A Homolog of the *armadillo* Protein in *Drosophila* (Plakoglobin) Associated with E-Cadherin

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Three cytoplasmic proteins, called catenins, bind to the cytoplasmic tail of the epithelial cell-cell adhesion molecule E-cadherin. The complementary DNA sequence was determined for the 92-kilodalton β catenin of *Xenopus laevis*. The sequence is homologous to mammalian plakoglobin, a protein of desmosomal and *zonula adherens* cell junctions, and to the plakoglobin homolog in *Drosophila melanogaster*, the product of the segment polarity gene *armadillo*. A monoclonal antibody to bovine plakoglobin recognizes the analogous β catenin in the Madin-Darby canine kidney (MDCK) cell line. *Armadillo* plakoglobin may link E-cadherin to the underlying actin cytoskeleton at cell-cell junctions; the E-cadherin–catenin protein complex may also participate in the transmission of developmental information.

THE CADHERINS ARE MEMBERS OF A family of Ca^{2+}-dependent cell-cell adhesion molecules involved in the morphogenesis of animal tissues (*1*). The functional activities of the cadherins involve not only specific (homotypic) intercellular recognition by their extracellular domains (*2, 3*) but also interaction with the cytoplasmic actin cytoskeleton by means of their intracellular domains (*4, 5*). The cytoplasmic tail domain is the most highly conserved region of the cadherin molecule (*6*). Three associated cytoplasmic proteins, called catenins (*7*), bind to this conserved cytoplasmic tail of E-cadherin to form a complex that is thought to interact directly or indirectly with actin filaments (*4, 5*). One of them (α catenin), was identified as a vinculin-like protein (*8*). The catenins seem to be required for E-cadherin function because deletions in the cytoplasmic tail of E-cadherin that result in a loss of catenin-binding also result in a loss of cell adhesion (*4, 9*). The *Xenopus laevis* β catenin, a 92-kD polypeptide, binds directly and tightly to the cytoplasmic tail of E-cadherin (*10*).

The β catenin was purified from the *Xenopus* epithelial cell line (A6) by coimmunoprecipitation with E-cadherin, selective elution from the immune complex, and preparative SDS–polyacrylamide gel electrophoresis (PAGE) (*10*). Amino acid sequences were determined for four tryptic peptides obtained from the purified protein (Fig. 1, overlines 1, 2, 3, and 4). A search against the Dayhoff database revealed that the peptides shared sequence identity or conservation with the *Drosophila* gene product *armadillo* (*11, 12*) and human plakoglobin (*13*).

We obtained a specific DNA probe for the *Xenopus* β catenin by amplifying the region between the sequences encoding peptides 1 and 2 with the polymerase chain reaction (PCR). We then used this PCR product to screen a λ-gt10 *Xenopus* embryonic cDNA library (*14*) at high stringency (*15*). The cDNA of a full-length clone was subcloned into the pBluescript vector for double-stranded sequencing (*16–18*).

The sequence of the *Xenopus* β catenin revealed that it is homologous to the *armadillo* protein in *Drosophila* (*11, 12*) and human plakoglobin (*13*), sharing 70% sequence identity with the *armadillo* protein and 63% identity with plakoglobin (Fig. 1). The four peptide sequences initially determined were found in the deduced β catenin protein sequence (Fig. 1, overlines 1, 2, 3, and 4).

To determine further whether the *Xenopus* β catenin is a homolog of mammalian plakoglobin, we tested whether it is recognized by the monoclonal antibody to bovine plakoglobin (*19*). Because antibody to plakoglobin did not crossreact with any polypeptide in *Xenopus* (Fig. 2), these experiments were performed with the Madin-Darby canine kidney (MDCK) cell line.

Immunoprecipitates from MDCK cells generated with antibody to E-cadherin (anti–E-cadherin) include the catenin polypeptides, as do anti–E-cadherin immunoprecipitates in all cell types and species examined so far (*7*). Under the same stringent detergent washing conditions used to coimmunoprecipitate the *Xenopus* E-cadherin–β catenin complex (*10*), a single ~90-kD polypeptide was associated with the

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b-cat  MATQADLMELDMAMEPD.....RKAAVSHWQQQSYL.DSGIHSGATTTAPSLSG  (48)
  arm  -SYM.PAQNRT-SHNNQynppdlppmvsA-EQTLM---N---g--------V-QV-----  (59)
 plak  -EVM.N---QPIK.....VTEW--T-Ty--------N-CV--V-S  (39)
            1
b-cat  KGNPEDEDVDTNQVLYEWEQGFSQSFTQDQVADIDGQYAMTRAQRVRAAMFPETLDEG.M  (107)
  arm  -...---EMEGDPLMFDLDT--P-N------D-MNQ-LSQ--S-----------E--.I  (115)
 plak  --IM-EDEACGR-YTLKKTTTYT-GVPPS-GDLEYQMSTTA--K---E--C-GVSG--qL  (99)
                                2
b-cat  QIPSTQFDSAHPTNVQRLAEPSQMLKHAVVNLINYQDDAELATRAIPELTKLLNDEDQVV  (167)
  arm  E-------PQQ--A----S-----------I----------  (175)
 plak  ALLA--VEGQ.A--L--------L--S-I-H----------V---L----------P--  (158)

b-cat  VNKAAVMVHQLSKKEASRHAIMRSPQMVSAIVRTMQNTNDVETARCTAGTLHNLSHHREG  (227)
  arm  -SQ--M---------------N-----A-L--AIS-S--L-STKAAV----------Q-  (235)
 plak  -T---MI-N---------R-L-G---L-A-V-------S-LD-----TSI----------  (218)
                          3
b-cat  LLAIFKSGGIPALVKMLGSPVDSVLFYAITTLHNLLLHQEGAKMAVRLAGGLQKMVALLN  (287)
  arm  ---------------L-S---E----------------D-S--------------T--Q  (295)
 plak  -------------R--S---E--------------Y-------CAGRRA.----P---  (277)

b-cat  KTNVKFLAITTDCLQILAYGNQESKLIILASGGPQALVNIMRTYSYEKLLWTTSRVLKVL  (347)
  arm  RN-------V----------NE--R---S-D--------------  (355)
 plak  -N-P----------L----------N-------Q---N----------------  (337)

b-cat  SVCSSNKPAIVEAGGMQALGLHLTDSSQRLVQNCLWTLRNLSDAATKQEGMEGLLGTLVQ  (407)
  arm  ----------D--------AM--GNM-P----------V--L-A--QS---  (415)
 plak  ---P----------K---SN-P----------V------L-SV-KI--N  (397)

b-cat  LLGSDDINVVTCAAGILSNLTCNNYKNKMMVCQVGGIEALVRTVLRAGDREDITEPAICA  (467)
  arm  V---T-V----------QR--AT------VD-----IIN-----E-----V--  (475)
 plak  Q-SV--V--L---T-T--------S---TL-T-NS-V---IHAI-----KD------V--  (457)

b-cat  LRHLTSRHQEAEMAQNAVRLHYGLPVVVKLLHPPSHWPLIKATVGLIRNLALCPANHAPL  (527)
  arm  --------VDS-L-------N---S-I--------R------VI----------  (535)
 plak  --------P-------S---N--I-AI----NQ-NQ---V---I---------  (517)

b-cat  REQGAIPRLVQLLVRAHQDTQRRTSIG...GTQQ..QFVEGVRMEEIVEGCTGALHILAR  (582)
  arm  --H---HH--R--M--F---E-QR-SIatt-S--psAYAD----------TV--------  (595)
 plak  Q-AAV---------K----A--HVAA....----p..YTD----------  (571)

b-cat  DIHNRIVIRGLNTIPLFVQLLYSPIENIQRVAAGVLCDVAQDKEAAEAIEAEGATAPLTE  (642)
  arm  ES---AL--QQSV--I--R--FNE----------EL-A---G--I--Q----G---D  (655)
 plak  -PM--ME-FR----------SV----------EL-------D--D----S---M-  (631)

b-cat  LLHSRNEGVATYAAAVLFRMSEDKPQDYKKRLSVELTSSLFRTEPMPWNEAADLG.....  (697)
  arm  ----------I---N--L-EDNNI-AN-.---mgpdl  (714)
 plak  --------T----------I----NP--R--V-----N---KHD-AA-EA-QSMipinep  (691)
                                  4
b-cat  ...LDIGAQGEPLGYRQDDSSYRS.....FHAAGYGQDAMGMDSM.....  (734)
  arm  qdm-GPEEAY-G-.-G-GPP-VH-shggra--QQ--..-TLPI---qgleisspvgggga  (771)
 plak  y.gD-MD-TYR-M.-S.....  (705)

b-cat  .....MDHDMGGHHPGADYPVDGLPDLSHAQ  (760)
  arm  ggapgnggavggasggggnigaippsgaptspys--M-V-EIDA--.....-NFDL  (822)
 plak  .....sdvpldple-HM--D-DY.....-IDTYSD  (730)

b-cat  DLMDGLPPGDSNQLAWFDTDL  (781)
  arm  -A-PTP-NDNN-LA--Y---C  (843)
 plak  G-RPPY-TA-HMLA.....  (744)
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Fig. 1. Alignment of the *Xenopus* β catenin (b-cat) protein sequence with the *armadillo* (arm) product from *Drosophila* (*11, 12*) and human plakoglobin (plak) (*13*) sequences (*16, 17*). Initially determined β catenin peptide sequences later revealed in the deduced amino acid sequence, are denoted by overlines (1, 2, 3, and 4) of the β catenin sequence. Uppercase letters are aligned, nonidentical amino acids; lowercase letters are unaligned amino acids; dashes are aligned identical amino acids; and dots represent gaps. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

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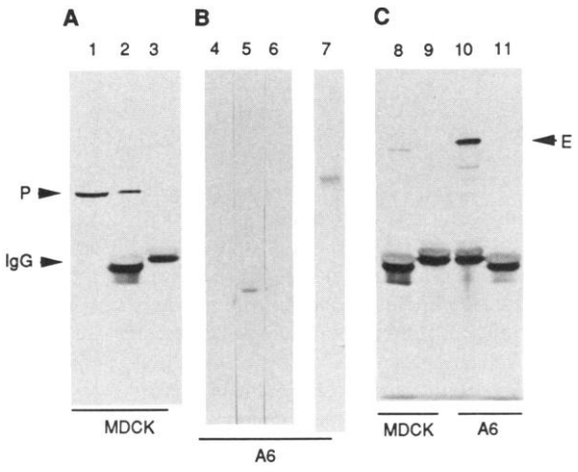

Fig. 2. Recognition by antibody to plakoglobin of β catenin associated with E-cadherin. (**A**) An MDCK whole-cell extract (lane 1), a stringently washed anti–canine E-cadherin immunoprecipitate of MDCK cells (lane 2), and a stringently washed negative-control immunoprecipitation (*21, 22*) (lane 3) were subjected to SDS-PAGE, blotted onto nitrocellulose, and reacted with antibody to plakoglobin. (**B**) An A6 (*Xenopus*) whole-cell extract (lane 4), a stringently washed anti–*Xenopus* E-cadherin immunoprecipitate of A6 cells (lane 5), and the purified *Xenopus* β catenin were subjected to SDS-PAGE, blotted onto nitrocellulose, and reacted with antibody to plakoglobin. Lane 7, silver stain of the purified β catenin in lane 6. (**C**) Lanes 8 and 9, samples correspond to those in lanes 2 and 3, respectively, but were immunoblotted with anti–canine E-cadherin. Lanes 10 and 11, immunoblots with anti–*Xenopus* E-cadherin of the sample run in lane 5 (lane 10) and of a stringently washed negative-control immunoprecipitation (*21, 22*) of a *Xenopus* whole-cell extract (lane 11). The electrophoretic mobility of MDCK E-cadherin is ~120 kD, and of *Xenopus* E-cadherin is ~140 kD. The abbreviations used are as follows: E, *Xenopus* E-cadherin; P, plakoglobin; and IgG, reduced heavy chains of immunoglobulin G used in immunoprecipitations (~55 kD).

canine E-cadherin (Fig. 3B); the α and γ catenins are removed (*10*). As would be anticipated for a cytoplasmic protein, this ~90-kD polypeptide was inaccessible to cell-surface labeling (Fig. 3A). In pulse-chase experiments, this newly synthesized polypeptide appeared rapidly in E-cadherin immunoprecipitates and underwent no detectable changes in gel mobility that might result from post-translational modifications (Fig. 3C). These properties are shared with the β catenin of *Xenopus*.

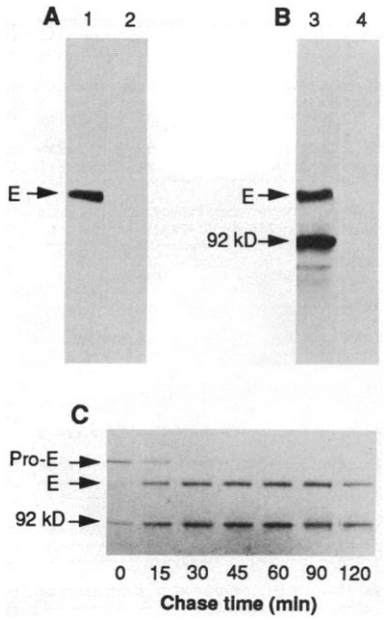

Fig. 3. The E-cadherin and β catenin complex in MDCK cells. (**A**) MDCK cell-surface iodination (^{125}I) (*33*), followed by immunoprecipitation with anti–canine E-cadherin (*20, 21*) (lane 1) or an irrelevant monoclonal antibody control (*21, 22*) (lane 2). (**B**) Stringently washed immunoprecipitates of metabolically labeled (steady-state ^{35}S-labeled methionine) MDCK cells with anti–canine E-cadherin (lane 3) or the irrelevant monoclonal antibody control (lane 4). (**C**) [^{35}S]methionine pulse-chase labeling of MDCK cells (15-min pulse). The abbreviations used are as follows: E, E-cadherin; and pro-E, E-cadherin precursor.

We therefore performed experiments to determine whether the β catenin polypeptide of MDCK cells was recognized by antibody to plakoglobin (Fig. 2A). A polypeptide comigrating with MDCK plakoglobin was present in the stringently washed immunoprecipitates isolated with the use of the monoclonal antibody to MDCK E-cadherin (*20*). But this polypeptide was not detected in negative-control precipitations when an irrelevant monoclonal antibody was used (*21, 22*) (Fig. 2A).

We conclude that the E-cadherin–associated β catenin is the homolog of the *armadillo* protein and plakoglobin because (i) *Xenopus* β catenin shares extensive sequence homology with the *armadillo* protein in *Drosophila* and human plakoglobin, and (ii) canine β catenin shares immunological crossreactivity with bovine plakoglobin. The sequence alignment data suggest that the *Xenopus* β catenin may be more closely related to the *armadillo* protein in *Drosophila* than to human plakoglobin (70% versus 63% identity, respectively), despite the greater phylogenetic distance. Although the *armadillo* gene product from *Drosophila* and human plakoglobin were initially reported to be homologs in different species [63% sequence identity (*12*)], they may turn out to be distinct members of a closely related family of proteins. So far, there is definitive evidence for only one species of plakoglobin in mammalian cells (*13*) and likewise only a single *armadillo*-like gene in *Drosophila* (*11*).

We consider it likely that some, if not all, of the immunoreactive plakoglobin-like molecules observed at the *zonula adherens* (*19*) are in fact β catenin and are bound directly to the cytoplasmic tail of E-cadherin. Plakoglobin has also been reported to interact directly with desmoglein I, a major glycoprotein component of the desmosomes (*23*). Desmoglein I is a member of the cadherin superfamily and contains the conserved catenin-binding domain of the cadherins (*24, 25*). Thus, plakoglobin-like molecules are probably components of both types of epithelial junctions because they are capable of binding to the cytoplasmic region of both desmoglein I and E-cadherin and may thereby mediate interactions with cytoskeletal components.

The *armadillo* gene is a member of the *wingless* class of *Drosophila* segment polarity genes (*12*). The *wingless* gene itself is the *Drosophila* homolog of the vertebrate oncogene *Wnt-1* (*26–28*) and encodes a secreted glycoprotein believed to interact with a plasma membrane receptor present on neighboring cells (*29, 30*). The amount of *armadillo* expression is sensitive to the *wingless* gene product secreted from nearby cells in developing *Drosophila* embryos (*31, 32*). These results and our demonstration that the β catenin bound to the cytoplasmic tail of E-cadherin is highly homologous to the *armadillo* protein suggest that the cadherin-catenin protein complex may participate in transducing developmental information between neighboring cells.

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subcloned into the Bluescript II KS+ vector (Stratagene, La Jolla, CA), and double-strand sequenced [Sequenase Version 2.0 protocol (United States Biochemical)], after both nested-deletion (Erase-a-Base, Promega), and oligonucleotide primer-walk methodologies (primers synthesized by personnel in the D. Julius laboratory, University of California, San Francisco). The deduced amino acid sequence was initiated from the first of four potential start methionines, giving a calculated molecular size of 85,434 daltons, somewhat lower than the ~92-kD of purified β catenin. We aligned protein sequences using the Doolittle multiple alignment algorithm (*17*) and made slight refinements by eye. The GenBank (*18*) accession number for β catenin is M77013.

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Folding of Circularly Permuted Transfer RNAs

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All of the ribose-phosphate linkages in yeast tRNA^{Phe} that could be cleaved without affecting the folding of the molecule have been determined in a single experiment. Circular permutation analysis subjects circular tRNA molecules to limited alkaline hydrolysis in order to generate one random break per molecule. Correctly folded tRNAs were identified by lead cleavage at neutral pH, a well-characterized reaction that requires proper folding of tRNA^{Phe}. Surprisingly, most of the circularly permuted tRNA molecules folded correctly. This result suggests that the tRNA folding motif could occur internally within other RNA sequences, and a computer search of Genbank entries has identified many examples of such motifs.

THE TERMINI OF PROTEINS OR RNA molecules can play an important role in defining their three-dimensional structure. Although an amino acid or nucleotide sequence can fold as a motif within the polymer chain, the termini can potentially either participate in unique interactions or be needed to promote a folding pathway. One method of evaluating the importance of the termini in macromolecular folding is to study the properties of the circularly permuted isomers. A circularly permuted polymer is produced by connecting the normal termini and cleaving the backbone at another site. If such a molecule folds normally, one can conclude that the termini are unnecessary for maintaining the structure or specifying a folding pathway. However, if the circularly permuted isomer misfolds, either the correct termini are required to maintain the structure, the new termini disrupt the structure, or the folding pathway is altered. The recent demonstration that two circularly permuted isomers of *Escherichia coli* phosphoribosyl anthranilate isomerase fold normally (*1*) prompted an investigation of the folding of circularly permuted RNA molecules.

Unmodified yeast tRNA^{Phe} was chosen for study because of its well-characterized three-dimensional structure (*2, 3*). Although the biological functions of tRNA require the correct termini, it is possible to monitor the folding of tRNA^{Phe} by measuring its specific cleavage reaction with lead (*4–7*). In this reaction, a Pb^{2+} ion is bound to the nucleotide bases U59 and C60 in the T loop and promotes a specific cleavage between U17 and G18 in the D loop. An analysis of the Pb^{2+} cleavage rates of more than 50 yeast tRNA^{Phe} mutants reveals that the rate of cleavage is very sensitive to the folded structure (*7*). Single mutations that disrupt tertiary interactions as far away as 16 to 20 Å from the lead binding site show a 3- to 20-fold reduction in the cleavage rate. The cleavage rate is restored when compensatory mutations allow the formation of an alternate tertiary interaction (*7*). The cleavage rate is not affected by mutations not expected to alter the folding of tRNA^{Phe} (*7*). Thus, this reaction is useful for examining the folding of circularly permuted tRNA molecules.

Circular permutation analysis (CPA) was carried out to determine which of the 76 possible circularly permuted tRNA^{Phe} molecules fold correctly (Fig. 1). The G1A mutant of tRNA^{Phe} was used because it has a mismatched terminal base pair that permits efficient formation of a circular tRNA by T4 RNA ligase (*8*) without affecting its ability to cleave with lead. The starting material for CPA was prepared by cleaving the circular tRNA with lead, introducing a ^{32}P label at the lead cleavage site between U17 and G18, and religating the tRNA (Fig. 1A). The uniquely labeled circular tRNA molecules were subjected to limited alkaline hydrolysis under denaturing conditions such that each molecule was cleaved no more than once and all bonds were cut with approximately equal frequency. The resulting collection of 76 different circularly permuted tRNAs was renatured and then treated with lead in the presence of Mg^{2+}. Those molecules that folded correctly, and therefore were cleaved with lead, produced an oligonucleotide with a 3′ ^{32}P label at the ribose of U17 and a 5′ terminus at the site generated by alkaline hydrolysis (Fig. 1B). For circularly permuted tRNAs that did not cleave with lead, no such shorter ^{32}P-labeled oligonucleotide was formed. Thus, separation of the reaction products on a sequencing gel and subsequent autoradiography identified all of the folding-permissive backbone breaks.

A typical CPA experiment is shown in Fig. 2A. The conditions chosen for alkaline hydrolysis resulted in a nearly uniform population of breaks when end-labeled linear tRNA was hydrolyzed. It is striking to find that a large number of circularly permuted tRNAs still cleaved with lead. We quantitated the radioactivity in each band and compared it to the band corresponding to the normal tRNA (break at ribose phosphate 1) to estimate the relative extent of lead cleavage at each position. Circularly permuted tRNAs with 5′ termini at 54 of 68 analyzable backbone positions had comparable or greater extents of lead cleavage than the native tRNA (Fig. 3). These positions include virtually the entire acceptor and T stems as well as most of the anticodon stem

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