Identification of a *Drosophila* Homologue of α-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. α-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* α-catenin gene by low stringent hybridization with a mouse αE-catenin probe. Isolated cDNAs encoded a 110-kD protein with 60% identity to mouse αE-catenin, and this protein was termed Dα-catenin. The gene of this protein was located at the chromosome band 80B. Immunostaining analysis using a mAb to Dα-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 β-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 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of the vertebrate cadherins (Mahoney et al., 1991; Hortsch and Goodman, 1991; S. Hirano, T. Uemura, and M. Takeichi, unpublished data).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, α, β, and γ-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 α-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 β-catenin, but not α-catenin. While PC9 cells cannot significantly aggregate (Shimoyama et al., 1992), their transfectants with α-catenin cDNA acquire the ability to form epithelioid aggregates using the E-cadherin-α-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*)[1], was found to encode a homologue of β-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 Ca^{2+}-dependent aggregating property, which resembles that described for vertebrate cells (Gratecos et al., 1990).

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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; Herrenknecht 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 Dα-catenin (*Drosophila* α-catenin), localized at cell–cell contact sites in many *Drosophila* tissues. Most interestingly, *arm* protein co-immunoprecipitated with Dα-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 Dα-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 λDASH *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× SCC at room temperature, then with 5× SSC at 42°C and finally with 2× SCC at 42°C for 20-min each. One positive clone (λD2-1) was obtained from 1.5 × 10^5 recombinants. Within a 14-kb insert of this phage clone, we found that the probe hybridized with a 0.7-kb AccI–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 λKZ was isolated from a library of 9–12-h-old embryos (K. Zinn, Pasadena, CA), λE8 from a library of 3–12-h-old embryos (L. Kauvar and T. Kornberg, University of California, San Francisco, CA), and λEYE 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 Dα-catenin gene, the genomic phage clone λD2-1 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 Dα-Catenin

Antibodies to Dα-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 Dα-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 7.6) 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 Biotrap elution chamber (Schleicher & Schuell Inc., Keene, NH). The recovered 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 Dα-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 Dα-catenin.

Immunoblot Analysis

Proteins were separated by SDS-PAGE (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 (Honen 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 Ig 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; 1.0% NP-40 was added to this solution when fixing larval tissues. The samples were blocked in TBSC containing 0.5% NP-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 7A1 (a gift of M. Peifer, University of North Carolina, Chapel Hill, NC). All secondary antibodies and fluorescent dye-coupled streptavidins were purchased from Amersham Corp.

Immunoprecipitation

To extract Dα-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 tran ³⁵S (ICN Biochemicals, Irvine, CA). They were lysed in 1 ml of an extraction buffer containing 1% Triton X-100, 1% NP-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 0–15 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

Figure 1. Nucleotide and deduced amino acid sequence of Dα-catenin cDNA. In the longest open reading frame, amino acids coordinates were measured from the first in-frame methionine which we predicted was the translational start codon (see text). The estimated translational product is a 105-kD protein containing 935 amino acid residues. Nucleotide 1-140 represents the sequence of cDNA clone λEYE, and the rest of the sequence is derived from clone λKZ. These sequence data are available from EMBL/Genbank/DDBJ under accession number D13964.

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 Dα-catenin. The alignment of the three α-catenins predicts that Dα-catenin has a longer amino terminus (Fig. 2 A). 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 Dα-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. 2 A (Nagafuchi et al., 1991; Herrenknecht et al., 1991; Hirano et al., 1992). Similarity of Dα-catenin to vinculin was comparable to those of vertebrate α-catenins. Dα-catenin showed 21, 29, and 33% identities to chicken vinculin in these three domains, respectively (Fig. 2 B).

The Dα-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 Dα-catenin cDNA probes.

Detection of the Dα-Catenin Protein

A 3.4-kb transcript of the Dα-catenin gene was detected throughout development (data not shown). To identify translation products of this gene, mAb DCAT-1 was prepared against a Dα-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 Dα-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 Dα-catenin.

To examine subcellular localization of Dα-catenin, we immunostained a Drosophila cell line MLDmBG-1 with DCAT-1. 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 cadherins in vertebrate cells.

Immunohistochemical Localization of Dα-Catenin in Embryos and Larvae

Embryos at various developmental stages and tissues of third instar larvae were immunostained with DCAT-1. 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 Dα-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

A

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Dα  MLQPALPLHCIYPSVKEFMLKPDKMGTLTDFGQIALKNDPKNLEIRTMSVEKTLEPLVLQ        60
αE          M TAVHAGNINFKWDPKSLEIRTLAVERLLEPLVTQ                        35
αN          M TSATSPII LKWDPKSLEIRTLTVERLLEPLVTQ                        34

    VTTLVNT KGPSKKKKGKSKRASALVAAVEKATENFIQKGEQIAYENPDITQEMLTAVD        118
    VTTLVNTNSKGPSNKRGRSKKAHVLAASVEQATENFLEKGDKIAKESQFLKEELVVAVE         95
    VTTLVNTSNKGPSGKKKGRSKKAHVLAASVEQATQNFLEKGDQIAKESQDLKEELVAAVE         94

    EVKKTGDAMSIAAREFSEDPCSSLKRGNMVRAARNLLSAVTRLLILADMVDVHLLLKSLH        178
    DVRKQGDLMKSAAGEFADDPCSSVKRGNMVRAARALLSPVTRLLILADMADVYKLLVQLK        155
    DVRKQGETMRIASSEFADDPCSSVKRGTMVRAARALLSAVTRLLILADMADVMRLLSHLK        154

    IVEDDLNKLKNASS QDELMDNMRQFGRNAGELIKQAAKRQQELKDFQLRDDLAAARAML        237
    VVEDGILKLRNAGNEQD LGIQYKALKPEVDKLNIMAAKRQQELKDVGNRDQMAAARGIL        214
    IVEEALEAVKNATNEQD LANRFKEFGKEMVKLNYVAARRQQELKDPHCRDEMAAARGAL        213

    KKHSTMLLTASKVYVRHPELDLAKVNRDFILKQVCDAVNTISDVAQGKSSQPTDIYS G        295
    QKNVPILYTASQACLQHPDVAAYKANRDLIYKQLQQAVTGISNAAQATASDDAAQHQGGS        274
    KKNATMLYTASQAFLRHPDVAATRANRDYVFKQVQEAIAGISNAAQATSPTDENKGHTG        272

    AGELAAALDDFDEGIVMDPMTYSEKRSRQLLEERLESIISAAALMADADCTRDERRERIV        355
    GGELAYALNNFDKQIIVDPLSFSEERFRPSLEERLESIISGAALMADSSCTRDDRRERIV        334
    IGELAAALNEFDNKIILDPMTFSEARFRPSLEERLESIISGAALMADSSCTRDDRRKRIV        332

    AECN AVRQALQDLLSEYMSNMSQKDNSPGLSRAIDQMCRKTRDLRRQLRKAVVDHVSDS        414
    AECN AVRQALQDLLSEYMGNAGRKERSDALNSAIDKMTKKTRDLRRQLRKAVMDHVSDS        393
    AECKRAVRQALQDLLSEYMNNTGRKEKGDPLNIAIDKMTKKTRDLRRQLRKAVMDHISDS        392

    FLETTTPLLDLIEAAKSGNEKKVREKSEIFTKHAEKLVEVANLVCSMSNNEDGVKMVRYA        474
    FLETNVPLLVLIEAAKNGNEKKVEYAQVFREHANKLIEVANLACSISNNEEGVKLVRMS        453
    FLETNVPLLVLIEAAKSGNEKEVKEYAQVFREHANKLVEVANLACSISNNEEGVKLVRMA        452

    AAQIESLCPQVINAASILTVRPNSKVAQENMTTYRQAHEVQVRILTEAVDDITTIDDFLA        534
    ASQLEALCPQVINAALALAAKPQSKLAQENMDLFKEQHEKQVRVLTDAVDDITSIDDFLA        513
    ATQIDSLCPQVINAALTLAARPQSKVAQDNMDVFKDQHEKQVRVLTEAVDDITSVDDFLS        512

    VSENHILEDVNKCVMALQVGDARDLRATAGAIQGRSSRVCNVVEAEMDNYEPCIYTKRVL        594
    VSENHILEDVNKCVIALQEKDVDGLDRTAGAIRGRAARVIHVVTSEMDNYEPGVYTEKVL        573
    VSENHILEDVNKCVIALQEGDVDTLDRTAGAIRGRAARVIHIINAEMENYETGVYTEKVL        572

    EAVKVLRDQVMMKFDQRVGAAVGALSNNSNKDVDENDFIDASRLVYDGVREIRRAVLMNR        654
    EATKLLSNTVMPCFTEQVEAAVEALSSDPAQPMDENEFIDASRLVYDGIRDIRKAVLMIR        633
    EATKLLSETVMPRFAEQVEVAIEALSANVPQPFEENEFIDASRLVYDGVRDIRKAVLMIR        632

    SSEDLDTDTEFEPVEDLTLETRSRSAHTGDQTVDEYPDISGICTAREAMRKMTEEDKQK        714
    TPEEL DDSDFET EDFDV RSRTSVQTED    DQLIAGQ SARAIMAQLPQEQKAK        683
    TPEELEDDSDFEQ EDYDV RSRTSVQTED    DQLIAGQ SARAIMAQLPQEEKAK        683

    IAQQVELFRRKLTFDSEVAKWDDTGNDIIFLAKHMCMIMMEMTDFTRGRGPLKTTMDVI        774
    IAEQVASFQEEKSKLDAEVSKWDDSGNDIIVLAKQMCMIMMEMTDFTRGKGPLKNTSDVI        743
    IAEQVEIFHQEKSKLDAEVAKWDDSGNDIIVLAKQMCMIMMEMTDFTRGKGPLKNTSDVI        743

    NAAKKISEAGTKLDKLTREIAEQCPESSTKKDLLAYLQRIALYCHQIQITSKVKADVQNI        834
    SAAKKIAEAGSRMDKLGRTIADHCPDSACKQDLLAYLQRIALYCHQLNICSKVKAEVQNL        803
    NAAKKIAEAGSRMDKLARAVADQCPDSACKQDLLAYLQRIALYCHQLNICSKVKAEVQNL        803

    SGELIVSGLDSATSLIQAAKNLMNAVVLTVKYSYVASTKYTR QGT VSSPIVVWKMKA        891
    GGELVVSGVDSAMSLIQAAKNLMNAVVQTVKASYVASTKYQKSQGMASLNLPAVSWKMKA        863
    GGELIVSGLDSATSLIQAAKNLMNAVVLTVKASYVASTKYQKVGTAAVNSPVVSWKMKA        863

    PEKKPLVRPEKPEEVRAKVRKGSQKKVQNPIHALSEFQSPADAV                      935
    PEKKPLVKREKQDETQTKIKRASQKKHVNPVQALSEFKAM DSI                      906
    PEKKPLVKREKPEEYQTRVRRGSQKKHISPVQALSEFKAM DSF                      906
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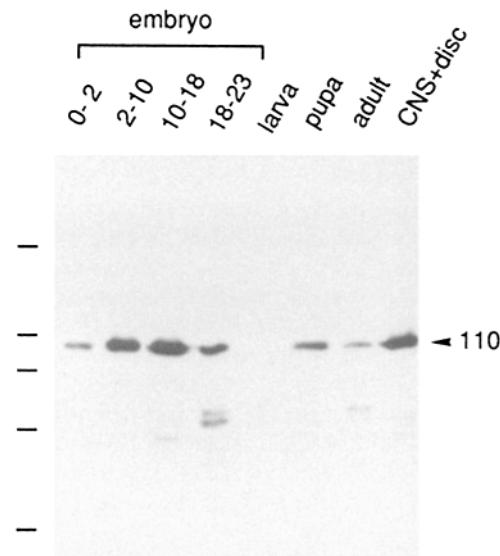

Figure 3. Immunoblot analysis of Dα-catenin during development. The same amount of total protein (100 μg) was loaded in each lane. Although the 110-kD band is hardly detectable in the extract of the whole bodies of late third instar larva (lane *larva*), a strong signal can be seen in the sample of CNS 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.
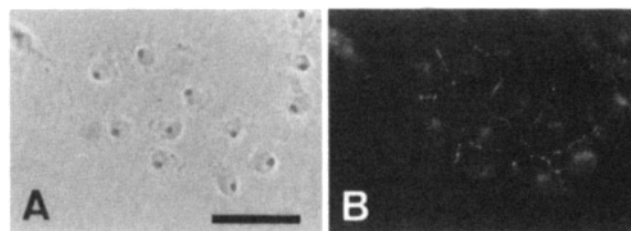

Figure 4. Subcellular localization of Dα-catenin in a *Drosophila* cell culture. MLDmBG-1 cells were stained with DCAT-1. (*A*) Phase-contrast micrograph. (*B*) Immunofluorescence image of the same field shown in *A*. Dα-catenin molecules are enriched at cell–cell boundaries, not in other parts of the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

B

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 (*a*, *b*, and *c*). Each α-catenin was divided

the anterior-posterior axis (Fig. 5 *D*), as reported (Riggleman et al., 1990). In contrast, Dα-catenin was distributed rather homogeneously (Fig. 5 *C*), although we could detect a subtle periodical change of the Dα-catenin level along the anterior-posterior axis. At stage 11, cells along the ventral midline strongly expressed both gene products (data not shown). Many differentiated organs of stage 16 embryos, which derived from different germ layers, also reacted with DCAT-1. These included the epidermis, the central nervous system (CNS) (Fig. 5 *E*), the peripheral nervous system and

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 *a*, *b*, and *c* are included in the three functionally distinct domains of vinculin, respectively (*black boxes*).

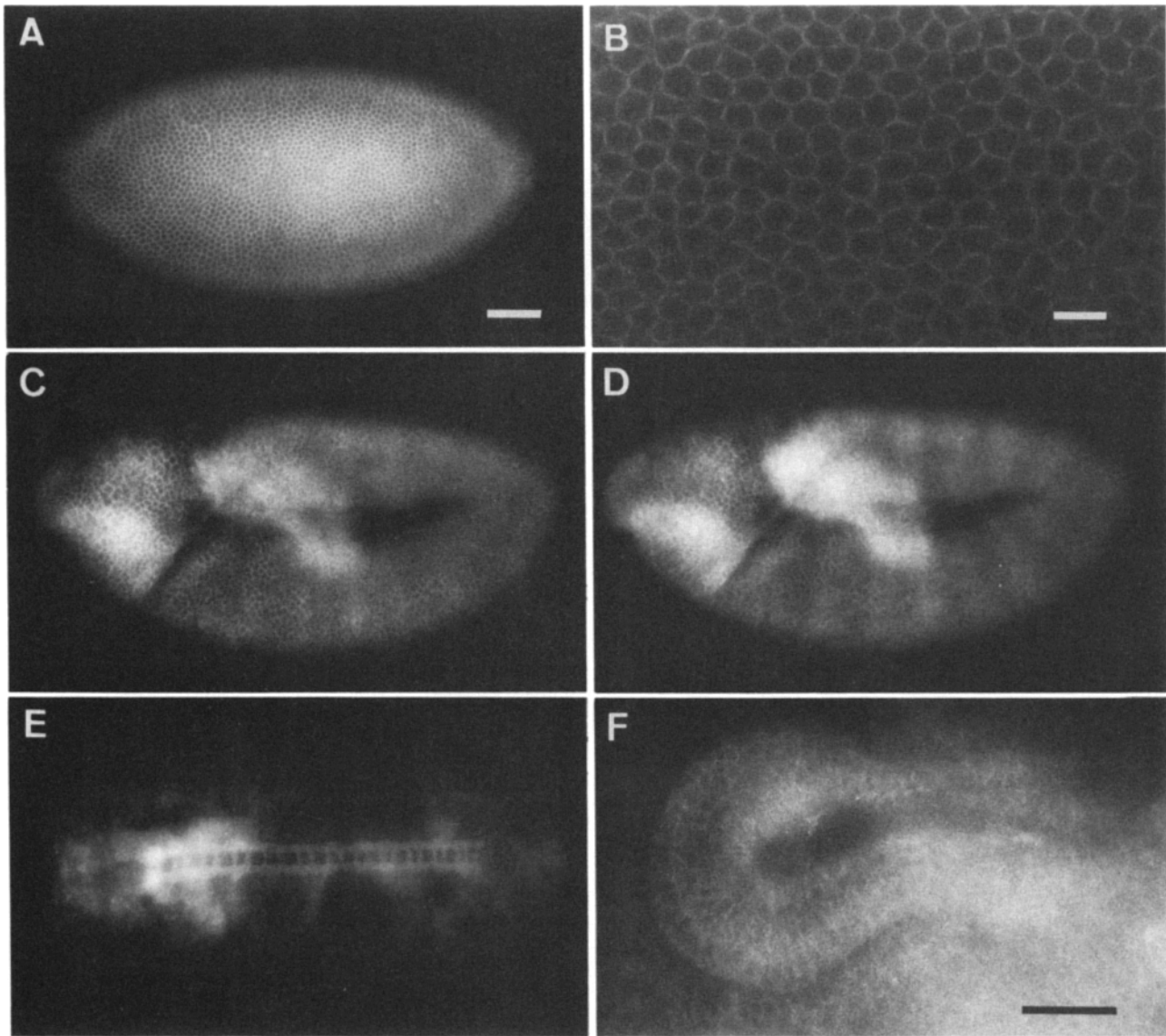*Figure 5.* Distribution of Dα-catenin during embryogenesis. (*A* and *B*) Cellular blastoderm stage (stage 5). A higher magnification clearly demonstrates the accumulation of Dα-catenin at cell–cell junctions (*B*). (*C* and *D*) Lateral view of an embryo at stage 9, double-stained with DCAT-1 (*C*), 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 Dα-catenin.

Dα-catenin was also found at cell–cell boundaries in many larval tissues. In the leg disc, interfaces of all epithelial cells stained for Dα-catenin (Fig. 6, *A* and *B*). The eye disc expressed Dα-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 Dα-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 Dα-catenin–positive cell boundaries were seen (Fig. 6 *D*). These expression patterns of Dα-catenin in larval

organs were similar to those previously reported for *arm* protein (Peifer and Wieschaus, 1990).

Co-Immunoprecipitation of Dα-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 Dα-catenin, we immunoprecipitated Dα-catenin using DCAT-1 from a detergent extract of MLDmBG-1 cells that had been metabolically labeled with [^{35}S]methionine. Electrophoresis of the immunoprecipitated materials showed several bands including 150-, 110-, and 106-kD bands (Fig. 7 *A*, *arrows*).
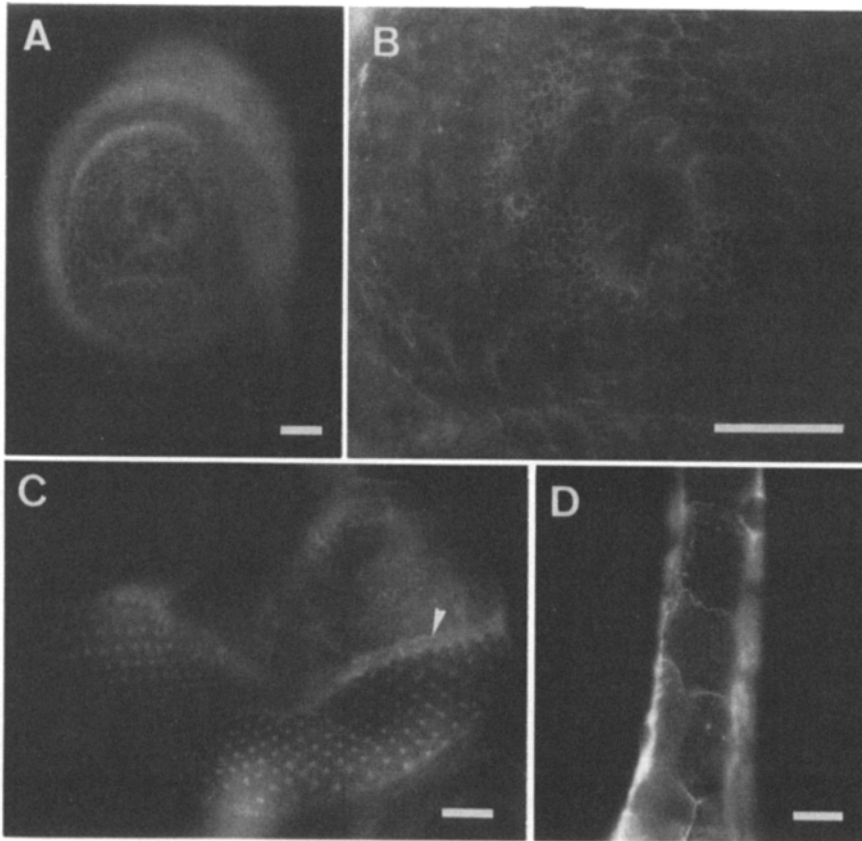
Figure 6. Dα-catenin localization in larval tissues. (A and B) Leg imaginal disc. A high magnification of a central region of the same sample is shown in B. (C) Eye imaginal disc. Arrowhead indicates the morphogenetic furrow (MF), which divides the disc into the posterior (below) and the anterior (above) regions. In the undifferentiated region anterior to MF, Dα-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 μm.
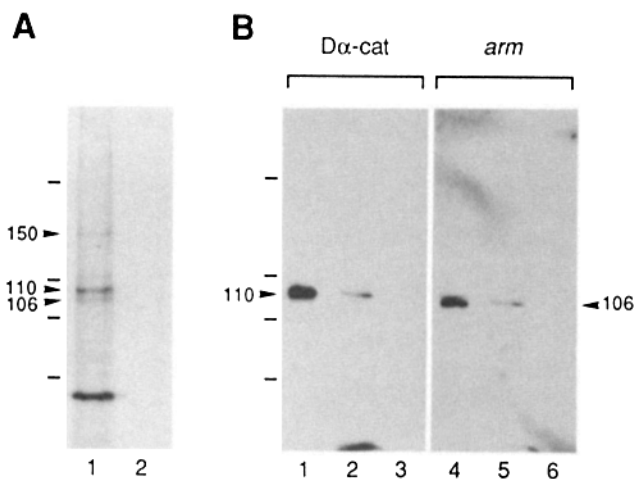
Figure 7. Co-immunoprecipitation of Dα-catenin and the *arm* protein from MLDmBG-1 cell lysates. (A) Autoradiogram made after SDS-PAGE of an immunoprecipitate obtained with DCAT-1 (lane 1) or with control medium (lane 2) from a lysate of [³⁵S]methionine–labeled MLDmBG-1 cells. Molecular masses of major proteins are shown. (B) Immunoblotting detection of Dα-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 Dα-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.
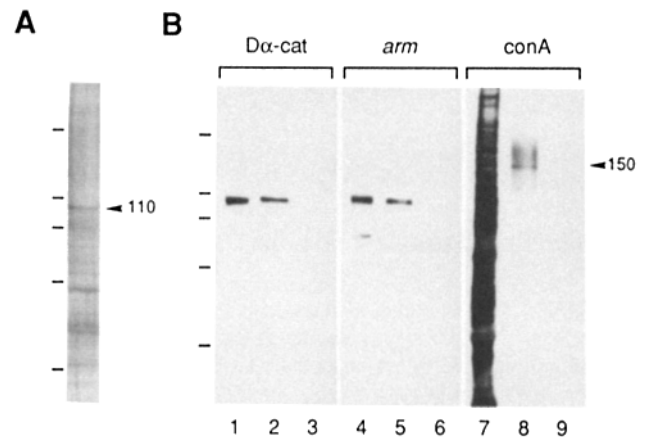
Figure 8. Co-immunoprecipitation of a 150-kD glycoprotein with Dα-catenin from 0–15-h-old embryonic lysates. (A) Silver-stained pattern of materials immunoprecipitated with DCAT-1. (B) Detection of Dα-catenin (*left*), the *armadillo* protein (*middle*), and Con A-binding proteins (*right*). Whole embryo 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.

7 *B*, lane *5*), that perfectly overlapped with the ^{35}S-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 Dα-catenin.

We also used extracts of 0–15-h-old embryos for immunoprecipitating Dα-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 Dα-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 Dα-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 Dα-catenin; cadherins are glycosylated and at least E-cadherin is known to be recognized by Con A (McCrea and Gumbiner, 1991). Anti-Dα–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

α-Catenins constitute a small molecular family in vertebrates. At least two members, αE and αN-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 α-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). α-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 α-catenin (Dα-catenin) which had 60 and 62% identities to the vertebrate αE and αN, respectively. This high similarity strongly suggests that this *Drosophila* molecule is functionally similar to the vertebrate homologues.

The cellular localization and tissue distribution of Dα-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 Dα-catenin was quite similar to that of the *Drosophila* β-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 Dα-catenin was rather uniform.

The identification of α- and β-catenin homologues in *Drosophila* strongly supports the idea that *Drosophila* may have a cell adhesion system involving cadherins. In vertebrate cells, αE or αN-catenin is always co-expressed with some type of cadherin. Furthermore, in the present study, we found that Dα-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, α-, and β-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 Dα-catenin and *arm* protein.

To test this possibility, we analyzed the materials that immunoprecipitated with the anti-Dα-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 Dα-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 Dα-catenin physically associates with *arm* protein suggests that Dα-catenin or the putative cadherin with which it associates might play a role in the *wg* signaling pathway. Double immunostaining for Dα-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 Dα-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 Dα-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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