Identification of a *Drosophila* Homologue of \(\alpha\)-Catenin and Its Association with the *armadillo* Protein

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**Abstract.** The cadherin cell adhesion system plays a central role in cell–cell adhesion in vertebrates, but its homologues are not identified in the invertebrate. \(\alpha\)-Catenins are a group of proteins associated with cadherins, and this association is crucial for the cadherins’ function. Here, we report the cloning of a *Drosophila* \(\alpha\)-catenin gene by low stringent hybridization with a mouse \(\alpha\)E-catenin probe. Isolated cDNAs encoded a 110-kD protein with 60% identity to mouse \(\alpha\)E-catenin, and this protein was termed \(\alpha\)c-catenin. The gene of this protein was located at the chromosome band 80B. Immunostaining analysis using a mAb to \(\alpha\)-catenin revealed that it was localized to cell–cell contact sites, expressed throughout development and present in a wide variety of tissues. When this protein was immunoprecipitated from detergent extracts of *Drosophila* embryos or cell lines, several proteins co-precipitated. These included the *armadillo* product which was known to be a *Drosophila* homologue of \(\beta\)-catenin, another cadherin-associated protein in vertebrates, and a 150-kD glycoprotein. These results strongly suggest that *Drosophila* has a cell adhesion machinery homologous to the vertebrate cadherin–catenin system.

**Cadherins** are a family of \(\text{Ca}^{2+}\)-dependent cell–cell adhesion receptors identified in vertebrates. Without cadherins, vertebrate cells dissociate and cannot maintain tissues, suggesting a central role in the organization of multicellular structures (for review see Takeichi, 1991). In the invertebrate, however, these molecules have not been identified, and it is even not known whether similar adhesion systems are present. Although some *Drosophila* genes encode proteins that contain sequences characteristic of the cadherins, their intracellular domains and overall size are quite different from those of the vertebrate cadherins (Mahoney et al., 1991; Hortsch and Goodman, 1991; S. Hirano, T. Uemura, and M. Takeichi, unpublished data). The *fat* product is one of these proteins, defined as members of the cadherin superfamily (Mahoney et al., 1991; for review see Magee and Buxton, 1991). *fat* appears to control the growth of imaginal discs, and whether its product has cell adhesion activity is not known. On the other hand, other types of vertebrate cell adhesion receptors or matrix proteins have been shown to be conserved in *Drosophila* (for review see Hortsch and Goodman, 1991). These include the position specific integrins (Bogaert et al., 1987; MacKrell et al., 1988; Leptin et al., 1989), laminin (Montell and Goodman, 1988), and immunoglobulin superfamily molecules (Seeger et al., 1988; Snow et al., 1989; Bieber et al., 1989; Grenningloh et al., 1990).

Vertebrate cadherins associate with a group of cytoplasmic proteins, \(\alpha\), \(\beta\), and \(\gamma\)-catenin and plakoglobin, via the cytoplasmic domain (Nagafuchi and Takeichi, 1989; Ozawa et al., 1989; McCrea et al., 1991; Knudsen and Wheelock, 1992). The association with \(\alpha\)-catenin is indispensable for cadherins to exhibit cell–cell binding activity. This was shown by using lung carcinoma PC9 cells that expressed E-cadherin and \(\beta\)-catenin, but not \(\alpha\)-catenin. While PC9 cells cannot significantly aggregate (Shimoyama et al., 1992), their transfectants with \(\alpha\)-catenin cDNA acquire the ability to form epithelioid aggregates using the E-cadherin–\(\alpha\)-catenin complex (Hirano et al., 1992). Obviously, the function of cadherins depends on the presence of these cytoplasmic proteins. Recently, one of the *Drosophila* segment polarity genes, *armadillo* (*arm*), was found to encode a homologue of \(\beta\)-catenin or plakoglobin (Peifer and Wieschaus, 1990; McCrea et al., 1991; Peifer et al., 1992). In addition to these findings, two pieces of other information suggest that the cadherin adhesion system may exist in *Drosophila*. First, *Drosophila* cells have well developed adherens junctions (Poodry and Schneiderman, 1970) which are similar to the vertebrate structures where cadherins are concentrated (for review see Tsukita et al., 1992). Those *Drosophila* adherens junctions are localized at the apical regions of cell–cell junctions, as found in the vertebrate. Secondly, *Drosophila* embryonic cells possess a \(\text{Ca}^{2+}\)-dependent aggregating property, which resembles that described for vertebrate cells (Gratecos et al., 1990).

1. Abbreviations used in this paper: arm, *armadillo*; wg, wingless.
In the present study, we attempted to identify Drosophila genes that were homologous to cDNA of mouse αE-catenin, a subtype of α-catenins (Nagafuchi et al., 1991; Herrnkecht et al., 1991). Low stringency screening of a Drosophila genomic DNA library allowed us to detect a gene encoding a protein which was similar to α-catenins. This protein, termed Δc-catenin (Drosophila α-catenin), localized at cell—cell contact sites in many Drosophila tissues. Most interestingly, arm protein co-immunoprecipitated with Δc-catenin. These results strongly support the hypothesis that the Drosophila has a cell adhesion machinery similar to the vertebrate cadherin system in its molecular organization.

Materials and Methods

Cloning of Δc-Catenin cDNA

The 1.5-kb HindIII—EcoRI fragment of mouse αE-catenin, originally termed CAP102, cDNA (Nagafuchi et al., 1991) was used as a probe to screen the XΔASH Drosophila genomic DNA library (made by L. and Y. N. Jan, University of California, San Francisco, CA). This fragment represents the carboxyl half of the molecule. Hybridization solution contained 5 × SSC, 1 × Denhardt's solution, 1% SDS, 20 mM sodium phosphate (pH 7.2), 100 μg/ml salmon sperm DNA, and 10 or 20% formamide. Filters were hybridized with the double-stranded DNA probe at 42°C overnight (longer than 16 h), and washed first with 5 × SSC at room temperature, then with 5 × SSC at 42°C and finally with 2 × SSC at 42°C for 20-min each. One positive clone (ΔD2-1) was obtained from 1.5 × 10⁵ recombinants. Within a 14-kb insert of this phase clone, we found that the probe hybridized with a 0.7-kb Accl—XhoI fragment specifically, and partial sequences of the 0.7 kb DNA were shown to be homologous to those of mouse αE-catenin. The 0.7-kb fragment was used to screen several cDNA libraries. cDNA clone Δcα was isolated from a library of 9-12-h-old embryos (K. Zinn, Pasaden, CA), ΔEβ from a library of 3-12-h-old embryos (L. Kauvar and T. Kornberg, University of California, San Francisco, CA), and ΔEBF from a library of eye imaginal discs (G. Rubin, University of California, Berkeley, CA). Sequencing double-stranded DNA was carried out using the Sequenase kit (U.S. Biochemical, Cleveland, OH).

For Northern blot analysis, total RNA was extracted by the CHAOS method (Jonas et al., 1985), and selection of poly(A)+ RNA was done batchwise using Oligotex (Takara Biochem., Inc., Japan). The RNA was run on formaldehyde gels, blotted onto Hybond N (Amersham Corp., Arlington Heights, IL) and ultraviolet cross-linked (Stratalinker, Stratagene, La Jolla, CA).

Chromosome In Situ Hybridization

To determine cytological position of the Δc-catenin gene, the genomic phage clone AD2-1 was digoxigenin labeled with random primers according to manufacturer's instructions (Boehringer Mannheim Biochemicals, Indianapolis, IN). For hybridization and signal detection, we followed a protocol of T. Tanimura, Kyushu University (T. Tanimura, personal communication).

Preparation of Antibodies to Δc-Catenin

Antibodies to Δc-catenin were produced by using a fusion protein as an antigen. The 2.0-kb BglII—EcoRI fragment of a cDNA clone, that includes amino acids 258 to 928 of Δc-catenin, was inserted into pGEMEX-1 vector (Promega Biotec, Madison, WI). Expression of the fusion protein was induced by adding isopropyl-β-D-thio-galactopyranoside (0.5 mM) to Escherichia coli cultures. Bacteria were disrupted by sonication in 50 mM Tris (pH 76) and 150 mM NaCl (TBS), and proteins in the extract were separated by SDS-PAGE. After brief staining with Coomassie blue, a band of the fusion protein was excised and the protein was electroeluted using a Bio- trap elution chamber (Schleicher & Schuell Inc., Keene, NH). The recover solution was dialyzed against PBS and emulsified with complete Freund adjuvant.

The antigen was intraperitoneally injected into Donryu rats (40-50 μg antigen per animal). These animals were boosted three times with the antigen mixed with incomplete Freund adjuvant at 3-wk intervals. 4 d after the last boost, splenocytes of these animals were fused with P3U1 myeloma according to the method of Köhler and Milstein (1975). Hybridoma culture supernatants were screened by immunoblotting for specific reactivity to another Δc-catenin fusion protein expressed using a fusion construct that was made by subcloning the 2.0-kb BglII—EcoRI fragment into pMALcRI vector (NEB). One of the mAbs obtained, DCAT-1, showed the highest affinity to Δc-catenin.

Immunoblot Analysis

Proteins were separated by SDSPAGE (7.5% polyacrylamide) and transferred to nitrocellulose sheets. After incubating the blots with primary antibodies and HRP-linked secondary antibodies, signals were detected by the ECL Western blotting detection system (Amersham Corp.). Blots were repeatedly used by removing antibodies according to manufacturer's instructions. Con A binding assay was performed as described by Brower et al. (1984). Blots were incubated with biotin-Con A (Hones Corp., Japan) at the concentration of 50 μg/ml. Polyvinylpyrrolidone-360 was used as a blocking agent.

Cell Culture

Drosophila cell line MLDmBG-1 (Hirano et al., 1991) was cultured with modified M3 (BF) medium (Ui et al., 1987) that was supplemented with 10% heat-inactivated FCS and 10 μg/ml insulin. Cells were incubated at 25°C under air.

Immunohistochemistry

MLDmBG-1 cells were fixed with 3.5% paraformaldehyde in C & G's balanced saline (55 mM NaCl, 40 mM KCl, 15 mM MgSO4, 5 mM CaCl2, 10 mM Tricine, 20 mM glucose, and 50 mM sucrose, pH 6.9). The fixed cells were rinsed with TBSC (50 mM Tris, pH 7.6, 150 mM NaCl, and 1 mM CaCl2), treated with 0.3% Triton X-100 in TBSC, and rinsed again with TBSC. The samples were blocked with 1% BSA in TBSC. These samples were then treated with primary antibodies, species-specific biotinylated anti-rat IgG and finally with fluorescent dye-coupled streptavidin. After washing, the samples were mounted in 90% glycerol containing 1 μg/ml paraphenylenediamine.

Fixation of embryos was performed in 3.5% paraformaldehyde, 0.1 M Pipes (pH 6.9), 2 mM MgSO4, and 1 mM EGTA; 10% NF-40 was added to this solution when fixing larval tissues. The samples were blocked in TBSC containing 0.5% NF-40 and 0.5% BSA. All incubations with antibodies were done in this buffer except that BSA concentration was reduced to 0.1%. For double-immunostaining embryos, Texas red-conjugated anti-rat IgG was used for detecting DCAT-1, and the combination of biotinylated anti-mouse IgG and FITC-conjugated streptavidin was used for anti-armadillo mAb TA1 (a gift of M. Peifer, University of North Carolina, Chapel Hill, NC). All secondary antibodies and fluorescent dye-coupled streptavidin were purchased from Amersham Corp.

Immunoprecipitation

To extract Δc-catenin from cultured cells or embryos, we followed the protocol described by Hirano et al. (1992). MLDmBG-1 cells, cultured in a 100 mm dish, were metabolically labeled overnight with 100 μCi/ml tritiated methionine (ICN Biochemicals, Irvine, CA). They were lysed in 1 ml of an extraction buffer containing 1% Triton X-100, 1% NF-40, 1 mM CaCl2, and mixtures of protease inhibitors, and incubated for 30 min. After centrifugation, 50 μl of 8% BSA and 60 μl of 5 M NaCl were added to the supernatant, and this solution was preincubated with 400-500 μl of Sepharose CL-4B (Pharmacia Fine Chemicals, Piscataway, NJ). After centrifugation, DCAT-1 hybridoma culture supernatant was added to one half of the extract in a ratio of 1:2, and, as a control, a fresh culture medium was added to the other half in the same ratio. After 1 h, 50 μl of anti-rat IgG-conjugated Sepharose 4B beads (Zymed, S. San Francisco, CA) were added and incubated for 1 h. The immune complexes were washed several times with the extraction buffer without protease inhibitors, and then proteins were released from the beads by boiling for 3 min in 100 μl of a SDS-PAGE sample buffer containing 5% β-mercaptoethanol. In case of extraction from embryos, about 0.4 g of 15-h-old dechorionated embryos were homogenized in 1 ml of the extraction buffer, and, to collect the antigens, Sepharose 4B beads to which DCAT-1 antibodies were directly coupled were used.
Results

Identification of a Drosophila Homologue of the αE-Catenin Gene and Its cDNA Cloning

To test whether Drosophila has a homologue of α-catenin, blots of Drosophila genomic DNA were hybridized with mouse αE-catenin cDNA probes under low stringent conditions. We could detect faint signals when probing with a fragment that covers the carboxy half of the mouse protein (data not shown). To clone homologous sequences, we screened a Drosophila genomic DNA library using the same probe, and obtained one positive clone. Then, cDNA clones that hybridized with this genomic sequence were isolated. The nucleotide sequences of the cDNAs were determined, and the open reading frame of 2,805 nucleotides was found. The predicted translational product of this reading frame was a 105 kD protein with 935 amino acid residues (Fig. 1). The amino acid sequence of this molecule showed 60 and 62% identities to mouse αE-catenin and chick αN-catenin, respectively (Fig. 2). Because this sequence similarity extended along almost the entire length of the vertebrate α-catenins, we concluded that the encoded molecule was a Drosophila homologue, and designated it as Dxα-catenin. The alignment of the three α-catenins predicts that Dxα-catenin has a longer amino terminus (Fig. 2A). Although we have not determined which of three methionines at positions 1, 19, and 25 was the real translational start codon, the methionine residue at position 1 may be the initiation site, because Dxα-catenin migrated slightly slower than mouse αE- and chick αN-catenins in SDS-PAGE (data not shown).

Vertebrate α-catenins are known to have similarity to vinculin, a major undercoat protein of the adherens junction (Geiger, 1979; Geiger et al., 1980), in three domains that are underlined in Fig. 2A (Nagafuchi et al., 1991; Herrenknecht et al., 1991; Hirano et al., 1992). Similarity of Dxα-catenin to vinculin was comparable to those of vertebrate α-catenins. Dxα-catenin showed 21, 29, and 33% identities to chicken αN-catenins in these three domains, respectively (Fig. 2B). The Dxα-catenin gene was located at the chromosome band 80B. The most recent genetic map of Drosophila (FlyBase version 9209) did not describe any mutations that exactly map to this cytological position. We were unable to detect other Drosophila α-catenin genes which hybridized with Dxα-catenin cDNA probes.

Detection of the Dxα-Catenin Protein

A 3.4-kb transcript of the Dxα-catenin gene was detected throughout development (data not shown). To identify translation products of this gene, mAb DCAT1 was prepared against a Dxα-catenin fusion protein expressed in E. coli. This antibody recognized a single 110-kD band whose size was similar to the predicted value (105-kD) in immunoblots of whole animal lysates (Fig. 3). The Dxα-catenin protein was detected at all stages of development, but its abundance varied. It increased in abundance during embryogenesis, but decreased at the late third instar larval stage. While detection of the protein was difficult in whole third instar larvae, the protein was fairly abundant in a fraction that includes imaginal discs and brain. Pupa and adult samples also contained Dxα-catenin.

To examine subcellular localization of Dxα-catenin, we immunostained a Drosophila cell line MLDmBG-1 with DCAT1. Signals were clearly observed at cell–cell boundaries, but not on other parts of the cell surface (Fig. 4). This localization pattern was reminiscent of those of α-catenins and adherins in vertebrate cells.

Immunohistochemical Localization of Dxα-Catenin in Embryos and Larvae

Embryos at various developmental stages and tissues of third instar larvae were immunostained with DCAT1. At the cellular blastoderm stage (stage 5), all cells stained positively, and the signal was restricted to their cell–cell boundaries (Fig. 5, A and B). This expression in the overlying ectoderm persisted through embryogenesis. We compared the expression of Dxα-catenin with that of the arm product, a homologue of β-catenin, by double immunostaining. At stage 9, expression of the arm protein showed a stripe pattern along...
Figure 3. Immunoblot analysis of Dα-catenin during development. The same amount of total protein (100 μg) was loaded in each lane. Although the 110-kD band is hardly detectable in the whole bodies of late third instar larva (lane larva), a strong signal can be seen in the sample of CNS and imaginal discs (lane CNS+disc) isolated from the larvae. A 70-kD band in the lane of 18–23-h-old embryos appeared to be a degradation product. 110, the 110-kD Dα-catenin band. Molecular size markers for 200, 116, 97, 66, and 45 kD are indicated with bars.

Figure 4. Subcellular localization of Dα-catenin in a Drosophila cell culture. MLDmBG-1 cells were stained with DCA1. (A) Phase-contrast micrograph. (B) Immunofluorescence image of the same field shown in A. Dα-catenin molecules are enriched at cell-cell boundaries, not in other parts of the cell surface. Bar, 20 μm.

Figure 2. Sequence similarity of Dα-catenin to vertebrate α-catenins and vinculin. (A) Alignment of the deduced amino acid sequences of Dα-catenin (Dα), mouse αE-catenin (αE; Nagafuchi et al., 1991), and chicken αN-catenin (αN; Hirano et al., 1992). Stippled are identical residues between Dα and either αE or αN. Three domains which are conserved among α-catenins and vinculin are indicated with underlines. (B) Schematic diagram showing the three conserved regions (α, β, and γ). Each α-catenin was divided into seven segments, and within every segment, identities were calculated between Dα and either αE or αN. Percentage figures on the left represent overall identities. Comparison of Dα-catenin with chicken vinculin (Coutu and Craig, 1988) are also shown. The domains α, β, and γ are included in the three functionally distinct domains of vinculin, respectively (black boxes).

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Figure 5. Distribution of Dot-catenin during embryogenesis. (A and B) Cellular blastoderm stage (stage 5). A higher magnification clearly demonstrates the accumulation of Dot-catenin at cell-cell junctions (B). (C and D) Lateral view of an embryo at stage 9, double-stained with DCAT-1 (C), and anti-armadillo antibody 7A1 (D). (E) Ventral view of an embryo at stage 16 stained with DCAT-1. Strong signals in the neuropile of CNS are observed. (F) Hindgut in an embryo at stage 16. Left is anterior in all panels. Bars: (A and C-E) 50 μm; and (B and F) 10 μm.

guts (Fig. 5 F). A prominent ladder-like structure in CNS was visualized by this antibody, suggesting that axons were enriched with Dot-catenin.

Dot-catenin was also found at cell-cell boundaries in many larval tissues. In the leg disc, interfaces of all epithelial cells stained for Dot-catenin (Fig. 6, A and B). The eye disc expressed Dot-catenin in a non-uniform fashion (Fig. 6 C). In the undifferentiated region in front of the morphogenetic furrow (Tomlinson and Ready, 1987), signals were present ubiquitously, while in the differentiating region, these were highly concentrated in the photoreceptor clusters in the ommatidia. The accumulation of Dot-catenin was much stronger at the photoreceptor junctions than at the interfaces of non-neuronal cells such as pigment cells. In the salivary gland duct, sharp Dot-catenin-positive cell boundaries were seen (Fig. 6 D). These expression patterns of Dot-catenin in larval organs were similar to those previously reported for arm protein (Peifer and Wieschaus, 1990).

Co-Immunoprecipitation of Dot-Catenin with Other Proteins

In vertebrate cells, α-catenin associates with cadherin, β-catenin and some other proteins, and these proteins can be co-immunoprecipitated as a molecular complex (Nagafuchi and Takeichi, 1989; Ozawa et al., 1989; Nelson et al., 1990; McCrea et al., 1991; Knudsen and Wheelock, 1992). To reveal proteins that may associate with Dot-catenin, we immunoprecipitated Dot-catenin using DCAT-1 from a detergent extract of MLDmBG-1 cells that had been metabolically labeled with [35S]methionine. Electrophoresis of the immunoprecipitated materials showed several bands including 150-, 110-, and 106-kD bands (Fig. 7 A, arrows).
Figure 6. D\(\alpha\)-catenin localization in larval tissues. (A and B) Leg imaginal disc. A high magnification of a central region of the same sample is shown in B. (C) Eye imaginal disc. Arrowhead indicates the morphogenetic furrow (MF), which divides the disc into the posterior (below) and the anterior (above) regions. In the undifferentiated region anterior to MF, D\(\alpha\)-catenin is present homogeneously in all cell-cell borders, while in the differentiated region posterior to MF, D\(\alpha\)-catenin is present homogeneously in all cell-cell borders, while in the differentiated region posterior to MF, it is condensed in the clusters of the photoreceptor cells in the ommatidia. (D) Salivary gland duct. Bars, 20 \(\mu\)m.

Figure 7. Co-immunoprecipitation of D\(\alpha\)-catenin and the arm protein from MLDMBG-1 cell lysates. (A) Autoradiogram made after SDS-PAGE of an immunoprecipitate obtained with DCAT-1 (lane 1) or with control medium (lane 2) from a lysate of [\(^{35}\)S]methionine-labeled MLDMBG-1 cells. Molecular masses of major proteins are shown. (B) Immunoblotting detection of D\(\alpha\)-catenin (left) and the armadillo protein (right). Whole MLDMBG-1 cell lysate (lanes 1 and 4), materials precipitated with DCAT-1 (lanes 2 and 5) and those prepared with control medium (lanes 3 and 6) were blotted, and probed with DCAT-1 (left) or anti-arm antibody 7A1 (right). The 106-kD form of the armadillo protein co-precipitated with D\(\alpha\)-catenin. The same immunoprecipitated samples were used for A and B. To determine the relationships between the immunoreactive and radiolabeled bands, the filters in B were autoradiographed (not shown). Molecular mass markers (200, 116, 97, and 66 kD) are indicated with bars.

Figure 8. Co-immunoprecipitation of a 150-kD glycoprotein with D\(\alpha\)-catenin from 0-15-h-old embryonic lysates. (A) Silver-stained pattern of materials immunoprecipitated with DCAT-1. (B) Detection of D\(\alpha\)-catenin (left), the armadillo protein (middle), and Con A-binding proteins (right). Whole embryo lysate (lanes 1, 4, and 7), immunoprecipitate with DCAT-1 (lanes 2, 5, and 8) and control precipitate (lane 3, 6, and 9) were blotted. A 150-kD Con A-reactive band is detected in the immunoprecipitate with DCAT-1. Smear in higher molecular mass regions is not reproducible. Molecular mass markers (200, 116, 97, 66, and 45 kD) are indicated with bars.

To identify these bands, an immunoblot of the precipitated materials was first autoradiographed, and then repeatedly probed with DCAT-1 and an anti-arm mAb. On this blot, DCAT-1 recognized a single 110-kD band (Fig. 7 B, lane 2) and the anti-arm antibody reacted with a 106-kD band (Fig.
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7 B, lane 5), that perfectly overlapped with the 35S-labeled 110- and 106-kD proteins, respectively. In crude lysates of MLDMbg-1 cells, the anti-arm antibody detected a doublet of 109- and 106-kD bands (Fig. 7 B, lane 4), but the 109-kD form was not reproducibly detected in the materials coprecipitating with Da-catenin.

We also used extracts of 0-15-h-old embryos for immunoprecipitating Da-catenin. Fig. 8 A shows a silver-stained gel pattern of proteins precipitated with DCAT-1. As demonstrated in the above experiments, the precipitated materials contained both Da-catenin and the arm product (Fig. 8 B, lanes 2 and 5). On the silver-stained gel, these proteins probably corresponded to the 110-kD band (Fig. 7 C, arrow) and a faint band migrating just in front of the 110-kD band, respectively. All these results suggested a direct or indirect association of Da-catenin with the arm protein. Although a 63-kD protein was efficiently recovered from extracts of both the cell line (Fig. 7 A) and embryos (Fig. 8 A), its identity remains to be studied.

Finally, we tested whether any glycoproteins coprecipitated with Da-catenin; cadherins are glycosylated and at least E-cadherin is known to be recognized by Con A (McCrea and Gumbiner, 1991). Anti-Da-catenin immunoprecipitates from embryos contained a prominent Con A-binding protein, the molecular mass of which was 150-kD (Fig. 8 B, lane 8). This protein was not clearly identified at the corresponding position in the silver-stained gel (Fig. 8 A). The sample obtained from MLDMbg-1 cells did not give any Con A-positive bands (data not shown).

Discussion

a-Catenins constitute a small molecular family in vertebrates. At least two members, aE and aN-catenins, have been identified, and vinculin is also a relative of this molecular family (Nagafuchi et al., 1991; Herrenknecht et al., 1991; Hirano et al., 1992). Our recent work demonstrated that a-catenins played crucial roles in cadherin function via an interaction with the cadherin's cytoplasmic domain (Nagafuchi and Takeichi, 1988, 1989; Ozawa et al., 1990). a-Catenin is important not only for cadherin function but also for organizing the multicellular structures (Hirano et al., 1992). In the present study, we identified a Drosophila homologue of a-catenin (Da-catenin) which had 60 and 62% identities to the vertebrate aE and aN, respectively. This high similarity strongly suggests that this Drosophila molecule is functionally similar to the vertebrate homologues.

The cellular localization and tissue distribution of Da-catenin were studied in a cell line, embryos, and larval organs, and all results showed that it was localized at cell-cell junctions. This expression pattern of Da-catenin was quite similar to that of the Drosophila ß-catenin homologue arm. The only difference we observed was during stage 9 of embryogenesis. While the arm protein showed a striped pattern in each parasegment, the distribution of Da-catenin was rather uniform.

The identification of a- and ß-catenin homologues in Drosophila strongly supports the idea that Drosophila may have a cell adhesion system involving cadherins. In vertebrate cells, aE or aN-catenin is always co-expressed with some type of cadherin. Furthermore, in the present study, we found that Da-catenin coprecipitated with the arm protein. This provided strong evidence, though circumstantial, for the presence of cadherin homologues in Drosophila. In the vertebrate cells so far studied, cadherin, a-, and ß-catenins are always co-precipitated as a complex when they are extracted under mild conditions (Nagafuchi and Takeichi, 1989; Ozawa et al., 1989; McCrea and Gumbiner, 1991). We thus suspected that a cadherin-like molecule might coprecipitate with Da-catenin and arm protein.

To test this possibility, we analyzed the materials that immunoprecipitated with the anti-Da-catenin antibody from two different sources, embryos and a cell line MLDMbg-1. The embryo-derived sample contained a glycoprotein recognized by Con A. Cadherins are glycosylated and at least E-cadherin binds to Con A (McCrea and Gumbiner, 1991). We consider this Con A-reactive protein as a candidate of Drosophila cadherin. The immunoprecipitates obtained from the cell line also contained a protein of similar size, but this did not react with Con A. This cell line might have other subclasses of cadherin, as multiple subclasses of cadherin have been identified in vertebrates (Takeichi, 1990). Similar results were obtained when immunoprecipitates with anti-arm antibodies were examined for co-precipitation of Da-catenin and Con A-binding proteins (M. Peifer, personal communication). Future characterizations of these proteins should be most intriguing for identification of hypothetical Drosophila cadherins.

armadillo belongs to a subclass of the segment polarity genes. This subclass (wingless [wg] class) consists of at least eight loci, and mutations in any of these genes cause replacement of the posterior regions of each segment by anterior structures, producing mirror-symmetrical denticle patterns in embryos (Nüsslein-Volhard and Wieschaus, 1980; Wieschaus et al., 1984; Perrimon and Mahowald, 1987; Perrimon et al., 1989). With respect to both axis determination in embryos and development of imaginal discs, wingless (wg) mutants exhibit a nearly identical phenotype to that of arm; therefore, a functional interaction has been assumed (Peifer et al., 1991). Our finding that Da-catenin physically associates with arm protein suggests that Da-catenin or the putative cadherin with which it associates might play a role in the wg signaling pathway. Double immunostaining for Da-catenin and the arm protein in stage 9 embryos showed that their distributions are similar but not identical, indicating that the function of these molecules could be differential. We are currently unable to investigate the function of Da-catenin by means of genetic analysis, because no promising mutant candidate of this gene is available. We hope in the future to pursue isolation of mutations in the Da-catenin gene. These mutations, together with those in arm, would provide a genetic approach to the roles of these proteins and also the putative cadherin adhesion system in development and multicellular organization of the animals.

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