Drosophila α-Catenin and E-cadherin Bind to Distinct Regions of Drosophila Armadillo*

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Adherens junctions are multiprotein complexes mediating cell-cell adhesion and communication. They are organized around a transmembrane cadherin, which binds a set of cytoplasmic proteins required for adhesion and to link the complex to the actin cytoskeleton. Three components of Drosophila adherens junctions, analogous to those in vertebrates, have been identified: Armadillo (homolog of β-catenin), Drosophila E-cadherin (DE-cadherin), and α-catenin. We carried out the first analysis of the interactions between these proteins using in vitro binding assays, the yeast two-hybrid system, and in vivo assays. We identified a 76-amino acid region of Armadillo that is necessary and sufficient for binding α-catenin and found that the N-terminal 258 amino acids of α-catenin interact with Armadillo. A large region of Armadillo, spanning six central Armadillo repeats, is required for DE-cadherin binding, whereas only 41 amino acids of the DE-cadherin cytoplasmic tail are sufficient for Armadillo binding. Our data complement and extend results obtained in studies of vertebrate adherens junctions, providing a foundation for understanding how junctional proteins assemble and a basis for interpreting existing mutations and creating new ones.

Cell-cell adhesion and communication are required for cells to form organized tissues. One structure used by cells for these purposes is the adherens junction, found near the apical surface of epithelial cells and found also in other cell types. Adherens junctions mediate calcium-dependent cell-cell adhesion and anchor the actin cytoskeleton (reviewed in Ref. 1). In addition, many signaling molecules are localized to adherens junctions, suggesting a role in the transmission of intercellular signals (reviewed in Ref. 2).

Adherens junctions consist of transmembrane cadherins and a set of cytoplasmic proteins associated with cadherin cytoplasmic domains (reviewed in Refs. 1 and 3). The extracellular domains of cadherins interact homotypically with cadherins of neighboring cells. The cytoplasmic proteins α-catenin, β-catenin, and plakoglobin (or γ-catenin) are required for cadherin adhesive function and anchor the actin cytoskeleton. The Src tyrosine kinase substrate p120<sup>ctn</sup> is also present in adherens junctions (4, 5); its function remains unknown. Changes in tyrosine phosphorylation of β-catenin (reviewed in Ref. 2) and p120<sup>ctn</sup> (6) correlate with transformation and associated changes in cell adhesion.

To understand the cell biological function of adherens junctions, we must determine how interactions among different adherens junction proteins mediate assembly. β-Catenin and plakoglobin bind directly to the E-cadherin cytoplasmic domain in a mutually exclusive fashion (7, 8). β-Catenin and plakoglobin are 70% identical in amino acid sequence; their central regions, containing ~13 copies of the 42-amino acid Arm<sup>4</sup> repeat (9), are particularly well conserved (~80% amino acid identity). These highly conserved Arm repeats mediate interaction with cadherin (10–12), suggesting that β-catenin and plakoglobin compete for the same binding site. The N-terminal regions of both β-catenin and plakoglobin bind to α-catenin; α-catenin does not bind cadherin directly (11, 13–15). α-Catenin, in turn, links adherens junctions to actin, directly (16) or via α-actinin (17). p120<sup>ctn</sup> also binds directly to E-cadherin (18), but likely to a site distinct from that bound by β-catenin/plakoglobin (4, 5). p120<sup>ctn</sup> does not interact with α-catenin (18), however, and thus does not appear to mediate interaction with actin. The core cadherin-catenin complex forms higher order assemblies such as the zona adherens. Both E- and N-cadherins dimerize (19, 20), and association with the cytoskeleton may help form larger assemblies.

Adherens junctions were first described in vertebrates, but precisely analogous structures exist in Drosophila. The Drosophila homolog of β-catenin is Armadillo, first discovered because of its role in transducing the Wingless cell-cell signal (reviewed in Ref. 3). Arm is structurally similar to β-catenin and plakoglobin (63% identical to β-catenin), with 13 Arm repeats (9) flanked by N- and C-terminal regions. Drosophila homologs of E-cadherin (DE-cadherin) and α-catenin have been identified (21, 22); no direct homolog of plakoglobin has been found. Both Arm and DE-cadherin are required for proper cell-cell adhesion in vivo (23–26).

We undertook a systematic study of the interactions between Drosophila Arm and its adherens junction partners, DE-cadherin and α-catenin. We localized binding sites for each of these proteins on Arm using in vitro binding assays, the yeast two-hybrid system, and in vivo binding assays. We also identified regions of α-catenin and DE-cadherin required for Arm binding. These experiments complement and extend analysis of the vertebrate homologs of Arm. Our parallel in vivo studies confirm the importance of these interactions for adherens junction function (27).

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**Plasmid Constructions**

GST-Arm Fusions—Arm fragments were PCR-amplified from E9 cDNA (28) with Vent DNA polymerase in 10 cycles. 5'-Primers contain a BamHI site and a methionine codon; 3'-primers contain EcoRI and BamHI sites and a stop codon (Tables I and II show end points of the constructs). PCR products were subcloned into both pBluescript KS+ and pLM1; pLM1 was generated by inserting oligomers creating BglII and EcoRI sites in the appropriate reading frame of pGEX2T128/129 (gift of Dr. M. Blanar (29)). The vector/insert junctions were sequenced; many inserts were entirely sequenced. pLM1-N1 and pLM1-N5 were generated by cloning BamHI/partial BclI digestion products of the arm E9 cDNA into the BglII site of pLM1.

PCR-mediated Site-directed Mutagenesis—Mutagenesis was performed as described (30) using PBS-N2 as a template. The fragments from the second PCR were digested with BamHI and subcloned into the pLM1 BamHI site. Mutants were screened for fusion protein expression and confirmed by sequencing.

**GST-α-Catenin Fusions**—The C-terminal two-thirds of α-catenin was subcloned from pBS-α-catenin (21) as a BglII/XhoI fragment, which was ligated with BglII/EcoRI-digested pLM1, followed by filling with Klenow fragment and a second ligation, creating pLM1-αC. The N-terminal end of α-catenin was made by PCR with Vent polymerase in 10 cycles. The 5'-primer contains BglII/EcoRI, and EcoRI sites, while the 3'-primer is just 3' to a BglII site in α-catenin. The PCR product was cut with BglII and cloned into pLM1-αC, creating pLM1-α-catRI. pLM1-α-catRI was generated by eliminating the C-terminal half of α-catenin by EcoRI digestion.

Two-hybrid Plasmids—pCK2 and pCK4 were generated from pBtM116 (gift of P. Bartel and S. Fields) and pACT2 (gift of S. Elledge), respectively, by inserting oligomers creating BamHI and EcoRI sites, while the other end is created by ligating BamHI/EcoRI sites. Mutant fragments were generated by PCR with full-length Arm mutant constructs (27) as templates. The α-catenin N terminus was isolated from pLM1-α-catenin by BglII digestion and cloned into the BamHI site of pCK2 and pCK4. The cytoplasmic domain of DE-cadherin and fragments thereof were amplified by PCR (with primers containing BamHI and EcoRI sites), digested with BamHI and EcoRI, and cloned into pCK4 (see Fig. 11A).

**Expression and Purification of GST Fusion Proteins and Bead and Blot Binding Assays**

Fusion proteins were expressed in Escherichia coli DH5α. Overnight cultures were diluted 1:10, grown for 1 h, and induced with 0.5 mM isopropyl-1-thio-β-D-galactopyranoside. After 2 h of further growth, cells were lysed in MTPBS (150 mM NaCl, 16 mM Na2HPO4, and 4 mM NaH2PO4) by sonication (20% output) twice for 30 s. 1% Tween 20 and isopropyl-1-thio-galactopyranoside were added, incubated for 30 min at room temperature, and then washed with MTPBS plus 0.1% Triton X-100 and 1% Tween 20. Wild-type Drosophila extracts were made from 0–2-h-old embryos. Embryos were rinsed with 0.1% Triton X-100, dechorionated in 50% bleach for 4 min, rinsed again, and then ground in RIPA buffer (23) or NET (400 mM NaCl, 5 mM EDTA, 50 mM Tris, pH 7.5, and 1% Nonidet P-40), both with 50 μg/ml phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 1.4 μg/ml pepstatin, 50 μM NaF, and 1 μM NaVO3. For bead binding assays, fusion protein bound to glutathione-agarose was mixed with wild-type embryo extract in RIPA buffer or NET at room temperature on a nutator for 2 h, and beads were washed with excess buffer four times for a total of 20 min. Samples were boiled for 5 min in SDS sample buffer and analyzed by SDS-PAGE and immunoblotting. For blot binding assays, fusion proteins were purified on glutathione-agarose, separated by SDS-PAGE, and transferred to nitrocellulose. Blots were stained with Ponceau S to detect total protein and incubated with wild-type embryo extract in RIPA buffer at room temperature for 2 h, followed by washing with excess RIPA buffer five times for a total of 1 h. Blots were immunoblotted with anti-α-catenin antibody and visualized by enhanced chemiluminescence (Amersham Corp.).

**Fly Stocks and ConA-Sepharose Fractionation**

Wild-type flies were Canton S; armH8.6, armCM17, armVP33, and arm62 are described in Ref. 31. arm255 was generated as a germ line transformant by injection into y w flies. To create pUAST-armRC, a fragment of Arm containing the repeats and the C terminus (amino acids 128–843) was excised from pLM1-RC with BamHI and EcoRI and ligated into pUAST-3 (a modified version of pUAST (32) with the EcoRI and BamHI sites inverted in the polylinker). ConA-Sepharose fractionation was done as described (33), but using NET instead of RIPA buffer.

**Two-hybrid System**

We used a version (33) of the yeast two-hybrid system (34) in which one protein is fused to the DNA-binding domain of E. coli LexA and its putative partner is fused to the transcriptional activation domain of yeast Gal4p. Interaction of the partners activates two reporter genes, HIS3, whose product can be quantitated, and 4-HIS3 activation paralleled the yeast HIS3 activation (data not shown). The yeast strain L40 (MATa hisΔ200 trp1-901 leu2-3,112 ade2 lys2::lexAop) HIS3 URA3::(lexAop)-lacZ (33) was used for all experiments. The strain was transformed (35) simultaneously with two plasmids encoding different fusion proteins (in pCK2 and pCK4); transformants were selected on synthetic complete medium lacking tryptophan and leucine. HIS3 activation was assayed by spotting dilutions of saturated liquid cultures onto synthetic complete medium lacking tryptophan, leucine, and histidine and containing 25 mm 3-aminotriazole; growth was compared with that of colonies on medium lacking only tryptophan and leucine. In all cases, HIS3 activation paralleled lacZ activation (data not shown).

**Expression of UAS-Arm**

Full-length Arm cDNA (21) was subcloned into pUAST as a BamHI/EcoRI fragment. The armΔ8.6 fragment is used as a control. A fragment containing the repeats in the appropriate reading frame of pGEX2T128/129 was excised from pLM1-RC with BamHI and EcoRI and ligated into pUAST-3. The yeast strain L40 (MATa hisΔ200 trp1-901 leu2-3,112 ade2 lys2::lexAop) HIS3 URA3::(lexAop)-lacZ was used as a partner.

**TABLE I**

Diagram of portions of Arm tested for association with α-catenin either as GST-Arm fusion proteins or in the two-hybrid system. Corresponding amino acid positions are indicated on the right. Binding activity is shown as plus and minus symbols. N.D., not determined.
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Liquid β-galactosidase assays were performed as described (36). Cells from 3 ml of late log phase culture were washed and resuspended in 1 ml of Z buffer (60 mM Na2HPO4, 40 mM NaH2PO4, 10 mM KCl, 1 mM MgSO4, and 40 mM 2-mercaptoethanol). 200-μl aliquots of this suspension were mixed with 600 μl of Z buffer, 20 μl of 0.1% SDS, and 3 drops of chloroform. Samples were vortexed for 15 s and incubated at 28 °C for 10–15 min. Reactions were initiated by adding 160 μl of 4 mg/ml o-nitrophenyl-β-D-galactosyranoside (in Z buffer) and stopped when the solution reached an appropriate yellow color (A420 ~ 0.4–0.7) by adding 400 μl of 1 M Na2CO3. β-Galactosidase activity (in Miller units) was calculated as follows: A420 × 1000 divided by (the A400 of the initial cell suspension × the volume of suspension used (0.2 ml) × the time in minutes of color development). All values shown are averages from assays in duplicate or triplicate on cultures of at least six independent transformants. Protein extracts for immunoblotting were made from samples of cultures grown for 23 amino acids from its N terminus (fusion protein N6). Other fragments of Arm such as the entire repeat region, do not interact with α-catenin (Fig. 4C).

We also mapped the in vivo α-catenin-binding site. We generated a mutant, arm314, with a 39-amino acid deletion in the N terminus (removing amino acids 101–139); these amino acids are within the 76-amino acid region required for α-catenin binding in vitro. This mutation was reintroduced into flies and abolishes the ability of Arm to bind to α-catenin in vivo (27). In contrast, the most N-terminal portion of Arm is not required for α-catenin binding in vivo. We generated and reintroduced in vivo a second mutant, arm210, with a 54-amino acid deletion in the N terminus (removing amino acids 34–87); this deletion falls outside the region defined as essential for binding in vitro. This mutant protein was tagged with a c-Myc epitope to distinguish it from wild-type endogenous Armadillo. Mutant protein can be specifically immunoprecipitated with anti-c-Myc antibody; α-catenin co-immunoprecipitates with this mutant protein (Fig. 5A).

Point Mutations inside the 76-Amino Acid Region Abolish α-Catenin Binding Activity—The 76-amino acid region sufficient for binding α-catenin in vitro is highly conserved between Arm and its vertebrate relatives β-catenin (39) and plakoglobin.
cadherin, it will bind to ConA-Sepharose and thus be included.

If a mutant protein forms a complex with DE-cadherin, a fraction of Arm binds to proteins that are recognized by this lectin (41); since wild-type cadherins are glycosylated, the N terminus. We used binding to ConA-Sepharose to measure association with DE-cadherin. Cadherins are glycosylated proteins (generated during purification or in bacteria) fail to bind. Molecular weight markers are shown on the left. HMW, high molecular weight markers; MMW, medium molecular weight markers; RI–13, Arm repeats 1–13.

Mutants, two to four positively charged amino acids that are conserved among Arm, β-catenin, and plakoglobin were changed to alanines (Fig. 6A). α-Catenin binding activity is abolished in each mutant (Fig. 6B). We also made point mutations in nonconserved residues, replacing Arm residues either with corresponding β-catenin residues or with alanines (Fig. 6A). Both alanine substitution mutants greatly reduced α-catenin binding activity. Putting β-catenin residues at the center of the 76-amino acid region reduced binding, while β-catenin residue substitutions at the N-terminal end of the binding region did not alter binding (Fig. 6B).

The Binding Site for Armadillo on α-catenin Is in the N-terminal 258 Amino Acids—We generated a full-length GST-α-catenin fusion protein and used it in both bead and blot binding assays. Full-length α-catenin bound Arm in a blot binding assay (Fig. 7 and data not shown), as did proteolytic breakdown products with molecular masses of >25 kDa (data not shown). A smaller GST fusion protein containing the N-terminal half of α-catenin (amino acids 1–526) binds to Arm as well as full-length α-catenin (Fig. 7). We extended these data using the two-hybrid system; in this assay, the N-terminal 258 amino acids of α-catenin bind Arm (100-fold better than the vector control) (Fig. 4).

The in Vivo DE-cadherin-binding Site on Armadillo Maps to the Central Repeat Region—Most of the original in vivo arm mutations truncate the coding sequence, yielding a series of C-terminally truncated mutant proteins (31). We examined the ability of some of these mutant Arm proteins to associate with DE-cadherin in vivo. We also assayed a protein lacking the entire N terminus. We used binding to ConA-Sepharose to measure association with DE-cadherin. Cadherins are glycoproteins that are recognized by this lectin (41); if a wild-type Arm associates with DE-cadherin, a fraction of Arm binds to ConA (23). If a mutant protein forms a complex with DE-cadherin, it will bind to ConA-Sepharose and thus be included in the bound fraction. In contrast, mutant protein that cannot form a complex will be exclusively in the unbound fraction. Arm<sup>ΔN</sup>, which lacks the entire N-terminal domain, binds DE-cadherin in vivo (Fig. 5B). arm<sup>H8.6</sup> mutant protein, lacking virtually the entire C-terminal domain, also binds DE-cadherin (Fig. 5, C and D). Proteins encoded by arm<sup>CM19</sup>, arm<sup>XP13</sup>, and arm<sup>ΔN2</sup>, with truncations in repeats 13, 10, and 9, respectively, also retain some binding activity (Fig. 5, C and D). Hence, the in vivo DE-cadherin-binding site on Arm lies at least in part between Arm repeats 1–9.

![Image](image-url)
The Central-most Arm Repeats Are Required for Binding to DE-cadherin in Vitro and in Yeast—To further narrow down the region of Arm interacting with DE-cadherin in vitro, we used GST-Arm fusion proteins containing portions of the Arm repeat region. Both full-length Arm and a fragment containing repeats 1–13 bind strongly to DE-cadherin. Repeats 3–10 and repeats 3–8 retain DE-cadherin binding activity, while the smaller fragment containing repeats 5 and 6 does not bind (Fig. 8).

Regions of Arm were also tested for interaction with DE-cadherin in the two-hybrid system. The N-terminal region of Arm does not interact with the cytoplasmic domain of DE-cadherin (Fig. 4C), but parts of the repeat region do (Fig. 9 and Table II). The smallest fragment of Arm capable of binding DE-cadherin is one containing Arm repeats 3–8 (R3–8). Smaller fragments (containing repeats 3–7 or 4–8) do not interact with cadherin, but repeats 4–13 and repeats 1–7 show substantial interaction; repeats 1–6 bind less well. (All LexA-Arm fusion proteins that do not interact with DE-cadherin are expressed in yeast to at least the same level as repeats 1–13, as assayed by Western blotting (data not shown).) We found a similar pattern of interactions between Arm and a vertebrate cadherin (mouse OB-cadherin; a gift of Dr. P. McCrea), except that the vertebrate cadherin shows reduced binding to repeats 1–7 (Fig. 9 and Table II).

We examined the effect of mutations in the Arm repeat region on the interaction with DE-cadherin both in yeast and in vivo. Five mutations (diagramed in Fig. 10A) were introduced into the full-length repeat region (repeats 1–13); three were also introduced into the smallest interacting fragment of Arm (repeats 3–8). These mutations (in the context of full-length Arm protein) were also tested in vivo (27): mutating repeat 1 or fusing repeats 10 and 11 did not affect DE-cadherin co-immunoprecipitation; removing repeat 5 or 8 greatly reduced cadherin binding; and removing repeats 3–6 eliminated cadherin binding in vivo. In the two-hybrid system, we observed similar but weaker effects (Fig. 10B). Mutating repeat 1 or fusing repeats 10 and 11 does not affect the interaction with cadherin, but removing repeats 3–6 eliminates binding. Removing repeat 5 (from repeats 1–13) does not substantially alter DE-cadherin binding, in contrast to the result in vivo; the high level of expression of the fusion protein in yeast may compensate for reduced binding affinity. We were not able to assess the effect of removing repeat 8 (from repeats 1–13) because this fusion activates the reporters by itself, in the absence of DE-cadherin. Removing one or more repeats from repeats 3–8 eliminates binding to DE-cadherin.

The Armadillo-binding Site on DE-cadherin Lies in a Small Segment of the Cytoplasmic Domain—We used the two-hybrid system to define the portion of DE-cadherin required for interaction with Arm. A series of constructs containing different parts of the DE-cadherin cytoplasmic domain (Fig. 11A) were tested for binding to the full repeat region of Arm. A 41-amino acid fragment (amino acids 1426–1466, DEC6) is sufficient for binding (Fig. 11B). A fragment consisting of amino acids 1350–1446 (DEC3) showed substantial interaction, but a shorter fragment (amino acids 1426–1444, DEC7) and all the fragments beginning at amino acid 1447 (DEC8, DEC9, and DEC10) did not interact with Arm.

DISCUSSION

Armadillo plays a central role in adherens junctions (3), linking the transmembrane adhesive protein DE-cadherin to α-catenin, which connects to the actin cytoskeleton. Depletion of Arm disrupts cell adhesion and actin integrity and thus disrupts organizational development (24, 42). We believe that Arm is a linker, joining together other junctional proteins. To characterize interactions between Arm and its junctional partners, we defined the regions of Arm responsible for each interaction. Both the sequence of Arm (the central two-thirds of the protein is composed of ~13 imperfect 42-amino acid Arm repeats (9, 28)) and the results of previous genetic analysis of mutant Arm proteins (3) suggested that Arm is modular in structure. We thus hypothesized that one might be able to define specific
regions of Arm responsible for interaction with individual protein partners.

To test this hypothesis, we used three different assays to map the regions of Arm responsible for α-catenin and DE-cadherin binding. We analyzed interactions in vitro using GST-

Arm fusion proteins, examined interactions in yeast using the two-hybrid system, and determined requirements for interaction with partners in vivo. Each system has its own inherent advantages and limitations, and these balance each other, at least in part. GST fusion proteins provide a simpler system by purifying one component and permit rapid assays, allowing

![Fig. 6. Point mutations inside the 76-amino acid region abolish α-catenin binding. A, shown is the amino acid sequence alignment of Arm family proteins in the 76-amino acid α-catenin-binding region, using the one-letter code. Identical residues are indicated with lines, while similar residues are indicated with colons. Residues altered in the different mutants are indicated above and beneath the alignment. βcat, β-catenin; plak, plakoglobin. B, wild-type and mutant fusion proteins were assayed for their ability to bind α-catenin using the bead binding assay as described for Fig. 2. Filters were subsequently immunoblotted with anti-Arm antibody to compare loading of the different fusion proteins (lower panel). N2, the wild-type fragment containing amino acids 25–165 of the N terminus; N2-MX, mutant X in the wild-type N2 fragment; CS, wild-type embryo extract (Canton S); GST, GST with no portion of Arm attached; MMW, medium molecular weight markers.

![Fig. 7. The N-terminal half of α-catenin binds to Arm. A, GST-α-catenin fusion proteins were assayed for binding activity for Arm in the bead binding assay as described for Fig. 2. Bound Arm was analyzed by SDS-PAGE and immunoblotting with anti-Arm antibody (anti-BicD antibody was used to control for nonspecific binding). α-cat, full-length GST-α-catenin fusion protein; α-catRI, the N-terminal half of α-catenin. B, the same blot was stained with Ponceau S to detect the amount of fusion protein in each reaction. Arrowheads indicate the positions of full-length fusion proteins. Molecular weight markers are indicated on the left. HMW, high molecular weight markers; MMW, medium molecular weight markers; CS, wild-type embryo extract (Canton S).

![Fig. 8. Mapping the region of Arm required for DE-cadherin binding. A, wild-type embryo extract in NET was incubated with GST-Arm fusion protein (diagramed in Tables I and II) bound to beads, and bound proteins were analyzed by SDS-PAGE and immunoblotting with anti-DE-cadherin antibody (upper panel). Anti-BicD antibody was used as a control for nonspecific binding (lower panel). B, blots were stained with Ponceau S to visualize the amount of fusion protein used in each reaction. Arrowheads indicate the positions of full-length fusion proteins. Molecular weight markers are indicated on the left. HMW, high molecular weight markers; CS, wild-type embryo extract (Canton S); R, Arm repeats.)
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Summary of Arm/cadherin interactions in the two-hybrid system.

A subset of the repeat region fragments tested for interaction with DE-cadherin (DE-cad) and mouse OB-cadherin (mOB-cad) are diagramed; amino acids present in each construct are indicated. Binding activities are given as plus and minus symbols. R, Arm repeats.

| N-terminal domain | Repeat region | C-terminal domain | Armadillo | DE-cad | mOB-cad |
|-------------------|---------------|-------------------|-----------|--------|---------|
|                   |               |                   | A          |        |         |

One discrepancy was noted: in the two-hybrid system, a few amino acids (amino acids 101–139) within the minimal interaction binding region blocks interaction with α-catenin in vivo (27). This latter mutant is embryonic lethal and completely deficient in adherens junction function (27).

Several studies examined the regions of the vertebrate relatives of Arm, β-catenin and plakoglobin, required for α-catenin binding (11–13, 15). These studies provide an excellent complement to our work. Comparison of these data with our own reveals general agreement as to the site of binding, although differences in the details may reveal subtleties of the in vivo situation. Arm, β-catenin, and plakoglobin share substantial amino acid sequence identity through the α-catenin-binding region, and thus, these similarities are not surprising. The most extensive previous study was that of Aberle et al. (15), who analyzed the region of plakoglobin involved in α-catenin binding. They found that amino acids 109–137 of plakoglobin (Arm amino acids 125–154) are necessary and sufficient for interaction with α-catenin (15); point mutations within this region identified amino acids critical to this interaction. In contrast, a larger region of Arm is required for full α-catenin binding; 76 amino acids of Arm are required for strong interaction with α-catenin (Figs. 2 and 3), while only 28 amino acids of plakoglobin are sufficient for binding (15). Our clustered point mutants provide strong support for a more extended binding site. Several mutations outside the minimal region defined by Aberle et al. (15) block binding of α-catenin to Arm (Fig. 6).

Several possible explanations exist for these differences. First, different assays are employed that may be more or less sensitive. Second, our experiments may identify regions that promote but are not essential for binding. Third, and less likely due to the sequence similarity between plakoglobin and Arm, these proteins may genuinely differ in the details of α-catenin binding.

We also obtained information about the region of α-catenin required to bind Arm. The Arm-binding site on α-catenin maps to its N-terminal third (Figs. 4 and 7). α-Catenin has three blocks of sequence similarity to vinculin, VH1, VH2, and VH3 (43, 44); the Arm-binding site roughly corresponds to VH1, while the actin-binding site of vinculin is found in the C-terminal region near VH3 (43, 44).

The Central Repeats of Armadillo Bind to DE-cadherin—We found that binding of Arm to DE-cadherin in vivo does not require the N- or C terminus of Arm, but does require a signif-
significant portion of the central Arm repeat region (Fig. 5) (27). Examination of DE-cadherin binding in vitro and in yeast allowed us to further narrow down the region required. A fragment carrying Arm repeats 3–8 is the smallest piece of Arm that interacts strongly with DE-cadherin. Removal of single repeats (repeat 3, 5, or 8) from this minimal fragment abolished interaction with DE-cadherin, although in the context of longer Arm fragments, these particular Arm repeats may not be essential for binding.

We confirmed the in vivo relevance of this binding site and compared the effects of mutations in the repeat region both on DE-cadherin binding in yeast and on DE-cadherin binding and adherens junction function in flies. Our two-hybrid data are largely consistent with the mutant phenotypes in vivo (27). Mutations in repeat 1 or in repeats 10 and 11 (S6 and S12) do not alter binding of Arm to DE-cadherin in yeast or in flies, while deletion of repeats 3–6 eliminates binding both in yeast and in flies. The one discrepancy involves the deletion of repeat 5 (S5), which reduces but does not eliminate DE-cadherin interaction and adherens junction function in flies (27); in the two-hybrid system, this mutation has little or no effect on binding of repeats 1–13 to DE-cadherin. Perhaps the two-hybrid system, where interacting proteins are expressed at high levels in a foreign environment, is less sensitive to reductions in the affinity of the interaction.

Our results suggest two models for the nature of the cadherin-binding site on Arm: either it is partially redundant, or the binding site is nonredundant, but must be present in the context of a minimum of six Arm repeats. Perhaps to form a proper binding site and even for individual repeats to fold into an appropriate tertiary structure, multiple repeats must fold together into a higher order structure. All Arm repeat proteins carry a block of six or more Arm repeats in tandem or near-tandem arrays (9). The DE-cadherin-binding site may span several repeats on the surface of a higher order structure, or it may be localized to a single repeat, but only recognized in the context of that higher order structure.

Our data complement and extend those obtained with the vertebrate relatives of Arm (10–12, 45). All studies agree that the central repeats are involved in binding. Plakoglobin truncated after repeat 8 interacts with N-cadherin, whereas shorter truncations progressively lose interaction (11). A plakoglobin variant lacking repeat 4 shows reduced but detectable binding to E-cadherin (12).

in vivo deletion analysis of β-catenin implicates the Arm repeats in E-cadherin binding; in one study, interaction of E-cadherin with a β-catenin protein truncated after repeat 10 was not observed (10), while in contrast, an analogous truncation of Xenopus β-catenin binds C-cadherin in vitro, whereas shorter fragments (repeats 1–9) do not (45). These results are consistent with our in vitro results; armXP33 mutant protein, truncated in repeat 10, partially colocalizes with DE-cadherin in vitro and retains a small amount of adhe-
suggesting that part of the
ated. Mutation of all eight serines to alanines blocks
eight serine residues, at least some of which are phosphoryl-
action (14, 46–49); this region of mouse E-cadherin contains
asterisks
E-cadherin mutated by Stappert and Kemler (49) are marked with
serine phosphorylation may be required for
binding (49). Stappert and Kemler (49) have suggested that
region of vertebrate cadherins is required for
domain are sufficient for Arm binding (Fig. 11). A similar
more extended region.

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REFERENCES
1. Kemler, R. (1993) Trends Genet. 9, 317–321
2. Kirkpatrick, C., and Peifer, M. (1995) Curr. Opin. Genet. & Dev. 5, 56–65
3. Reynolds, A. B., Daniel, J., McCrea, P., Wheelock, M. J., Wu, J., and Zhang, Z.
(1994) Mol. Cell. Biol. 14, 8333–8342
4. Aberle, H., Schwartz, H., Hoschuetzky, H., and Kemler, R. (1996) J. Cell Biol.
76, 789–791
5. Kinch, M. S., Clark, G. J., Der, C. J., and Burridge, K. (1995) J. Cell Biol. 130,
461–471
6. Nakonechny, I., Sacco, P. A., McGranahan, T. M., Wheelock, M. J., Matsuyoshi, N.,
Takeichi, M., and Ito, F. (1995) J. Cell Sci. 107, 3655–3663
7. Hinck, L., Naehke, I. S., Papkoff, J., and Nelson, W. J. (1994) J. Cell Biol. 125,
1341–1352
8. Peifer, M., Berg, S., and Reynolds, A. B. (1994) Cell 76, 789–791
9. Hu¨lsken, J., Birchmeier, W., and Behrens, J. (1994) J. Cell Biol. 127,
20201–20206
10. Sacco, P. A., McGranahan, T. M., Wheelock, M. J., and Johnson, K. R. (1995)
J. Biol. Chem. 270, 8813–8817
11. Aberle, H., Butz, S., Stappert, J., Weissig, H., Kemler, R., and Hoschuetzky, H.
(1994) J. Cell Sci. 107, 3655–3663
12. Orosz, K. A., Soler, A. P., Johnson, K. R., and Wheelock, M. J. (1995) J. Cell
Biol. 130, 67–77
13. Daniel, J. M., and Reynolds, A. B. (1995) Mol. Cell. Biol. 15, 4819–4824
14. Shapiro, L., Fannon, A. M., Kwong, P. D., Thompson, A., Lehmann, M. S.,
Kitamura, N., Johnson, K. R., Wheelock, M. J., Matsuyoshi, N., Takeichi,
M., and Ito, F. (1995) J. Cell Sci. 107, 3655–3663
15. Aberle, H., Schwartz, H., Hoschuetzky, H., and Kemler, R. (1996) J. Biol.
Chem. 271, 1520–1526
16. Rimm, D. L., Koslov, E. R., Kebrina, S., Giancetta, C. D., and Morrow, J. S. (1995)
Proc. Natl. Acad. Sci. U. S. A. 92, 8813–8817
17. Kitamura, N., Johnson, K. R., Wheelock, M. J., Matsuyoshi, N., Takeichi, M.,
and Ito, F. (1995) J. Cell Biol. 128, 949–957
18. Daniel, J. M., and Reynolds, A. B. (1995) Mol. Cell. Biol. 15, 4819–4824
19. Shapiro, L., Fannon, A. M., Kwong, P. D., Thompson, A., Lehmann, M. S.,
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REFERENCES
1. Kemler, R. (1993) Trends Genet. 9, 317–321
2. Kirkpatrick, C., and Peifer, M. (1995) Curr. Opin. Genet. & Dev. 5, 56–65
3. Reynolds, A. B., Daniel, J., McCrea, P., Wheelock, M. J., Wu, J., and Zhang, Z.
(1994) Mol. Cell. Biol. 14, 8333–8342
4. Aberle, H., Schwartz, H., Hoschuetzky, H., and Kemler, R. (1996) J. Cell Biol.
76, 789–791
5. Kinch, M. S., Clark, G. J., Der, C. J., and Burridge, K. (1995) J. Cell Biol. 130,
461–471
6. Nakonechny, I., Sacco, P. A., McGranahan, T. M., Wheelock, M. J., Matsuyoshi, N.,
Takeichi, M., and Ito, F. (1995) J. Cell Biol. 128, 949–957
7. Hinck, L., Naehke, I. S., Papkoff, J., and Nelson, W. J. (1994) J. Cell Biol. 125,
1327–1340
8. Nakonechny, I. S., Hinck, L., Swedlow, J. R., Papkoff, J., and Nelson, W. J. (1994)
J. Cell Biol. 125, 1341–1352
9. Peifer, M., Berg, S., and Reynolds, A. B. (1994) Cell 76, 789–791
10. Hu¨lsken, J., Birchmeier, W., and Behrens, J. (1994) J. Cell Biol. 127,
20201–20206
11. Sacco, P. A., McGranahan, T. M., Wheelock, M. J., and Johnson, K. R. (1995)
J. Biol. Chem. 270, 20201–20206
12. Orosz, K. A., Soler, A. P., Johnson, K. R., and Wheelock, M. J. (1995) J. Cell
Biol. 130, 67–77
13. Daniel, J. M., and Reynolds, A. B. (1995) Mol. Cell. Biol. 15, 4819–4824
14. Shapiro, L., Fannon, A. M., Kwong, P. D., Thompson, A., Lehmann, M. S.,
Assembling Fly Adherens Junctions

Grübel, G., Legrand, J.-F., Als-Nielsen, J., Colman, D. R., and Hendrickson, W. A. (1995) *Nature* **374**, 327–337, and references therein

20. Nagar, B., Overduin, M., Ikura, M., and Rini, J. M. (1996) *Nature* **380**, 360–364, and references therein

21. Oda, H., Uemura, T., Shiomi, K., Nagafuchi, A., Tsukita, S., and Takeichi, M. (1993) *J. Cell Biol.* **121**, 1133–1140

22. Oda, H., Uemura, T., Harada, Y., Iwai, Y., and Takeichi, M. (1994) *Dev. Biol.* **165**, 716–726

23. Peifer, M. (1993) *J. Cell Sci.* **105**, 993–1000

24. Cox, R. T., Kirkpatrick, C., and Peifer, M. (1996) *J. Cell Biol.* **134**, 133–148

25. Uemura, T., Oda, H., Kraut, R., Hatashi, S., Kataoka, Y., and Takeichi, M. (1996) *Genes Dev.* **10**, 659–671

26. Tepass, U., Gruzynski-DeFeo, E., Haaag, T. A., Omatyar, L., Torsik, T., and Hartenstein, V. (1996) *Genes Dev.* **10**, 672–685

27. Orsulic, S., and Peifer, M. (1996) *J. Cell Biol.* **134**, 1283–1301

28. Riggleman, B., Wieschaus, E., and Schell, P. (1989) *Genes Dev.* **3**, 96–113

29. Blanar, M. A., and Rutter, W. J. (1992) *Science* **256**, 1014–1018

30. Landt, O., Grunert, H.-P., and Hahn, U. (1990) *Gene (Amst.)* **96**, 125–128

31. Peifer, M., and Wieschaus, E. (1999) *Cell* **83**, 1167–1178

32. Brand, A. H., and Perrimon, N. (1993) *Development (Camb.)* **118**, 401–415

33. Vojtek, A. B., Hollenberg, S. M., and Cooper, J. A. (1993) *Cell* **74**, 205–214

34. Fields, S., and Sternglanz, R. (1994) *Trends Genet.* **10**, 286–289

35. Schiestl, R. H., and Gietz, R. D. (1989) *Curr. Genet.* **16**, 329–346

36. Guarente, L. (1983) *Methods Enzymol.* **101**, 181–191

37. Smith, D. B., and Johnson, K. S. (1988) *Gene (Amst.)* **76**, 31–40

38. Suter, B., and Steward, R. (1991) *Cell* **67**, 917–926

39. McCrea, P., Turck, C., and Gumbiner, B. (1991) *Science* **254**, 1359–1361

40. Cowin, P., Kapprell, H. P., Franke, W. W., Tamkun, J., and Hynes, R. O. (1986) *Cell* **46**, 1063–1073

41. McNear, P. D., and Gumbiner, B. M. (1991) *J. Biol. Chem.* **266**, 4514–4520

42. Peifer, M., Orsulic, S., Sweeton, D., and Wieschaus, E. (1993) *Development (Camb.)* **118**, 1191–1207

43. Nagafuchi, A., Takeichi, M., and Tsukita, S. (1991) *Cell* **65**, 849–857

44. Herrenknecht, K., Ozawa, M., Eckerskorn, C., Löttspeich, F., and Kemler, R. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88**, 9156–9160

45. Pagotto, F., Funayama, N., Glück, U., and Gumbiner, B. M. (1996) *J. Cell Biol.* **132**, 1105–1114

46. Nagafuchi, A., and Takeichi, M. (1988) *EMBO J.* **7**, 3679–3684

47. Ozawa, M., Baribault, H., and Kemler, R. (1989) *EMBO J.* **8**, 1711–1717

48. Ozawa, M., Ringwald, M., and Kemler, R. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87**, 4246–4250

49. Stappert, J., and Kemler, R. (1994) *Cell Adhesion Commun.* **2**, 319–327

50. Robinfield, B., Souza, B., Albert, I., Munemitsu, S., and Polakis, P. (1995) *J. Biol. Chem.* **270**, 5549–5555

51. Robinfield, B., Albert, I., Proff, R., Fiol, C., Munemitsu, S., and Polakis, P. (1996) *Science* **272**, 1023–1026