

The product of the *Drosophila* segment polarity gene *armadillo* is part of a multi-protein complex resembling the vertebrate adherens junction

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SUMMARY

Sequence similarity between the *Drosophila* segment polarity protein Armadillo and the vertebrate adherens junction protein β -catenin raised the possibility that adherens junctions function in transduction of intercellular signals like that mediated by Wingless/Wnt-1. To substantiate the sequence similarity, properties of Armadillo were evaluated for consistency with a junctional role. Armadillo is part of a membrane-associated complex. This complex includes Armadillo, a glycoprotein similar in size to vertebrate cadherins, and the

Drosophila homolog of α -catenin. Armadillo co-localizes with junctions that resemble vertebrate adherens junctions in morphology and position. These results suggest that *Drosophila* and vertebrate adherens junctions are structurally similar, validating use of Armadillo and its associated proteins as a model for vertebrate adherens junctions.

Key words: *armadillo*, β -catenin, adherens junctions, cadherins, *Drosophila*

INTRODUCTION

The epithelial sheet is the fundamental structural unit of many tissues. When individual cells form an epithelium they associate via a set of molecules that constitute adherens junctions. These junctions are thought to mediate both cell adhesion and communication between cells, and also to integrate the cytoskeletons of individual cells. Vertebrate adherens junctions components have been identified (reviewed by Magee and Buxton, 1991), and the genes encoding them cloned. The central component of the junction is a member of the cadherin family of transmembrane cell adhesion molecules. The extracellular domains of these molecules are thought to interact homotypically to mediate adhesion between adjacent cells. Cadherins also share a conserved intracellular domain that is thought to organize the formation of a complex of proteins that include the molecules β -, γ -, and δ -catenin. A distinct but analogous complex of proteins forms in the other cell-cell adhesive junction type, the desmosome (reviewed by Magee and Buxton, 1991).

The functions of adherens junctions as a whole and of some of the individual components have been analyzed in cultured mammalian cells, and the results of these experiments support some postulated roles of adherens junctions. Cell lines have been found that lack either cadherins (Nagafuchi et al., 1987; Nose et al., 1988; McNeill et al., 1990) or β -catenin (Hirano et al., 1992). These cell lines exist as individual non-adherent cells. When these cell lines are transfected with a gene encoding the missing component, the cells gain the ability to adhere and at times can assume many, if not all, of the other properties of epithelial

cells. These experiments further suggest that the cadherins alone do not serve a 'glue' function in mediating adhesion, but rather that the complex as a whole is responsible for the entire suite of cellular responses to cell-cell contact. Lacking in this analysis, however, are genetic tools allowing an in vivo structure/function study of adherens junctions during development. In addition, these studies have not addressed the function of β -catenin, due to the lack of a cell line missing this component.

A possible solution to this problem was provided by realization that the *Drosophila* segment polarity gene product Armadillo (Arm) is related in sequence to two different components of cell-cell adhesive junctions, plakoglobin (Peifer and Wieschaus, 1990; Franke et al., 1989) and β -catenin (McCrea et al., 1991). Plakoglobin and β -catenin associate with the cytoplasmic domains of different cell adhesion molecules of the cadherin super-family (reviewed by Magee and Buxton, 1991). β -catenin-containing complexes make up the adherens junctions that anchor the actin cytoskeleton, while plakoglobin-containing complexes form desmosomes and anchor intermediate filaments. The sequence similarity suggested that Arm might function in adhesive junctions in *Drosophila*. The experiments reported here were designed to test this potential connection.

A role for Arm in adhesive junctions would be particularly intriguing due to the connection between Arm function and the transmission of the *wingless* intercellular signal (Klingensmith et al., 1989; Peifer et al., 1991). *wingless* and its vertebrate homolog *wnt-1* encode secreted cell-cell signaling molecules critical for pattern formation in insects and vertebrates (reviewed by Peifer and Bejsovec, 1992; McMahon, 1992). If Arm is the functional homolog of β -catenin

or plakoglobin, adhesive junctions may function in *wing-less/wnt-1* signal transduction in vertebrates.

Low resolution immunofluorescence analysis had revealed that, like its vertebrate relatives (Cowin et al., 1986; Peifer et al., 1992), Arm is concentrated at the cell surface and that its distribution in certain cell types is polarized (Riggleman et al., 1990; Peifer and Wieschaus, 1990). To evaluate further whether sequence similarity between Arm, β -catenin and plakoglobin implies similar function, experiments were performed to determine (1) whether *arm* protein is a component of *Drosophila* cell-cell adhesive junctions, and (2) whether these junctions resemble more closely vertebrate adherens junctions or desmosomes.

MATERIALS AND METHODS

Membrane fractionation

Drosophila embryos (0- to 16-hours-old) were collected, rinsed with 0.1% Triton X-100, dechorionated with 50% bleach for 4 min, rinsed with 0.1% Triton X-100, and fractionated into soluble and membrane fractions as in the method of Resh and Erikson (1985). Equal aliquots of each fraction (here 25% of each fraction for the immunoblot, 10% of each fraction for the Coomassie-stained gel) were run on SDS-polyacrylamide gels, and the gels then either stained with Coomassie blue or immunoblotted (see Riggleman et al., 1990) and probed with monoclonal anti-Arm antibody 7A1 (Peifer et al., unpublished) at a 1:500 dilution, using the conditions described by Peifer et al. (1992).

Sucrose gradients

Ozawa and Kemler (1992) used sucrose gradients to determine sedimentation behavior of the vertebrate cadherin-catenin complex. The experiments described here were done using a modified version of their protocol, as follows: *Drosophila* embryos were washed and dechorionated as above, homogenized in one of the buffers described below, spun for 5 min at 1,000 g to remove eggshells, nuclei, and unlysed embryos, and the supernatant loaded onto 5.5 ml 5 to 20% sucrose gradients in an SW50.1 rotor. They were spun at 30,000 r.p.m. for 26 h at 4°C. Then, 12-drop fractions (~300 μ l each) were collected. For each gradient, a parallel gradient was run using BSA, aldolase, and catalase as markers. For each gradient fraction a 30 μ l sample was analyzed by SDS-PAGE. Markers were assayed by Coomassie staining. Gradients of *Drosophila* extracts were assayed by immunoblotting with the 7A1 monoclonal anti-Arm antibody. Arm had a similar mobility when extracts were made in RIPA buffer (300 mM NaCl, 50 mM Tris-HCl, pH 8.5, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) with or without 2 mM CaCl₂, in PBS+ 1% Triton X-100 + 1% NP-40, or in RIPA buffer without SDS and with only 0.5% NP-40. For the experiment in Fig. 2b, embryos were ground in RIPA buffer, spun for 5 min at 1,000 g, and SDS was added to the supernatant to a final concentration of 1% before loading onto the sucrose gradient.

ConA-Sepharose fractionation

Drosophila embryos were prepared, homogenized in RIPA buffer, and the supernatants cleared as above. Extracts were incubated for 2 h at 4°C with 50 μ l ConA-Sepharose (Sigma). An aliquot of the supernatant was saved as the unbound fraction, while the beads were washed three times with excess RIPA buffer. Both the unbound fraction and the beads were boiled for 5 min after addition of SDS-PAGE sample buffer, and aliquots were analyzed by SDS-PAGE and immunoblotting as described above.

Immunoprecipitations

For the experiment in Fig. 3c, *Drosophila* embryos were prepared, homogenized in RIPA buffer, supernatants cleared as above, and incubated at 4°C for 1 h with the anti-Arm monoclonal antibody N2-7A1 (at 1:20), or with a control antibody (used at 1:5). The control antibody is a monoclonal antibody recognizing a mammalian c-myc epitope, which does not recognize any *Drosophila* protein. For the experiment in Fig. 4 *Drosophila* embryos were prepared, homogenized in RIPA buffer, supernatants cleared as above, and incubated at 4°C for 1 h. with an anti-Arm monoclonal antibody (N2-7A1 (1:20), N2-4D7 (1:20), or N1-7C7 (1:5)), or with the control anti-c-myc antibody (used at 1:5). 50 μ l Protein A-Sepharose (Sigma) was added to each mixture, and incubated for 90 min. The beads were washed 4 times with excess RIPA buffer, resuspended in SDS-PAGE sample buffer and boiled for 5 min. Samples were run on an SDS-polyacrylamide gel and immunoblotted. In the experiment described in Fig. 3c, the blot was reacted with concanavalin A, which was then detected by HRP (as in Gauger et al., 1987). In the experiment in Fig. 4, rat polyclonal anti- β -catenin (a gift from H. Oda, T. Uemura and M. Takeichi) was diluted 1:50 and used as the primary antibody.

Electron microscopy and immunofluorescence

Samples were prepared for electron microscopy as described by Wieschaus and Sweeton (1988), with the following exceptions. Vitelline membranes were removed while embryos were in PBS, and embryos were post-fixed in 1% OsO₄ in 1% KFeCN, stained for 2 h with 2% uranyl acetate, and embedded in Embed 812. Embryos for immunofluorescence were fixed as described by Miller et al. (1989), and after fixation treated as described by Peifer and Wieschaus (1990).

RESULTS

The majority of cellular Arm is associated with the membrane

Arm's vertebrate homologs, β -catenin (Ozawa et al., 1989; Nagafuchi and Takeichi, 1989; Peifer et al., 1992) and plakoglobin (Korman et al., 1989; Peifer et al., 1992; Knudsen and Wheelock, 1992) are peripheral membrane proteins existing both as soluble molecules and associated with transmembrane proteins of the cadherin super-family. When vertebrate cells are fractionated into membrane-associated and soluble fractions, the majority of both β -catenin and plakoglobin are found in the membrane fraction, though a significant fraction of each protein is soluble. To test whether Arm is membrane-associated, *Drosophila* embryo extracts were separated into membrane and soluble fractions (see Material and Methods). While the soluble fraction contains most cellular proteins (Fig. 1a), about three-quarters of Arm is membrane-associated (Fig. 1b), similar to the fraction of membrane-associated β -catenin in vertebrate cells (Peifer et al., 1992). Arm exists in two forms in the cell (both derived from the *armadillo* gene (H. MacLeod, M. P. and M. Casey, unpublished data)). The predominant form in most tissues is a set of post-translationally modified isoforms of 105-110 kDa (Riggleman et al., 1990; Peifer and Wieschaus, 1990). The smaller isoform (~82 kDa) is nervous system-specific (H. MacLeod, M. P. and M. Casey, unpublished data). Both forms of Arm are enriched in the membrane fraction (Fig. 1b). These results are consistent with both the previous results of

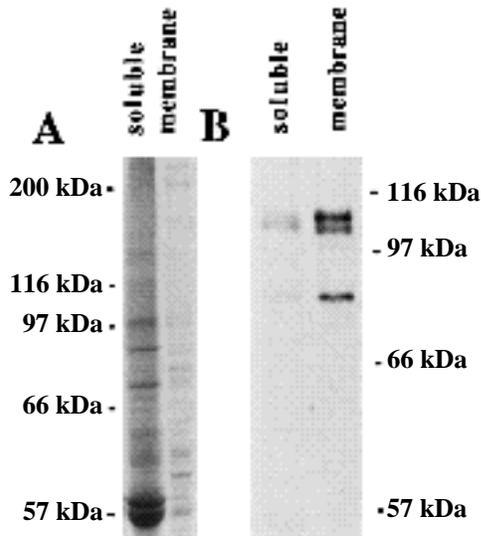


Fig. 1. Arm exists in membrane-associated and soluble forms. *Drosophila* embryos were fractionated into membrane-bound and soluble fractions, fractions analyzed by SDS-PAGE and either stained with Coomassie blue (A) or immunoblotted with anti-Arm antibody (B). Samples represent equivalent fractions of the soluble or membrane-associated proteins isolated from a given number of embryos. (A) The majority of cellular proteins are found in the soluble fraction. (B) About three-quarters of Arm is membrane-bound.

immunofluorescence analysis (Riggleman et al., 1990; Peifer and Wieschaus, 1990), and with the higher resolution immunofluorescence analysis reported below.

Arm protein is part of a larger protein complex

Both β -catenin and plakoglobin are found within the cell as part of a membrane-associated multi-protein complex. In the case of β -catenin, biochemical analysis has been used

to determine the size and the nature of the components of this complex. When vertebrate cells are lysed in a buffer containing non-ionic detergents, β -catenin is found associated in a three protein complex (Ozawa and Kemler, 1992). To determine whether, like β -catenin, Arm exists in cells as part of a larger complex, the sedimentation of Arm in sucrose gradients was examined.

Drosophila embryo extracts were run on 5 to 20% sucrose gradients in parallel with proteins of known mobility (see Ozawa and Kemler, 1992; also see Material and Methods). These extracts were made under a variety of different conditions, in which the concentrations of non-ionic detergents were varied. Virtually all of the Arm in these extracts runs at ~ 8 S (Fig. 2a), even when extracts are made under relatively stringent conditions. This sedimentation coefficient is similar to that of the vertebrate complex of N-cadherin, β - and γ -catenin (Ozawa and Kemler, 1992). In contrast, when extracts are treated with 1% SDS, Arm runs at ~ 5 S, similar to the predicted size of an Arm monomer (Fig. 2b). Thus in vivo the majority of Arm is part of a larger complex that is stable to non-ionic detergents but is dissociated by 1% SDS. The putative nervous system-specific Arm isoform, while smaller in apparent molecular mass, runs at a slightly higher S value (Fig. 2a). This may suggest that the nervous system-specific form of Arm may associate with a slightly different complex of associated proteins than the other Arm isoform.

Arm associates both with a glycoprotein and with α -catenin

To determine what other molecules associate with Arm in embryos, possible candidates were examined. Vertebrate β -catenin (Ozawa et al., 1989; Nagafuchi and Takeichi, 1989; Peifer et al., 1992) and plakoglobin (Korman et al., 1989; Peifer et al., 1992; Knudsen and Wheelock, 1992) associate with transmembrane glycoproteins of the cadherin superfamily. The lectin Concanavalin A (ConA) binds to

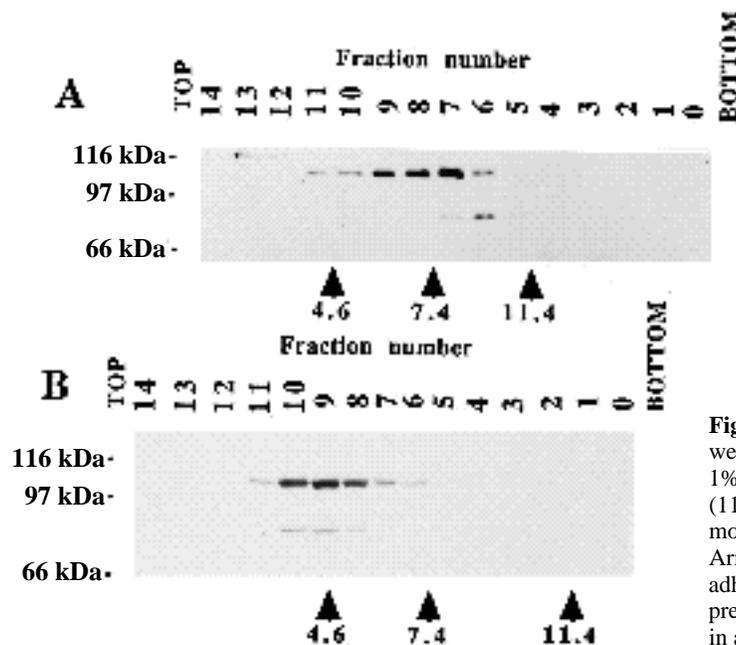


Fig. 2. Arm is part of a larger complex. *Drosophila* embryos were lysed in RIPA buffer in the absence (A) or presence (B) of 1% SDS, and separated on 5 to 20% sucrose gradients. Catalase (11.4 S), aldolase (7.4 S), and BSA (4.6 S) were used as mobility markers (arrowheads). In the absence of 1% SDS (A), Arm has a mobility of about 8 S, similar in size to the vertebrate adherens junction complex (Ozawa and Kemler, 1992). In the presence of 1% SDS (B), Arm migrates with a mobility of 5 S, in agreement with its molecular mass.

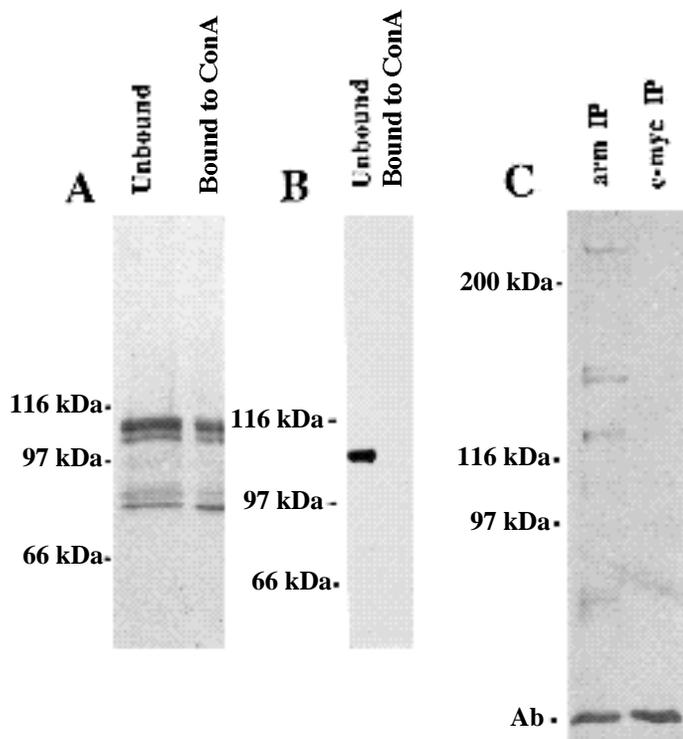


Fig. 3. Arm associates with a glycoprotein similar in size to vertebrate cadherins. (A, B) *Drosophila* embryos were fractionated using ConA-Sepharose. A substantial portion of Arm associates with ConA (A), while a control protein, Bicaudal-D, remains almost entirely in the unbound fraction (B). (C) Arm immunoprecipitates were probed with ConA, to determine what mediates association with ConA-Sepharose. Several glycoproteins co-immunoprecipitate with Arm using anti-Arm monoclonal N2-7A1. The most consistently prominent are proteins of ~140 kDa and >200 kDa.

vertebrate cadherins, allowing use of ConA-Sepharose as an affinity reagent to purify both vertebrate cadherins and the molecules that associate with them (McCrea and Gumbiner, 1991; Peifer et al., 1992). A similar cell fractionation was used to test for an association between Arm and a glycoprotein in *Drosophila*.

When *Drosophila* embryo extracts are fractionated with ConA-Sepharose (see Materials and Methods) most cellular protein remains unbound (data not shown). In contrast, a substantial amount of Arm is found in the bound fraction (Fig. 3a), similar to results seen with vertebrate β -catenin (Peifer et al., 1992). To control for non-specific association, extracts were probed with antibody to the *Drosophila* protein Bicaudal-D (Suter and Steward, 1991); in contrast to Arm, virtually all of the Bicaudal-D is found in the ConA-Sepharose supernatant (Fig. 3b).

Binding to ConA-Sepharose suggests that either Arm is a glycoprotein (unlikely due to its cytoplasmic localization) or that Arm associates with a glycoprotein. To investigate these different possibilities, Arm immunoprecipitates were made, subjected to SDS-PAGE, and western blotted. The blot was reacted with ConA, and sites of ConA binding were identified by labeling with horseradish peroxidase (as in Gauger et al., 1987). Several glycoproteins are found in

these Arm immunoprecipitates. The major glycoprotein is a molecule of ~145-150 kDa that runs as a doublet (Fig. 3c). Other species are also often seen; in particular, a glycoprotein(s) of >200 kDa is present. Similar patterns of glycoproteins are seen in immunoprecipitates with two anti-Arm monoclonal antibodies (data not shown) and are absent in immunoprecipitates with two control antibodies (Fig. 3c and data not shown). Arm itself is not labeled with ConA under these conditions (Fig. 3c), and thus as expected, its association with ConA seems to be mediated by its association with one or more glycoproteins. The most prominent glycoprotein in anti-Arm immunoprecipitates is similar in size to vertebrate cadherins (120-140 kDa; Peyrieras et al., 1983; Vestweber and Kemler, 1984), making it tempting to speculate that this represents a *Drosophila* cadherin homolog. No *Drosophila* molecules have thus far been identified that are similar to standard vertebrate cadherins. Molecules with extracellular domains similar to cadherins have been found (Mahoney et al., 1991), but they are several times larger than vertebrate cadherins. It is possible that one of these larger members of the cadherin superfamily might represent the very large glycoprotein in the immunoprecipitates.

The other molecule tightly associated with vertebrate cadherins is β -catenin (Ozawa et al., 1989; Nagafuchi and Takeichi, 1989; Kemler and Ozawa, 1989; Nagafuchi et al., 1991; Heerenknecht et al., 1991). The *Drosophila* homolog of β -catenin has recently been identified and antibodies have been raised against it (Oda et al., 1993). These antibodies (kindly provided by Takeichi and colleagues) were used to determine whether β -catenin co-immunoprecipitates with Arm. Immunoprecipitates were made using three different anti-Arm antibodies and with a control monoclonal that does not recognize any *Drosophila* protein; immunoblots of these immunoprecipitates were reacted with anti- β -catenin antibody. Under several different extraction conditions, β -catenin co-immunoprecipitates with Arm but does not co-precipitate with the control monoclonal (Fig. 4; data not shown). It is worth noting that these results do not demonstrate a direct association between β -catenin and Arm - they may associate directly or by virtue of their association with a third molecule, such as a cadherin.

Together, these results support the presence of Arm as a component of a membrane-associated multi-protein complex similar to the vertebrate adherens junction. Arm and its vertebrate homologs are all membrane-associated. The *Drosophila* and vertebrate complexes have similar apparent mobilities on a sucrose gradient. Finally, like the vertebrate β -catenin/ β -catenin/cadherin complex, the *Drosophila* complex contains at least three components: Arm; a glycoprotein similar in size to vertebrate cadherins; and the *Drosophila* β -catenin homolog.

Arm is localized to adherens-like junctions in *Drosophila* cells

To further the comparison between invertebrate and vertebrate junctions, the morphology of Arm-containing *Drosophila* junctions was examined. In the electron microscope, vertebrate adherens junctions appear as regions of close membrane apposition that are fuzzy both externally

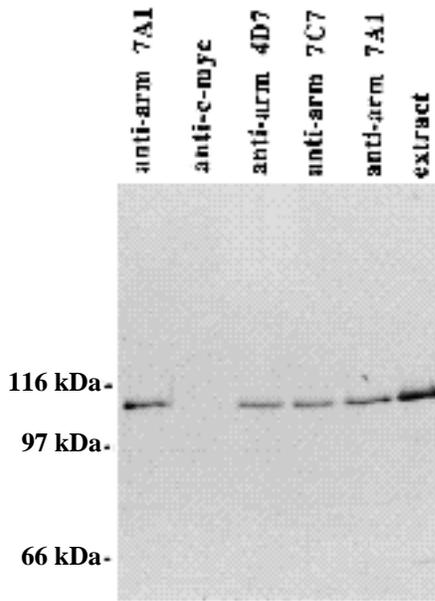


Fig. 4. Arm and the *Drosophila* homolog of β -catenin co-immunoprecipitate. Arm immunoprecipitates were analyzed by SDS-PAGE, and immunoblotted with anti- β -catenin antibody. β -Catenin co-immunoprecipitates with Arm using several anti-Arm monoclonal antibodies, while β -catenin is not co-precipitated by a control anti-c-myc antibody.

and internally; they are located at the junction between the apical and lateral surfaces of epithelial cells. Zonula adherens-like junctions have been reported in several epithelial tissues of *Drosophila* imaginal discs and embryos (Poodry and Schneiderman, 1970; Eichenberger-Glinz, 1979). If Arm is a component of adherens junctions, it ought to be localized to these junctions within the cell. To test

this, the position and morphology of adherens-like junctions and the localization of Arm were compared in a simple epithelial tissue, the developing *Drosophila* gut.

Gut cells in the *Drosophila* embryo form a simple single-cell thick tube, with an external basal lamina and apical surfaces facing the lumen. Sections were cut through the developing gut and examined using the electron microscope. Gut cells examined in this way resemble typical vertebrate epithelia - in particular, fuzzy regions of the membrane are visible where the apical and lateral cell surfaces meet (Fig. 5a,b). These regions closely resemble vertebrate adherens-type junctions in position and morphology.

Previous immunofluorescence analysis of Arm had demonstrated that it is found localized near the cell periphery, and in some cell types is polarized (Riggelman et al., 1990; Peifer and Wieschaus, 1990). Cytoplasmic Arm is also seen in many cell types. While this resembles the localization of cadherins and catenins in vertebrate cells, which are found all along the lateral surface of cells though they are enriched in adherens junctions, we desired to examine Arm localization at higher resolution, so analysis was carried out using immunofluorescence and the confocal microscope. To determine whether Arm is an integral part of adherens junctions in *Drosophila* an alternate cell fixation procedure was used that was developed to examine cytoskeletal complexes (Miller et al., 1989). This fixation procedure fixes only the most tightly bound Arm, allowing much of the soluble and loosely bound Arm to wash away (Fig. 5c). When these conditions are used, the previously observed polarization of the Arm protein is greatly enhanced. When the high resolution images provided by the confocal microscope are compared with the electron micrographs, they provide strongly suggestive support for a localization of Arm in adherens junctions. This analysis suggests that Arm is a tightly associated component of the adherens junction in the *Drosophila* gut.

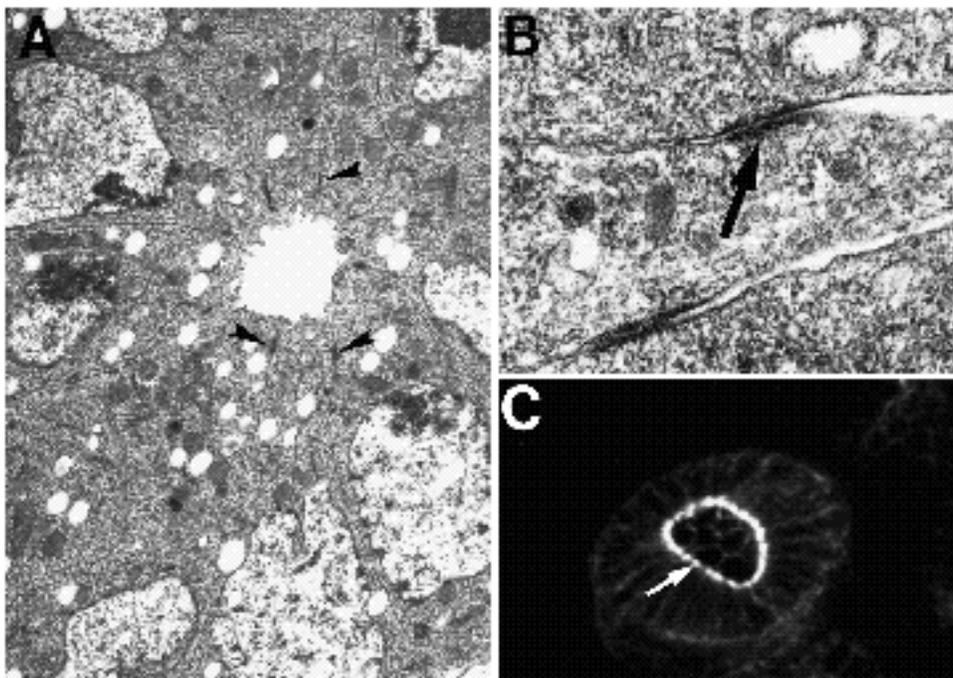


Fig. 5. Arm co-localizes with adherens-like junctions. (A) Electron microscope section through the developing embryonic gut of *Drosophila*. These epithelial cells have apparent cell-cell adhesive junctions at the apical end of their lateral surfaces, near the lumen (arrowheads). (B) Close-up of a *Drosophila* cell-cell junction (arrow). These junctions closely resemble vertebrate adherens-type junctions in morphology. (C) An optical cross section of *Drosophila* embryonic gut stained with anti-Arm antibody, under conditions that remove all but the most tightly bound Arm. Arm localizes in a dot-like pattern to the apical end of the lateral cell surface, where cell-cell junctions are seen in (A).

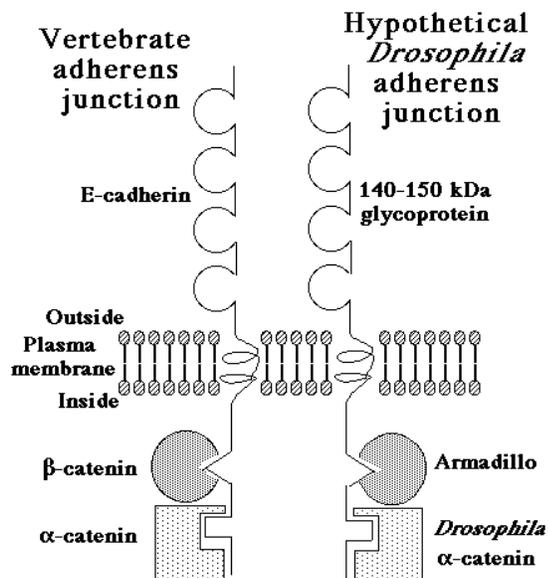


Fig. 6. A comparison of the structure of a vertebrate adherens junction and the hypothetical multi-protein complex of the *Drosophila* adherens junction. The structure of the vertebrate junction is based on the results of a large number of labs (reviewed by Magee and Buxton, 1991).

DISCUSSION

Among the most surprising and exciting recent findings in developmental biology has been the realization that many molecules involved in the regulation of key developmental processes, originally identified during the examination of mutations with interesting developmental consequences, are homologs of molecules identified by scientists investigating questions in vertebrate cell and molecular biology. These connections have resulted in a synergistic relationship between experimenters in these different fields, often leading to rapid advances. These connections are usually first made on the basis of sequence similarity between different molecules. One such example is the sequence similarity between the *Drosophila* segment polarity gene *armadillo* and the vertebrate adhesive junction components β -catenin and plakoglobin (Peifer and Wieschaus, 1990; Franke et al., 1989; McCreath et al., 1991).

This surprising connection between a *Drosophila* gene involved in the establishment of cell fate during development and two vertebrate molecules thought to mediate association between different cells in epithelia has raised several interesting issues. It suggested the possibility of using genetic analysis to analyze junctional function during development. In addition, genetic and molecular analysis suggests that Arm is required for transmission of the *wingless* intercellular signal (Klingensmith et al., 1989; Peifer et al., 1991). *wingless*, and its vertebrate homolog *wnt-1*, are key cell-cell signaling molecules involved in a number of cell fate decisions in both insects and vertebrates (reviewed in Peifer and Besjovec, 1992; McMahon, 1992) - the connection between Arm and its vertebrate homologs suggested that adhesive junctions might be required for the reception or transmission of these signals.

Sequence similarity alone, however, is not sufficient to prove a functional connection between Arm and its vertebrate homologs. To substantiate the suggestion provided by the sequence data, the properties of Arm have been examined to see if they are consistent with a role in junctions. The results of these experiments support the idea that Arm is a key component of *Drosophila* cell-cell adhesive junctions, and suggest that these junctions are structurally more analogous to adherens-type junctions than to desmosomes (Fig. 6).

Arm is part of a multi-protein complex similar to the vertebrate adherens junction

It has recently become apparent that different vertebrate cell-cell adhesive junctions share analogous and in some cases homologous components (reviewed by Magee and Buxton, 1991). Both adherens junctions and desmosomes are multi-protein complexes organized around transmembrane cell adhesion molecules of the cadherin super family. The extracellular domains of these molecules are thought to associate homotypically and thus form the adhesive component of the junction. The cytoplasmic domains of the cadherins associate with several peripheral membrane proteins that are thought to both transmit a signal into the cell that adhesion has occurred and to mediate anchoring of the actin (in adherens junctions) or intermediate filament (in desmosomes) cytoskeletons.

Among the cytoplasmic components of this complex are the proteins β -catenin and plakoglobin. β -catenin associates with true cadherins as part of the adherens junction (Ozawa et al., 1989; Nagafuchi and Takeichi, 1989; Peifer et al., 1992), while plakoglobin is found associated both with desmosomal cadherin homologs in the desmosome and with true cadherins in the adherens junction (Cowin et al., 1986; Korman et al., 1989; Peifer et al., 1992; Knudsen and Wheelock, 1992). Each junctional complex also contains at least one junction-specific component. The vinculin-related protein γ -catenin is found in the adherens junction (Ozawa et al., 1989; Nagafuchi and Takeichi, 1989; Nagafuchi et al., 1991; Heerenknecht et al., 1991), where it has been suggested it may help anchor the actin cytoskeleton. In the desmosome, desmoplakin appears to be involved in anchoring the intermediate filaments (Stappenbeck and Green, 1992).

Elegant cell biological experiments in vertebrate tissue culture cells have provided compelling evidence that the adherens junction functions as hypothesized to initiate the organization of individual cells into epithelia (Nagafuchi et al., 1987; Nose et al., 1988; McNeill et al., 1990; Hirano et al., 1992). These same experiments have provided evidence that specific adherens junction components are required for these processes. However, lack of the appropriate reagents and cell lines has made it as yet impossible to test the function of β -catenin by this sort of approach. More critical, these experiments in tissue culture do not allow one to assay the role of the adherens junction and its components during development. Certain clever experiments have provided tantalizing hints that junctions likely play an important role in a variety of important developmental processes (e.g. see Vestweber and Kemler, 1984; Fujimora et al., 1990; Detrick et al., 1990; Kintner, 1992),

but the lack of the ability to apply genetics to the function of these junctions makes further analysis difficult.

It was thus of some interest to learn that the product of the *Drosophila* segment polarity gene *armadillo* (Arm) is quite similar in sequence to the β -catenin and plakoglobin proteins (Peifer and Wieschaus, 1990; Franke et al., 1989; McCrea et al., 1991). Many mutations in the *armadillo* gene exist, and the effects of these mutations on a variety of developmental processes are known (Wieschaus et al., 1984; Klingensmith et al., 1989; Peifer et al., 1991). If Arm is an adhesive junction component, this offers the opportunity to use genetic analysis to dissect the function of adherens junctions, and also provides a surprising connection between the function of these junctions and the transmission of an important class of cell-cell signaling molecules, the *wingless/wnt-1* proteins. However, this connection rested largely if not solely on the strength of the sequence identity between Arm and its vertebrate homologs. While this sequence relationship is quite close, implying similarities in the biochemical function of the proteins, this alone could not prove that Arm is part of an adhesive junction complex in *Drosophila*.

To strengthen this connection, a preliminary characterization of the biochemical and cell biological properties of Arm was carried out. The results reported above confirm that Arm is part of a membrane-associated multi-protein complex, and that this complex has a similar sedimentation coefficient to the vertebrate complex of N-cadherin with β - and γ -catenin (Figs 1 and 2). By use of a co-immunoprecipitation assay, it was demonstrated that this complex contains Arm, a glycoprotein of a size similar to vertebrate cadherins, and the *Drosophila* homolog of β -catenin (Figs 3 and 4). Finally, in an epithelial tissue like the developing embryonic gut, Arm protein is highly enriched in and tightly associated with the region of the cell where electron microscopy reveals cell-cell junctions similar in morphology and position to vertebrate adherens junctions (Fig. 5). Together, these results strongly support the idea that *Drosophila* epithelial cells are joined together by adherens junction complexes similar in structure to those in vertebrates (Fig. 6).

In addition to these structural similarities, *Drosophila* junctions may also share functions proposed for vertebrate adherens junctions, such as mediating adhesion and anchoring the actin cytoskeleton. Arm co-localizes with actin in many (though not all) cell types (Riggleman et al., 1990; Peifer and Wieschaus, 1990; Peifer et al., in press). Further, if Arm is completely depleted from the *Drosophila* germline using null mutations in *arm*, both cell adhesion and integrity of the actin cytoskeleton are affected (Peifer et al., in press). The morphological, biochemical and functional similarities between vertebrate and invertebrate junctions strengthen the case that these two sorts of junctions may play similar roles in cells, in epithelia, and in the entire organism. These similarities also support the use of Arm as a genetic tool to examine the function of β -catenin/plakoglobin and of adherens junctions during development.

The complexity of adhesive junctions in *Drosophila*

One might conclude from these results that Arm is the

structural and functional homolog of the adherens junction component β -catenin rather than of the desmosomal protein plakoglobin. This would be consistent with the greater sequence identity between Arm and β -catenin (71%; McCrea et al., 1991) than between Arm and plakoglobin (63%; Peifer and Wieschaus, 1990). The increased identity between Arm and β -catenin is concentrated in the N-terminal region (Peifer et al., 1992), raising the possibility that this domain mediates a possible shared function lacked by plakoglobin. However, in interpreting these results, one must remember that while desmosomes seem to contain exclusively plakoglobin (Peifer et al., 1992), the reverse is not true for adherens junctions. Both β -catenin and plakoglobin are found in the adherens junction, though the latter is more loosely bound (Cowin et al., 1986; Peifer et al., 1992; Knudsen and Wheelock, 1992). It thus seems safest to conclude for the moment that Arm is an adherens junction rather than a desmosomal component in *Drosophila*, but that its precise relationship to β -catenin and plakoglobin remains an open question. The other question that remains open is whether *Drosophila* contains only adherens-type junctions, or whether there may also be desmosomes. If the latter are present, there may be a plakoglobin-like molecule in *Drosophila*.

In vertebrates, different tissues contain both different cadherins (e.g. Takeichi, 1991; Suzuki et al., 1991) and different β -catenins (Hirano et al., 1992); proteins related to β -catenin may also exist (Peifer et al., 1992). The cadherin-catenin complex can assemble on different cadherins (Ozawa et al., 1989; Nagafuchi and Takeichi, 1989; Ozawa and Kemler, 1992; Wheelock and Knudsen, 1991). The presence of a complex of a slightly different size in association with the putative nervous system form of Arm (Fig. 2a), and the presence of several distinct glycoproteins in anti-Arm immunoprecipitates (Fig. 3c) suggests that this sort of diversity may also exist, at least to some extent, in *Drosophila*.

These initial biochemical and cell biological results confirm predictions from sequence analysis about Arm's cellular role and lay the groundwork for using genetic analysis to study adherens junction function during development. The parallels between insects and vertebrates strengthen the idea that vertebrate adherens junctions may be important for transduction of the *wingless/wnt* intercellular signal, crucial to pattern formation in both vertebrates and invertebrates. We can now take advantage of the genetic, biochemical and cell biological tools available in *Drosophila* to investigate the role of adherens junctions in cell adhesion and signal transduction.

H. Oda, T. Uemura, and M. Takeichi very kindly provided anti- β -catenin antibody before publication and generously shared unpublished results. B. Suter kindly provided *Bic-D* antibody. I am grateful to M. Casey, D. Sweeton, and R. Knittel for valuable technical assistance, and to S. Whitfield for photography. I thank A. Comer, G. Maroni, C. A. McCormick, H. Oda, R. Quatrano, M. Takeichi, and T. Uemura for critically reviewing the manuscript, and members of the Peifer and Searles labs for valuable discussions. I am especially grateful to D. Sweeton and E. Wieschaus for help during early stages of these experiments and for continuing helpful discussions. These experiments were sup-

ported in part by NIH grant 1-R01 GM47857 to M. P., who is a Searle Scholar.

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