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Uvomorulin–catenin complex formation is regulated by a specific domain in the cytoplasmic region of the cell adhesion molecule

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ABSTRACT **We have recently found that the cytoplasmic region of the cell adhesion molecule uvomorulin associates with three proteins named catenin α, β, and γ. Here we show by analysis of various mutant uvomorulin polypeptides expressed in mouse L cells that this association is mediated by a specific domain in the cytoplasmic region. A specific recognition site for catenins is located in a 72-amino acid domain. Interestingly, 69 of the 72 amino acid residues are encoded by a single exon of the uvomorulin gene. To demonstrate the direct interaction between catenins and the 72-amino acid domain, cDNA constructs composed of H-2K^d cDNA and various 3′ sequences of uvomorulin were expressed in L cells. Chimeric proteins between H-2K^d and the 72-amino acid domain of uvomorulin were shown, by immunoprecipitation with anti-H-2K^d antibodies, to complex with catenin α, β, and γ. Catenins connect uvomorulin to cytoskeletal structures. We provide biochemical evidence for an association of the uvomorulin–catenin complex with actin bundles. Our results suggest that catenin α plays a key role in the association with actin filaments, whereas catenin β binds more directly to the cytoplasmic region of uvomorulin. In cell aggregation assays with transfected cells expressing normal or mutant uvomorulin, the adhesive function was expressed only when uvomorulin was associated with catenins. From these results we conclude that the cytoplasmic anchorage of uvomorulin is of major biological importance.**

Ca^{2+}-dependent cell adhesion molecules (CAMs) are a group of structurally and functionally related molecules (1, 2). Molecular cloning and determination of the primary structure of several CAMs from different species revealed that they are structurally related integral membrane proteins. Primary and secondary structure analysis and sequence comparison of CAMs led to the identification of domains that might be of functional importance (3).

The cytoplasmic region represents the most conserved part of all Ca^{2+}-dependent CAMs, which suggested a common functional role (2, 3). Such a view found experimental support when we showed (4) that the cytoplasmic domain of uvomorulin is associated with three structurally independent proteins with molecular masses of 102, 88, and 80 kDa, which were named catenin α, β, and γ, respectively. Catenins seem to be present rather ubiquitously in different cell types. When recombinant uvomorulin is expressed in uvomorulin-negative mouse, avian, or human cells, the introduced protein associates with endogenous proteins that are structurally related in the three species.

The association of catenins with the cytoplasmic region of uvomorulin has been demonstrated by studying mutant uvomorulin polypeptides with deletions in the cytoplasmic or extracellular part of the protein (4). This work allowed no further insights into the molecular interactions since changes in the protein conformation of uvomorulin resulting from the deletions could influence possible interactions with catenins. In this report we have identified a specific domain at the carboxyl terminus of uvomorulin that mediates the interaction with catenins. Direct proof for a specific catenin binding site is shown by the analysis of chimeric molecules composed of the class I major histocompatibility antigen (MHC) H-2K^d and various parts of the cytoplasmic region of uvomorulin. Our results indicate that this complex formation connects uvomorulin to actin and also regulates the adhesion of uvomorulin.

MATERIALS AND METHODS

Antibodies. Affinity-purified rabbit anti-uvomorulin antibodies against the extracellular part of uvomorulin were obtained as described (4). DECMA-1 is a rat monoclonal antibody that perturbs the adhesive function of uvomorulin (5). Monoclonal antibody anti-H-2K^d (K9-18) was a kind gift of B. Arnold (DKFZ, Heidelberg) (6).

cDNA Constructs. All cDNA constructs were inserted into the expression plasmid pSV2tk-neoβ (7) as described (4). To construct a cDNA coding for a uvomorulin polypeptide with a cytoplasmic deletion of 70 amino acid (aa) residues (pSUMΔC9), the 1711-base-pair (bp) *Eco*RI–*Ban* II fragment of clone F5 (3), which contains coding sequences for the extracellular part and the transmembrane domain with an additional 9 aa residues of the cytoplasmic region, was connected to the 246-bp *Cla* I–*Nco* I fragment of clone F5, which encodes most of the carboxyl-terminal 72 aa residues of uvomorulin, by using the synthetic oligonucleotide CGCCCCCAT as a linker. This cDNA construct was subcloned into pM3 (4) to add the missing sequences for the signal sequence, the precursor region, and a small portion of the amino-terminal part of uvomorulin.

The same strategy was used to construct cDNAs encoding uvomorulin polypeptides with other deletions in the cytoplasmic part. For this the 1711-bp *Eco*RI–*Ban* II fragment was linked to a 433-bp *Sma* I–*Nco* I, a 365-bp *Pvu* II–*Nco* I, or a 139-bp *Sac* I–*Nco* I fragment of F5 using synthetic oligonucleotides, CGCCC, CGCCCCCCCC, or CGCCCCGAGCT, as linkers to generate vectors pSUMΔC7, pSUMΔC8, and pSUMΔC10, respectively. These cDNAs code for mutant uvomorulin polypeptides with deletions in the cytoplasmic region of 8, 31, or 105 aa residues, respectively (see Fig. 1*A*). To obtain cDNAs encoding H-2K^d–uvomorulin chimeric polypeptides the 980-bp *Eco*RI–*Hin*dIII fragment of H-2K^d cDNA (8) was either linked to a 602-bp *Hin*cII–*Nco* I fragment of F5 or a 398-bp *Hin*cII–*Nco* I fragment of pSUMΔC9 using the synthetic oligonucleotide AGCTTGTC as a linker. These cDNAs code for chimeric polypeptides that contain the extracellular part of H-2K^d

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Abbreviations: CAM, cell adhesion molecule; aa, amino acid; NP-40, Nonidet P-40; MHC, major histocompatibility complex.

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linked to a small part (16 aa residues) of the extracellular region and the transmembrane domain of normal uvomorulin as well as the cytoplasmic region of normal or mutant uvomorulin (see Fig. 3*A*). To produce a chimeric protein that contains the extracellular part and the transmembrane region of H-2K^d with the most carboxyl-terminal 72 aa residues of uvomorulin, a 1180-bp *Eco*RI–*Bsp*HI fragment of H-2K^d cDNA, which encodes 341 aa of the 347 aa of the H-2K^d polypeptide, was linked to the 246-bp *Cla* I–*Nco* I fragment of clone F5 using a synthetic oligonucleotide CATGGGCCCAT as a linker.

DNA Transfection. Purified plasmids were introduced into mouse L-TK$^-$ cells by cotransfection with pSVtk-neoβ at a 10:1 molar ratio using the calcium phosphate precipitation method as described (4).

Determination of Exon Boundaries. Oligonucleotides complementary to uvomorulin cDNA sequences were synthesized (Applied Biosystems) and used as primers in dideoxynucleotide sequencing (9) of genomic clone UG4c (10). Complete exon and adjacent intron sequences were determined.

Biochemical Analysis of Transfectants. Transfectants were isolated by fluorescence-activated cell sorting and analyzed by immunoprecipitation or with immunoblots as described (4). For metabolic labeling experiments, 1×10^6 cells were cultured in [^{35}S]methionine (50 μCi/ml; 1 Ci = 37 GBq; Amersham) for 16 hr and solubilized in isotonic phosphate-buffered saline (PBS) containing 1% Triton X-100, 1% Nonidet P-40 (NP-40), 1 mM $CaCl_2$, and 1 mM phenylmethylsulfonyl fluoride (PMSF). After centrifugation, supernatants were incubated with affinity-purified rabbit anti-uvomorulin or monoclonal anti-H-2K^d antibodies and the immunocomplexes were collected by protein A-Sepharose CL4B (Pharmacia). The separation of uvomorulin into the detergent-soluble and insoluble fractions was studied as described (4). To analyze the interaction of the uvomorulin–catenin complex with actin, 2–5 $\times 10^6$ cells were solubilized with 1% NP-40 in 10 mM Tris·HCl (pH 8.0) containing 30 mM NaCl, 0.2 mM $CaCl_2$, 0.2 mM ATP, 0.5 mM PMSF, and 0.5 mM 2-mercaptoethanol. After centrifugation, supernatants (600 μl) were incubated for 1 hr at 4°C with 50 μl of DNase I-Sepharose, which was prepared by coupling 20 mg of DNase I (Boehring grade II) to 1 ml of BrCN-Sepharose (Pharmacia). The DNase-Sepharose was washed three times with 1 ml of 10 mM Tris·HCl (pH 8.0) containing 0.05% NP-40, 30 mM NaCl, 0.2 mM $CaCl_2$, 0.5 mM PMSF, and 0.1% ovalbumin. Bound material was eluted with 10 mM Tris·HCl (pH 8.0) containing 0.05% NP-40, 160 mM NaCl, 0.2 mM $CaCl_2$, and 0.5 mM PMSF and collected by ethanol precipitation. The precipitates were boiled in SDS/PAGE sample buffer and subjected to immunoblot analysis.

Cell Aggregation Assay. Cells were washed with PBS and treated with 0.01% trypsin in Hepes-buffered saline (HBS = 37 mM NaCl/5.4 mM KCl/0.34 mM Na_2HPO_4/5.6 mM glucose/10 mM Hepes) containing 2 mM $CaCl_2$ for 10 min at 37°C. After washing with HBS containing 2 mM $CaCl_2$, cells were resuspended in the same buffer at 5×10^5 cells per ml. The cells were allowed to aggregate for 30 min at 37°C with a constant rotation of 70 rpm. The extent of cell aggregation was calculated according to ref. 11 by the index $(N_0 - N_e)/N_0$, where N_e is the total particle number after the incubation (30 min) and N_0 is the total particle number at the initiation of incubation.

RESULTS

Mutant uvomorulin lacking as little as 37 aa residues at the carboxyl terminus cannot associate with catenins (4). Such deletions may generate changes in the protein conformation that inhibit the uvomorulin–catenin interaction. Therefore, a set of cDNAs were constructed that code for uvomorulin polypeptides with various deletions in the cytoplasmic region. This was achieved by using appropriate restriction sites and connecting different parts of uvomorulin 3′ sequences with oligonucleotides. The different cDNA constructs had coding sequences for the signal peptide, the precursor region, the extracellular part, and the transmembrane domain of uvomorulin in common. The deletions in the cytoplasmic domain are schematically shown in Fig. 1*A*. Vector pSUM1 codes for intact uvomorulin with a 151-aa cytoplasmic region. Vector pSUMΔC5 codes for a mutant uvomorulin with a 37-aa deletion at the carboxyl terminus. This deletion was already shown to impair the association with catenins and was included as a control. Vectors pSUMΔC7, -ΔC8, -ΔC9, and -ΔC10 encode mutant cytoplasmic regions with deletions of 8, 31, 70, and 105 aa residues, respectively. The uvomorulin cDNA construct plus the neomycin-resistance gene were

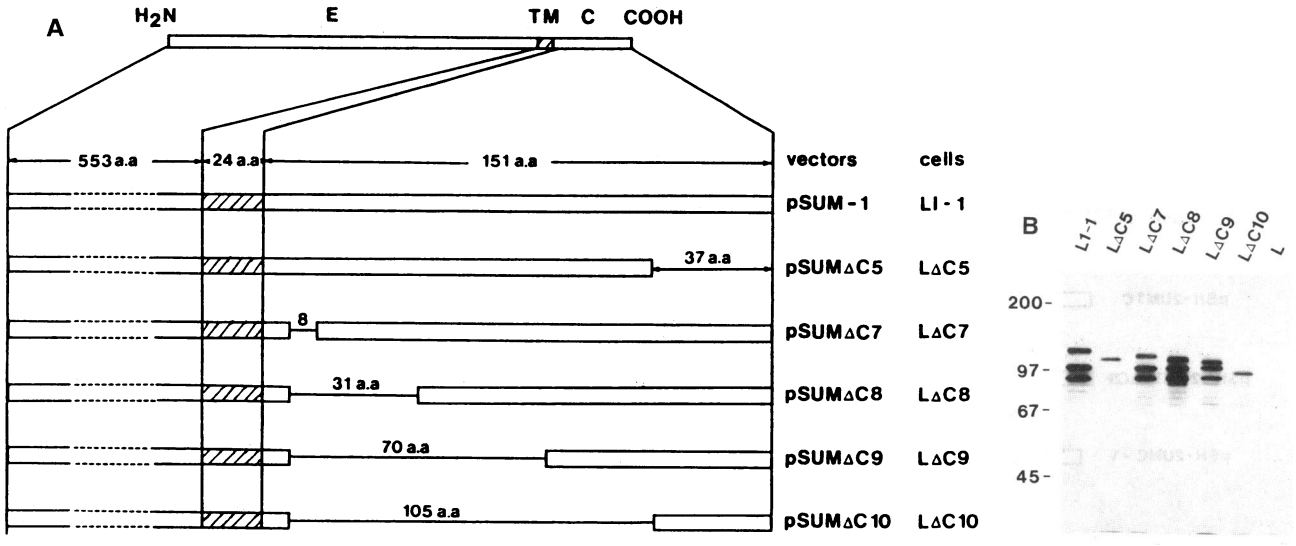

FIG. 1. (*A*) Uvomorulin structure and various deletion mutants of the cytoplasmic region. Expression vectors containing various cDNA constructs were introduced into mouse L cells. E, extracellular part; TM, transmembrane domain; C, cytoplasmic region. (*B*) Transfectant L cells (as indicated) expressing normal or mutant uvomorulin were analyzed in immunoprecipitation experiments with anti-uvomorulin antibodies (L, untransfected L cells as control). In cells expressing pSUMΔC5 and pSUMΔC10, the mutant uvomorulin is not associated with catenins, indicating that most of the carboxyl-terminal 72 aa residues of uvomorulin are necessary for uvomorulin–catenin complex formation.

introduced into mouse L cells, and transfectants with cell surface expression of uvomorulin were isolated by fluorescence-activated cell sorting. Transfectants expressing either intact uvomorulin or the mutant forms shown in Fig. 1*A* were subjected to immunoprecipitation. Catenin polypeptides α, β, and γ were detected in cells expressing pSUM1 or pSUMΔC7, -ΔC8, and -ΔC9 (Fig. 1*B*). No catenins were detected in cells expressing pSUMΔC5 and -ΔC10. These results demonstrate that the most carboxyl-terminal 72 aa residues of uvomorulin are a prerequisite for the association of uvomorulin and catenin α, β, and γ.

To test whether the 72 carboxyl-terminal aa residues are sufficient for the association with catenins, chimeric proteins between a class I MHC molecule and the cytoplasmic region of uvomorulin were expressed in L cells. For this, cDNA sequences coding for various parts of the transmembrane and cytoplasmic region of uvomorulin were linked to H-2K^d cDNA. These constructs are schematically represented in Fig. 2*A*. Transfectant cell lines expressing chimeric proteins were isolated by fluorescence-activated cell sorting and subjected to immunoprecipitation experiments using anti-H-2K^d monoclonal antibodies. As shown in Fig. 2*B*, the addition of the 72 aa residues is a minimal requirement to complex the chimeric protein and catenin α, β, and γ. Peptide pattern analysis of the catenins detected by using the chimeric proteins and of those associated with normal uvomorulin revealed that they were identical (data not shown). These results demonstrate unambiguously that the 72 aa residues represent a recognition site for catenins.

The cytoplasmic part of uvomorulin that interacts with catenins is most likely to represent one functional domain. This is supported by data about the genomic organization of the uvomorulin gene. The cytoplasmic region of uvomorulin is encoded by three exons. The last exon (exon 16) codes for the 69 aa of the 72 aa residues necessary for the complex formation (Fig. 3*A*). Thus the intron–exon structure in this region of the gene corresponds with the functional properties of the cytoplasmic domain. Additional support for such an interpretation was obtained when the genomic organization of mouse uvomorulin and chicken liver CAM (12) was compared. In both proteins the 69 aa residues are encoded by a single exon, and the intron/exon boundaries in both genes are highly conserved (Fig. 3*B*).

Catenins most likely connect uvomorulin with cytoskeletal structures. A considerable amount of uvomorulin cannot be solubilized in non-ionic detergents, Triton X-100 or NP-40 (4). The separation of uvomorulin into detergent-soluble and -insoluble nuclear and cytoskeletal fractions was analyzed

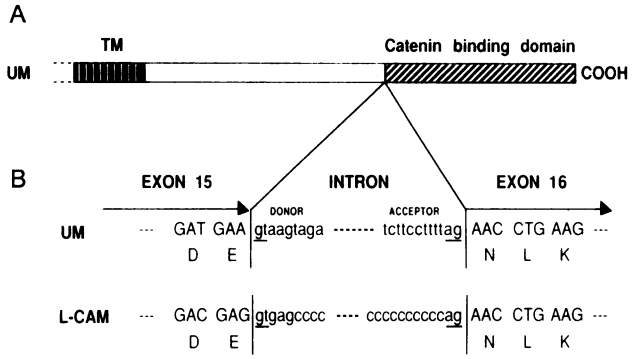

FIG. 3. Single exon of the uvomorulin gene codes for the catenin binding domain. (*A*) Carboxyl-terminal part of uvomorulin. UM, uvomorulin; TM, transmembrane region. (*B*) Exon/intron sequences of exon 15 and 16 from the uvomorulin gene (UM) and chicken liver CAM (L-CAM) (12). The exon/intron boundary is highly conserved in both genes. Exon 16 contains in both genes the stop codon and respective 3′ untranslated sequences (data not shown). The single-letter amino acid code is used.

using transfected cells carrying the cDNA constructs shown in Fig. 1*A*. Uvomorulin was only detected in the detergent-insoluble fractions of cells expressing normal uvomorulin or cells with constructs pSUMΔC7, -ΔC8, or -ΔC9 (data not shown). Thus, the 72 aa residues at the carboxyl terminus of uvomorulin, which mediate the association with catenins, also determine the separation of uvomorulin into the detergent-insoluble fraction.

Indirect evidence (13) suggested that uvomorulin might be associated with the actin filament network. Thus we initiated experiments to demonstrate directly an interaction with actin. A number of actin-binding proteins have been purified by using the specific affinity of globular actin for DNase I, such as barbed-end-capping proteins that bind to globular actin as well as to filamentous actin (14). L cells expressing normal or mutant uvomorulin were lysed in low-ionic-strength detergent buffer and incubated with DNase I-Sepharose. Bound material was eluted with buffer containing 160 mM NaCl and subjected to immunoblot analysis (Fig. 4). Affinity-purified anti-uvomorulin antibodies recognized only uvomorulin in samples from cells expressing either normal (L1-1) or mutant uvomorulin (LΔC8) that could complex with catenins. No uvomorulin was detected in the DNase I-bound fraction from cells expressing mutant uvomorulin that could not associate with catenins (LΔC5 and LΔC10). In control

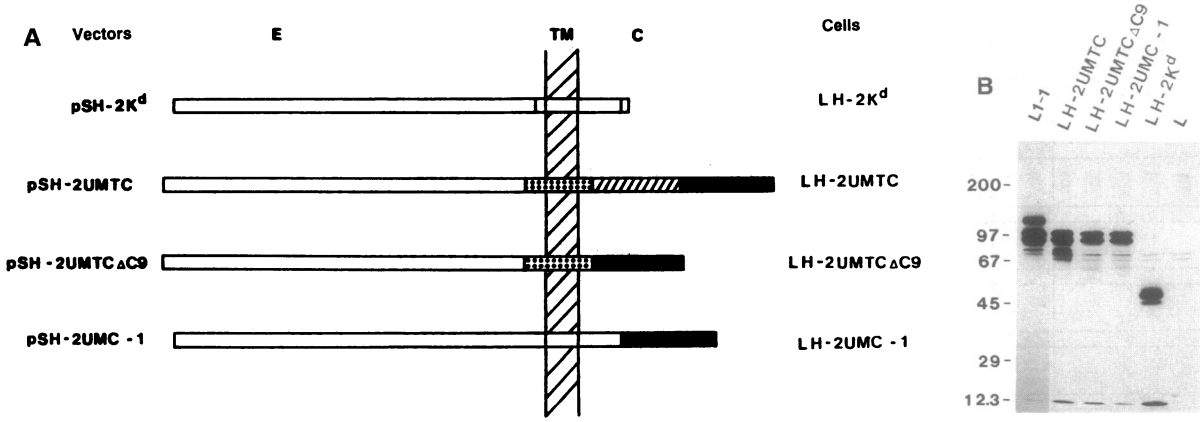

FIG. 2. (*A*) MHC class I, H-2K^d antigen (open boxes) and three chimeric proteins containing the transmembrane domain (stippled boxes), the cytoplasmic region, or both of uvomorulin (hatched and solid boxes, respectively). Expression vectors containing the various cDNA constructs were introduced into mouse L cells. E, extracellular part; TM, transmembrane domain; C, cytoplasmic region. (*B*) Immunoprecipitation analysis with anti-H-2K^d of detergent extracts from transfected cells, as indicated. Immunoprecipitation with anti-uvomorulin from L1-1 cell lysates is included as control.

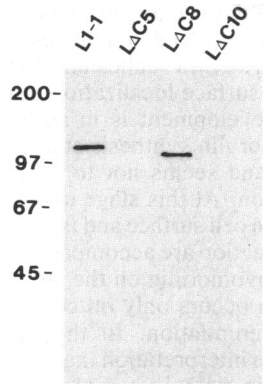

FIG. 4. Immunoblot experiments with anti-uvomorulin analyzing DNase I-Sepharose-bound material. Cell lysates were incubated with DNase I-Sepharose. Bound material was subjected to immunoblot analysis. Uvomorulin is detected in the DNase I-bound fraction from cells expressing normal or mutant uvomorulin that can complex with catenins.

experiments where the column-bound fraction of bovine serum albumin-Sepharose was analyzed, no uvomorulin was detected (data not shown). These experiments provide biochemical evidence that catenins mediate the connection of uvomorulin and actin. They do not identify, however, which catenin binds directly to uvomorulin or which catenin connects uvomorulin to actin bundles.

The uvomorulin-catenin complex is routinely detected after solubilization of cells in a non-ionic detergent, Triton X-100 or NP-40. To define experimental conditions where the sequential binding of individual catenins to uvomorulin could be studied, cell lysates extracted with various ionic or non-ionic detergents were immunoprecipitated. Uvomorulin associated with catenin α, β, and γ (all catenins) was constantly found in these cell extracts with one exception. After solubilization with octyl glucoside catenin α was no longer detected in the uvomorulin–catenin complex (Fig. 5*A*). These results suggest that catenin β is more closely associated with uvomorulin than catenin α. Earlier observations after pH treatment of cell lysates indicated that catenin γ might bind to the periphery of the complex (4). Although preliminary, a

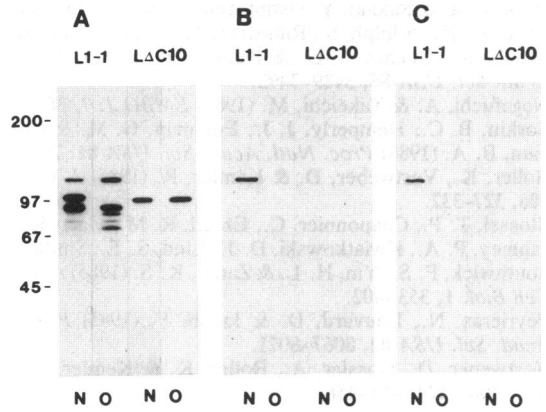

FIG. 5. Immunoprecipitation and immunoblot analysis of uvomorulin–catenin complex from cell extracts solubilized with 1% NP-40 (lanes N) or 40 mM octyl glucoside (lanes O). L1-1, cells expressing normal uvomorulin; LΔC10, cells expressing mutant uvomorulin not complexed with catenins (control). (*A*) Immunoprecipitations demonstrating that catenin α is not found in the complex after octyl glucoside solubilization. (*B*) Immunoblots of detergent-insoluble material. After octyl glucoside solubilization, uvomorulin is not found in the detergent-insoluble material. (*C*) Immunoblots of the DNase I-bound fraction. No uvomorulin is found in the DNase I-bound fraction after octyl glucoside solubilization of cells.

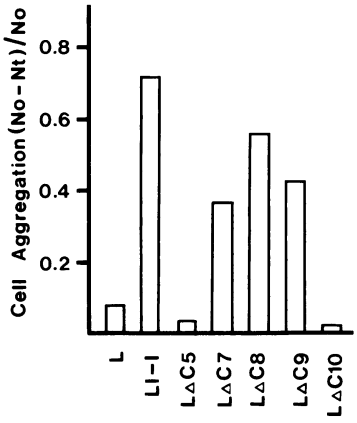

FIG. 6. Ca^{2+}-dependent cell aggregation assay with transfected L cells expressing normal uvomorulin or various mutant polypeptides (L, untransfected L cells). Uvomorulin-dependent adhesiveness correlates with the presence of catenins. N_0, total particle number at the initiation of incubation; N_t, total particle number after a 30-min incubation.

sequential order of binding in the complex might be uvomorulin, catenin β, catenin α, and catenin γ. The amount of solubilized uvomorulin seems to be higher in octyl glucoside than in NP-40 or Triton X-100 extracts. More important, after octyl glucoside extraction uvomorulin was no longer found in the detergent-insoluble material (Fig. 5*B*) and was also absent from the DNase I-bound fraction (Fig. 5*C*). Thus, the presence of catenin α correlates with the actin-bound properties of uvomorulin indicating that catenin α plays a key role in mediating the connection of uvomorulin with the actin filament network.

Cytoplasmic anchorage of uvomorulin–catenin complexes to actin regulates the cell adhesion function of uvomorulin. L cells expressing intact uvomorulin or the various mutant forms shown in Fig. 1*A* were studied in functional cell aggregation assays. Since in these experiments the amount of uvomorulin on various transfectants could influence their aggregation behavior, only transfectant cell clones with a similar number of monoclonal antibody DECMA-1 binding sites were compared. The aggregation of cells expressing vector pSUMΔC7, -ΔC8, or -ΔC9 was nearly as efficient as cells expressing intact uvomorulin (Fig. 6). The aggregation of cells expressing vector pSUMΔC5 or -ΔC10 was reduced although these cells express similar amounts of uvomorulin on their cell surfaces.

Thus the results presented show that catenins associate at a specific site in the cytoplasmic domain of uvomorulin, that this association links uvomorulin to the actin filament network where catenin α seems to be an important mediator, and that this cytoplasmic anchorage influences strength of the uvomorulin-mediated adhesiveness.

DISCUSSION

Earlier studies on uvomorulin gave initial indications that uvomorulin is associated with structurally independent polypeptides but this association was not well understood (15, 16). The molecular cloning of uvomorulin enabled us to demonstrate that three proteins of 102, 88, and 80 kDa, named catenin α, β, and γ, complex with the cytoplasmic region of uvomorulin (4). Similar observations on the association of proteins with E-cadherin have been described (17). We report here that catenins bind to a specific domain of the cytoplasmic region. The analysis of mutant uvomorulins with different cytoplasmic deletions indicated that complex formation is mediated by a distinct recognition site located within a specific 72-aa region. Further evidence for this came

from the analysis of chimeric proteins. A chimeric protein between a MHC class I (H-2K^d) and the 72-aa domain can complex with catenin α, β, and γ, indicating that this domain of uvomorulin is sufficient to mediate the uvomorulin–catenins complex formation. Catenins are only detected in association with uvomorulin and they seem to represent proteins identified in ref 4. An 82-kDa protein, named radixin, has been shown to be localized at the cell-to-cell adherens junction in various cell types (18). It remains to be seen whether radixin and one of the catenins might be related. Our experiments do not determine the order or stoichiometry with which catenins bind to uvomorulin. The fact that catenin α, β, and γ are either present or absent as a group in all deletion mutants and chimeric proteins makes multiple binding sites for each catenin less likely. Our results favor a sequential mode of interaction between uvomorulin and catenins, where catenin β may interact more directly with uvomorulin and catenin α may mediate the anchorage to the actin filament network. Catenin γ is thought to be located in the periphery of the complex (4). Further analysis is needed to dissect the molecular basis of uvomorulin–catenin interaction. Because uvomorulin is phosphorylated in the cytoplasmic region at serine and threonine residues and the 72-aa domain has a high serine content, perhaps phosphorylation plays a role in the uvomorulin–catenin complex formation.

The 72-aa domain appears to be a unique functional domain. When compared with other Ca^{2+}-dependent CAMs, this domain represents a hot spot of homology (19). In uvomorulin the domain is encoded almost entirely by one exon, which coincides with the hypothesis that exons code for functional units. Furthermore, in mouse uvomorulin and chicken liver CAM genes (12), the carboxyl-terminal 69 aa residues are encoded by a single exon. Thus, this domain may have a similar function in other Ca^{2+}-dependent CAMs.

It has been proposed that catenins connect uvomorulin to cytoskeletal structures (4), because uvomorulin is only found in the detergent-insoluble cytoskeletal and nuclear fraction when it is complexed with catenins. Here we report more direct evidence by demonstrating that only uvomorulin complexed with catenins was observed in the fraction bound to DNase I-Sepharose. Catenin α seems to be of major importance to mediate this connection. These results suggest a uvomorulin–catenin–actin filament complex. It remains to be seen whether additional polypeptides are involved in this complex, as discussed (20).

The association of catenins with uvomorulin affects the adhesive function of uvomorulin. In a functional cell aggregation assay, uvomorulin is fully adhesive only when complexed with catenins. Our results clearly demonstrate that the presence of catenins correlates with the adhesion of uvomorulin. This could give catenins a central role in regulating the strength of uvomorulin-mediated cell adhesion and has prompted our present working model (2). It is assumed that the adhesiveness of uvomorulin is different in developing tissues and in adult tissues. During development uvomorulin is involved in condensation, pattern formation, and cell sorting. Uvomorulin-mediated adhesion should be less stringent in these processes, since cells migrate and reorganize. For the integrity of an epithelial sheet however, uvomorulin should promote stronger cell–cell adhesion. This function of uvomorulin is mediated by the attachment of the uvomorulin–catenin complex to actin filaments and by clustering the complex in the zonula adherens. During development, the relative amount of free uvomorulin–catenin complex vs. complex bound to actin filaments would then regulate the adhesive state of a given cell, a high amount of free complex would correlate with low adhesion. Earlier work on the synthesis and cell surface localization of uvomorulin during preimplantation development is in agreement with such a model (16). Uvomorulin synthesis starts at the late two-cell embryonic stage and seems not to be correlated with the onset of compaction. At this stage uvomorulin is uniformly distributed over the cell surface and is not fully adhesive. The first signs of compaction are accompanied by a redistribution and clustering of uvomorulin on the surface of blastomeres. This redistribution occurs only on cells determined for trophectodermal differentiation. In the light of our present results we favor the interpretation that during the compaction process uvomorulin–catenin complex becomes attached to the actin filament network and that this attachment is a prerequisite for establishing a trophectodermal cell layer. Whether additional proteins are required in this process, as discussed (2), remains elusive. If so, the preimplantation embryo represents a unique system for the study of the uvomorulin–catenin complex formation during the transition of a nonpolarized cell to a polarized epithelial cell.

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