Signaling and Adhesion Activities of Mammalian β-Catenin and Plakoglobin in *Drosophila*

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Abstract. The armadillo protein of *Drosophila* and its vertebrate homologues, β-catenin and plakoglobin, are implicated in cell adhesion and wnt signaling. Here, we examine the conservation of these two functions by assaying the activities of mammalian β-catenin and plakoglobin in *Drosophila.* We show that, in the female germ line, both mammalian β-catenin and plakoglobin complement an *armadillo* mutation. We also show that *shotgun* mutant germ cells (which lack *Drosophila* E-cadherin) have a phenotype identical to that of *armadillo* mutant germ cells. It therefore appears that armadillo's role in the germ line is solely in a complex with *Drosophila* E-cadherin (possibly an adhesion complex), and both β-catenin and plakoglobin can function in *Drosophila* cadherin complexes. In embryonic signaling assays, we find that plakoglobin has no detectable activity whereas β-catenin's activity is weak. Surprisingly, when overexpressed, either in embryos or in wing imaginal disks, both β-catenin and plakoglobin have dominant negative activity on signaling, an effect also obtained with COOH-terminally truncated armadillo. We suggest that the signaling complex, which has been shown by others to comprise armadillo and a member of the lymphocyte enhancer binding factor-1/T cell factor–family, may contain an additional factor that normally binds to the COOH-terminal region of armadillo.

Wnt genes encode secreted glycoproteins required for a large number of developmental processes in a variety of species (for reviews see Nusse and Varmus, 1992; Klingensmith and Nusse, 1994; Parr and McMahon, 1994). In the developing fly, the wnt-1 homologue, wingless, is used at many times and places. Most notably, it is required for the patterning of each segment, and for pattern formation and cell proliferation in imaginal discs (Nusslein-Volhard et al., 1984; Peifer et al., 1991; Neumann and Cohen, 1996). Downstream members of the wingless signaling pathway have been identified and characterized genetically. They include *disheveled*, *shaggy*/*zeste-white-3*, *armadillo*, and *pangolin.* Vertebrate homologues of all four genes have also been identified, and recent biochemical work has identified a potential wingless receptor (*D*-frizzled*2*) (Bhanot et al., 1996), which also has vertebrate homologues (Wang et al., 1996). Most relevant to this study, armadillo is homologous to both β-catenin and plakoglobin, also known as γ-catenin (Peifer and Wieschaus, 1990; Peifer et al., 1992).

In *Xenopus laevis*, overexpression of β-catenin leads to ectopic axis formation, an effect also obtained with overexpressed *Xenopus* wnt, implying that β-catenin is part of the wnt pathway (Sokol et al., 1991; Funayama et al., 1995). β-Catenin is clearly required for axis formation as depletion of β-catenin messages leads to ventralization and loss of dorsal structures (Heasman et al., 1994). Overexpression of plakoglobin also induces ectopic axes in *Xenopus* (Karnovsky and Klymkowsky, 1995), suggesting that it too can act in the wnt pathway.

In addition to their roles in wnt signaling, β-catenin and plakoglobin are components of adherens junctions. β-Catenin forms complexes linking classical cadherins to the actin cytoskeleton via α-catenin (Kemler, 1993; Aberle et al., 1994; Oyama et al., 1994), and formation of such complexes is required for cell–cell adhesion (Nagafuchi and Takeichi, 1988; Ozawa et al., 1990; Hirano et al., 1992). Mice missing E-cadherin (Larue et al., 1994; Riethmacher et al., 1995) or β-catenin (Haegel et al., 1995) exhibit defects in adherens junction function during embryogenesis. Plakoglobin can also form similar cadherin complexes which might be functionally significant (Franke et al., 1989; Knudsen and Wheelock, 1992; Sacco et al., 1995; Chitaev et al., 1996). Plakoglobin is best known for being a major component of desmosomes, junctional structures mediating the interaction between desmosomal cadherins (desmogleins and desmocollins) and intermediate filaments, and is the only known junctional component found in both desmosomes and adherens junctions (Cowin et al., 1986; Garrod, 1993; Koch and Franke, 1994; Chitaev et al., 1996). In contrast, β-catenin does not form complexes with desmosomal cad-

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herins despite strong sequence similarity between classical and desmosomal cadherins (Collins et al., 1991; Mechanic et al., 1991; Wheeler et al., 1991). There seems to be only one *armadillo* gene in *Drosophila.* Apparently two distinct adhesion functions have evolved in vertebrates whereas one suffices in *Drosophila.*

As expected from its homology with β-catenin, armadillo appears to be required for cell–cell adhesion in *Drosophila*, and evidence so far suggests that the roles in signaling and cadherin complexes are separable (Peifer, 1995; Orsulic and Peifer, 1996; Sanson et al., 1996). Armadillo interacts biochemically with both *Drosophila* E-cadherin (DE-cadherin)[1] (Oda et al., 1994) and α-catenin (Dα-catenin) (Oda et al., 1993) and is believed to participate in the adherens junction complex. Defects in cell–cell adhesion and epithelial integrity can be seen in embryos depleted for maternal and zygotic armadillo (Cox et al., 1996). Likewise, an adhesion role has been suggested in the germline; females lacking armadillo activity in the germ line do not lay eggs and their ovaries are abnormal (Peifer et al., 1993; and this work). Because the *shaggy*/*zeste-white-3* and *dishevelled* genes are not required for oogenesis, it appears that these defects are not due to lack of wingless signaling. Therefore, it was suggested that the defects of *armadillo* mutant germ cells are due only to a lack of cell adhesion or a cadherin-related function. In this paper we add support to this conclusion by showing that an *armadillo* mutant germline displays defects identical to those of a DE-cadherin (*shotgun*) mutant.

The sequence similarity between armadillo and its vertebrate counterparts is strong (71% overall identity with β-catenin and 63% with plakoglobin). Most of the similarity lies in a central "core" repeat region containing 13 42–amino acid repeats, and in the α-catenin binding site at the end of the NH_2-terminal domain. The central repeats mediate interactions with a variety of proteins including adenomatous polyposis coli (Rubinfeld et al., 1993), fascin (Tao et al., 1996), pangolin/T cell factor (TCF)/lymphocyte enhancer binding factor-1 (LEF) (Behrens et al., 1996; Brunner et al., 1997; Riese et al., 1997), and DE-cadherin (Oda et al., 1994). The sequence similarity is significantly lower in the NH_2- and COOH-terminal domains, the COOH-terminal region of plakoglobin being only 36% identical to armadillo with large intervening gaps. (The plakoglobin COOH-terminal domain is less than half as long as armadillo's and lacks the gly/pro rich region.)

In this paper we examine the functional homology between armadillo, β-catenin, and plakoglobin in vivo, assaying both their signaling and adhesion activities. As there is only one *armadillo* gene in *Drosophila* compared with two in vertebrates, encoding β-catenin and plakoglobin, we can assess the activities of these proteins individually without the potential complication arising from the presence of other functional homologues. We find that despite their normal participation in different junctions, both mammalian proteins can fulfill the adhesion (or cadherin-related) role of armadillo. In terms of signaling activity in the wingless pathway, we find that, in the absence of endogenous armadillo, plakoglobin shows none whereas that of β-catenin is low. Surprisingly, in an overexpression assay, both proteins act as dominant negatives on wingless signaling. Because these proteins diverge most at their carboxy termini, we asked whether this dominant negative activity resulted from the absence of a functional COOH-terminal domain. We tested the effect of overexpressing a COOH-terminally truncated form of armadillo. We found that this mutant also has dominant negative activity, thus implying a role for the COOH terminus in the regulation of signaling.

1. *Abbreviations used in this paper*: DE-cadherin, *Drosophila* E-cadherin; LEF, lymphocyte enhancing binding factor; MBP, maltose-binding protein; TCF, T cell factor; XTCF, xenopus T cell factor.

Materials and Methods

Protein Interaction Assays

Expression and purification of maltose-binding protein (MBP)–fusion proteins including β-catenin and plakoglobin have been described in Aberle et al. (1996). Dα-catenin was cloned in the prokaryotic expression vector pQE60. Construction was as follows: *Drosophila* α-catenin carrying a COOH-terminal histidine tag was cloned from pBS-DCT-EK (kind gift of T. Uemura and M. Takeichi, Kyoto University, Kyoto, Japan) by PCR using Pwo-polymerase (Boehringer Mannheim, Mannhein, Germany). An NH_2-terminal fragment was amplified with primer Dα53 5′-TCAAGATCTATGCTGCAGCCAGCTCTC. One primer was sufficient for amplification since dα53 has a false priming site 18 bp in length on the sense strand at position 942 of the cDNA. A COOH-terminal fragment was amplified with primers Dα758 5′ATGCTTAAAAAACATTCAACTATGC and Dα2856r 5′AGTCGGCCGTTAAGATCTAACAGCGTCAGCAGGACTC. The NH_2-terminal fragment was cut with BglII/MroI and the COOH-terminal fragment with MroI/MscI and MscI/BglII. All three fragments were cloned simultaneously into the BglII site of the prokaryotic expression vector pQE60 (Qiagen Inc., Hilden, Germany). Dα-catenin was expressed in *Escherichia coli* strain M15.

Cells were lysed in buffer H (PBS, pH 7.4, 10 mM imidazole, 0.25% Triton X-100, 10 μ/ml of each DNaseI, PMSF, and leupeptin) using a French pressure cell. His-tagged proteins were isolated from the soluble fraction by affinity chromatography on a Ni^{2+} chelate resin equilibrated with lysis buffer. After absorption of the proteins, the resin was washed with buffer H containing 10, 20, 40, and 80 mM imidazole, pH 7.4. Dα-catenin was eluted stepwise with 250 mM imidazole in PBS. Protein fractions were pooled, adjusted to 50% glycerol, and stored at −20°C.

For in vitro reconstitution, recombinant proteins were mixed in PBS, pH 7.4, containing 0.01% Triton-X100. Protein complexes were collected with amylose agarose, as described in Aberle et al. (1996).

Immunoprecipitations to assay in vivo interactions were done according to Hoschuetzky et al. (1994), except that the lysates were obtained by homogenizing the embryos in a Dounce homogenizer.

Drosophila Expression Constructs (under armadillo Promoter Control)

To express armadillo, β-catenin, and plakoglobin under *armadillo* control, the expression vector pCASV40 was constructed. An *armadillo* promoter fragment was excised from pCaSpeR-arm-β-Gal (Vincent et al., 1994) with EcoRI and KpnI and transferred to pCaSpeR-4 (Thummel and Pirrotta, 1992) digested with EcoRI and KpnI. An SV-40 polyA termination sequence was ligated into the BamHI/StuI(blunt) sites of this vector. cDNAs encoding armadillo and human plakoglobin were cut from the prokaryotic expression vectors pGEXArm (Aberle et al., 1996) and pGEXPlako (Aberle et al., 1994) as BamHI/NotI fragments and ligated blunt ended (reverse orientation) into CASV40 digested with BamHI and NotI. Mouse β-catenin cDNA was cut from pGEXBeta (Aberle et al., 1994) as an NdeI/NotI fragment and ligated blunt ended (reverse orientation) into pCASV40 digested with BamHI and NotI. These P-element constructs were injected, and 10 homozygous transformant lines were established for each construct.

Drosophila Expression Constructs (under UAS/Gal4 Control)

UAS-armadillo. An armadillo cDNA was transferred from the E9 plasmid (E9 cDNA cloned into Bluescript KS(+); Riggleman et al., 1989) as a

KpnI/NotI fragment into pUAST (Brand and Perrimon, 1993) digested with KpnI and NotI.

UAS-β–catenin. A cDNA encoding β-catenin was transferred from pGEXBeta as an NdeI(klenow)/NotI fragment and ligated into pUAST digested with EcoRI(klenow) and NotI.

UAS-plakoglobin. A cDNA encoding plakoglobin was transferred from pGEXPlako as a BamHI(klenow)/NotI fragment and ligated into pUAST as for β-catenin.

UAS-armΔC. A truncated form of an *armadillo* cDNA was inserted in pUAST in such a way as to introduce an in frame stop codon. The site of truncation was at the NdeI at position 2102 downstream of the ATG. This leaves the first 31 residues of repeat 13 unaffected (the XM19 mutation introduces a stop codon at nucleotide 2043). The end generated by NdeI was treated with Klenow and ligated blunt to the T4-polished KpnI end of pUAST. This construction results in one unrelated amino acid (leu) and a stop codon being introduced at the amino terminus of residue 31 of armadillo repeat 13.

UAS-wg^{ts}. As described in Wilder and Perrimon (1995).

Stocks, Constructs, and Generation of Germline Clones

Phenotypes of *arm*^{XM19}, *arm*^{XP33}, and *arm*^{XK22} have already been described (Peifer et al., 1993; Cox et al., 1996). *yw* flies were used in transformation and as controls. Generation of germline clones to produce germlines and embryos depleted of maternal product and containing *armadillo*^{XM19}, *armadillo*^{XP33}, *armadillo*^{XK22}, or *shotgun*^{IG29} mutant product only were performed using the FLP recombinase–dominant female sterile technique (Chou and Perrimon, 1992). The stocks, *XXy f/ovo^{D1} FRT101*; *hsflp-38*, *y w hsflp-12*; *CyO/ScO*, and *FRT-G13 ovo^{D1}/CyO* are as described in Chou and Perrimon (1992).

For the generation of *armadillo*^{XM19} and *armadillo*^{XP33} germline clones, second to third instar larvae generated from the cross between *arm FRT101/FM7* females and *ovo^{D1} FRT101*; *hsflp* males were heat shocked in an air flow incubator at 37°C for 4 h. This induces site-specific homologous mitotic recombination at the FRT sequences. Due to the presence of the ovo^{D1} female sterile mutation, which allows only germ cells homozygous for the *armadillo* mutation to develop, the only fertile females hatching from this cross will have *armadillo* mutant germlines.

Germline clones homozygous for *armadillo*^{XK22} cannot complete oogenesis. Therefore, to assess the rescue of *armadillo*^{XK22} germline clones by any transgene, the following stock was constructed: *arm*^{XK22} *FRT101/FM7*; ** (where * denotes the required transgene). These stocks were then used as above to generate *arm*^{XK22} *FRT101/ovo^{D1} FRT101*; */*hsflp-38* females with *armadillo*^{XK22} mutant germlines and one copy of the required transgene. This method was also used with *armadillo*^{XM19} to provide the rescuing transgene or the *arm*-gal-4 driver maternally in *armadillo*^{XM19} germline clones.

For the generation of *shotgun* germline clones, a recombinant stock *y w*; *shg*^{IG29} *FRT-G13/CyO* was constructed. Second to third instar larvae from the cross between *y w hsflp-12/Y;FRT-G13 ovo^{D1}/CyO* males and *y w*; *shg^{IG29} FRT-G13/CyO* females were heat shocked for 4 h at 37°C as above. From this cross, females lacking CyO (genotype *y w*; *hsflp-12/+*; *FRT-G13 ovo^{D1}/FRT-G13 shg^{IG29}*) will contain only *shotgun*^{IG29} germ cells.

Whole-Mount Immunocytochemistry and Actin/DNA Visualization

Embryos were dechorionated and fixed for 30 min at the interface of a heptane/7.5% formaldehyde in PBS fix. (For staining with antiarmadillo, anti–β-catenin or antiplakoglobin, the fix also contained Tween-20 to a final concentration of 0.1%.) The aqueous phase was removed and the embryos devitellinized by adding equal amounts of methanol and shaking vigorously. Embryos were then washed for 30 min in PBS/0.1% Triton-X/1%BSA/0.05% azide (PTX), and incubated with the primary antibody overnight at 4°C. For antiengrailed staining, a biotinylated secondary followed by peroxidase histochemistry was used. Antiarmadillo, anti–β-catenin, or antiplakoglobin were detected with fluorescent secondaries. All secondary antibodies were from Jackson Laboratory (West Grove, PA). The following primary antibodies were used: antiengrailed (mAb 4D9 from the Developmental Studies Hybridoma Bank, University of Iowa, Des Moines, IA), antiarmadillo (mAb N2 7A1 from the Developmental Studies Hybridoma Bank), anti–β-catenin (mAb C19220 from Transduction Laboratories, Lexington, KY), and antiplakoglobin (mAb C26220 from Transduction Laboratories).

For actin/DNA visualization, ovaries were fixed in 4% paraformaldehyde (PFA) in PBS, 0.1% Tween-20 (PBT) for 20 min, washed in PBT, and stained for 20 min in rhodamine-phalloidin (5% in PBT; Sigma Chemical Co., Poole, UK). They were then washed again in PBT, then in 0.1% Tween-20 in H_2O, and stained for 3 min in Hoechst (Sigma B-2883, bis-BENZIMIDE, 10 mg/ml in H_2O/0.1% Tween-20) and mounted in Fluoromount-G (Southern Technologies Associates, Birmingham, AL).

Cuticle and Wing Preparations

Embryos collected overnight and aged 24 h were dechorionated, washed in PTX, and mounted in Hoyers' medium. In some cases the vitelline membranes were removed manually after attaching the embryos to double-sided sticky tape.

Wings were dissected in isopropanol and mounted in Euparal.

Results

The core experiments described below involve testing the ability of various transgenes to rescue *armadillo* mutations. Three *armadillo* alleles (Fig. 1) were used to separate the extent to which the transgenes could rescue either the adhesion and/or the signaling defects of *armadillo* mutations. *armadillo*^{XM19} encodes the longest protein of the three and is the weakest allele. Evidence suggests that it has little (if any) signaling activity in the absence of wild-type maternal product (Peifer and Wieschaus, 1990). However, the armadillo^{XM19} protein contains fully intact DE-cadherin and Dα-catenin–binding sites, and mutants show no adhesion defects even in the absence of the wild-type maternal contribution. *armadillo*^{XP33} and *armadillo*^{XK22} are intermediate and strong alleles, respectively. Homozygotes show a strong segment polarity phenotype indicating no signaling function. In addition, they show adhesion defects in the germline (*armadillo*^{XK22}; Peifer et al., 1993) and embryo (*armadillo*^{XP33}; Cox et al., 1996) when wild-type maternal contribution is removed. These adhesion defects are be-

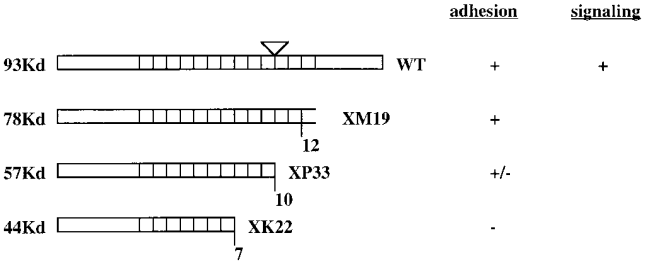

Figure 1. armadillo alleles. Schematic diagram of armadillo protein and alleles showing activity in wingless signaling and adhesion (adapted from Peifer and Wieschaus, 1990). All three alleles consist of point mutations leading to premature stop codons (Peifer and Wieschaus, 1990). *armadillo*^{XM19} encodes a protein truncated in the 13th repeat and shows a weak zygotic segment polarity phenotype that is enhanced to a severe phenotype on removal of wild-type maternal contribution. It maintains full adhesion presumably because its binding domains to Dα-catenin and DE-cadherin are intact. *armadillo*^{XP33} encodes a protein truncated at the end of the 10th repeat and exhibits a strong zygotic segment polarity phenotype. In the absence of wild-type maternal product adhesion defects similar to those of a *shotgun* mutant are observed in embryos (Cox et al., 1996). However, the viability of eggs from *armadillo*^{XP33} germlines indicates sufficient activity to complete oogenesis. *armadillo*^{XK22} exhibits a severe zygotic segment polarity phenotype and females with *armadillo*^{XK22} germlines do not complete oogenesis, with egg chambers exhibiting defects identical to cadherin (*shotgun*) mutants and no eggs being laid.

lieved to arise because of the inability of the mutant proteins to bind DE-cadherin.

In all experiments that required removal of maternal armadillo or DE-cadherin, we used the FLP recombinase–dominant female sterile germline clone technique (Chou and Perrimon, 1992). Females generated with this technique have mutant germlines but are viable because they are somatically wild-type. These females were used either to analyze the rescue of ovary phenotypes or to generate embryos lacking wild-type maternal contribution.

Loss of armadillo or DE-Cadherin (shotgun) Function Leads to Identical Phenotypes in the Germline

Localization of armadillo to adherens junctions in the follicle cells and the phenotype of mutant egg chambers has suggested an adhesion function for armadillo in oogenesis (Peifer et al., 1993). If *armadillo* mutant germ cells are truly deficient for adhesion, we would expect germ cells lacking DE-cadherin function (mutant for *shotgun*) to look identical to *armadillo* mutant germ cells. We have examined the germline defects of a strong *shotgun* mutant, *shotgun*^{IG29} (Nusslein-Volhard et al., 1984), and compared them to those of a strong *armadillo* mutant (*armadillo*^{XK22}).

Egg chambers lacking armadillo function (*armadillo*^{XK22} germlines) show a variety of defects (Fig. 2; see also Peifer et al., 1993). Defects include random positioning of the oocyte within the egg chamber, irregular shape and size of nurse cells and nuclei, floating ring canals, and actin inclusions (Fig. 2, *D* and *E*). Cytoplasmic actin filaments in the nurse cells do form, although they are somewhat disorganized and nurse cell dumping (the rapid transfer of nurse cell contents into the oocyte occurring after stage 10) is inhibited. Egg chambers derived from *shotgun*^{IG29} germline clones (lacking DE-cadherin function) show strikingly similar phenotypes (Fig. 2, *F–I*). The oocyte is often mispositioned, and nurse cells have an irregular size and shape and can be multinucleate. Similar disruptions of the actin cytoskeleton, such as floating ring canals (Fig. 2, *arrowheads*) and actin inclusions, are also visible. Again nurse cell dumping is inhibited despite the formation of cytoplasmic actin filaments. Identical phenotypes exhibited by germ cells mutant for *armadillo* and *shotgun* suggests that the two proteins act in the same complex there. This complex is likely to function in cell adhesion, although we cannot exclude yet another activity such as, for example, in organizing the cytoskeleton (see below). In accordance with our analysis of mutant germ lines, it has been recently reported that embryos carrying weaker alleles of *armadillo* and *shotgun* also display similar phenotypes. Germ cells carrying either the *armadillo*^{XP33} mutation or *shotgun*$^{P34-1}$ mutations can survive oogenesis, but the embryos display

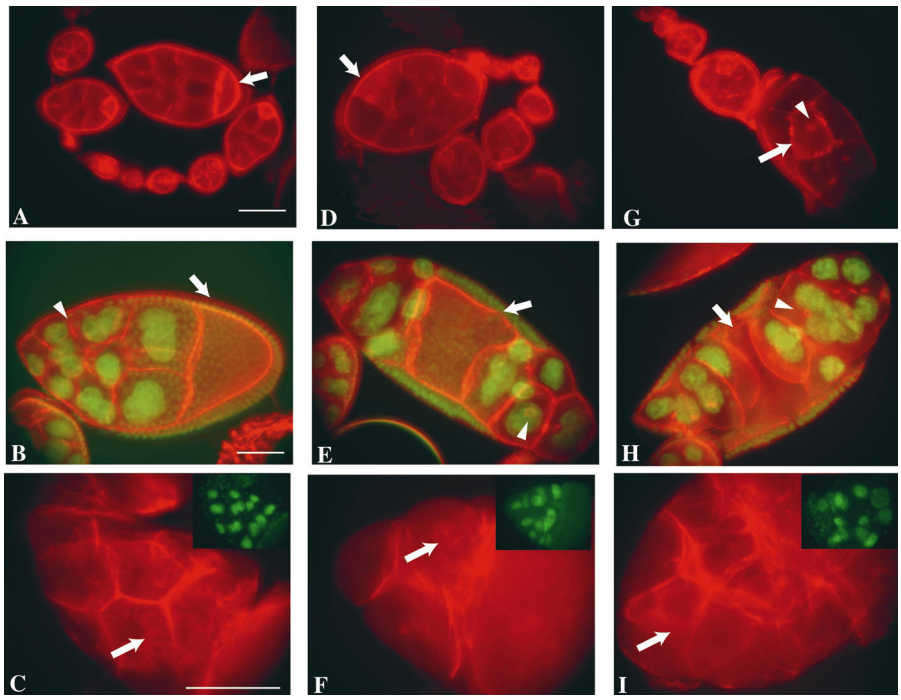

Figure 2. armadillo and *shotgun* have similar phenotypes in the germline. (*A*, *D*, and *G*) Egg chamber progressions (stages 1–8) stained with rhodamine-phalloidin to detect filamentous actin. (*B*, *E*, and *H*) Stage 10 egg chambers double labeled with rhodamine-phalloidin to detect F-actin and Hoechst to show DNA. (*C*, *F*, and *I*) Stage 10 egg chambers at the onset of nurse cell dumping stained with rhodamine-labeled phalloidin to detect filamentous actin. Insets show Hoechst (Sigma B-2883) staining. In wild-type egg chambers (*A–C*), the cortical actin cytoskeleton surrounds the germ cells and follicle cells with a heavy accumulation around the oocyte (*A* and *B*). The nurse cells are joined by ring canals (*arrowheads*). Throughout successive egg chambers of the ovariole the oocyte is always localized to the posterior of the egg chamber (*arrows*). Note also the regular size and shape of the nurse cell nuclei (*B*). Cytoplasmic actin filaments polymerize in the nurse cells at stage 10 before the onset of nurse cell dumping (*C*). In *armadillo*^{XK22} egg chambers (*D–F*), the position of the oocyte appears random, often mislocalized away from the posterior of the egg chamber (*D* and *E*). The nurse cells have irregular shape and size, often becoming multinucleate (*E*), and we see floating ring canals (*arrowhead*) and actin inclusions. Cytoplasmic actin filaments appear at stage 10 (*F*, *arrow*), but they are somewhat disorganized and nurse cell dumping fails to occur. However, the nurse cell nuclei maintain their positions within the nurse cells and do not appear to block the ring canals (a common feature of other "dumpless" mutants, see text; *F*, *inset*). *shotgun*^{IG29} egg chambers display a similar mislocalization of the oocyte (*G* and *H*, *arrows*). In addition, the presence of floating ring canals (*arrowheads*) and actin inclusions within misshaped nurse cells suggests a similar disruption of the actin cytoskeleton as seen in *armadillo* mutant egg chambers. Cytoplasmic actin filaments also appear at stage 10, but they are disorganized as in *armadillo*^{XK22} egg chambers (*I*, *arrow*). Again nurse cell dumping fails to occur, although the nurse cell nuclei do not appear to block the ring canals (*I*, *inset*). Bar, 100 μm.

a similar cuticle phenotype due to disruption of epithelial integrity by loss of adhesion (Cox et al., 1996; Tepass, 1996; Tepass et al., 1996).

The random positioning of the oocyte in *armadillo* and *shotgun* mutants suggests that cell adhesion may play a role in oocyte localization within the egg chamber. At the same time, actin disruption in these mutants points to a role for cadherin/catenin complexes in organizing the actin cytoskeleton. Actin disruption is not universal, however, because intact ring canals are still present. Maybe disruption occurs only in regions that contain DE-cadherin/catenin complexes, such as the cell membrane. The absence of nurse cell dumping in *armadillo* germ lines might thus be due to the failure of the cortex to provide the driving force for dumping as it is believed to do in the wild-type (Gutzeit, 1986). It should be pointed out here that a common reason for a dumpless phenotype is the blockage of ring canals by floating nuclei as seen in mutants such as *chickadee*, *singed*, and *quail* (encoding *Drosophila* profilin, fascin, and villin, respectively). In such mutants, the cytoplasmic actin filaments that anchor the nurse cell nuclei to the cell membrane fail to polymerize (Cooley et al., 1992; Cant et al., 1994; Mahajan-Miklos and Cooley, 1994). However, the phenotype of *armadillo* and *shotgun* germ cells is distinct as we never observed nuclei blocking ring canals (Fig. 2, *F* and *I*). Clearly further work is required to assess the role of cadherin complexes, if any, in organizing the actin cytoskeleton. To leave open this possibility, we will refer below to the "cadherin-related activity" of armadillo instead of its "adhesion function."

Mammalian β-Catenin and Plakoglobin Can Form Functional Complexes with Drosophila E-Cadherin and α-Catenin

Before attempting to rescue *armadillo* mutations with the mammalian homologues, we considered whether the cadherin–catenin and catenin–catenin interactions are conserved across species. First, we investigated the ability of Dα-catenin to interact with mammalian β-catenin and plakoglobin in in vitro reconstitution assays. As can be seen in Fig. 3 *A*, Dα-catenin interacts with both β-catenin and plakoglobin. This was expected because Dα-catenin and mouse α-catenin are 60% identical. The binding sites themselves seem to be conserved, because plakoΔ128/139, which contains an in frame deletion of its α-catenin binding site, showed a strongly reduced affinity to Dα-catenin. Second, we determined whether mammalian β-catenin and plakoglobin can interact with Dα-catenin and DE-cadherin in vivo. We expressed β-catenin and plakoglobin in transgenic *Drosophila* embryos, prepared extracts from such embryos, and assayed protein interactions with immunoprecipitation experiments. Fig. 3 *B* shows that immunoprecipitates obtained with anti–β-catenin or antiplakoglobin antibodies do contain endogenous Dα-catenin (Fig. 3 *B*, lanes *4* and *6*), although, in lesser amounts than immunoprecipitates obtained with antiarmadillo. Likewise, as can be seen in Fig. 3 *C*, both β-catenin and plakoglobin expressed in transgenic flies interact in vivo with endogenous DE-cadherin.

Next, we asked whether the interactions demonstrated above are functional. We assayed the ability of transgenes

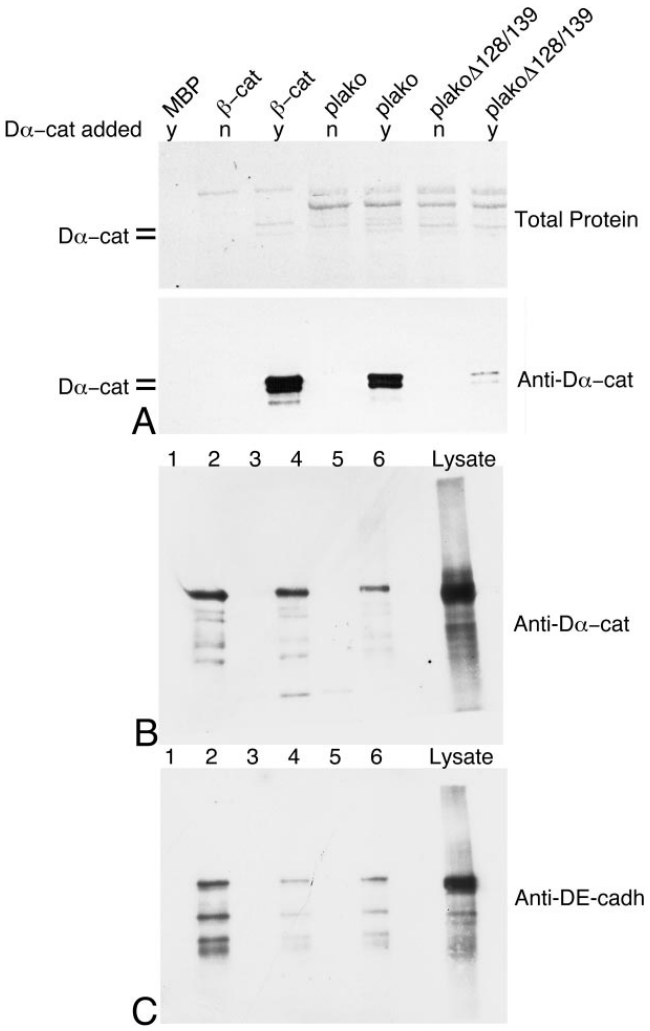

Figure 3. Mammalian β-catenin and plakoglobin interact with Dα-catenin and DE-cadherin. (*A*) The interaction between bacterially expressed β-catenin or plakoglobin and Dα-catenin. Recombinant MBP-tagged β-catenin and plakoglobin fusion proteins were incubated with or without Dα-catenin (as indicated) in association buffer. Formed complexes were affinity precipitated with amylose beads, washed and analyzed by SDS-PAGE (*top*) or by immunoblotting with polyclonal anti–α-catenin antibodies (M12K; Aberle et al., 1994) that cross-react with Dα-catenin (*bottom*). As can be clearly seen in the lower panel, both β-catenin and plakoglobin pull down α-catenin while PlakoΔ128/139 does so only very weakly. MBP-plakoglobin appears as a double band due to proteolytic degradation. *MBP*, maltose-binding protein; *PlakoΔ128/139*, deletion of amino acids 128–139. (*B* and *C*) Protein interactions in *Drosophila* embryos. For B, extracts were prepared from embryos carrying one of three transgenes: one encoding armadillo (lanes *1* and *2*), one encoding β-catenin (lanes *3* and *4*), or one encoding plakoglobin (lanes *5* and *6*). Lysates were immunoprecipitated with the appropriate antibody, antiarmadillo in lane *2*, anti–β-catenin in lane *4* and antiplakoglobin in lane *6* or with antiMyc as a control (lanes *1*, *3*, and *5*), and the immunoprecipitates were loaded on an SDS gel. The lane labeled "Lysate" was loaded with extract from wild-type embryos. Clearly, armadillo, β-catenin, and plakoglobin expressed from the transgenes interact with endogenously expressed Dα-catenin. Note that four times more material was loaded in lanes *3–6* indicating a weaker interaction of Dα-catenin with β-catenin and plakoglobin than with armadillo. The experiment in *C* is the same as in *B* except that the blot was probed with anti–DE-cadherin. Here, twice as much material was loaded in lanes *3–6*. Again, the interaction is weaker with β-catenin and plakoglobin than with armadillo.

encoding β-catenin and plakoglobin to rescue the germline and embryonic cadherin-related defects of *armadillo* mutants. As a positive control, we used a transgene encoding wild-type armadillo that brings about complete rescue (data not shown). First, to look at rescue in the germline, we used females with germlines carrying the strong allele *armadillo*^{XK22} (see Materials and Methods for details) containing one copy of the relevant transgene. Transgenes encoding either β-catenin or plakoglobin completely rescue the defects observed in *armadillo*^{XK22} ovarioles (>90% egg chambers appeared wild-type). The oocyte is localized at the posterior of the egg chamber (Fig. 4, *A* and *B*, β-catenin; *D* and *E*, plakoglobin). The nurse cells show regular positioning and shape and nurse cell dumping occurs normally.

Secondly, to look at rescue of adhesion in embryos, we used the intermediate allele *armadillo*^{XP33} which, in the absence of wild-type armadillo (maternal and zygotic) can complete oogenesis, but displays an embryonic phenotype (Fig. 4, *B–G*) similar to that of intermediate *shotgun* mutants (also lacking wild-type maternal and zygotic contribution) (Cox et al., 1996; Tepass, 1996; Tepass et al., 1996). We looked at the ability of transgenes encoding β-catenin and plakoglobin to rescue this embryonic "adhesion" phenotype (see Materials and Methods for details). Both β-catenin and plakoglobin bring about complete rescue (Fig. 4, *H* and *J*): there are no holes in either the dorsal or ventral cuticle faces. However, although the integrity of the cuticle is restored, patterning is abnormal: the ventral epidermis forms a denticle lawn resembling that of *wingless* mutants (Fig. 4, *H–J*, *arrows*; *wg*^{cx4}). Thus, when provided zygotically, both β-catenin and plakoglobin have enough activity to form adherens junctions in the embryo and rescue the adhesion phenotype while not appearing to rescue signaling.

Although β-catenin and plakoglobin clearly have the ability to function as adhesion molecules, they do not appear to be as active as wild-type armadillo. This is apparent when we consider the rescue of the germline cadherin-related defects of the strong *armadillo*^{XK22} allele. In addition to this rescue, females can lay eggs, and a small percentage of them (2% β-catenin, 1% plakoglobin) are fertilized. (These embryos [Fig. 4, *C* and *F*] cannot usually be obtained because of the requirement for armadillo in the germ line.) Although development begins in these embryos, the transgenes are not sufficient for a normal pattern. The head regions and parts of the cuticle are missing much like in a strong zygotic *shotgun* mutant, suggesting a possible lack of adherens junction function during embryonic development. Thus, whereas there is sufficient activity from β-catenin and plakoglobin to rescue the cadherin-related defects of *armadillo*^{XK22} germ lines, there appears to be insufficient activity for the formation of wild-type adherens junctions in the embryo. This is reminiscent of what is seen with the *armadillo*^{XP33} allele. In the absence of maternal and zygotic wild-type contribution, the *armadillo*^{XP33} allele encodes a protein that has sufficient activity to support oogenesis but insufficient activity for embryogenesis leading to a phenotype similar to that of strong *shotgun* mutants. This implies that there is a higher requirement for the cadherin-related function of armadillo in the embryo than in the germline. Adding extra doses of the transgene does not improve the phenotype, indicating that expression level of the transgene is probably not the limiting factor. Rather, it is likely that cross-species protein–protein interactions are not as stable as those within a species.

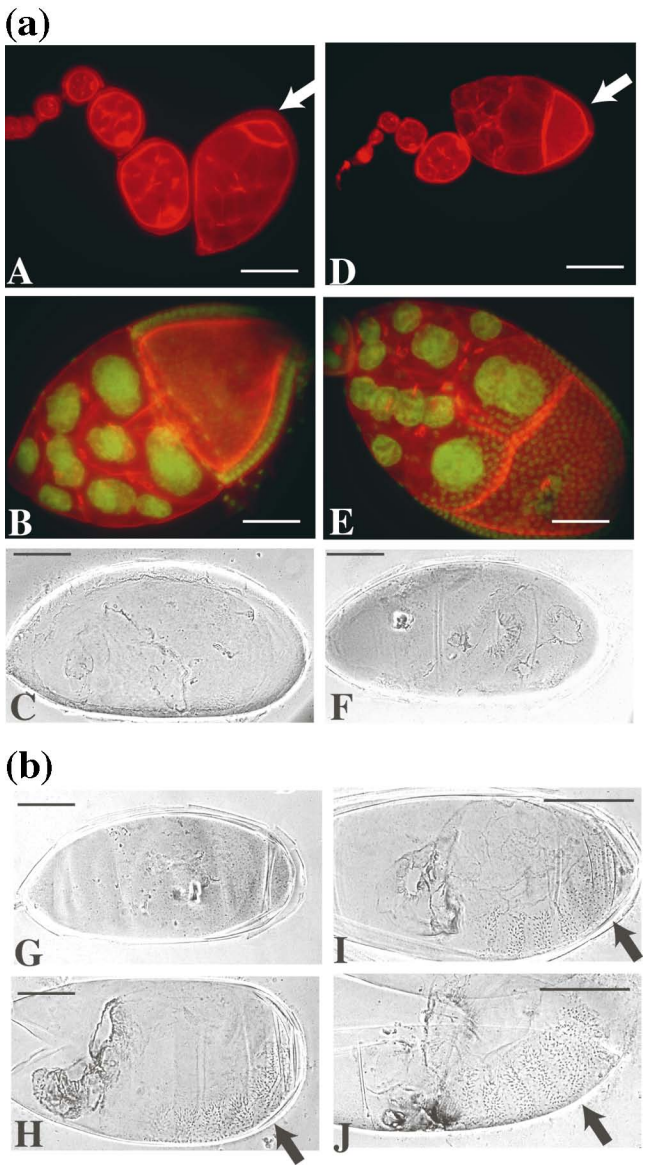

Figure 4. Rescue of cadherin related function. β-catenin and plakoglobin rescue both germline and embryonic adhesion defects. Rescue of an *armadillo*^{XK22} germline by β-catenin (*A–C*) and plakoglobin (*D–F*). For both β-catenin and plakoglobin, ~90% of egg chambers containing one copy of the rescuing transgene show posterior localization of the oocyte (*A* and *D*, *arrows*) at all stages of development. The nurse cells show regular size and shape. Fertilized eggs are laid by both β-catenin (*C*) and plakoglobin (*F*) rescued *armadillo*^{XK22} germline clone females. In both cases the cuticles show some denticle formation (*C* and *F*, *arrows*) but have severe defects resembling a strong zygotic *shotgun* mutant. Rescue of the *armadillo*^{XP33} germline clone adhesion ("crumbs-like") phenotype (*G–J*). Embryos from germlines containing *armadillo*^{XP33} protein only display a "crumbs" phenotype (*G*) similar to that of strong *shotgun* mutants. This phenotype is rescued by one copy of β-catenin (*H*) or plakoglobin (*J*) provided paternally. Both cuticles show the denticle lawn (*arrows*) typical of a *wingless* null mutant (*wg*^{CX4}; *I*).

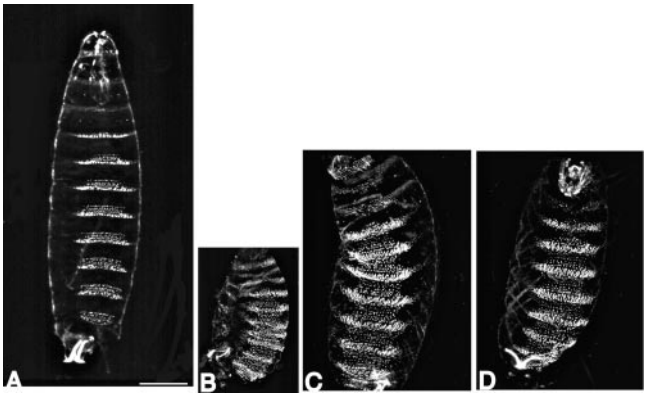

Figure 5. Rescue of signaling in the presence of maternal armadillo. Zygotic rescue of the *armadillo*^{XK22} strong segment polarity phenotype. Wild-type (*A*). *armadillo*^{XK22}/Y embryos show a strong segment polarity phenotype characterized by cuticles approximately 1/3 wild-type length, loss of head structures, and a lawn of denticles (*B*). Both β-catenin (*C*) and plakoglobin (*D*) slightly rescue *armadillo*^{XK22}/Y embryos. Most notably some length of the cuticle is restored.

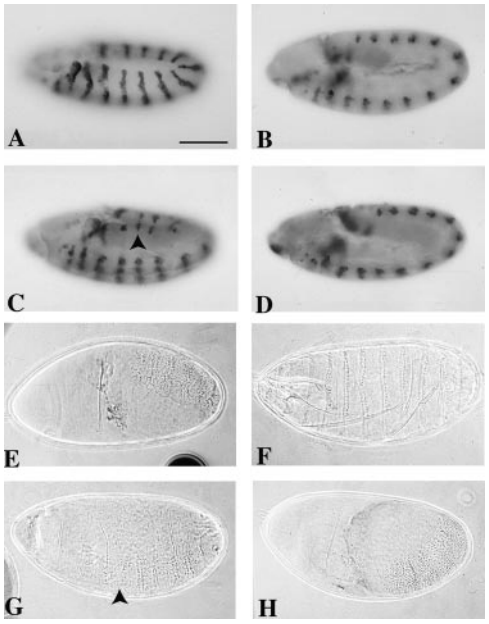

Figure 6. Rescue of signaling in the absence of maternal armadillo. Plakoglobin shows no signaling function in the absence of maternal wild-type armadillo and β-catenin shows only slight function. (*A–D*) Engrailed staining in embryos from *armadillo*^{XM19} germline clones. Engrailed expression in wild-type embryos (*A*). Embryos containing only *armadillo*^{XM19} mutant protein show a complete loss of engrailed expression in the ectoderm (*B*). One copy of β-catenin provided paternally rescues expression slightly in patches of dorsal/lateral ectoderm (*C*, *arrow*), but one copy of plakoglobin shows no rescue (*D*). (*E–H*) Cuticle phenotypes of embryos from *armadillo*^{XM19} germline clones with β-catenin and plakoglobin transgenes provided maternally. *armadillo*^{XM19} germline clones show a strong signaling phenotype (*E*), which is rescued to wild-type by one copy of a control *armadillo* transgene (*F*). One copy of β-catenin shows partial rescue including a slight increase in length of the cuticle and occasional regions of naked cuticle (*G*, *arrowhead*), whereas plakoglobin shows no rescue (*H*).

Indeed, the cytoplasmic domain of DE-cadherin is only 30% identical to mouse E-cadherin, and this may explain why it binds less tightly to β-catenin and plakoglobin than to armadillo (as shown in Fig. 3).

β-Catenin and Plakoglobin as Signaling Molecules

We looked at the ability of β-catenin and plakoglobin to replace the second function of armadillo—its transduction of the wingless signal. We assayed their ability to rescue a strong segment polarity phenotype. This phenotype was obtained either by removing zygotic function with a strong allele (*armadillo*^{XK22}; as in Fig. 5) or by removing wild-type maternal function in a weak allele (germline clones of *armadillo*^{XM19}; as in Fig. 6). Neither of these conditions seem to lead to adhesion defects (Peifer and Wieschaus, 1990).

We first looked at the rescue of *armadillo*^{XK22} embryos by zygotic expression of transgenes encoding β-catenin or plakoglobin. Both β-catenin (Fig. 5 *C*) and plakoglobin (Fig. 5 *D*) show rescue of the strong segment polarity phenotype to that of a weak phenotype. For β-catenin, 86% of the embryos are rescued to the phenotype shown and 58% for plakoglobin, indicating that β-catenin is slightly more active. Adding an extra dose of the transgene does not ameliorate the phenotype, suggesting that the level of expression is not a determining factor in the degree of rescue observed. We also considered the length of the embryos as a measure of pattern rescue (see Lawrence et al., 1996) and noted that both β-catenin and plakoglobin can rescue the length of *armadillo*^{XK22} embryos from about 1/3 of wild-type length to at least 3/4. β-Catenin often rescues to full wild-type length.

We wanted to test whether the activity observed above depends on the presence of wild-type maternal armadillo. To address this issue we analyzed embryos from germline clones containing only *armadillo*^{XM19}, which appears to have no signaling activity (see Materials and Methods for details), and assayed the rescue of their strong segment polarity phenotype by zygotic expression of β-catenin or plakoglobin (Fig. 6). Expression of engrailed in the ectoderm (used as an indicator of wingless signaling) is completely lost in embryos derived from *armadillo*^{XM19} germline clones (Fig. 6 *C*, expression in the central nervous system is still present), confirming that *armadillo*^{XM19} has no signaling function. (The strong segment polarity cuticle phenotype also indicates this [Fig. 6 *E*].) One copy of the plakoglobin transgene provided zygotically does not rescue engrailed expression (Fig. 6 *D*), and one copy of β-catenin shows a slight rescue indicated by patches of engrailed expression on the lateral surfaces of the embryo (Fig. 6 *B*). When these embryos are allowed to make cuticle, the pattern seen is in accordance with the analysis of engrailed expression: no rescue by plakoglobin and slight rescue by β-catenin (data not shown). To eliminate the possibility that the lack of rescue by zygotically provided plakoglobin might be due to expression of the rescuing transgene occurring too late, we repeated the experiments, but this time providing the rescuing transgene maternally (Fig. 6, *E–H*). Results support the evidence obtained from the zygotic rescue experiments and show that whereas a

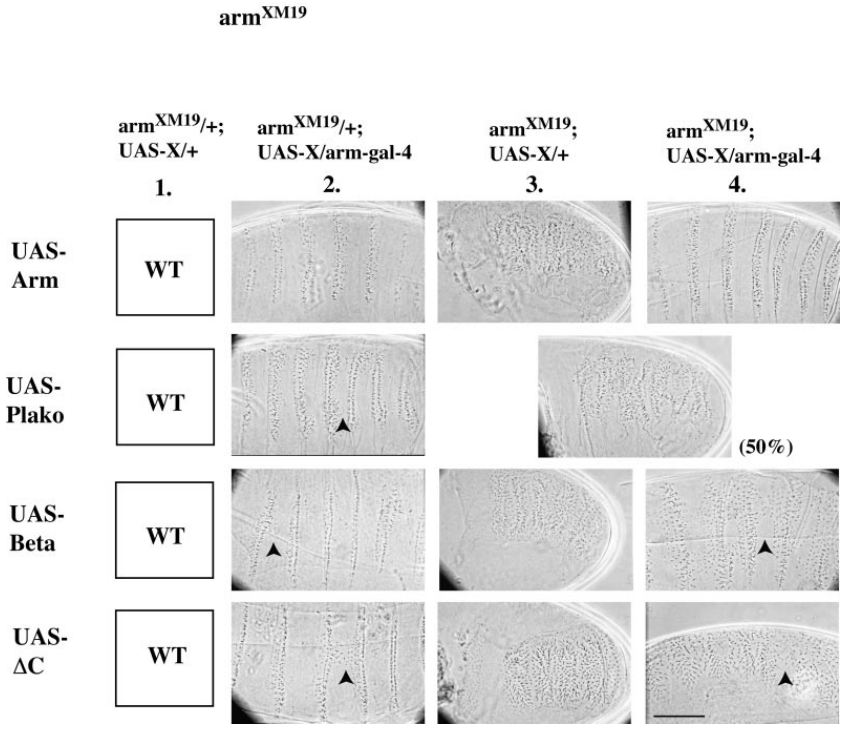

Figure 7. Overexpression of β-catenin, plakoglobin, or armΔC in embryos. Phenotypes observed from the cross between *armadillo*^{XM19} germline clone females carrying one copy of the arm-gal-4 driver and males homozygous for either UAS-plakoglobin, UAS-β-catenin, or UAS-armΔC. There are four equally possible outcomes to this cross. As expected, with no transgene overexpression, *armadillo*^{XM19} heterozygotes are phenotypically wild-type (column *1*), and *armadillo*^{XM19} embryos show a strong segment polarity phenotype (column *3*). Overexpression of a UAS-armadillo wild-type transgene (used as a control) in *armadillo*^{XM19} heterozygotes leads to slight loss of denticles from the denticle belts (column *2*; consistent with enhanced activation of the wingless pathway), and in *armadillo*^{XM19} embryos there is complete rescue of the strong segment polarity phenotype (column *4*). In contrast, for plakoglobin, we observe that ~50% of the progeny have the strong segment polarity phenotype of *armadillo*^{XM19} (column *3* and *4*), therefore we conclude that plakoglobin shows no rescue. For both β-catenin and armΔC we observe slight rescue of the *armadillo*^{XM19} phenotype leading to regions of naked cuticle (column *4*, *arrows*). Plakoglobin, β-catenin, and armΔC all show ectopic denticles (column *2*, *arrows*) in the naked cuticle regions of *armadillo*^{XM19} heterozygotes suggesting dominant negative activity.

control armadillo transgene rescues to wild-type (Fig. 6 *F*), plakoglobin does not rescue the strong segment polarity cuticle phenotype (Fig. 6 *H*), and β-catenin shows only partial rescue (Fig. 6 *G*).

The evidence from rescue of *armadillo*^{XM19} mutants suggests that, in the absence of wild-type maternal contribution, plakoglobin cannot function in the *Drosophila* wingless pathway, and β-catenin has only slight activity. This evidence is further supported by the rescue of *armadillo*^{XP33} germline clones (see above; Fig. 4 *b*) where both β-catenin and plakoglobin can fully rescue the adhesion phenotype, but the cuticles obtained are identical to that of *wingless* null mutants.

To ensure that the near lack of rescue by β-catenin and plakoglobin is not due to insufficient expression, we also assayed the activity of these two proteins expressed at high levels with the Gal-4/UAS system (Brand and Perrimon, 1993). We made females with a germline carrying *armadillo*^{XM19} as well as one copy of the arm-gal-4 driver and crossed them to males homozygous for UAS-β-catenin or UAS-plakoglobin (Fig. 7; see Materials and Methods for detail). This enables us to add high levels of β-catenin or plakoglobin in embryos lacking wild-type *armadillo* activity. There are four equally possible genotypes arising from this cross (Fig. 7). These include heterozygote *armadillo*^{XM19}, which are viable (column *1*), and hemizygote *armadillo*^{XM19} males, which show a strong segment polarity phenotype (column *3*, maternal contribution is absent). In the case of overexpressed β-catenin, we observe four phenotypic classes, each making up ~25% of the progeny. To each we can assign a genotype as indicated in Fig. 7. We find that, as seen in column *2*, overexpressed β-catenin leads to a weak segment polarity phenotype (extra denticles in the naked cuticle regions) in *armadillo*^{XM19} heterozygous embryos. These embryos are normally identical to wild-type; therefore this indicates a dominant negative effect. Nevertheless, *armadillo*^{XM19} mutant embryos are partially rescued by overexpressed β-catenin (column *4*) showing that β-catenin has some positive signaling activity as well. In the case of plakoglobin, this phenotypic class (partial rescue) is absent while the proportion of *armadillo*^{XM19}-like embryos goes to ~50%, indicating that plakoglobin has no rescuing activity but like β-catenin, it has dominant negative activity (column *2*).

These results support our earlier conclusion that in the absence of maternal contribution, plakoglobin has no signaling activity and β-catenin has only slight activity. They also suggest that, when overexpressed, both β-catenin and plakoglobin have a dominant negative effect.

Activation of the wingless pathway leads to the stabilization and cytoplasmic accumulation of armadillo (Van Leeuwen et al., 1994). A 54–amino acid region of armadillo containing a glycogen synthase kinase-3 consensus phosphorylation site has been shown to be required for the control of stability by wingless (Pai et al., 1997). Cytoplasmic accumulation can be seen in stripes in embryos stained with antiarmadillo antibodies (Peifer et al., 1994). To find out if β-catenin and plakoglobin are also stabilized in wingless-responding cells, we stained embryos carrying a β-catenin or plakoglobin transgene with the appropriate antibodies. As can be seen in Fig. 8 *B*, β-catenin does accumulate in stripes like armadillo does (although not as clearly), indicating that β-catenin responds to the upstream components of the pathway despite its relative

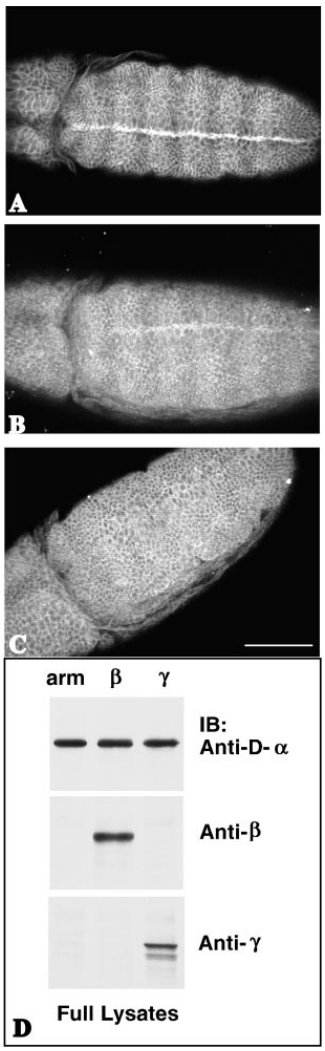

Figure 8. Effect of wingless on the cytoplasmic levels of armadillo, β-catenin, and plakoglobin. Wild-type embryo stained with antiarmadillo (*A*) shows stripes of cytoplasmic accumulation in cells responding to wingless as shown previously by Peifer et al. (1994). Transgenic embryo expressing β-catenin and stained with anti–β-catenin also shows cytoplasmic accumulation in stripes although not as clearly (*B*). In contrast, no plakoglobin stripes are detected in embryos expressing it from a transgene (*C*). Note also that plakoglobin is not stabilized in the ventral midline as armadillo and β-catenin are. All embryos are around stage 9 and in roughly the same orientation. No signal was detected in control (nontransgenic) embryos stained with anti–β-catenin or antiplakoglobin (not shown). Extracts made from the transgenic embryos shown in *A–C* were run on polyacrylamide gel (one lane per transgene as indicated; γ refers to plakoglobin), blotted, and probed with antibodies as shown (*D*). Anti–α-catenin was used as a control for loading. These immunoblots confirm that the antibodies we used do not cross-react. They also show that both β-catenin and plakoglobin are abundantly expressed from the transgenes. The weaker signal from plakoglobin could in part be explained by the lower affinity of antiplakoglobin (with bacterially produced protein, antiplakoglobin consistently gives a weaker signal than anti–β-catenin at equal amounts of antigen loaded; not shown).

inability to transduce the signal further downstream. In contrast, we were unable to detect stripes of plakoglobin accumulation in the cytoplasm. This was surprising since the region surrounding the glycogen synthase kinase-3 consensus is highly conserved between armadillo and plakoglobin (even more so than between armadillo and β-catenin). Our antibody against plakoglobin clearly recognizes plakoglobin in situ since we do see membrane staining. It may be that the antibody does not recognize modified, stabilized plakoglobin. Alternatively, plakoglobin may not be stabilized by wingless because another, less conserved region of the protein, possibly in the COOH teminus, is required for stabilization. If the second alternative is correct, one would conclude that plakoglobin does not respond to upstream components of the wingless pathway. In any case, we suggest that it cannot act downstream since it has no rescuing activity even when overexpressed in embryos at levels known to lead to dominant negative effects on endogenous armadillo activity.

Overexpression of β-Catenin and Plakoglobin in Wing Primordia

Studies from *Xenopus* have shown that both β-catenin (Funayama et al., 1995) and plakoglobin (Karnovsky and Klymkowsky, 1995) can induce ectopic axes when overexpressed in early (4–32 cell) embryos, and that the central core region is both necessary and sufficient for this activity. Considering the high degree of homology in the core region, we were surprised by the poor signaling activity of the mammalian proteins in *Drosophila.* To confirm our findings in embryos, we have assayed the activity of these proteins in another region of the fly. We asked whether β-catenin and plakoglobin could activate the wingless pathway when overexpressed in wild-type *Drosophila* wings. We used the MS1096-Gal-4 driver (Capdevila and Guerrero, 1994) to direct expression of UAS-β-catenin and UAS-plakoglobin in wing discs. For comparison, we also overexpressed armadillo and wingless with the same driver.

Overexpression of both wingless and armadillo in the wing induces ectopic vein material and the formation of margin bristles in the blade (Fig. 9). These effects are also observed upon overexpression of Dishevelled suggesting that they represent activation of the wingless pathway. For both wingless and armadillo, we have assessed the dose response; in the case of wingless by driving the expression of a temperature-sensitive protein at different temperature (UAS-wg^{ts}) and in the case of armadillo by driving transgenes of various strengths (as determined by position in a phenotypic series). At a low activity, wingless overexpression induces ectopic vein material, whereas at high activity, ectopic bristles form (Fig. 9 *D*). In the case of armadillo, ectopic veins and bristles appear simultaneously throughout the phenotypic range (Fig. 9 *G*). The effects of overexpressed β-catenin (Fig. 9, *B* and *C*) and plakoglobin (Fig. 9, *E* and *F*) differ somewhat from those of overexpressed wingless and armadillo. We do see ectopic vein formation throughout the wing blade, but instead of extra bristles, there is a loss of both anterior and posterior margin bristles (Fig. 9, *C* and *F*, *arrows*). At high levels of overexpression, we see loss of the margin altogether leading to a smaller wing blade (Fig. 9 *E*). The loss of margin structures, including bristles, is a characteristic of weak *wingless* loss of function (Couso et al., 1994) and therefore confirms our previous conclusion that β-catenin and plakoglobin have dominant negative activity on wingless signaling.

It is difficult to explain the ectopic vein formation seen in wings expressing either β-catenin or plakoglobin. All the evidence we have obtained so far suggests that, in the absence of maternal contribution, plakoglobin has no signaling activity in the wingless pathway. It is possible, however, that plakoglobin and β-catenin can potentiate the pathway when overexpressed in the presence of wild-type maternal contribution, and that this ability is reflected by the ectopic vein material (see also Discussion). Or, it could be that another signaling pathway affecting veins is at work, possibly mediated by the core domain, which exhibits the

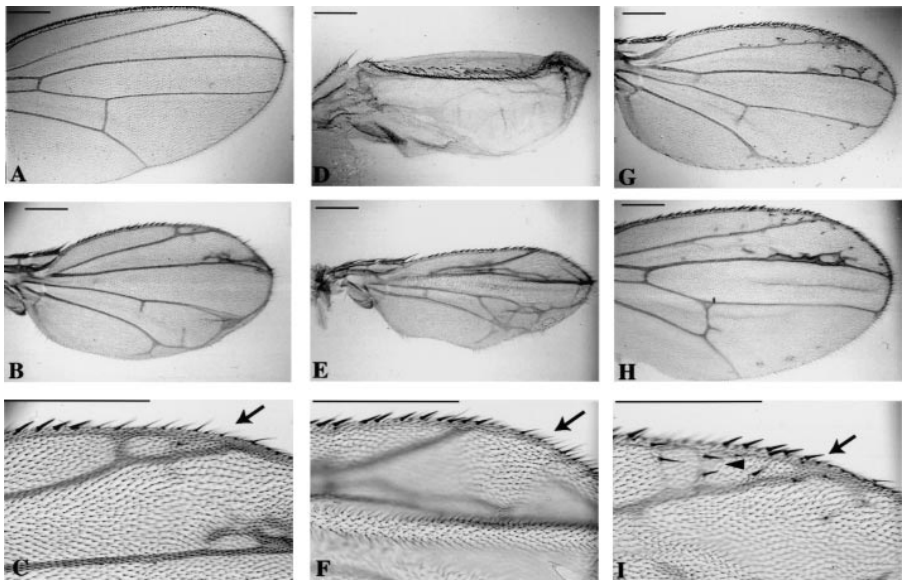

Figure 9. Overexpression of β-catenin, plakoglobin, and armΔC in wing primordia. Using the Gal-4/UAS system (Brand and Perrimon, 1993), β-catenin and plakoglobin (and armadillo and wingless for comparison) were overexpressed in wild-type wing discs. (*A*) Wild-type. Both UAS-wingless (*D*) and UAS-armadillo (*G*) induce ectopic veins and bristles in the wing blade. Overexpressed β-catenin (*B*) and plakoglobin (*E*) both cause ectopic vein formation but no ectopic bristles are seen (*C*, catenin; *F*, plakoglobin higher magnification). However, unlike with UAS-wingless and UAS-armadillo, there is loss of margin bristles in both cases (*C* and *F*, *arrows*). UAS-armΔC (*H* and *I*) causes ectopic veins and bristles in the wing blade (*H*, *arrowhead*) similarly to UAS-armadillo. However, UAS-armΔC also leads to loss of margin bristles as seen with UAS-β-catenin and UAS-plakoglobin.

highest sequence similarity within the armadillo/β-catenin/plakoglobin family.

The COOH-terminal Domain of Armadillo Is Required for Signaling

The sequence similarity between plakoglobin/β-catenin and armadillo is significantly lower in the COOH-terminal domain. To ask whether this divergence might explain the dominant negative activity (and thus indicate a requirement for the COOH terminal in signaling), we overexpressed a COOH terminally deleted form of armadillo (armΔC; see Materials and Methods) (Fig. 9, *H* and *I*). Like β-catenin and plakoglobin, overexpression of armΔC in wing discs causes a loss of margin bristles indicating dominant negative activity. However, armΔC also induces ectopic bristles and veins in the wing blade, although at a frequency lower than wild-type armadillo (Fig. 9 *I*, *arrowhead*). Thus we conclude that, like β-catenin, armΔC does have a dominant negative effect on wingless signaling while at the same time and somewhat paradoxically, retaining some positive activity. This result is confirmed in embryos where overexpression of armΔC in *armadillo*^{XM19} germline clones showed slight rescue of the strong segment polarity phenotype (Fig. 7, column *4*), but also a dominant negative effect (column *2*) similar to that seen with β-catenin and plakoglobin.

Discussion

The Role of Armadillo in the Germline

In the germline, the function of armadillo seems to be solely as part of a complex with E-cadherin insofar as *shotgun* (DE-cadherin) mutants look identical to *armadillo* mutants. This, and the fact that the wingless pathway does not seem to operate in the germline (Baker, 1988; Peifer et al., 1993) is consistent with an adhesion function for armadillo in the germ line. We have suggested that, in addition to the potential role of cadherin-mediated adhesion in localizing the oocyte, the armadillo/cadherin complex may be required for a functional actin network in the cortex of germline cells. The observation that germline cells lack well-defined adherens junctions and that armadillo protein shows only a low level punctate distribution along the cell surfaces (Peifer et al., 1993) instead of the expected dense staining at sites resembling classical adherens junctions also suggests a more diverse role for armadillo in the cortical actin network.

β-Catenin and Plakoglobin Can Act as Adhesion Molecules in Drosophila

Both β-catenin and plakoglobin provide a link between junctional complexes and the cytoskeleton in vertebrate cells. Plakoglobin is believed to be primarily a component of desmosomes that link to intermediate filaments (Cowin et al., 1986) whereas β-catenin participates in adherens junctions that connect to the actin cytoskeleton. Because neither desmosomes nor intermediate filaments have yet been identified in *Drosophila* (Tepass and Hartenstein, 1994), it may be that plakoglobin arose from a β-catenin–like molecule and took up a specialized role in desmosomes, in the process retaining its ability to participate in adherens junctions. In vitro binding studies have shown that in addition to interacting with desmosomal cadherins, plakoglobin can bind to the adherens junction components E-cadherin (Ozawa et al., 1989; McCrea et al., 1991) and α-catenin (Hinck et al., 1994), although with considerably lower affinity than β-catenin (McCrea and Gumbiner, 1991; Aberle et al., 1994). We have assayed the ability of plakoglobin and β-catenin to form functional adherens junction complexes in *Drosophila.* We show that both plakoglobin and β-catenin bind in vitro to Dα-catenin as well as to DE-cadherin suggesting that the molecular assembly at adhesion complexes has been extensively conserved

through evolution. We next applied functional tests of conservation in the *armadillo*^{XK22} (strong allele) female germline and in embryos derived from *armadillo*^{XP33} (intermediate allele) germline clones. Both conditions lead to phenotypes believed to reflect defects in a cadherin-related function. In accordance with our in vitro results, we find that both β-catenin and plakoglobin rescue these *armadillo* mutations in flies. Therefore, plakoglobin has the ability to interact functionally with DE-cadherin and Dα-catenin, most likely in a similar way as has been shown for vertebrate cadherin complexes. It has never been demonstrated that E-cadherin/plakoglobin/α-catenin complexes are sufficient for adhesion in the absence of β-catenin; our results show that plakoglobin could replace β-catenin if expressed appropriately, although its main functional requirement would be in desmosomes.

Signaling Activity of β-Catenin and Plakoglobin

We have assayed the signaling activity of β-catenin and plakoglobin in *Drosophila* by looking at their ability to rescue the segment polarity phenotype of *armadillo* mutants. In the absence of wild-type armadillo, plakoglobin has no signaling activity regardless of whether it is provided maternally or zygotically and in the same assay, β-catenin has weak activity. In the presence of maternal armadillo (zygotic rescue of the strong *armadillo*^{XK22} allele), plakoglobin does show some activity. It could be that, by virtue of its ability to form functional adhesion complexes, plakoglobin competes with wild-type maternal armadillo for binding to DE-cadherin creating a larger cytoplasmic pool of armadillo that can act in the signaling pathway (see also below). Alternatively, or in addition, it could compete with armadillo for its negative regulator shaggy/zeste-white-3 thus preventing phosphorylation/degradation of maternal armadillo, leading to a cytoplasmic enrichment available for signaling. The weak signaling activity of β-catenin in *Drosophila* (even in the absence of wild-type armadillo) as opposed to the total lack of signaling by plakoglobin confirms the suggestion, based on sequence comparison that β-catenin is the true homologue of armadillo (Peifer et al., 1992). However, from the high sequence identity between β-catenin and armadillo, and the demonstrated ability of *Drosophila* dishevelled to induce ectopic axis formation in *Xenopus* (Rothbacher et al., 1995), one might have expected more complete rescue. Homologues of upstream components such as dishevelled and shaggy/zeste-white-3 have been identified and shown to be conserved in frogs and mice. Recently, potential downstream components (LEF-1, XTCF-3 [xenopus T cell factor]) have been uncovered in frogs (Behrens et al., 1996; Molenaar et al., 1996) and a fly homologue, pangolin, has been shown to be involved in wingless signaling (Brunner et al., 1997).

LEF-1 and XTCF-3 are transcription factors that have been shown in vitro to interact with the central repeat region of β-catenin and induce its translocation to the nucleus. Interestingly, LEF-1 and XTCF-3 also interact with plakoglobin and translocate it to the nucleus in a similar fashion (Behrens et al., 1996; Molenaar et al., 1997) suggesting that plakoglobin might be a signaling molecule as well. Moreover, wnt signaling in mammalian tissue culture cells leads to an increase in plakoglobin levels (and, of course, of β-catenin as well; Bradley et al., 1993; Hinck et al., 1994). Consistent with this, plakoglobin contains a region matching a consensus GSK-3 phosphorylation site implying that it might be under wnt control. This contrasts with the complete lack of signaling by plakoglobin (even when overexpressed) that we report as well as with the apparent insensitivity of plakoglobin levels to wingless in *Drosophila* (Fig. 8). Also, the loss of plakoglobin function in mouse or *Xenopus* does not indicate a signaling function. In mouse, plakoglobin knock-out leads to a loss of desmosomes in heart tissues and skin defects (Bierkamp et al., 1996; Ruiz et al., 1996) whereas depletion of plakoglobin in *Xenopus* results in partial adhesion defects (Kofron et al., 1997). More work is needed to determine whether plakoglobin is indeed regulated by GSK-3 and whether its interaction with LEF-1 is functionally significant.

We found that, in an overexpression assay, both β-catenin and plakoglobin have a dominant negative effect in embryos and on bristle formation in the wing margin (where wingless signaling is required). This was surprising in the case of β-catenin because it partially rescues the signaling defect of *armadillo* mutations. A similarly paradoxical result was obtained with the overexpression of a COOH-terminal deletion of armadillo (armΔC). ArmΔC encodes a protein that is truncated within the 13th (and last) armadillo repeat, the same repeat where the *armadillo*^{XM19} mutation introduces a nonsense codon (although the truncation is not at an identical position and armΔC is longer by 21 residues; see Materials and Methods). Homozygous *armadillo*^{XM19} embryos display a rather weak segment polarity phenotype, indicating that in the presence of wild-type maternal armadillo, this truncated protein makes some positive contribution to wingless signaling. Likewise, armΔC shows a positive effect on signaling in adult precursors where, when overexpressed, it induces margin bristles in the wing blade. However, in apparent contradiction with these positive effects, overexpressed armΔC has a dominant negative effect at the margin where it suppresses bristle formation. We have suggested that an adhesion-competent protein (such as β-catenin, plakoglobin, or armΔC) could have a positive effect at low level by freeing up residual maternal armadillo from the membrane, making it available for signaling. However, β-catenin and armΔC clearly have signaling activity even in the absence of maternal wild-type armadillo. This activity is weak, and when either protein is expressed at a high level it brings down the level of signaling, probably by titrating away one or more components required for a fully active signaling complex. For example, β-catenin, plakoglobin, and armΔC are likely to have retained the ability to bind *Drosophila* LEF-1/TCF/pangolin, as this interaction is mediated by the central repeat region. But, this complex may not be sufficient to fully activate transcription of target genes due to loss of, or a nonfunctional COOH-terminal domain. In this way, pangolin would be titrated out of the wingless pathway and into a nonsignaling (or weakly signaling) complex leading to the dominant negative effects observed.

Our results suggest that a factor binding to the COOH-terminal domain of armadillo is required for maximum signaling activity. We anticipate that the COOH-terminal domain of β-catenin is equally important in vertebrates,

although this proposal appears inconsistent with the findings in *Xenopus* that the core region is sufficient to activate the pathway when overexpressed. One possible explanation for the discrepancy is that at very high expression levels, as often attained in *Xenopus* (e.g., Heasman et al., 1994), the core is weakly active on its own and that once the axis-specifying process has been initiated, it is self-enhanced by endogenous wild-type protein. Nevertheless, it is clear from the low signaling activity and dominant negative effect of armΔC that the COOH-terminal region of armadillo is crucial to transduce the signal.

It appears that the different domains of armadillo and β-catenin required for signaling have been differentially conserved in evolution: The central core region and its interaction with pangolin is highly conserved (*Drosophila* armadillo core binds to mouse LEF-1; Riese et al., 1997), while the COOH-terminal domain and its interaction with other factors is more diverged. In contrast, the various domains required for cell adhesion (such as the domains of interaction with α-catenin and cadherin) have been coordinately conserved. The signaling domains that have not been conserved may correspond to those that are not required for adhesion. This appears true for the COOH-terminal region that, while being required for signaling, is poorly conserved and completely dispensable for cell adhesion (Orsulic and Peifer, 1996).

We are grateful to Jose Casal, Matthew Freeman (both, Medical Research Council), and Daniel St. Johnston (Wellcome Institute, Cambridge, UK) for critical reading of the manuscript, and Acaimo Gonzalez-Reyes (Wellcome Institute) for advice. We would also like to thank Mariann Bienz (MRC), Anthony Bretscher (Cornell University, Ithaca, New York), Peter Lawrence, Hugh Pelham, and Benedicte Sanson (all, MRC) for comments.

Received for publication 24 March 1997 and in revised form 31 October 1997.

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