The uvomorulin-anchorage protein α catenin is a vinculin homologue

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ABSTRACT The cytoplasmic region of the Ca²⁺dependent cell-adhesion molecule (CAM) uvomorulin associates with distinct cytoplasmic proteins with molecular masses of 102, 88, and 80 kDa termed α , β , and γ catenin, respectively. This complex formation links uvomorulin to the actin filament network, which seems to be of primary importance for its cell-adhesion properties. We show here that antibodies against α catenin also immunoprecipitate complexes that contain human N-cadherin, mouse P-cadherin, chicken A-CAM (adherens junction-specific CAM; also called N-cadherin) or Xenopus U-cadherin, demonstrating that α catenin is complexed with other cadherins. In immunofluoresence tests, α catenin is colocalized with cadherins at the plasma membrane. However, in cadherin-negative Ltk⁻ cells, α catenin is found uniformly distributed in the cytoplasm, suggesting some additional biological function(s). Expression of uvomorulin in these cells results in a concentration of α catenin at membrane areas of cell contacts. We also have cloned and sequenced murine α catenin. The deduced amino acid sequence reveals a significant homology to vinculin. Our results suggest the possibility of a new vinculin-related protein family involved in the cytoplasmic anchorage of cell-cell and cell-substrate adhesion molecules.

The cadherin gene family of Ca^{2+} -dependent cell adhesion molecules (CAM) was originally composed of a rather limited number of transmembrane glycoproteins of which the best studied examples were uvomorulin/E-cadherin, liver CAM (L-CAM), N-cadherin, and P-cadherin (for a review, see refs. 1 and 2). Each member was found to regulate cell adhesion of particular cell types, and this was thought to be fundamental for the organization of multicellular organisms. More recently new members of this family have been described including M-cadherin on mouse myoblasts (3), E/P-, U-, and XB-cadherin in early *Xenopus* development (4–6), and a new subgroup of more distantly related desmosomal glycoproteins (7–9).

It has been shown that the cytoplasmic region of uvomorulin associates with defined proteins of 102, 88, and 80 kDa termed α , β , and γ catenin, respectively (10). The linkage of uvomorulin to actin filaments via these proteins seems to be of crucial importance for the cell-binding function of uvomorulin (11). Moreover, catenins connect uvomorulin to other integral membrane proteins, such as Na⁺/K⁺-ATPase, or to cytoplasmic structures, such as fodrin or ankyrin. This indicates that uvomorulin is part of a cyto-cortical network (12). These findings support the concept that uvomorulinmediated adhesion induces redistribution of cytoplasmic and membrane proteins which, in turn, may initiate the molecular events that result in the transition of a nonpolarized to a polarized epithelial cell (2, 12). If so, catenins might play a central role in these processes. Since the catenin-binding

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domain is well conserved in other cadherins, it is possible that catenins may also complex with other members of this gene family (13, 14). Here we have produced antibodies against α catenin and show that α catenin is indeed associated with cadherins from human, mouse, and *Xenopus*. We have cloned and sequenced[¶] the cDNA coding for α catenin and have established the primary protein structure. Sequence comparison reveals homology to vinculin, a well-known adherens-type and focal contact protein.

MATERIALS AND METHODS

Cell Lines. Mouse fibroblasts Ltk⁻, human HeLa, chicken fibroblasts CEF38, and their respective transfectants expressing mouse uvomorulin, L1-1, H1-3, and C1-4 (10, 15) were used as well as embryonal carcinoma cells F9, PCC₄, and PAS5E. Porcine kidney LLC-PK₇ and *Xenopus* A6 cells were gifts from H. Hoschützky (Freiburg, F.R.G.) and D. Wedlich (Berlin), respectively. The A6 cells were grown in Leibovitz L-15 medium containing 8% (vol/vol) fetal calf serum (FCS) at 24°C. All other cells were cultured in Dulbecco's modified Eagle's medium containing 10% FCS at 37°C in an atmosphere containing 10% CO₂. For the generation of F9 tumors, about 1×10^7 cells were injected subcutaneously in 129/SV mice, and solid tumors were removed 12–15 days later and stored at -80°C.

Purification of α **Catenin.** Ten grams of solid F9 tumor was homogenized in 50 ml of Nonidet P-40 (NP-40)/TBS (2% NP-40/20 mM Tris·HCl, pH 7.5/0.15 M NaCl/2 mM CaCl₂/1 mM phenylmethylsulfonyl fluoride) at 4°C for 15 min. Insoluble material was pelleted (48,000 × g for 30 min at 4°C), and the supernatant was preincubated on a protein A-sepharose column (Pharmacia). Unbound material was incubated with 200 mg of rabbit anti-uvomorulin IgG covalently coupled to 20 ml of protein A-sepharose and washed with 250 ml of NP-40/salt buffer (0.1% NP-40/1 M NaCl/20 mM Tris·HCl, pH 7.5/2 mM CaCl₂). Uvomorulin–catenin complexes were eluted with 100 ml of acidic pH buffer (0.1% NP-40/0.1 M glycinehydrochloride, pH 2.5/2 mM CaCl₂), and after neutralization proteins were precipitated with 4 vol of 100% ethanol at -20°C.

Protein Microsequencing. Uvomorulin-catenin complexes collected from 100 g of tumor were separated on a SDS/6% polyacrylamide gel and, after Coomassie-staining, bands containing α catenin ($\approx 20 \ \mu$ g) were excised and digested with 5 μ g of endoproteinase LysC (Boehringer Mannheim) as described (16). Peptides were eluted with 75% (vol/vol) trifluoroacetic acid and separated by reversed-phase HPLC

Abbreviations: CAM, cell adhesion molecule; A-CAM, adherens junction-specific CAM (also called N-cadherin).

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[¶]The sequence reported in this paper has been deposited in the GenBank/EMBL data base (accession no. X59990).

as described (16). Amino acid sequence analysis of the peptides was performed with a gas-phase sequenator (model 477 A; Applied Biosystems) as described (17).

Antibodies. Rabbit antiserum against α catenin was produced by using a synthetic peptide, His-Val-Asp-Pro-Val-Gln-Ala-Leu-Ser-Glu-Phe-Lys (localized near the carboxyl terminus, see Fig. 4), coupled to keyhole limpet hemocyanin (Sigma) by glutaraldehyde. After five subcutaneous immunizations in intervals of 3 weeks, specific antibodies were isolated on a peptide- ε -aminohexanoyl (EAH)-Sepharose (Pharmacia) column (5 mg of peptide coupled to 1 ml of EAH-Sepharose 4B as described by Pharmacia). Rabbit antibodies against human P-cadherin and mouse N-cadherin (cross-reacting with mouse P-cadherin and human N-cadherin, respectively) have been raised against Escherichia coli TrpE-cadherin fusion proteins, containing approximately the carboxyl-terminal half of each cadherin protein. The respective cDNAs have been cloned by the polymerase chain reaction (PCR) technique (M.L. and Dietmar Vestweber, unpublished data). Monoclonal antibodies FA-5 and GC-4 against chicken A-CAM (adherens junction-specific CAM; also called N-cadherin) (18) were purchased from Sigma, and the monoclonal antibody 6D5 reacting with Xenopus U-cadherin (5) was a generous gift of B. Angres (Tübingen, F.R.G.). Immunoblot and immunoprecipitation experiments were carried out as described (10). For indirect immunofluorescence tests, cells grown on cover slips were fixed with methanol (-20°C), and anti- α -catenin antibodies were detected with goat fluorescein isothiocyanate-conjugated F(ab)₂ anti-rabbit immunoglobulin (Dynatech).

cDNA Isolation and Sequencing. An oligo(dT)-primed phage λ gt11 cDNA library prepared from poly(A)⁺ RNA of mouse embryonal carcinoma cells PCC₄-aza.1 was screened by using affinity-purified anti- α -catenin antibodies. Seven initial clones were obtained from 500,000 phage plaques, and additional 5' sequences were cloned by using a random primed (Amersham; RPN.1601Z) [α -³²P]dCTP-labeled cDNA fragment (positions 2056–2334; see Fig. 4). All cDNA inserts were subcloned into the *Eco*RI site of Bluescript



FIG. 1. Immunoprecipitations (lanes 1-4) and immunoblots (lanes 5-10) show that α catenin associates with different cadherins. Anti- α -catenin (lane 3) and anti-uvomorulin (lane 2) (control is lane 1) precipitate the characteristic uvomorulin-catenin complex from cell lysates of [³⁵S]methionine-labeled HeLa (lane 4) or H1-3 cells (lanes 1-3). This is confirmed by the cross-wise staining of the immunoprecipitates in immunoblots with anti- α -catenin (lane 5) or anti-uvomorulin (lane 6). Immunocomplexes collected with anti- α -catenin from human HeLa (lane 7), mouse PSA 5E, chicken CEF38 (lane 9) and Xenopus A6 cells (lane 10) were stained with antibodies against N- and P-cadherin, A-CAM, and U-cadherin, respectively, showing that α catenin associates with different cadherins from human, mouse, or Xenopus. The arrowhead indicates the position of α catenin. Molecular mass markers are given in kDa.



FIG. 2. Immunofluorescence staining of permeabilized PCC₄ (*a* and *b*), HeLa (*c*), Ltk⁻ cells (*e*), and the corresponding mouse uvomorulin-expressing transfectants H1-3 (*d*) and L1-1 (*f*) with anti-uvomorulin (*a*) and anti- α -catenin (*b*-*f*). Uvomorulin and α catenin are colocalized at the plasma membrane (*a* and *b*). Membrane staining for α catenin is observed in cells expressing N-cadherin (*c*), uvomorulin (*f*), or both (*d*). However, in cadherin-negative Ltk⁻ cells, α catenin is localized throughout the cytoplasm (*e*). (×200.)

KS(+) II (Stratagene) and sequenced by using either the Klenow fragment of DNA polymerase I (19) or the Sequenase system (United States Biochemical). Sequencing was done on both strands of cDNA on either double-stranded or single-stranded template DNA by using the dideoxy chaintermination method (19). The full-length clone is designated pBsACAT-1.

Sequence Analysis. All analyses were done with programs of the GCG package (University of Wisconsin).

RESULTS

Conventional approaches to generate antibodies against α catenin were unsuccessful, most likely because of the low antigenicity of the protein. Therefore F9 tumors were generated to obtain large amounts of material from which to purify α catenin. Proteolytic fragments were then produced and subjected to microsequencing analysis. Several internal protein sequences were obtained (see Fig. 3), and the respective peptides were synthesized for antibody production in rabbits. One anti-peptide serum was found to recognize α catenin equally well in immunoprecipitation, immunoblot, and immunofluorescence experiments. These antibodies precipitated, as did anti-uvomorulin, the characteristic uvomorulin-catenin complex (Fig. 1, lanes 2 and 3). The identity of both complexes was confirmed by the cross-wise staining of the immunoprecipitates with both antibodies in immunoblot experiments (Fig. 1, compare lanes 2 and 5 with lanes 3 and 6), as well as by peptide pattern analysis (not shown). Anti- α -catenin antibodies cross-reacted with a protein of similar size from cell lysates of different cell types and species including human, swine, rabbit, and Xenopus (not shown). In immunoblot experiments uvomorulin-negative cells had very little α catenin compared with the corresponding transfectants expressing uvomorulin. This suggests that uvomorulin might affect the rate of synthesis or protein stability of α catenin. Immunoprecipitates collected with anti- α -catenin antibody from different cell types were subjected to immunoblot experiments. In each instance specific staining with antibodies to human N-cadherin, mouse P-cadherin, chicken A-CAM, and Xenopus U-cadherin was observed (Fig. 1, lanes 7-10). This strongly suggests that the complex formation with α catenin is a general feature of all cadherins. The immunoprecipitation experiments led to an additional observation that might be of biological importance. Complexes collected with anti- α -catenin from HeLa cells contained more N-cadherin than those collected from H1-3 cells, which express in addition uvomorulin (Fig. 1, lanes 3 and 4). This might indicate that α catenin has a stronger affinity for uvomorulin than for N-cadherin, although other explanations are possible.

Immunofluorescence tests on permeabilized mouse PCC_4 cells revealed colocalization of uvomorulin and α catenin

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with the respective antibodies (Fig. 2 *a* and *b*). Like uvomorulin, α catenin was concentrated in membrane areas of cell-cell contacts. Anti- α -catenin antibodies stained equally the plasma membrane of permeabilized HeLa (positive for N-cadherin) and H1-3 cells (expressing uvomorulin and N-cadherin, Fig. 2 *c* and *d*). In contrast, Ltk⁻ cells deficient for any cadherin, showed α catenin staining throughout the cytoplasm (Fig. 2*e*). However, α catenin became localized at the plasma membrane in L1-1 cells expressing uvomorulin (Fig. 2*f*). This redistribution of α catenin is most likely due to complex formation with uvomorulin.

The peptide antibodies were used to screen a λ gt11 cDNA library from embryonal carcinoma cells PCC₄. Seven initial cDNA clones were analyzed, and 5' sequences were used to isolate a sequence of 3712 base pairs that hybridized to a single 3.7-kb mRNA in Northern blot analysis (not shown). An open reading frame encoding 906 amino acid residues can be defined with a calculated molecular mass of 100 kDa, which is very close to the relative molecular mass of 102 kDa

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2461	AA K	GGC A	E E	V	TAC		AAC N	L	rGG: G	rGGJ G	AGA(E	GCT L	GGT V	CGT V	S	TGG G	GGT V	D	S	CGC Å	CATG M	816
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2701	GA E	GAC T	Q Q	NGA T		KAG.	ATT I	K	R	AGC'	S	Q	K	GAA K		CGT V	GAA M	200	AGT V	Q	A COCC	696
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2941	GT	TA	AG	CTG	GT	FAG	Ň		~	ICC	TTT	TAG	AAT	TÇA	***	ATA	TAT	TTC	TAG	CTA	TATT	
3001	TT TC	TA1	(AA) (T T	STT FC#	TA.	ATG.		AT.		NGT:	TCT	G TA CTP	ACC	360	GAG TAG	TAC	CAC	ATT	2440	TTC	TTGG	
3121	GĂ	GG	TG	CTI	TT	FAG	ccc	TA	CTG	TTG	GGC	AGA	TGG	ANT	TAA	ATG	ACC	ANG	IGG1	GGC	CCAG	
3181	TG	TA	AT	CAC	AGO	GC.	ACC	TT	AGA	CGG	TCT	GGA		GGA	AGG	TAC	CAG		CCT	CTI	CTGA	
3301	TT	AT	322	GCA	GN	AGG	GGI	AT	rta/ Frta	CAC	GCA	TGG	CTA	CAG	TTA	AGG CTA	ATC	GM	AT7	CC1	GCAC	
3361	Â	CA	TA	ATG	GA	STT	GCI	TT	ATT	CGG	ÇCT	CGT	GTG	TTG	TGA	TGC	TGT	AAT	CAC	ANC	ATOC	
3421	TT	CCC	TG	TTA CRC	TTO	CCA	GC1	TG		rgc.	***	TTC.	ACC	ATC	GGG	CTC	ATT	TCI	-	CG	CGCA	
3541	GT TA	GT7 CT	TC	LTG	CM	rtg CCG	GGC TG	CA	GCA STC	AG	AAG	UCT ACA	GCZ	GAC	TGC	ATÀ Atà	666	TTI	GCT	TAG	GCAA	
3601	TT	AC	GT	CTT	AT	TTA		TT	TAT	-	TCT	cic	TGT	TIT	TTA	CAC	TAN	TTT	TCC	CA	COAL A	
3661	GT	cca	TT	MG		CCA	**			NA.		۸Ň	***			***		à.				

FIG. 3. Nucleotide and amino acid sequence of mouse α catenin. Nucleotides are numbered on the left, and amino acids (single letter notation), on the right. The cDNA sequence reveals an open reading frame of 906 amino acids flanked by 72 nucleotides of 5' and 922 nucleotides of 3' untranslated sequences with a typical polyadenylylation signal 14 nucleotides before the poly(A) tail. The deduced amino acid sequence contains peptide sequences (underlined) determined by direct protein sequencing analysis. The anti- α catenin antiserum was raised against a peptide near the carboxyl terminus (bold letters, boxed).

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 ************************************	a-Catenin Chicken Human Nematode Consensus		1 MTAVHAGNIN 1	FKWDPKSLEI MPVF MPVF	RTLAVERLLE HTRTIESILE HTRTIESILE HTRTIESILE DThE-hLE	PLVTQVTTLV PVAQQISHLV PVAQQISHLV PVAQQVSRLV PhQho-LV	NTNSKGPSNK Imheegevdg Imheegevdg Ilheean.dg 	50 34 34 33	a-CATENIN CHICKEN HUMAN NEMATODE CONSENSUS	377 582 582 478	DLRRQLRKAV DLKARNQEAN DLKARNQEAN DLGDFNRRVL DLhh	HDHVSDSFLE TGEVSDVFSD TGEVSDVFSD TDRVVDOFAD V-D-F-A	TNVPLLVLIE TTTPIKLLAV TTTPIKLLAV ITTPLKQFVE Ph	AAKNGN AATAPSDŤPN AATAPPDAPN AVHADPYDPN A	. EKEVKEYAQ Rijevfeeraa Rijevfderaa Rijevfderaa Rijevfderaa Rijevfderaa Rijevfderaa Rijevfderaa	421 631 631 527
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 ************************************	a-Catenin Chicken Human Nematode Consensus	9 8 8	9 KQGDLMKSÅJ 3 NACTKLVRAJ 3 NACTKLVQAJ 2 GSSKLLEESS h	GEFADDPCSS QHLQADPYSV QHLQSDPYSV SYSLKHDPYSV DP-S-	VKRGNMVRAA PARDYLIDGS PARDYLIDGS PARKKLIDGA Rhh-s-	RALLSAVTRL NGI LSGTSDL NGI LSGTSDL NGI LQGTSAL RahL-a-o-L	LI LADMADVY LLTFDEAEVR LLTFDEAEVR LLCFDESEVR LhDaV-	148 132 132 131	a-CATENIN Chicken Human Nematode Consensus	470 681 681 578	ALAARPQSKL ILLRNIPGNQA ILLRNIPGNQA VRLHNIPGSE	Agenndlfke Ayerfetnen Ayerfetnen ghfenihk a	QMEKQVRVLT QMIDNVEKNT QMIDNVEKNT QYADALHRLR Qrhh-	DAVDDITSID GLVDEAIDTK GLVDEAIDTK SHVDDAIDTG VDa	DFLAVŠENHI SLLDASERAI SLLDASERAI EFVRASETAM hSEh	519 730 730 624
$ \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} -\text{CATENIN}\\ \text{HURKEN }\\ \begin{array}{c} 199 \\ \text{HURKEN }\\ \begin{array}{c} 193 \\ 183 \\ \text{HURKEN }\\ \begin{array}{c} 198 \\ 183 \\ \text{HURKEN }\\ \begin{array}{c} 183 \\ 183 \\ 183 \\ \text{HURKEN }\\ \begin{array}{c} 183 \\ 183 \\ 183 \\ \text{HURKEN }\\ \begin{array}{c} 183 \\ 183 \\ 183 \\ \text{HURKEN }\\ \begin{array}{c} 183 \\ 183 \\ 183 \\ 183 \\ 183 \\ 183 \\ 183 \\ 183 \\ 183 \\ 183 \\ 183 \\ 113$	a-C ATENIN Chicken Human Nematode Consensus	149 133 133 133	9 KLLVQLKVVE 3 KIIRVCKGII 3 KIIRVCKGII 2 KIIRVCRAN Khhb	DGILKLRNAG EYLTVAEVVE EYLTVAEVVE DYVAVSEVIE a-h	NEQDIGIQYE THEDLVTYTE THEDLVTYTE SHADLQQFVE DLE	ALKPEVDKLN NLGPGMTKMA NLGPGMTKMA DISPVLHDVT -h-P-hh-	IMAAKROOEL KMIDEROOEL KMIDEROOEL NDVNLROOEL	198 182 182 182	«-CATENIN CHICKEN HUMAN NEMATODE CONSENSUS	520 731 731 625	LEDVNKCVIA KKDLDKCKVA KKDLDKCKVA RRYTNHCEGA	LQEKDVDGLD NANHQPQHLV NANIQPQHLV INGADAHGLV hL-	RTAGAIRGRA AGATSIARRA AGATSIARRA NNSSQIARLG I9	ARVIHVVTSB NRILLVAKRE NRILLVAKRE NRVLMTAQNE -RhhE	MDNYEPGVYT VENSEDPKFR VENSEDPKFR ADNSEEPSFV - an-E-s-r-	569 780 780 780 7674
III ************************************	a-CATENIN Chicken Human Nematode Consensus	199 183 183 183	KDVGNRDQMA THQEHRVMLV THQEHRVMLV THQVHREILI	AARGILQKNV NSHNTVKELL NSHNTVKELL RCHDSIKVIA	PILYTA 224 PVLISA 208 PVLISA 208 PILICS 207 PhL				«-C ATE NIN Chicken Human Nematode Consensus	570 781 781 675	EKVLEATKLL EAVKAASDEL EAVKAASDEL SRVRNAADQL VAL	SNTVMP 585 Sktisp 796 Sktisp 796 HNAIPP 690 h-P				
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	a-CATENIN CHICKEN HUMAN NEMATODE CONSENSUS	889	KHVNPVQALS	EFKAMDSI 90	06											

FIG. 4. Comparison of α catenin and vinculin. Multi-alignment of homologous regions (I = amino-terminal region, II = central region, and III = carboxyl-terminal region) of mouse α catenin and vinculin from human, chicken, and *C. elegans*. The consensus sequence is given: upper-case letters depict absolute homology and lower-case letters depict conservative differences where h = hydrophobic, a = acidic, o = oxygen-containing side chain, r = aromatic, b = basic, and s = small side chain. (*Right Lower*) Schematic comparison of mouse α catenin and chicken vinculin according to dot-blot matrix analysis. Numbering of vinculin was done according to ref. 20. Homologous regions are indicated by dotted boxes; the percentage identity is indicated.

determined in SDS/polyacrylamide gels for α catenin. Several peptide sequences obtained from direct protein sequencing, including the one used for immunization, are present in the deduced amino acid sequence (Fig. 3). The protein shows no internal homology, and secondary structure prediction analysis indicates a high content of α -helices and overall strong hydrophilicity. As expected from the protein analysis, no signal peptide or hydrophobic membrane-spanning domain could be identified.

Nucleic acid and protein data-base searches identified α catenin as a vinculin-related protein. The homology between vinculin and α catenin is restricted to three major regions in the amino-terminal, central, and carboxyl-terminal part of both proteins. The homologous amino acid sequences of human, chicken, and *Caenorhabditis elegans* vinculin and α catenin are aligned in Fig. 4. The strongest degree of homology was found in the respective carboxyl-terminal regions. A schematic comparison of vinculin and α catenin is given in Fig. 4.

The most significant difference between the two proteins is the absence in α catenin of the repeated units and the proline-rich sequence characteristic of vinculin. Regions of α catenin that are nonhomologous to vinculin have no significant homology to any other proteins.

DISCUSSION

Here we show that α catenin is a ubiquitous cellular component since anti-peptide antibodies detect homologues in different cell types from *Xenopus* to man. This confirms earlier observations that the heterotypic expression of mouse uvomorulin in human or chicken cells results in the formation of a complex between uvomorulin and endogenous proteins, one of which is α catenin (10).

In addition we show that various members of the cadherin gene family from different cell types and species all associate with α catenin. For uvomorulin the association with α catenin is a prerequisite for its connection to the actin filament network (11). A similar transmembrane linkage of other cadherins to cytoskeletal structures has not yet been reported. Although the function of the association between these cadherins and α catenin remains elusive, it is reasonable to assume that such a conserved molecular interaction might reflect some common functional properties one of which might be to mediate the cytoplasmic anchorage of all cadherins. These complex formations might also regulate other cellular functions of cadherins, and it will be interesting to see whether, similar to the uvomorulin-catenin complex, β and γ catenin can also be found in association with other cadherins. If so, it may be that catenins play a key role in the function of all cadherins.

It is becoming increasingly evident that a given cell can express more than one cadherin at a time. This raises the question of how this cell can regulate the selectivity of adhesion by one type of cadherin interaction. For uvomorulin it has been shown that complete adhesive function is possible only when uvomorulin complexes with catenins (10, 11). It is tempting to speculate that a differential complex formation of catenins with one or the other cadherin could direct the selectivity of cell adhesion. Although preliminary, such a view is supported by our results concerning the different amounts of N-cadherin associated with α catenin in Hela and H1-3 cells.

Our immunofluorescence studies demonstrate that α catenin is localized close to the plasma membrane and is concentrated in membrane areas of cell contacts whenever cadherins are coexpressed in the same cell. However, α catenin can be detected in cells such as Ltk⁻ that express no cadherin molecule as determined by PCR analysis (unpublished observations). In these cells no association of α catenin with the plasma membrane is detected; rather, the protein seems to be uniformly distributed in the cytoplasm. This observation may reflect some additional biological properties of α catenin. The α catenin detected in Ltk⁻ cells is functional, at least with respect to its interaction with uvomorulin, since α catenin becomes concentrated at the plasma membrane after expressing uvomorulin in these cells.

Nucleic acid and protein data-base searches identified α catenin as a vinculin-related protein. Vinculin is a cytoskeletal protein associated with both cell-cell and cellextracellular matrix adherens-type junctions (21, 22). Biochemical binding studies indicate an interaction of vinculin with talin (23, 24) and α actinin (25, 26), which is thought to be part of the apparent transmembrane linkage of the extracellular matrix to the cytoplasmic microfilament system (22). The homology between vinculin and α catenin is restricted to three major regions in the amino-terminal, central, and carboxyl-terminal parts of both proteins, the strongest degree of homology being found in the C-terminal portion (20, 27–29). For vinculin this region is thought to exhibit self-association properties (30); however, it is not yet known whether α catenin undergoes similar di(oligo)merization. Another appealing possibility is that α catenin might interact directly with vinculin. Vinculin is found to be associated with the adherens-type junctions, but its exact function in this subcellular structure is not known. It may well be that α catenin and vinculin interact with each other and are part of a multiprotein complex.

Perhaps most significantly, α catenin contains a short peptide, Arg-Gln-Gln-Glu-Leu, which is conserved in all vinculins and located in a region thought to regulate the binding of vinculin to talin (31). This suggests that α catenin could also associate with talin. Although there is no experimental evidence for such an association, the detection of α catenin in cadherin-negative Ltk⁻ cells suggests such a possibility. This might indicate that α catenin exhibits multiple biological properties. For example, it may exhibit similarity to vinculin in its interaction with talin on one side and

 α -actinin or F-actin (32) on the other. In conclusion, our work suggests the existence of a new vinculin-related family of proteins involved in the cytoplasmic anchorage of cell-cell and cell-substrate adhesion molecules.

Note. Since the submission of this manuscript, Nagafuchi et al. (33) have reported the molecular cloning of the 102-kDa cadherinassociated protein, termed CAP 102. Sequence comparison revealed that CAP 102 is identical to α catenin.

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