory structures of the peripheral nervous sytem (Fig. 10, F–H). Relative enrichment of p120 in the CNS versus the epidermis did not appear as high as that of Arm; this may be real, but the high levels of *p120* mRNA in the CNS (Fig. 7 F) suggest that it may be due to differences in penetration of anti-p120 and anti-Arm antibodies. We also used p120-GFP to examine its subcellular localization in select postembryonic tissues. In eye imaginal discs, p120-GFP accumulates uniformly at cell–cell boundaries of undifferentiated cells ahead of the morphogenetic furrow (Fig. 10 M, arrowheads), and at elevated levels where differentiating photoreceptors abut one another (Fig. 10 M, arrows), resembling Arm. In larval brains, p120-GFP localizes to cell boundaries between neuroblasts and ganglion mother cells (Fig. 10 N) and to axon

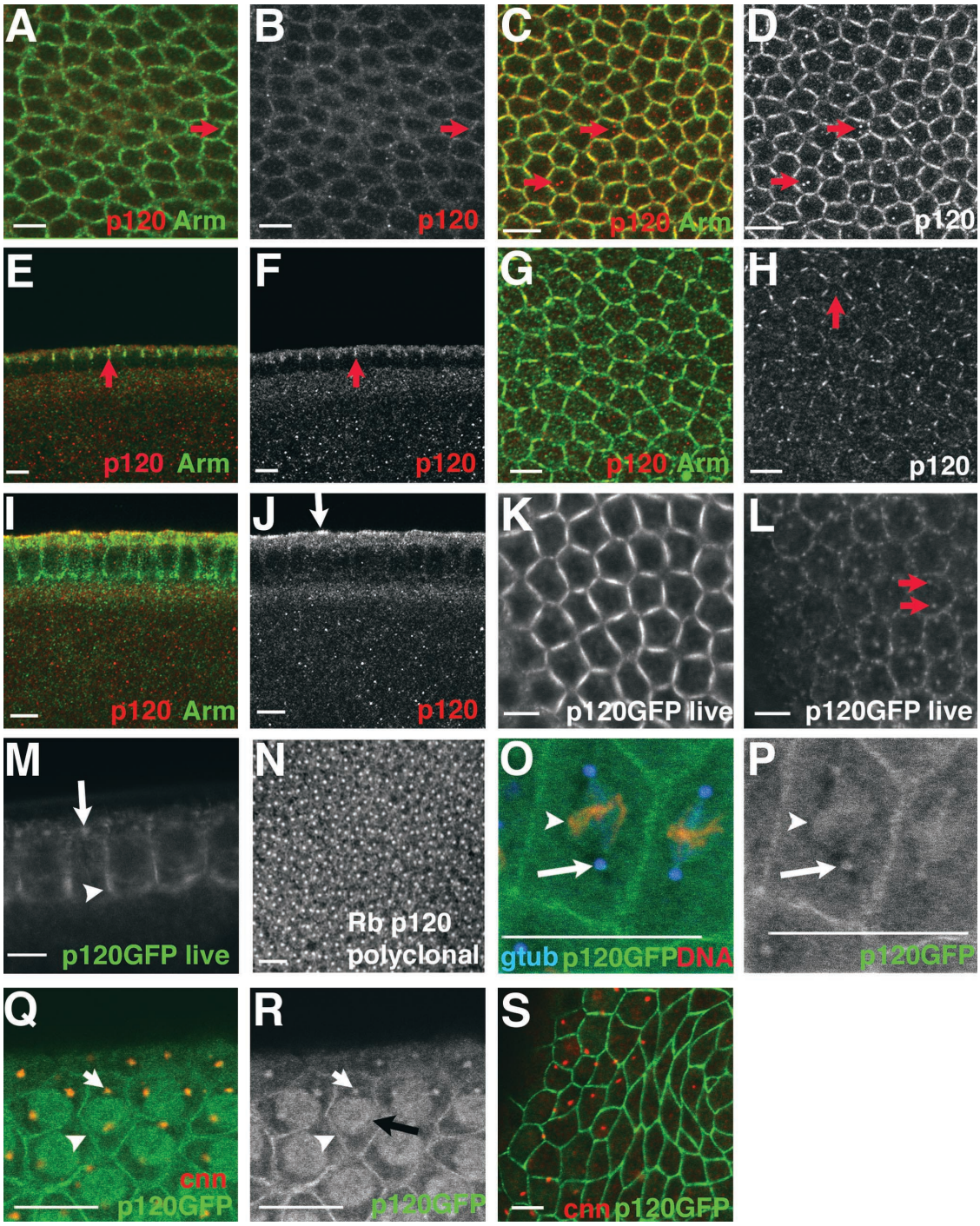

Figure 8. **p120 localization during the syncytial development and cellularization differs from that of Arm.** (A–J) Syncytial blastoderm (A and B) and cellularizing embryos (C–J). p120 (red); Arm (green). (A–D, G, and H) Surface sections. (E, F, I, and J) Optical cross sections. (A and B) Arm localizes strongly to pseudocleavage furrows, whereas p120 staining is much less intense (arrow). (C–F) Early cellularization. p120 colocalizes with Arm at basal junctions (E and F, arrows) and also stains paired structures in the cytoplasm (C and D, arrows). (G–J) Mid-late cellularization. p120 is reduced at cell junctions (H, arrow) compared with Arm (G). Arm labels basal junctions, lateral membranes, and nascent AJs (I). p120 localizes to an apical domain (J, arrow). (K–M and O–S) Embryos expressing p120-GFP. (K–M) Live images, cellularization. p120-GFP localizes to basal junctions (K and M, arrowhead), nascent AJs (L), and centrosomes (L and M arrows). (N) Syncytial embryo, rabbit anti-p120. (O and P) p120-GFP (green), propidium iodide to label DNA (red), γ-tubulin (gtub, blue). p120-GFP and γ-tubulin colocalize (arrow) and p120-GFP is on mitotic spindles (arrowhead). (Q–S) p120-GFP (green); centrosomin (cnn, red). p120-GFP is enriched at cell junctions (arrowhead) and with condensing DNA (black arrow), and colocalizes with centrosomin (white arrow). Centrosomal p120-GFP is absent by stage 9 (S). Bars, 5 μm.

bundles emerging from the neuroblast's progeny (Fig. 10 O), paralleling AJ proteins (Akong et al., 2002). In ovaries, p120-GFP colocalizes with Arm in AJs of epithelial follicle cells (Fig. 2 P and Fig. 10 P), junctions between migrating border cells (Fig. 10 P, arrow) and, more weakly, in junctions between germ cells.

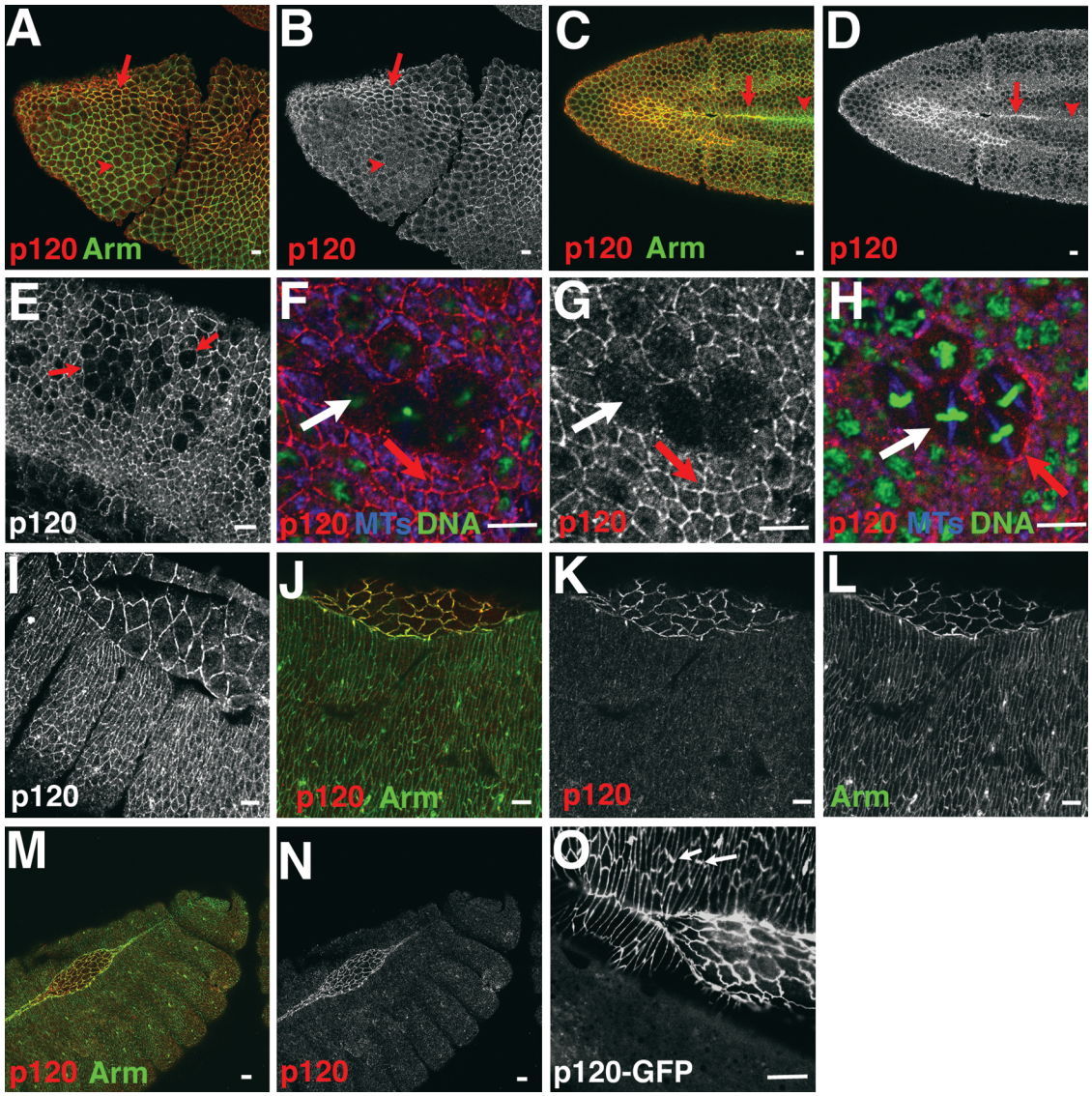

Figure 9. **p120 localizes to embryonic cell junctions but is not as uniformly distributed as core AJ proteins.** (A–D) Stage 8. p120 (red); Arm (green). (Arrows) Cell junctions accumulating both p120 and Arm; (arrowheads) cell junctions depleted for p120. (E) Stage 9. p120 junctional accumulation is becoming more uniform. In mitotic cells, it is absent from the cytoplasm (arrows). (F–H) Stage 9. Histone-GFP (green); p120 (red); microtubules (MT; blue). (F and G) Apical planes; (H) section through the middle of the cells. In nonmitotic cells, p120 localizes in the cytoplasm and at cell junctions (F and G, red arrow). In mitotic cells, junctional and cytoplasmic p120 is reduced (E, arrows; F–H white arrows). Some junctional staining remains at the cell mid-plane (H, red arrow). (I) Stage 12/13. (J–N) Stage 14. p120 (red); Arm (green). (O) Stage 14. Live image, p120-GFP. (Arrows) Accumulation at the ends of stretched cells. Bars, 5 μm.

As mammalian p120 binds to the transcription factor Kaiso and can accumulate in nuclei (for review see Anastasiadis and Reynolds, 2000), we also looked carefully for nuclear accumulation of either endogenous p120 or p120-GFP. In most cases, we saw no strong nuclear accumulation and in fact sometimes saw apparent nuclear exclusion (Fig. 10 P, arrowhead). One exception was the syncytial embryo where we saw weak nuclear accumulation of p120-GFP (Fig. 8, P and R).

Discussion

The cadherin–catenin complex plays an essential role in cell–cell adhesion (for reviews see Yap et al., 1997; Tepass et al., 2001). The classic cadherins, β-cat/Arm and α-cat are core components of AJs, which are required for adhesion, assembly, and maintenance of epithelia. It is not clear whether these are the only core proteins of AJs or whether other proteins are essential for junctional assembly or function. Other junctional proteins are also likely to regulate adhesion, allowing cells to behave dynamically during development.

A growing body of evidence (summarized in the Introduction) supports the idea that the JM domain of classic cadherins is a target of mechanisms that modulate adhesion. One model suggests that p120 family members act as critical regulators of adhesion by binding to the JM region. Other experiments suggested possible cadherin-independent roles of p120, as a Rho regulator (Anastasiadis et al., 2000; Noren et al., 2000; Magie et al., 2002) and as a transcriptional modulator (for review see Anastasiadis and Reynolds, 2000).

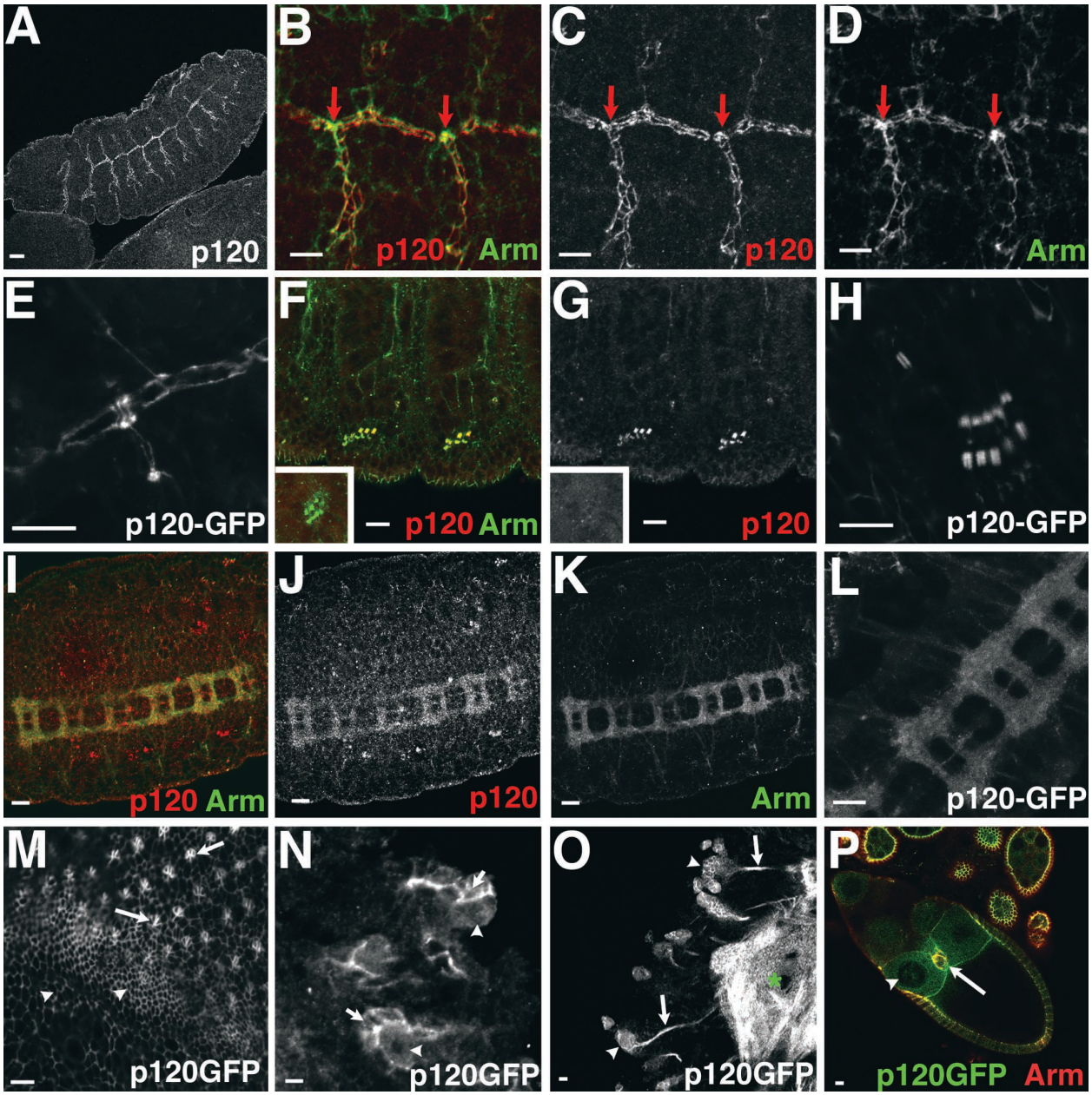

Figure 10. **p120 colocalizes with Arm in other tissues.** (A–D) Stage 15. p120 (red); Arm (green). Arm is enriched in fusion cells (red arrows). (E) p120-GFP. (F and G) Stage 15–16. p120 (red) and Arm (green) colocalize to peripheral nervous system chordotonal organs. (F and G, insets) Similar stage *p120* mutant. Arm localizes to chordotonals; p120 is lost. (H) p120-GFP in chordotonals. (I–K) Stage 17. p120 (red); Arm (green). (L) p120-GFP. (M–N) p120-GFP. (M) Eye imaginal disc. Undifferentiated cells (arrowheads), photoreceptor cells (arrows). (N and O) Larval brain. (N) p120-GFP at cell borders between neuroblasts (arrowheads) and ganglion mother cells (arrow). (O) Progeny of neuroblasts (arrowheads) sending bundled axons (arrows) to the neuropil. (P) Stage 10 egg chamber. p120-GFP (green) and Arm (red) colocalize at cell junctions of migrating border cells (arrow). In nurse cells, p120-GFP is cytoplasmic but excluded from nuclei (arrowhead). Bars, 5 μm.

To date, these hypothesized functions of p120 are largely based on indirect arguments. The postulated roles in adhesion regulation rest largely on the effects of mutating the JM region. The effects on Rho regulation rest largely on effects of p120 overexpression, and the interaction with Kaiso remains without a known biological function. Furthermore, most studies of p120 were performed in cultured cells, and thus little is known about its expression or localization during the complex events of embryogenesis. We tested models of p120 function by characterizing the expression and function of the single *Drosophila* p120.

Drosophila p120 is not an essential component of adherens junctions

When we began this work, we hypothesized that p120 would be an essential gene, reflecting its proposed roles as a core AJ component and/or a critical regulator of adhesion, Rho, and transcription. However, *Drosophila* p120 is not essential: null mutants are viable and fertile under laboratory conditions. Thus, fly p120 is not an essential core component of cell–cell AJs, as are classic cadherins and the other catenins.

The AJs of flies and mammals are remarkably similar in

structure and function. Thus, we think that it is likely that mammalian p120 subfamily members are also not essential AJ components; in other words, we predict that functional AJs can be formed in their absence. However, this does not necessarily mean that mammalian family members are nonessential in development. Cell–cell adhesion in mammals is more elaborate than in *Drosophila*. The greater size and complexity of the mammalian body and the substantially longer life span mean that tissues are subjected to greater mechanical stress than are insect tissues. As a result, mammals evolved mechanisms to strengthen cell–cell adhesion; for example, desmosomes strengthen adhesion in the heart and skin (for review see Green and Gaudry, 2000). Further, the relative contributions of different AJ regulators may be different in mammals, leading p120-depleted AJs in mammals to retain less function. The p120 family has expanded and diversified in mammals, and the organismal functions of family members remain to be tested by genetic analysis. Our data also leave open the question of the function of the cadherin JM region, since essential p120-independent functions remain possible. However, in this issue Pacquelet et al. (2003) present evidence that cadherins with mutations in the JM domain can fully rescue *DE-cadherin* function in *Drosophila*, suggesting that this region may not play an irreplaceable function.

p120 has also been suggested to be a Rho regulator and thus a regulator of the actin cytoskeleton. Disruption of a key Rho regulator would be predicted to have severe consequences for cytoskeletal regulation and thus morphogenesis: alterations in *Drosophila* Rho function disrupt many developmental events (for review see Settleman, 2001). Our data suggest that p120 is not an essential Rho regulator, since mutants are viable and we do not see drastic defects in the actin cytoskeleton in its absence (Fig. 5). However, we did see subtle defects in the progress of cells during dorsal closure that may reflect subtle underlying defects in actin organization. The initial studies suggesting that p120 regulates Rho were done in cells expressing elevated levels of p120 (Anastasiadis et al., 2000; Noren et al., 2000) and might not reflect a normal physiological function. However, we think it is more likely that p120 is one of several Rho regulators and that loss of p120 alone does not result in Rho misregulation. This is supported by the work of Magie et al. (2002) who found that both p120 and α-cat bind to Rho. If these two proteins are redundant Rho regulators, loss of one may be compensated for by the other. In one respect, our data conflict with those of Magie et al. (2002) who used double-stranded RNA interference (RNAi) to remove p120. They report severe morphogenetic defects that we did not see. RNAi removal of p120 might fail to trigger a compensatory mechanism that does come into play in our mutant. However, in Pacquelet et al. (2003) report that p120 RNAi has no effect. We also attempted *p120* RNAi and did not observe morphogenetic defects in *p120* RNAi-injected embryos that were not also seen in control RNAi experiments with *ftz* (unpublished data).

p120 is an important positive modulator of cell–cell adhesion

Although animals homozygous mutant for *p120* are viable and fertile, its conservation through 600 million yr of evolutionary time suggests it plays a role sufficient for natural selection to act on it. Our data suggest that one function of p120 is as an important positive modulator of adhesion. In its absence, cells and animals are much more sensitive to reductions in cadherin or catenin function (Fig. 6; Tables I–IV). These data are consistent with data generated in earlier experiments in cultured mammalian cells, though not necessarily consistent with models derived from these data that suggested an essential role for p120 in cell adhesion. Mutational alteration of the JM domain could often be compensated for by overexpressing cadherins (Thoreson et al., 2000) or by treating the cells with reagents altering tyrosine phosphorylation (Aono et al., 1999; Ohkubo and Ozawa, 1999). These data are consistent with the idea that the JM domain, and by extension p120, play modulatory roles rather than essential ones.

While this paper was under review, a paper was published that casts further light on this issue. Ireton et al. (2002) examined a mammalian cell line that expresses only mutant forms of p120 and expresses these at low levels. They found that this cell line exhibited impaired cell–cell adhesion and that this was rescued by restoration of wild-type p120. Further, they could also restore adhesion by overexpressing either wild-type cadherin or a cadherin that could not bind p120. These data are quite consistent with our own, suggesting that p120 positively promotes adhesion but that this deficit can be overcome by elevating cadherin levels.

The mechanism by which p120 modulates adhesion remain less clear. We saw no clear or consistent differences in the levels or localization of other AJ proteins in animals lacking p120 (Fig. 5) (though our data were consistent with the possibility that junctional localization of these proteins might be mildly reduced). The lack of a developmental phenotype further suggests that any difference in adhesion is likely to be subtle. The defects we saw in the dorsal closure front (Fig. 5 O) may reflect such subtle defects. In their cultured cell system, Ireton et al. (2002) observed that loss of p120 reduced the stability of E-cadherin. Although we did not observe differences in steady-state levels of DE-cad, cadherin stability may not be a limiting factor in wild-type *Drosophila.* Ireton et al. (2002) also observed reduced junctional accumulation of both β-cat and α-cat. Although we did not observe striking effects, the genetic interaction we observed with *arm* is also consistent with reduced assembly of AJ complexes. Given our data and those of Ireton et al. (2002), p120 could act in a wide variety of ways. For example, it might modulate assembly of cadherin–catenin complexes or their lateral clustering, it might alter cadherin stability (e.g., by competing for binding with presenilins), it might alter trafficking of cadherin–catenin complexes to or from the membrane, or it might modulate actin assembly at junctions via Rho or other mechanisms. Further work will be needed in flies and cultured mammalian cells to address these issues.

p120 localization suggests a possible role in strengthening adhesion during morphogenesis

Our data also provide the first comprehensive look at the localization of a p120 family member during embryogenesis. Overall, *Drosophila* p120 localization is largely consistent with what is known about its mammalian homologues. In most tissues, p120 localized both to AJs and the cytoplasm (Figs. 2 and 9), largely paralleling DE-cad and Arm. In addi-

tion to looking for junctional localization, we also looked for localization of p120 to other cellular structures. We did not note accumulation of p120 in nuclei, with the exception of the accumulation of p120-GFP in nuclei of syncytial embryos. However, our tissue survey was not exhaustive, and this does not rule out a shuttling role in which steady-state levels of nuclear p120 are low. p120-GFP localizes to centrosomes in early embryos (Fig. 8). The meaning of this remains to be determined.

Perhaps the most striking observation was that accumulation of p120 in AJs and other junctional structures was not as uniform as that of the core AJ proteins (Figs. 8 and 9). These data are consistent with our suggestion that p120 is not an essential AJ component as are Arm, DE-cad, and α-cat. Instead, p120 appears to be targeted to a subset of AJs and junctional structures, suggesting that cells differ in their requirements for p120. Consistent with this, *p120* mRNA accumulates at elevated levels in some cells undergoing morphogenetic movements (Fig. 7). Thus, p120 may confer upon junctions in which it accumulates properties that facilitate certain morphogenetic events, though our genetic analysis suggests it does so partially redundantly with other regulators. Our challenge in the future will be to determine the mechanisms by which p120 regulates adhesion and the identity of other regulators with which it may be redundant, and use this information to understand why some cells accumulate high levels of junctional p120 while others do not.

Materials and methods

Molecular biology

Degenerate PCR was done using the *Drosophila* Schneider cell plasmid cDNA library as template, with cycles as follows: 95°C for 3 min, 35 cycles of 95°C for 1 min, 44, 48, or 50°C for 1 min, and 72°C for 2 min, and 1 cycle at 72°C for 5 min. Successful primers: forward 5′-TAYYY-NCARCAYYTNTG-3′ encoding YLQHLC, reverse 5′-CANACRCARTTY-TCNAG-3′ encoding VENCVC). Genomic DNA was isolated as in http://www.fruitfly.org/about/methods/inverse.pcr.html and amplified in 50 μl with 60°C annealing. Primer sequences are available upon request. For RT-PCR, RNA was isolated from 20 larvae using TRIzol reagent and the manufacturer's protocol (Invitrogen Life Technologies), cDNA was generated using oligo-dT primers (Promega Reverse Transcription System), and 5 of 100 μl used for PCR. P element transformation constructs were generated with a fragment of *p120* cDNA extending from the start to stop codons. Six copies of the myc epitope (MEQKLISEEDLNE) were added, and this was cloned into the P element vectors UASg (UAS promoter) and pCasper3 UP2 RX polyA (ubiquitin promoter). p120GFP was generated by cloning p120 aa 1–728 in front of GFP in pNEGFPX.1 and then transferring into UASg and pCasper3 UP2 RX polyA. RNA in situ hybridizations were as in Duronio and O'Farrell (1994) using digoxigenin-labeled *p120* antisense RNA probe made using Boehringer Mannheim's Dig Labeling kit.

Two-hybrid analysis

pCK2, pCK4, pCK4 DEC, pCK4 DEC 1–4, and β-galactosidase assays are described in Pai et al. (1996). To make pCK4 DEC 14, 15, 16, 17, 19, 20, and 21, primers were designed to add a BamHI site to the 5′ end of the fragment and a stop codon and an EcoRI site to the 3′ end for cloning into pCK4. A similar approach was used to generate portions of p120 cloned into pCK2. Amino acid coordinates are as follows: pCK2-p120full (1–781), pCK2-p120-NT (1–194), pCK2-p120-CT (651–781), pCK2-p120-R1–8 (173–567), pCK2-p120-R1–10 (173–664), pCK2-p120-R3–10 (241–664), and pCK2-p120-R3-C (241–781).

Protein work and immunolocalization

p120 antisera were raised by the Pocono Rabbit Farm against a maltose-binding protein–p120 fusion containing its COOH-terminal 96 aa. For affinity purification, this region was expressed as a GST fusion, separated by SDS-PAGE and blotted to nitrocellulose. Protein extract preparation and immunoblotting were as in Peifer (1993). Antibody dilutions: rabbit or rat polyclonal anti-p120 (1:1,000, or 1:10 if affinity purified), mAb Arm N27A1 (1:250; Developmental Studies Hybridoma Bank [DSHB]), mAb myc9E10 (undiluted), mAb BicD (1:500; DSHB), mAb Pnut (1:30; DSHB), anti–DE-cad (DCAD1, 1:500), and anti–α-cat (1:1,000; both from T. Uemera and M. Takeichi (Kyoto University, Kyoto, Japan). IPs were as in Peifer (1993) with a buffer from Reynolds et al., (1994). For IPs, Myc or control BicD mAbs were concentrated fivefold by incubation with protein A–sepharose before extract addition. Fixation conditions: embryos (4% formaldehyde in 1× PBS, 1 mM $CaCl_2$:heptane [1:1], 20 min), brains (4% PFA in 1× PBS); ovaries (2% PFA in 1× PBS). All were blocked and stained in 1× PBS, 1% goat serum, 0.1% Triton X-100. Antibody dilutions for immunolocalization: affinity-purified rat anti-p120 (1:20 or 1:50), mAb Arm N27A1 (1:250), mAb myc9E10 (1:250), anti–β-tubulin E7 (1:500; DSHB), anti–γ-tubulin (1:500; Sigma-Aldrich), anti–DE-cad (DCAD2, 1:50; from T. Uemera and M. Takeichi, Kyoto University), and anti-Cnn (1:500; from T. Kaufman, Indiana University, Bloomington, IN). Tissue was mounted in Aquapolymount (Polysciences, Inc.) and visualized on Zeiss LSM 410 or 510 confocal microscopes (Carl Zeiss MicroImaging, Inc.). Digital images were prepared with Adobe Photoshop®.

Fly work

Mutations are described in Flybase (http://flybase.bio.indiana.edu/). Work was done at 25°C. Stocks were from the following: *Df(2R)M41A8*, shg^2, KG01086, prd-Gal4 (Bloomington Stock Center); shg^{G119}, and shg^{R69} (provided by U. Tepass, University of Toronto, Toronto, Ontario, Canada); His-GFP (provided by R. Saint, Adelaide University, Adelaide, Australia). The generation of *p120* deletions will be described in detail elsewhere (unpublished data). For genetic interaction tests, unbalanced male and females heterozygous for both $p120^{308}$ and *shg* were mated. To remove maternal p120, $p120^{308}$, *shg* /$p120^{308}$ females were crossed to $p120^{308}$, *shg/Cy* males. To assess interactions with Arm, arm^{YD35}/FM7; $p120^{308}/p120^{308}$ females were crossed to *FM7/Y;* $p120^{308}/p120^{308}$ males. Cuticles of lethal progeny were processed as in Cox et al. (1996).

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