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The cytoplasmic domain of the cell adhesion molecule uvomorulin associates with three independent proteins structurally related in different species

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Uvomorulin belongs to the group of Ca^{2+}-dependent cell adhesion molecules, which are integral membrane proteins with several structural features in common. In particular, the cytoplasmic part of these proteins is highly conserved in different species, suggesting a common biological function. To test this assumption we transfected a uvomorulin full-length cDNA into uvomorulin-negative mouse NIH 3T3 and L cells. Immunoprecipitations with anti-uvomorulin antibodies detected, in addition to uvomorulin, three independent proteins of 102, 88 and 80 kd which are of host origin and which form complexes with uvomorulin. Using cDNA constructs coding for uvomorulin with cytoplasmic or extracellular deletions it is shown that the 102, 88 and 80 kd proteins complex with the cytoplasmic domain of uvomorulin. Peptide pattern analysis revealed that these three proteins are identical in different mouse cells. When uvomorulin cDNA was introduced into cell lines from other species, such as human HeLa and avian fibroblasts, the expressed uvomorulin was also associated with endogenous 102, 88 and 80 kd proteins and, moreover, each of these proteins showed structural similarities to the respective mouse molecule. A panel of antibodies specific for known cytoplasmic proteins of mol. wts similar to those of the three proteins did not react with any of the described components. This suggests that the 102, 88 and 80 kd proteins constitute a new group of proteins for which we propose the nomenclature of catenin α, β and γ respectively. The characterization of these proteins provides a first molecular basis for a possible cytoplasmic anchorage of uvomorulin to the cytoskeleton.

Key words: uvomorulin/cell adhesion molecules/cytoplasmic anchorage

Introduction

The availability of full-length cDNAs coding for cell adhesion molecules (CAMs) has opened new possibilities for detailed studies on the molecular mechanisms of cell adhesion and the role that these molecules play during development and in tissue organization.

Uvomorulin is an integral membrane glycoprotein of 120 kd which is involved in the aggregation of embryonal and epithelial cells and which belongs to the group of Ca^{2+}-dependent CAMs (Vestweber and Kemler, 1984a; Ekblom *et al.*, 1986). Members of this group express their adhesive properties only in the presence of Ca^{2+}, and Ca^{2+} protects these proteins from proteolytic degradation. Some of the best-studied examples of this group, besides uvomorulin, are chicken L-CAM (Gallin *et al.*, 1987), human cell-CAM 120/80 (Damsky *et al.*, 1983), mouse E- (Nagafuchi *et al.*, 1987), P- (Nose *et al.*, 1987) and N-cadherin (Hatta *et al.*, 1988), canine Arc-1 (Behrens *et al.*, 1985) and rr.1 antigen (Gumbiner and Simons, 1986) and chicken A-CAM (Volk and Geiger, 1986). A more detailed description of these proteins is given in a recent review (Takeichi, 1988).

Comparison of the primary structure revealed that Ca^{2+}-dependent CAMs are evolutionarily conserved and genetically related molecules. More precisely, from sequence data, uvomorulin (identical to E-cadherin) shows 80% identity to human uvomorulin (human cell-CAM 120/80; Mansouri *et al.*, 1988). By antibody cross-reactivity uvomorulin is homologous to canine Arc-1 and rr-1 antigen. The amino acid sequences of uvomorulin (E-cadherin) show 58 and 62% homology to P- and N-cadherin respectively. More striking is that these proteins have a similar domain structure with defined regions of higher identity. This holds true also when chicken L-CAM and uvomorulin are compared (Ringwald *et al.*, 1987). It seems likely that regions with higher homology represent units of functional importance. For example, the extracellular part of uvomorulin and L-CAM is largely composed of a multi-domain structure with internal homology. Within each domain, clustered arrangements of putative Ca^{2+}-binding sites can be defined in both proteins. These common structural features might have been generated by gene duplication during evolution under a strong selection for adhesive function (Ringwald *et al.*, 1987).

We have been attracted by the fact that the amino acid sequences of all Ca^{2+}-dependent CAMs show the highest degree of homology in their cytoplasmic domains. The cytoplasmic parts of mouse uvomorulin and chicken L-CAM are 90% identical, which could be indicative of a common functional role. Possibly, these proteins might interact with cytoplasmic components as is suggested from studies on colocalization of CAMs and cytoskeletal structures. In adult epithelial cells uvomorulin expression is restricted to the baso-lateral membrane where it is concentrated in the adherens junctions known to be associated with the cortical actin belt (Burgess, 1982; Boller *et al.*, 1985). Colocalization of A-CAM (Volk *et al.*, 1986) and cadherins (Hirano *et al.*, 1987) with actin bundles also suggested an association of these CAMs with cytoskeletal components.

In this report we describe three independent proteins of 102, 88 and 80 kd which associate with the cytoplasmic domain of uvomorulin by transfecting uvomorulin-negative mouse cells with full-length cDNA and different deletion constructs. These proteins have already been observed in immunoprecipitation experiments with anti-uvomorulin antibodies and were found to be structurally not only different from uvomorulin but also different from each other (Vestweber and Kemler, 1984b; Peyrieras *et al.*, 1985;

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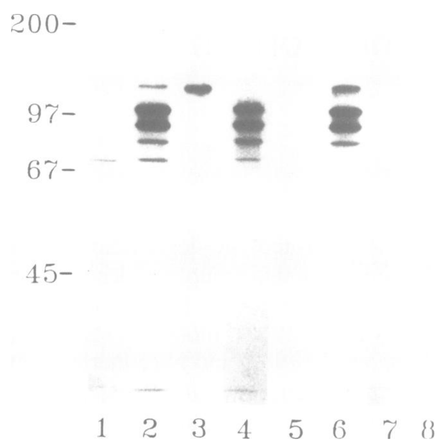

Fig. 1. Analysis of uvomorulin complex by lentil lectin–Sepharose. Cell lysates were precipitated with affinity-purified antibodies. Immune complexes were boiled in sample buffer and either directly analyzed by SDS–PAGE (**lane 2**, control = **lane 1**) or fractionated by lentil lectin–Sepharose (**lanes 3** and **4**). Uvomorulin separated into the bound (lane 3) and the associated proteins into the unbound fraction (lane 4). When whole-cell lysates were first fractionated by lentil lectin–Sepharose and unbound and specifically eluted material was immunoprecipitated, uvomorulin together with the associated proteins were found in the specifically eluted (**lane 6**) and not in the unbound fraction (**lane 8**); respective controls = lanes 5 and 7.

Vestweber *et al.*, 1987). In addition, by heterotypic expression of uvomorulin we found analogous proteins to the 102, 88 and 80 kd proteins in chicken and human cells associated with the introduced uvomorulin. Peptide pattern analysis demonstrates that there is structural similarity of the respective proteins in human, mouse and chicken cells.

Results

Uvomorulin-associated proteins

As already pointed out, only in immunoprecipitation experiments, but not in immunoblots, anti-uvomorulin antibodies detect in addition to uvomorulin independent proteins of 102, 88 and 80 kd (Vestweber and Kemler, 1984b; Peyrieras *et al.*, 1985; Vestweber *et al.*, 1987). The exact relation of the three proteins to uvomorulin was not clear and cross-reactivity of some anti-uvomorulin antibodies was discussed. To demonstrate an association between uvomorulin and these proteins two series of experiments were performed.

Firstly, based on earlier observations that the 102, 88 and 80 kd proteins are not glycosylated (Peyrieras *et al.*, 1985), whole-cell lysates and immunoprecipitates were separated by lentil lectin–Sepharose into bound and unbound material. When immunoprecipitates were separated, only uvomorulin bound to lectin–Sepharose while the lower mol. wt proteins separated in the unbound fraction (Figure 1, lanes 3 and 4). In contrast, when whole-cell lysates were separated by lentil lectin–Sepharose and immunoprecipitations were performed from the unbound and specifically eluted fractions, the latter contained uvomorulin and the associated proteins (Figure 1, lanes 6 and 8).

Another series of experiments was performed using cross-linking reagent dithiobis(sulfosuccinimidyl)propionate (DTSSP), which has an internal disulfide bond and which reacts covalently with proteins of close spatial relationships

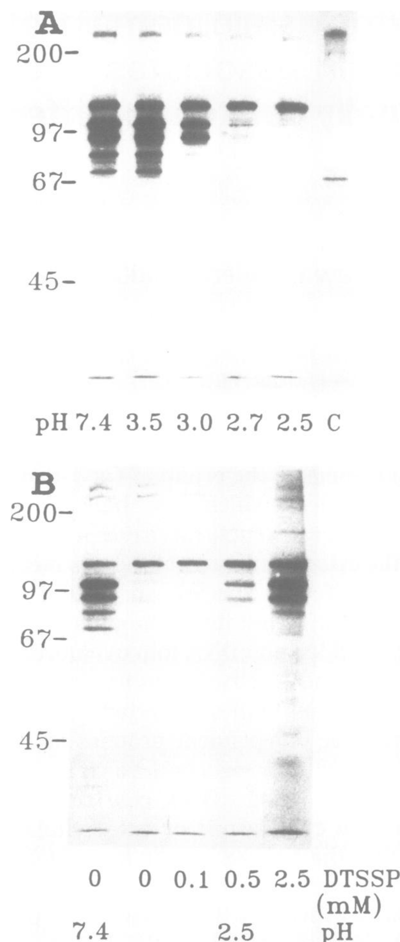

Fig. 2. Immunoprecipitation analysis of uvomorulin and associated proteins. (**A**) Cell lysates were treated with different pHs as indicated for 30 min at 4°C, neutralized and immunoprecipitated with affinity-purified antibodies, control = c. After treatment at pH 2.5 only uvomorulin was precipitated. (**B**) Cell lysates were incubated with indicated concentration of cross-linker DTSSP for 30 min at 4°C. The samples were exposed to pH 7.4 or pH 2.5, neutralized and immunoprecipitated with affinity-purified antibodies. At concentration of 0.5–2.5 mM DTSSP the associated proteins were detected even after pH 2.5 treatment.

(~12 Å). Cell lysates were treated with DTSSP and immunoprecipitates were analyzed by SDS–PAGE under reducing and non-reducing conditions. Under non-reducing conditions uvomorulin appeared in a high mol. wt complex which hardly entered 4% acrylamide gels (not shown). This result impaired the analysis of the cross-linked material by two-dimensional non-reduced and reduced SDS–PAGE.

For further analysis, experimental conditions were defined where uvomorulin and the associated proteins could be separated. This was achieved by exposing cell lysates to different acidic pHs and subjecting them to immunoprecipitations after neutralization. As can be seen in Figure 2A, after pH shift to pH 2.5–2.7 the lower mol. wt proteins were no longer detectable, indicating that the association is sensitive to acidic pH. At pH 3.0 the amount of 80 kd protein was reduced as compared to the other two components. When cross-linked cell lysates were shifted to pH 2.5, neutralized and immunoprecipitated, the lower mol. wt proteins were clearly detected (Figure 2B). From these experiments we conclude that the 102, 88 and 80 kd proteins

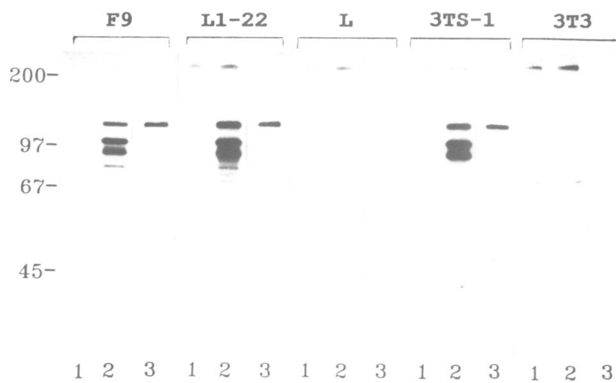

Fig. 3. Analysis of uvomorulin expressed in transfected cells. Transfectant cell lines L1-22 and 3TS-1 were isolated from L and 3T3 cells respectively, after introducing uvomorulin full-length cDNA (pSUM1) and the neomycin-resistance gene. Cell lysates from transfectants, parental cells and F9 cells as positive control were analyzed in immunoblot (**lanes 3**) and immunoprecipitation experiments (**lanes 2**, control = **lanes 1**). Uvomorulin is correctly expressed in transfected cells and is associated in immunoprecipitations with lower mol. wt proteins which are of host origin.

form a complex with uvomorulin. We tested whether immunoprecipitated and blotted lower mol. wt proteins were recognized by antibodies against chicken vinculin and α-actinin, bovine plakoglobin, human adducin, pig and mouse band 4.1 protein. All results were negative, although the respective positive control experiment for each antibody gave a clear reaction (not shown).

Further experiments were performed to elucidate which part of the uvomorulin protein mediates this association.

Expression of uvomorulin in mouse cells

Uvomorulin cDNA was introduced into uvomorulin-negative NIH 3T3 and L cells together with the neomycin-resistance gene by Ca^{2+}-phosphate cotransfection. About 40% of G-418 resistant cells expressed uvomorulin on their cell surface (not shown). Five independent transfectants of 3T3 and L cells were analyzed by immunoprecipitation and immunoblot experiments with affinity-purified anti-uvomorulin antibodies. The results of one representative of each (L1-22 and 3TS-1) are given in Figure 3 and are compared to those obtained with the respective untransfected cells and with embryonal carcinoma cells (F9) as a positive control. The antibodies were negative on cell lysates from untransfected 3T3 and L cells. In immunoblots the antibodies recognize exclusively the 120 kd uvomorulin protein (Figure 3, lanes 3). In immunoprecipitations, additional proteins of 102, 88 and 80 kd were reproducibly observed (Figure 3, lanes 2). Peptide pattern analysis was carried out to compare uvomorulin and the lower mol. wt proteins in transfected cells and F9 cells (Figure 4). The peptide pattern of uvomorulin was different from those of the associated proteins and is shown in Figure 4A. The respective peptide pattern of the 102 kd (Figure 4B), 88 kd (Figure 4C) and 80 kd (Figure 4D) protein from transfected cells was very similar or even identical to those obtained with F9 cells.

Thus, heterotypic expression of uvomorulin leads to an association with endogenous proteins that are structurally identical in different mouse cells.

Expression of mutant uvomorulin

From earlier protein studies it was known that the 102, 88 and 80 kd proteins are not glycosylated and cannot be labeled

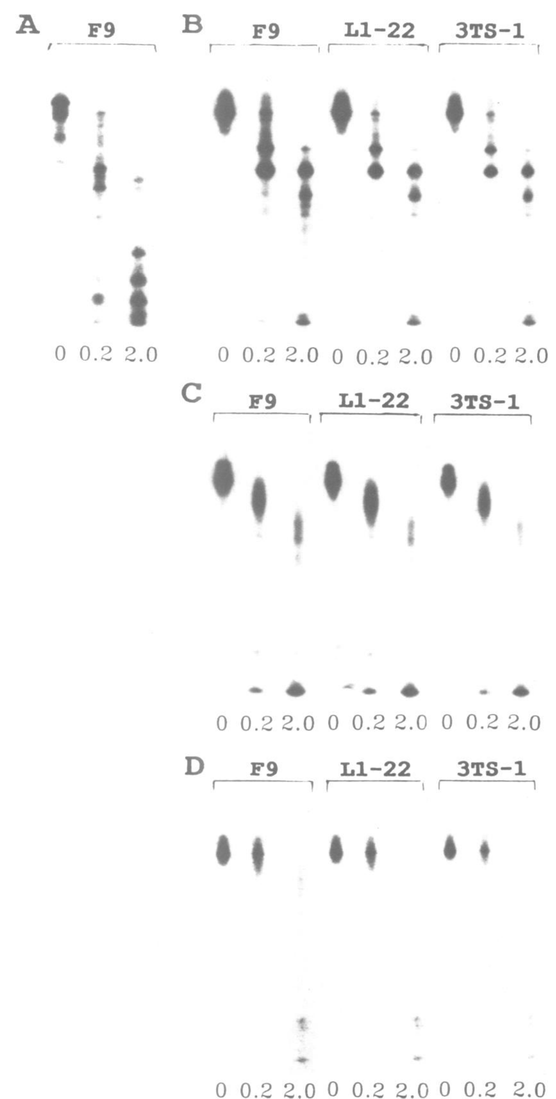

Fig. 4. Comparison of the peptide pattern of uvomorulin and the associated proteins. Cleveland digest analysis was performed from uvomorulin and the 102, 88 and 80 kd proteins isolated from F9 cells and transfectants L1-22 and 3TS-1. The concentration of V8 protease (ng/μl) is indicated in the figure. (**A**) Uvomorulin from F9 cells; (**B**) 102 kd protein from F9, L1-22 and 3TS-1 cells; (**C**) 88 kd protein from F9, L1-22 and 3TS-1 cells; (**D**) 80 kd component from F9, L1-22 and 3TS-1 cells. The peptide pattern of uvomorulin, the 102, 88 and 80 kd protein was different from each other. More important, each protein was structurally similar in F9, L1-22 and 3TS-1 cells.

when intact cells are iodinated by the lactoperoxidase method (unpublished observations). This result offered the possibility that the proteins might be linked to the cytoplasmic domain of uvomorulin. To test this hypothesis, two truncated uvomorulin cDNA constructs (pSUMΔC5 and pSUMΔC4) with different deletions at their 3′ ends (Figure 5A) were transfected into L cells. Transfected cell lines with cell surface expression of mutant uvomorulin (LΔC5-14 and LΔC4-17) were established and analyzed using anti-uvomorulin antibodies directed against lines expressing uvomorulin with a size roughly proportional to that expected from the deletion constructs (Figure 5B, lanes 3). More importantly, the associated 102, 88 and 80 kd proteins were not detected in LΔC5-14 and LΔC4-17 cells (Figure 5B, lanes 2). Thus, deletion of 37 amino acid residues at the carboxy terminus interfered with the complex formation

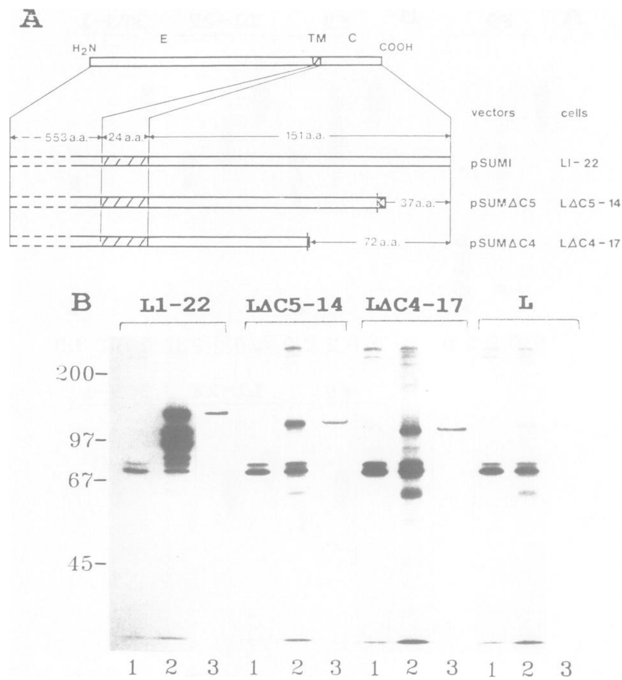

Fig. 5. Expression of carboxy-terminal deleted uvomorulin in mouse L cells. (**A**) Schematic representation of uvomorulin primary structure (E, extracellular; TM, transmembrane; and C, cytoplasmic domain) and deleted uvomorulin encoded by different cDNA constructs: pSUM1, full-length cDNA; pSUMΔC5 and pSUMΔC4, deleted cDNAs encoding for cytoplasmic deletions as indicated. (**B**) Transfection of construct pSUMΔC5 and pSUMΔC4 together with the neomycin-resistant gene gave rise to cell line LΔC5-14 and LΔC4-17 respectively. Immunoblot (**lanes 3**) and immunoprecipitation (**lanes 2**, control = **lane 1**) results were compared with parental L and L1-22 cells expressing normal uvomorulin. The associated proteins are not detected in cells expressing uvomorulin with cytoplasmic deletions (cf. lanes 2 of L1-22, LΔC5-14 and LΔC4-17 cells).

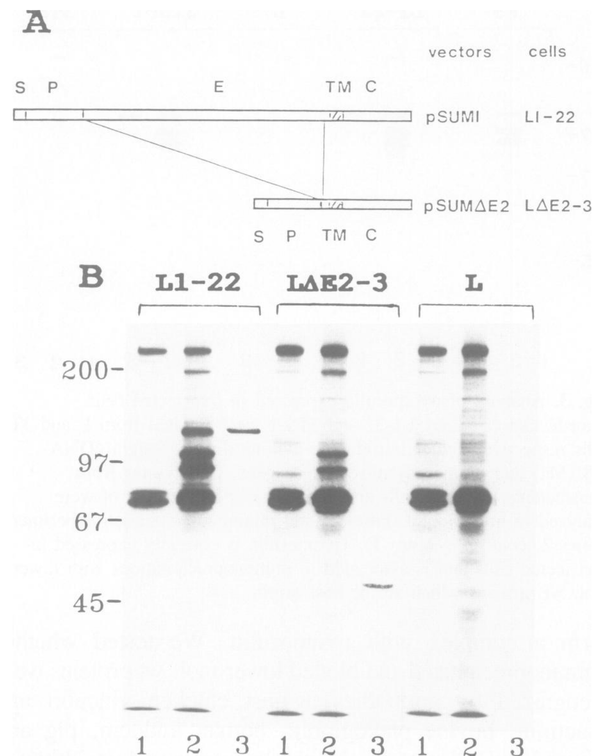

Fig. 6. Expression of extracellular deleted uvomorulin in mouse L cells. (**A**) Schematic representation of uvomorulin and of a truncated protein where most of the extracellular part is deleted. (S, signal peptide; P, precursor region; E, extracellular; TM, transmembrane; and C, cytoplasmic domain.) Plasmid pSUM1, full-length cDNA; pSUMΔE2, construct encoding for extracellular deletion.
(**B**) Transfection of construct pSUMΔE2 together with the neomycin-resistance gene gave rise to cell line LΔE2-3. Immunoblot (**lanes 3**) and immunoprecipitation (**lanes 2**, control = **lanes 1**) results were compared with those of parental L and L1-22 cells, which express normal uvomorulin. Affinity-purified antibodies against the cytoplasmic domain recognized a truncated protein of 49 kd (**lanes 2** and **3** of LΔE2-3). The associated proteins were detected in immunoprecipitates of LΔE2-3 cell lysates.

between uvomorulin and the set of proteins. As a control, a cDNA clone (pSUMΔE2) was constructed that encoded a truncated uvomorulin where most of the extracellular part of the protein was deleted. In this construct, sequences coding for the cytoplasmic and the transmembrane domains were left intact (Figure 6A). Cells were transfected with plasmid pSUMΔE2 and a transfected cell line LΔE2-3 was established and analyzed using anti-uvomorulin antibodies directed against the cytoplasmic domain (Figure 6B). In immunoblots, the antibodies reacted with a truncated protein of 49 kd (Figure 6B, lanes 3). In immunoprecipitations the truncated protein was hardly visible, which is most likely due to its low methionine content. However, the associated proteins were clearly detected in these experiments (Figure 6B, lane 2). This indicates that the cytoplasmic domain of uvomorulin mediates the complex formation with the three proteins.

First indications about a possible functional role of this complex came from cell aggregation studies. The uvomorulin-dependent cell aggregation of L1-22 cells was compared to those of LΔC5-14 and LΔC4-17 cells. Only L1-22 cells expressing normal uvomorulin aggregated in an uvomorulin-dependent manner that can be blocked by anti-uvomorulin antibodies while the aggregation of cells expressing the mutant uvomorulin was at the level of untransfected L cells (not shown). These results confirm similar observations described by Nagafuchi and Takeichi (1988) where cytoplasmic deletions of E-cadherin also affected cell adhesion. This suggests that the complex formation of the 102, 88 and 80 kd proteins with the cytoplasmic domain may regulate the cell-binding function of uvomorulin.

More information about the possible functional role of the associated protein was obtained when the partitioning of normal and mutant uvomorulin into detergent-soluble and -insoluble fractions was studied. It was already known that after detergent solubilization of embryonal carcinoma or epithelial cells, a significant amount of uvomorulin remained in the insoluble fraction. This was also the case when L1-22 cells expressing normal uvomorulin were examined (Figure 7). On the other hand, in LΔC5-14 and LΔC4-17, the mutant uvomorulin was found exclusively in the soluble fraction (Figure 7). These results indicate that the 102, 88 and 80 kd proteins might be involved in connecting uvomorulin to cytoskeletal structures not soluble in non-ionic detergents.

Expression of uvomorulin in avian and human cells

The amino acid sequences of uvomorulin, chicken L-CAM and human uvomorulin show a high degree of homology (Ringwald *et al.*, 1987; Mansouri *et al.*, 1988). This fact and the results presented above prompted us to analyze human and avian cells transfected with mouse uvomorulin

Fig. 7. Separation of uvomorulin in detergent-soluble and -insoluble fractions. L1-22 cells express normal uvomorulin, LΔC5-14 and LΔC4-17 cells expressing deleted constructs of plasmids pSUMΔC5 and pSUMΔC4 respectively (see Figure 5A). Cells were lysed in 1% NP-40/1% Triton X-100 in PBS, washed three times in the same buffer and the detergent-soluble (S) and -insoluble (IS) material was subjected to immunoblot experiments. Only in L1-22 cells did uvomorulin separate into the detergent-insoluble fraction. This correlates with the presence or absence of uvomorulin-associated proteins (see Figure 5B).

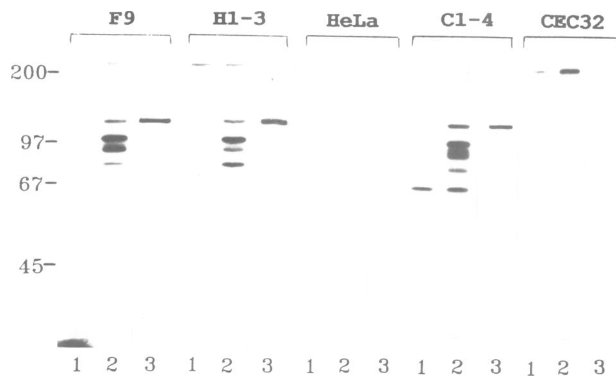

Fig. 8. Expression of uvomorulin in human HeLa and chicken CEC 32 cells. Uvomorulin full-length cDNA and the neomycin-resistance gene were introduced into HeLa and CEC 32 cells and the respective transfectant cell lines H1-3 and C1-4 were isolated. Immunoblot (**lanes 3**) and immunoprecipitation (**lanes 2**, control = **lanes 1**) results were compared with those of parental HeLa and CEC 32 and with F9 cells as positive control. Uvomorulin is expressed with the correct size in human and chicken cells. In immunoprecipitation, the associated proteins are detected in H1-3 and C1-4 cells that are of host origin.

cDNA. Human (HeLa), quail (QT-6) and mouse (CEC 32) cells were transfected with uvomorulin cDNA as described, generating cell lines H1-3, Q2-2 and C1-4 respectively. Transfectants and the respective parental cells were subjected to immunoblot and immunoprecipitation analysis. The general outcome of these experiments was identical to the results obtained with transfected mouse cells. In immunoblots only the uvomorulin protein was recognized (Figure 8, lanes 3). Following immunoprecipitation, additional polypeptides of 102, 88 and 80 kd were detected (Figure 8, lanes 2) and

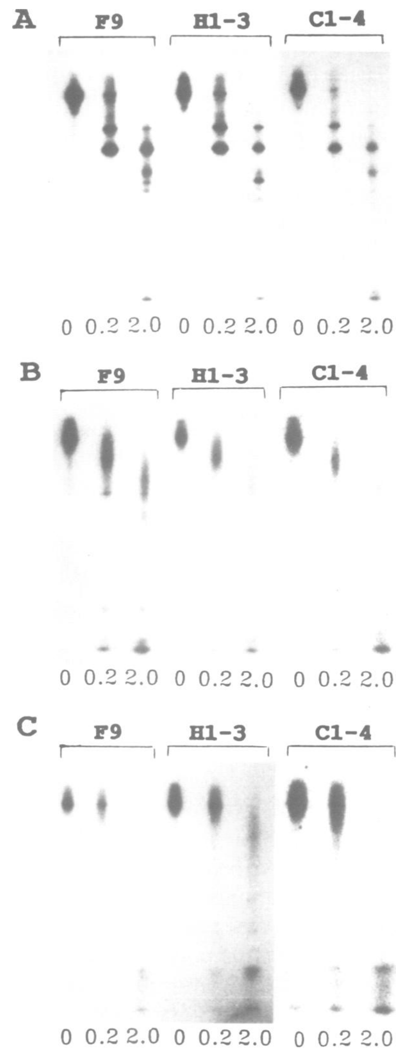

Fig. 9. Comparison of peptide patterns of the 102, 88 and 80 kd proteins of mouse, human and chicken cells. Cleveland digest analysis was carried out with the concentration of V8 protease (ng/μl) as indicated in the figure. (**A**) 102 kd protein; (**B**) 88 kd protein; (**C**) 80 kd protein. Each of the uvomorulin-associated proteins shows structural similarity in mouse, human and chicken cells.

peptide pattern analysis revealed that these proteins are structurally related to the protein found associated with uvomorulin in mouse cells (Figure 9). The results obtained with transfectant Q2-2 were similar to those obtained with C1-4 cells (not shown). These results show that mouse uvomorulin can complex with endogenous avian and human proteins.

Discussion

In this report we have identified three independent proteins with molecular masses of 102, 88 and 80 kd which associate with the cytoplasmic domain of the cell adhesion molecule uvomorulin. This association is shown directly by cross-linking experiments and by analyzing the heterotypic expression of normal and mutant uvomorulin.

We show here that the uvomorulin-associated 102, 88 and 80 kd proteins are structurally related in different species. Cleveland digest analysis revealed for each protein an identical peptide pattern in different mouse cells and a high degree of similarity in chicken and human cells. Thus, a set

of structurally related proteins from different species is able to form complexes with the cytoplasmic domain of mouse uvomorulin. These findings are consistent with the fact that several Ca^{2+}-dependent CAMs from different species are most homologous in their respective cytoplasmic domains, suggesting that this region has a conserved function. The identification of the 102, 88 and 80 kd proteins in different cell types of various species supports such a view. Further experiments should determine how the three proteins associate with uvomorulin and in what stoichiometry. The binding of the 80 kd protein to uvomorulin seems to be weaker compared to the two other molecules and the relative amount of the 80 kd protein is apparently different in F9 and transfected mouse cells (see Figure 3), which might indicate some variability for this component.

Transfection experiments show that uvomorulin associates with endogenous 102, 88 and 80 kd proteins. It is likely that these proteins are already pre-existing in these cells but other explanations are possible. This raises questions about the function of these proteins in untransfected cells. Do these proteins interact only with uvomorulin or with other as yet unknown Ca^{2+}-dependent CAMs in untransfected cells or do they also associate with other (membrane) proteins in a more general way? With the availability of molecular probes for the three proteins this could be investigated. We have tested antibodies against different cytoplasmic proteins that could be candidates for the uvomorulin-associated proteins because of similar mol. wt, known association with the cytoskeleton or distribution in the peripheral cytoplasm. Since these antibodies have been raised against proteins from different species, we examined transfectants from different species, as well as cell types indicative for the respective proteins to obtain unambiguous results. None of the antibodies recognized the uvomorulin-associated proteins.

We have named the 102, 88 and 80 kd proteins catenin α, β and γ respectively (*catena*, the Latin name for chain) since one major function of catenins might be to link Ca^{2+}-dependent CAMs with cytoskeletal structures. Separation of uvomorulin from the transfected cells into detergent-soluble and -insoluble material revealed that cytoplasmic-deleted uvomorulin—which does not complex with catenins—is found only in the detergent-soluble fraction, while normal uvomorulin—which complexes with catenins—separates in both detergent-soluble and -insoluble material. Since in these experiments the complex of uvomorulin and catenins was also found in the detergent-soluble fraction, this indicates that additional unknown proteins are required to link the uvomorulin–catenin complex to cytoskeletal structures. Together with the observations that only L cells expressing normal uvomorulin exhibit a uvomorulin-dependent cell adhesion mechanism, the interaction between the uvomorulin cytoplasmic domain and catenins might be necessary for the regulation of cell adhesion. Concordant with this interpretation are results recently described by Nagafuchi and Takeichi (1988). In this study different cytoplasmic deletions of E-cadherin also affected cell adhesion. Although no immunoprecipitation analyses were performed we would predict from our results that in the case of deleted E-cadherin, no catenins would have been detected.

There is at present no indication for molecular differences of uvomorulin during development and in adult tissues as has been reported for other CAMs (Cunningham *et al.*, 1987). However, the biological function of uvomorulin is assumed to be slightly different during development and in adult tissues. During development, uvomorulin is involved in the condensation, the pattern formation and the sorting-out of cells and might well act as a 'morpho-regulator' (Edelman, 1984). In these processes the uvomorulin-mediated adhesiveness must be controlled, since cells reorganize and migrate during development. On the other hand, for the maintenance of the histo-architecture in adult tissues, uvomorulin function should be more 'mechanostatic', since it is important for preserving the integrity of an epithelial sheet (Boller *et al.*, 1985; Vestweber and Kemler, 1985; Gumbiner, 1988). From our work we would like to propose that differences in strength of adhesiveness might be regulated by catenins. Cell adhesion might be a multistep mechanism where the extracellular domain establishes the initial contact. Maintenance and strength of adhesiveness is subsequently regulated via catenins and their connection to cytoskeletal structures. Although in this view catenins have a central functional role in regulating adhesiveness, this might not be their sole function. Catenins are already associated with uvomorulin in the endoplasmic reticulum as monitored by pulse–chase experiments (unpublished observations). Since uvomorulin is expressed on the baso-lateral membrane of epithelial cells, catenins might be involved in the intracellular sorting machinery. Catenins could also control correct assembly and transport of uvomorulin to the plasma membrane in a way similar to the multimeric complexes reported for the T cell antigen receptor (Sussman *et al.*, 1988) and for the hemagglutinin glycoprotein of influenza virus (Copeland *et al.*, 1988). Although the cytoplasmic-deleted uvomorulin molecules are expressed on the cell surface, significant amounts of protein are also accumulated in the cytoplasm (unpublished observations).

Moreover, catenins may be involved in signal transduction controlled by cell adhesion. Following the ideas about the morpho-regulatory role of CAMs in development where CAM-mediated cell adhesion drives cells into particular cell lineages (Edelman, 1984), a communication between the cell surface and the nucleus has to be posulated. In conclusion, by identifying catenins as structurally related proteins in mouse, chicken and human cells that form complexes with the cytoplasmic domain of uvomorulin, we might have taken a first step towards a more general understanding not only of the connection of CAMs with the cytoskeletal but also of the as yet unknown adhesion-dependent signal transduction.

Materials and methods

Cells

The following cell lines were used: mouse embryonal carcinoma cell line F9 (Artzt *et al.*, 1973), NIH 3T3, L-tk$^-$ and human HeLa cells were obtained from ATCC. Two avian cell lines, mouse CEC 32 (Kaaden *et al.*, 1982) and quail QT-6 (Moscovici *et al.*, 1977), were a gift from Dr Th.Graf, EMBL, Heidelberg. The cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (15% for F9 cells) fetal calf serum (FCS) in a 10% CO_2 atmosphere at 37°C.

For metabolic labeling experiments 5×10^5 cells were grown in DMEM without methionine (dialyzed 10% FCS) for 1 h and subsequently labeled with [^{35}S]methionine (Amersham, sp. act. 1100 Ci/mmol) at 50 μCi/ml for 16 h.

Gene transfer experiments were carried out using the Ca^{2+}-phosphate transfection method essentially as described (Gorman, 1985). Briefly, 15 μg DNA and 1.5 μg DNA of pSVtk-neoβ (Nicolas and Berg, 1983) were mixed and added to 5×10^5 cells that were plated 1 day in advance. After 48 h, selection was started with medium containing 1 mg/ml G-418 (Gibco) for

2 weeks and continued with 0.25 mg G-418/ml medium. Uvomorulin-positive transfectants were isolated by immunofluorescence tests or fluorescence-activated cell sorting (FACS) using affinity-purified rabbit anti-uvomorulin antibodies and FITC-labeled goat $F(ab)_2$ anti-rabbit IgG (Jackson Immunoresearch Lab. Inc.).

Biochemical methods

Cell lysates were prepared in 1% NP-40/1% Triton X-100 in PBS, pH 7.4, 1 mM PMSF (Vestweber and Kemler, 1984a). For pH shifts, cell lysates were treated with acidic pH (0.1 M glycine–HCl, pH 2.5) for 30 min at 4°C and subsequently neutralized. Immunoprecipitations were done with affinity-purified rabbit antibodies directed either against the extracellular part (Vestweber and Kemler, 1985) or against the cytoplasmic domain of uvomorulin (Ringwald *et al.*, 1987) and analyzed by SDS–PAGE. For immunoblots, cells were boiled directly in sample buffer, separated by SDS–PAGE and transferred to nitrocellulose (Schleicher and Schüll). The following antibodies against different cytoplasmic components were included: rabbit anti-chicken gizzard vinculin and α-actinin (a gift of Dr B.Jokusch, Bielefeld), guinea-pig serum and mouse monoclonal antibodies against bovine plakoglobin (a gift of Dr W.Franke, DKFZ, Heidelberg), rabbit antibodies against human adducin and against pig and mouse band 4.1 (a gift of Dr D.Drenckhahn, Marburg and Dr M.Lehnert-Wilzewski, EMBL).

Cross-linking experiments

To 90 μl of [^{35}S]methionine-labeled cell lysate, 10 μl of the cleavable bifunctional cross-linking dithiobis(sulfosuccinimidyl)propionate (DTSSP, Pierce) in PBS was added. The mixture was incubated for 30 min at 0°C, treated with 200 μl 0.1 M glycine–HCl, pH 2.5, for 30 min at 0°C, neutralized (125 μl 0.75 M Tris–HCl, pH 8.8) and subjected to immunoprecipitation.

Lentil lectin–Sepharose

Immunocomplexes were boiled in 20 μl sample buffer, centrifuged and the supernatant was diluted 20 times with PBS containing 1% Triton X-100 and 1% NP-40. After incubation with 200 μl (packed volume) of lentil lectin–Sepharose (Pharmacia) for 1 h at 4°C under rotation, unbound material was collected. This procedure was repeated twice and the lectin–Sepharose was washed several times in PBS. Lectin-bound material was eluted with PBS containing 0.1 M α-methyl mannoside, 0.1% Triton X-100 or boiled in sample buffer and analyzed by SDS–PAGE. Similar separation experiments in bound and unbound fraction were carried out with whole cell lysates and unbound and specifically eluted material was subjected to immunoprecipitation experiments.

Peptide pattern analysis

Peptide mapping experiments were performed essentially as described by Cleveland *et al.* (1977). Immunoprecipitates from [^{35}S]methionine-labeled cells were separated on 8% polyacrylamide gels. The unfixed gel was dried and subjected to autoradiography. Bands corresponding to uvomorulin and 102, 88 and 80 kd proteins were excised, transferred into the slots of a new gel and proteins were digested with 0.2–2.0 ng/μl of *Staphylococcus aureus* V8 protease (Miles).

Construction of expression vectors

A full-length uvomorulin cDNA was constructed using the cDNA clone F5 (Ringwald *et al.*, 1987) containing most of the uvomorulin coding sequence and the cDNA clone M2 obtained from a primer extension cDNA library in λgt10 containing further 5′ sequences of uvomorulin. A *Bgl*II site was introduced in the 5′ untranslated region of M2 using synthetic oligonucleotides. The resulting 537 bp *Bgl*II–*Eco*RI fragment (pM3) was ligated to the 2.1 kb *Eco*RI–*Nco*I fragment of F5 that is lacking part of the 3′ untranslated region and subcloned into *Bgl*II–*Sma*I site of the expression vector pSVtk-neoβ (pSUM1).

To construct expression vectors encoding uvomorulin with cytoplasmic deletions, a synthetic oligonucleotide CTGCAGTAATTAATTAAGATA-TCA was introduced into the *Eco*RI–*Nco*I subclone of F5. The *Cla*I and *Sac*I restriction sites of the nucleotide sequence encoding the cytoplasmic portion of uvomorulin and the *Pst*I site of the introduced oligonucleotide were used to make the deletions. The *Cla*I and *Sac*I correspond to sites 72 and 37 amino acid residues from the carboxy terminus respectively. The deleted constructs were ligated to the pM3 fragment and subcloned into the *Bgl*II–*Sma*I site of the pSVtk-neoβ expression vector. The resulting vectors were named pSUMΔC4 (lacking 72 amino acids) and pSUMΔC5 (lacking 37 amino acids).

An expression vector with mutant uvomorulin lacking most of the extracellular portion was constructed as follows. A 465 bp *Bgl*II–*Rsa*I fragment encoding the leader sequence and the first 120 amino acid residues of the precursor portion of uvomorulin was isolated from pM3. To this, a 625 bp fragment that contains the sequence encoding the transmembrane and cytoplasmic domains as well as 16 amino acid residues from the carboxy terminus of the extracellular portion including 17 bp at the 5′ end derived from the pUC polylinker (GGGGATCCTCTAGAGTC) was introduced in frame at the 3′ end of the pM3 insert and subcloned into the *Bgl*II and *Sma*I site of pSVtk-neoβ (pSUMΔE2). The integrity of all constructs was controlled by direct DNA sequencing (Hattori and Sakaki, 1986).

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