A specific domain in α-catenin mediates binding to β-catenin or plakoglobin

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SUMMARY

The E-cadherin-catenin adhesion complex has been the subject of many structural and functional studies because of its importance in development, normal tissue function and carcinogenesis. It is well established that the cytoplasmic domain of E-cadherin binds either β-catenin or plakoglobin, which both can assemble α-catenin into the complex. Recently we have identified an α-catenin binding site in β-catenin and plakoglobin and postulated, based on sequence analysis, that these protein-protein interactions are mediated by a hydrophobic interaction mechanism. Here we have now identified the reciprocal complementary binding site in α-catenin which mediates its interaction with β-catenin and plakoglobin. Using in vitro association assays with C-terminal truncations of α-catenin expressed as recombinant fusion proteins, we found that the N-terminal 146 amino acids are required for this interaction. We then identified a peptide of 27 amino acids within this sequence (amino acid positions 117-143) which is necessary and sufficient to bind β-catenin or plakoglobin. As shown by mutational analysis, hydrophobic amino acids within this binding site are important for the interaction. The results described here, together with our previous work, give strong support for the idea that these proteins associate by hydrophobic interactions of two α-helices.

Key words: E-cadherin-catenin-complex, α-Catenin binding site, Site-directed mutagenesis

INTRODUCTION

The Ca²⁺-dependent cell adhesion molecule E-cadherin plays an important role during mouse embryonic development (Larue et al., 1994; Riethmacher et al., 1995) and in the proper organization of epithelia (for review see Gumbiner, 1996). For its function in epithelial cell polarity and epithelial tissue integrity, the cytoplasmic tail of E-cadherin must be associated with three proteins termed α-, β- and γ-catenin (plakoglobin) (for review see Kemler, 1993). Catenins link cadherins to the actin microfilament network, but they also interact with other transmembrane or cytoplasmic proteins and have been considered as more general linker or adapter proteins (for review see Huber et al., 1996a). β-Catenin and plakoglobin are members of the armadillo protein superfamily (Peifer et al., 1994) and have recently attracted much attention because of their possible signaling function besides their demonstrated involvement as structural components in adherens junctions and desmosomes (Gumbiner, 1995; Huber et al., 1996a,b; Miller and Moon, 1996; Behrens et al., 1996; Molenaar et al., 1996).

The α-catenin primary sequence reveals homology to vinculin, a protein first described as a component of focal contacts, where it may be part of a molecular network connecting the extracellular matrix with the intracellular cytoskeleton. The sequence homology is restricted to three regions within the molecules: an N-terminal region, a central domain, and the C-terminal region (Herrenknecht et al., 1991; Nagafuchi et al., 1991). α-Catenin mediates the contact of the cadherin-catenin complex with the actin cytoskeleton (Ozawa et al., 1990) and may bind to actin microfilaments directly and/or in association with α-actinin (Rimm et al., 1995; Knudsen et al., 1995). Similar actin binding properties have been reported for vinculin, and based on the sequence homology the interaction of these two proteins with actin filaments may be regulated in a similar way (for review see Jockusch and Rüdiger, 1996).

The essential function of α-catenin for the cadherin-catenin complex was shown in mouse development by mutational analysis (Torres et al., 1997), as well as in cancer cell lines (PC-9 and PC-3) which exhibit impaired adhesion due to a lack of a large portion or of the complete α-catenin gene (Shimoyama et al., 1992; Morton et al., 1993; Ewing et al., 1995). In human esophageal cancer tissue, loss of α-catenin expression also correlates with the degree of infiltration and the extent of lymph node metastasis (Kadowaki et al., 1994).

Biochemical analysis of the cadherin-catenin complex revealed the presence of two mutually exclusive complexes within the same cell, composed of either E-cadherin, β-catenin and α-catenin, or E-cadherin, plakoglobin and α-catenin (Butz and Kemler, 1994; Näthke et al., 1994). β-Catenin and plakoglobin bind to amino acid (aa) positions 832-862 in the cytoplasmic domain of E-cadherin (Ozawa et al., 1990; Stappert and Kemler, 1994). The sites in β-catenin and plakoglobin which mediate the interaction with E-cadherin appear to be less defined and are located in the multiple armadillo repeats (Hülslken et al., 1994). α-Catenin associates with the complex by interacting with either β-catenin or plakoglobin (Aberle et al., 1994; Hülslken et al., 1994). The sequence comprising aa
positions 120-151 in β-catenin is necessary and sufficient for the interaction of β-catenin with α-catenin (Aberle et al., 1994, 1996). A homologous amino acid sequence in plakoglobin was identified which mediates its interaction with α-catenin (Aberle et al., 1996; Sacco et al., 1995). No detailed information was available on the amino acid sequences in α-catenin necessary for the interaction with β-catenin and plakoglobin, although it had been found, using a two-hybrid assay, that the N-terminal 606 aa of α-catenin bind to β-catenin (Jou et al., 1995). Here we have characterized the binding sites in α-catenin more precisely, identifying a peptide of 27 aa in the α-catenin sequence which is sufficient to bind β-catenin or plakoglobin.

MATERIALS AND METHODS

Chemicals and reagents

Molecular biological reagents and enzymes were purchased from Boehringer Mannheim or New England Biolabs. Oligonucleotides were synthesized on an Applied Biosystems model 394A synthesizer. The peptide S27D was synthesized on an Applied Biosystems model 431A synthesizer using Fmoc (N-(9-fluorenylmethoxycarbonyl) chemistry. For purification of the peptide a reversed-phase column (Vydac 218 TP) connected to a BioCade Sprint perfusion chromatography system (Perceptive Biosystems) was used. For biotinylation the purified peptide was incubated at pH 9 with Biotin-XNHS ester (Calbiochem). To remove excess biotinylation reagent the reaction mixture was acidified with trifluoroacetic acid (TFA), applied to a C18 Sep-Pak cartridge (Waters Millipore) equilibrated with 5% (v/v) acetonitrile + 0.1% (v/v) TFA, and washed with the same solvent. The peptide was eluted with 70% (v/v) acetonitrile + 0.1% (v/v) TFA, dried in a Speedvac and dissolved in water (1 mg/ml). The peptide was coupled to tosyl-activated magnetic beads (Dynal) in 0.15 M NaHCO₃ + 10% (w/v) PEG 8000. Non-reacted tosyl-groups were directly blocked with ethanolamine.

Antibodies and detection systems

Antibodies against α-catenin, β-catenin and plakoglobin have been described previously (Butz and Kemler, 1994) or were purchased from Transduction Laboratories (Lexington, USA). Chicken antibodies against MBP and anti-IgY monoclonal antibody were obtained from Nanotools (Denzlingen, Germany). Peroxidase-labeled secondary antibodies were from Dianova (Hamburg, Germany) or Sigma (Deisenhofen, Germany). The ECL detection kit and Hyperfilm-ECL were purchased from Amersham.

Construction of vectors for maltose binding protein (MBP)-α-catenin fusion proteins and expression in Escherichia coli

The deletion constructs MBPβ1-246 and MBPα1-146 were generated by PCR using Pwo DNA-polymerase with forward primer 5’-CA-GGCTCCGGATCCATGACTGCCGTGCACAGCA-3’ and reverse primers 5’-CGGATCTCCCTGCTGCTGCCGTCA 5’-GCCGGTATCCCTGGCTGCTGCCGTCA 5’-CTCTCTGCCGTCA CCGCGCTGCTGA TTCTGGCT -3’. These primer pairs generate BamHI sites to use for cloning the PCR product into a modified pMalC2 vector (New England Biolabs). pMalC2 was digested with HindIII, blunt ended with the Klenow fragment of DNA polymerase I, and religated to obtain an in-frame stop codon. Ligation and transformation in E. coli strain XL-1 were done by standard procedures (Sambrook et al., 1989). Transformants were checked for correct orientation of the insert by colony-PCR. For expression plasmids were transformed in E. coli BL21DE4 carrying the pREP4 plasmid (Qiagen) containing the lac repressor. A 20 ml volume of an overnight culture was used to inoculate 1 l of LB medium + 10 g/l glucose + antibiotics. Bacteria were grown at 30°C to an OD₅₇₈ of 0.5 and expression of the fusion protein was induced by addition of 1 mM IPTG (Biomol). After 1 hour bacteria were harvested and washed with PBS. For ultrasonic lysis bacteria were resuspended at an OD₅₇₈ of 200 in 20 mM Hepes-NaOH, pH 7.4 + Complete™ protease inhibitor mixture (Boehringer Mannheim). The lysate was adjusted to 10 ml with 20 mM Hepes-NaOH, pH 7.4, and ultracentrifuged for 30 minutes at 4°C at 45,000 rpm in an AH650 swing-out rotor. The supernatant was applied to 2 ml amylose resin (New England Biolabs) and incubated for 15 minutes at 4°C on a rotatory shaker. To achieve complete binding the flow-through was reapplied to the settled beads. After washing the amylose beads 2× with 10 ml PBS, the fusion protein was eluted with 20 mM maltose in PBS, dialysed against 20 mM Tris-HCl, pH 8.0, and stored after shock freezing in liquid nitrogen. pMalα129 was constructed by digestion of pMalα146 with XhoI and SalI and religation. pMalα98 was generated by digestion of pMalα146 with NspV and XhoI. Overhanging ends were filled by Klenow polymerase treatment to allow blunt-end ligation. Expression of these constructs was done as described for MBPα146. β-Catenin and plakoglobin, each carrying a C-terminal His₆-tag, were expressed with pQE60 (Qiagen) and purified on Ni²⁺-chelating Sepharose (Pharmacia). GST-β-catenin was expressed as described previously (Aberle et al., 1994).

Construction of eukaryotic α-catenin expression vectors and expression in Neuro2A cells

α-Catenin cDNA was cloned into the EcoRI site of pCIneo (Promega). To obtain His₆-tagged α-catenin, pCIneo α-cat was digested with BspEI and Smal. This fragment was replaced by a corresponding fragment obtained from pQE60 α-cat (Aberle et al., 1994) by digestion with HindIII. Klenow DNA-polymerase treatment to create a blunt end and subsequent digestion with BspEI. A Koszak sequence was introduced by PCR amplification with Pwo-polymerase using the oligonucleotide 5’-CCGGTATCCCTGGCTGCTGCCGTCA 5’-CGGAAATTCGCCATGGCGATGTTC-3’ and a pCIneo T3 primer. The amplification product was digested with EcoRI and NotI and cloned into pCIneo. pCIneo α-catα143 was generated by an identical procedure using the oligonucleotide 5’-CGGGAATTCGCCATGCGATGTTC-3’ and the T3 primer.

Transient transfections of α-catenin and β-catenin cDNAs in Neuro2A cells and immunoprecipitation experiments with Neuro2A cell lysates were performed as described (Huber et al., 1996b).

Site-directed mutagenesis

Site-directed mutagenesis was performed as described (Stappert et al., 1992). For the primer extension reaction the forward primer 5’-CA-GGCTTCGGATCCATGACTGCCGTGCACAGCA-3’ containing the tag sequence necessary for subsequent PCR amplification was used. The following mutagenic primers containing mismatches were used to replace specific amino acids and to introduce or remove restriction sites by silent mutation for colony identification: V125A, 5’-CA-GAGGCAACATGCGCGCGAGCTGAGCTTGTGTTT-3’; R126A, 5’-CA-GAGGCAACATGCGCGCGAGCTGAGCTTGTGTTT-3’; R129A, 5’-ATGGTGGCAAGACGTGGCAGGCTTGTGTTT-3’; S133A, 5’-GC TCGAGATTGGCTGCTGGCTGCCAACGGGCTG-3’; T136A, 5’-TTGGCTTTGGCTGCCAACGGGCTG-3’; R137A, 5’-CTCTCCTGCTGCCAACGGGCTGCGTATGCTGC-3’; L131A, 5’-CGGGCAATTCGCCATGGCGATGTTC-3’; L132A, 5’-CGACGCTAGCTGAGCTTGTGTTTG-3’; V135A, 5’-TGTATGCTGCCAACGGGCTGCGTATGCTGC-3’; L133A, 5’-TGTATGCTGCCAACGGGCTGCGTATGCTGC-3’; L134A, 5’-TGTATGCTGCCAACGGGCTGCGTATGCTGC-3’. The mutated genes were inserted into these constructs. To verify correct orientation of the insert by colony-PCR. For expression mutants were transformed in E. coli BL21DE4 carrying the pREP4 plasmid (Qiagen) containing the lac repressor. A 20 ml volume of an overnight culture was used to inoculate 1 l of LB medium + 10 g/l glucose + antibiotics. Bacteria were grown at 30°C to an OD₅₇₈ of 0.5 and expression of the fusion protein was induced by addition of 1 mM IPTG (Biomol). After 1 hour bacteria were harvested and washed with PBS. For ultrasonic lysis bacteria were resuspended at an OD₅₇₈ of 200 in 20 mM Hepes-NaOH, pH 7.4, and ultracentrifuged for 30 minutes at 4°C at 45,000 rpm in an AH650 swing-out rotor. The supernatant was applied to 2 ml amylose resin (New England Biolabs) and incubated for 15 minutes at 4°C on a rotatory shaker. To achieve complete binding the flow-through was reapplied to the settled beads. After washing the amylose beads 2× with 10 ml PBS, the fusion protein was eluted with 20 mM maltose in PBS, dialysed against 20 mM Tris-HCl, pH 8.0, and stored after shock freezing in liquid nitrogen. pMalα129 was constructed by digestion of pMalα146 with XhoI and SalI and religation. pMalα98 was generated by digestion of pMalα146 with NspV and XhoI. Overhanging ends were filled by Klenow polymerase treatment to allow blunt-end ligation. Expression of these constructs was done as described for MBPα146. β-Catenin and plakoglobin, each carrying a C-terminal His₆-tag, were expressed with pQE60 (Qiagen) and purified on Ni²⁺-chelating Sepharose (Pharmacia). GST-β-catenin was expressed as described previously (Aberle et al., 1994).
Reconstitution assays and affinity purification

For in vitro reconstitution, recombinant proteins were mixed in 250 μl association buffer (10 mM Hepes-NaOH, pH 7.4, 0.1 M KCl, 1 mM MgCl₂, 0.1% Triton X-100). After incubation for 30 minutes at room temperature precipitated material was removed by centrifugation for 10 minutes at 14,000 rpm. For isolation of the reconstituted heterodimers 250 μl of association buffer and 50 μl of a 50% slurry of glutathione-agarose beads (Sigma) were added. After 30 minutes gentle agitation at 4°C the beads were pelleted (1 minute, 2,000 g) and washed four times with 2x association buffer + 20 mM imidazole.

Bound protein complexes were eluted from the beads by incubation in Laemmli sample buffer for 3 minutes at 95°C, separated by SDS-PAGE, and analyzed by immunoblotting or Coomassie Blue staining. For peptide binding assays, magnetic beads were incubated for 1 hour at 4°C with recombinant β-catenin-Hs6 and washed 5x in 2x association buffer + 20 mM imidazole. The collected beads were resuspended in 20 ml sample buffer and heated for 3 minutes at 95°C. Bound proteins were analyzed by immunodetection with M14M polyclonal antibody against β-catenin.

SW480 cells were metabolically labeled with 71.5 μCi/ml [35S]-Promix (50 mCi/ml [35S]methionine + 21.5 mCi/ml [35S]cysteine; >1,000 Ci/mmol) (Amersham) for >12 hours, washed 3x with PBS, and extracted with 0.8 ml lysis buffer (10 mM Hepes-NaOH, pH 7.4, 0.1 M KCl, 1 mM MgCl₂, 2 mM EGTA, 0.2% (v/v) Triton X-100, 1 mM NaVO₄, 1 mM NaF). The soluble extract was precleared with the appropriate beads and incubated with 2 μg of MBP-β-catenin 1-146 for 10 minutes. Then Protein G-Sepharose beads (Pharmacia) preincubated with 2 μg of chicken anti-MBP polyclonal antibody and 2 μg monoclonal anti-IgY were added, and after incubating for 1 hour at 4°C under gentle agitation, beads were washed 5x with lysis buffer. Bound protein complexes were eluted with Laemmli buffer, separated by SDS-PAGE and analyzed by fluorography or immunoblotting.

RESULTS

Identification of the β-catenin binding site in α-catenin

Previous experiments have shown that β-catenin and α-catenin expressed in E. coli form heterodimers in solution with a 1:1 stoichiometry (Aberle et al., 1994). An E-cadherin-catenin complex was even assembled in vitro upon addition of the cytoplasmic domain of E-cadherin. Therefore, this assay system was used to identify the binding site for β-catenin in α-catenin. MBP-α-catenin fusion constructs coding for a series of C-terminally truncated α-catenins were expressed in E. coli, taking care to optimize bacterial growth conditions and the controlled induction of protein expression (see Materials and Methods). The structure of the fusion proteins is schematically summarized in Fig. 1. Recombinant β-catenin tagged with glutathione-S-transferase (GST) was mixed in solution with various mutated MBP-α-catenin proteins, the resultant protein complexes were purified on glutathione-agarose beads and subjected to immunoblot analysis (Fig. 2). Full-length α-catenin and a truncated protein encoding the N-terminal 246 amino acids were both able to associate with GST-β-catenin (Aberle et al., 1994; not shown). Of all the C-terminal truncations of α-catenin only MBP-α-catenin 1-146 was able to interact with β-catenin (Figs 1, 2). No interaction was observed between α- and β-catenin when N-terminal deletion constructs of α-catenin were analyzed (not shown).

MBP-α-catenin 1-146 binds native β-catenin

SW480 cells, which exhibit a large pool of cytoplasmic β-catenin (Munemitsu et al., 1995; Jou et al., 1995), were analyzed to test whether MBP-α-catenin 1-146 is able to bind endogenous β-catenin. Cell lysates from 35S-metabolically labeled SW480 cells were mixed with each of the truncated α-catenin fusion proteins depicted in Fig. 1, and protein complexes were immunoprecipitated with antibodies against the MBP fusion partner. Only MBP-α-catenin 1-146 was able to bind endogenous β-catenin (Fig. 3A); this was confirmed by subjecting the immunoprecipitates to immunoblot analysis with anti-β-catenin antibodies (Fig. 3B). These results demonstrate that a sufficient β-catenin binding site is encoded within the N-terminal 146 amino acids of α-catenin.

α-CateninΔ143 is unable to bind to β-catenin in Neuro2A cells

To study the interaction between α-catenin and β-catenin in living cells transient expression experiments with subsequent immunoprecipitations were performed. Neuro2A cells possess no endogenous E-cadherin and catenins (Hülsken et al., 1994). They were co-transfected with expression vectors encoding β-catenin and α-catenin or α-catenin deleted for the N-terminal 143 aa (α-cateninΔ143). In immunoprecipitates collected with anti-β-catenin antibodies full-length α-catenin, but not α-cateninΔ143, was found associated with β-catenin (Fig. 4).

β-Catenin binds to α-catenin peptide S27D

Amino acid sequence analysis of the first 150 aa of α-catenin revealed that aa 117-143 sequence is rich in hydrophobic amino acid residues, which could represent a complementary motif to the recently mapped hydrophobic binding site in β-catenin and plakoglobin (Sacco et al., 1995; Aberle et al., 1996). To provide experimental support for this possibility, the corresponding peptide S27D (aa 117-143) was synthesized, HPLC-purified, coupled to tosyl-activated magnetic beads, and

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![Fig. 1. Schematic representation of MBP-α-catenin fusion proteins and peptide used. The ability of the constructs to interact with β-catenin is indicated at the right. The numbers refer to aa positions in α-catenin according to the published sequence (Herrenknecht et al., 1991).](image-url)
tested for interaction with His6-tagged β-catenin. Binding of β-catenin was found only to beads coupled with peptide S27D but not to control beads blocked with ethanolamine (Fig. 5).

**Plakoglobin binds to peptide S27D**

Homologous domains in β-catenin (aa positions 120-151) and plakoglobin (aa positions 109-137) are interaction sites for α-catenin (Aberle et al., 1996). Therefore, it seemed likely that β-catenin and plakoglobin could bind both to the same amino acid sequence in α-catenin. Binding experiments with recombinant GST-plakoglobin and SW480 cell extracts indicated that plakoglobin also binds to the MBP-α-catenin 1-146 fusion protein, but not to MBP-α-catenin 1-129 or MBP-α-catenin 1-98, as shown in Fig. 6A,B. Since plakoglobin is only a minor component in SW480 cells, plakoglobin was not easily detected in binding experiments with 35S-metabolically labeled cell lysates (not shown), but an interaction was unambiguously demonstrated by subjecting the immunoprecipitates to immunoblot analysis with anti-plakoglobin antibodies (Fig. 6B). More importantly, peptide S27D coupled to magnetic beads was able to bind His6-tagged recombinant plakoglobin (Fig. 6C), demonstrating that there is a unique binding site in α-catenin for both β-catenin and plakoglobin.

**Site-directed mutagenesis**

Mutational analysis revealed that hydrophobic amino acids in both plakoglobin and β-catenin are indispensable for their interaction with α-catenin. Within the binding site hydrophobic aa residues are spaced appropriately for an α-helical interaction mechanism (Aberle et al., 1996). This suggested that the hydrophobic aa residues in the binding site in α-catenin identified here might have a similar importance. The DNAstar software package and the PHD PredictProtein mail server program (Rost, 1996; Rost and Sander, 1993, 1994), predicted a secondary structure of α-catenin with two α-helical regions separated by a loop at aa positions 113-122. The second α-
helical domain, starting at aa position 123, was within the β-catenin binding site (aa positions 117-143). In an α-helical wheel model of aa sequence 124-143, all hydrophobic amino acids except Ile140 are located on one side of the helix (Fig. 7). In an approach comparable to that used by Aberle et al. (1996), hydrophobic as well as hydrophilic aa residues shown in the helical wheel model were individually mutated to Ala in the MBPα1-146 construct. Fusion proteins carrying the single aa substitutions were mixed with β-catenin from metabolically 35S-labeled SW480 cell lysates. Bound β-catenin was quantified by Phospholmager analysis. Association with β-catenin was reduced whenever hydrophobic aa residues were mutated. Introducing a hydrophilic Ser (mutation A142S) into the hydrophobic part of the α-helix also greatly reduced the binding of β-catenin binding. Comparably hydrophilic and charged aa located on the opposite side of the α-helix were exchanged to Ala. Interestingly, three of these mutant proteins (R126A, S133A and T136A) exhibited an increased binding to β-catenin. These results, summarized in Fig. 7B, suggest that the association with β-catenin indeed is mediated by a hydrophobic interaction mechanism.

**DISCUSSION**

Originally, immunoprecipitation experiments with antibodies against E-cadherin led to the identification of α-, β-catenin and plakoglobin complexed to E-cadherin via its cytoplasmic domain (Ozawa et al., 1989; Nagafuchi et al., 1989). Subsequently, two major lines of research led to a better understanding of the role of catenins. First, establishing the primary structure of catenins provided important insights into their possible biological function and opened up new perspectives, for example revealing β-catenin and plakoglobin to be homologous to the *Drosophila* armadillo protein. Second, biochemical analysis on the assembly of the cadherin-catenin complex and heterotypic expression studies of normal and mutant proteins elucidated the stoichiometry of the complex and the mode of interaction between these proteins (Aberle et al., 1994; Hulsken et al., 1994).

Here we report on one heretofore missing piece of information, namely the binding site in α-catenin for its association with β-catenin or plakoglobin. Our results show that the N-terminal region of α-catenin harbors the β-catenin/plakoglobin-binding site. Further analysis let us identify peptide S27D corresponding to aa 117-143 of α-catenin as being sufficient to bind β-catenin or plakoglobin. These results are consistent with data recently reported by Nieset et al. (1997) where the domain in α-catenin that interacts with β-catenin and plakoglobin was mapped to aa 97-148 by two-hybrid and in vitro association assays.

Detailed mutational analysis had previously shown that mostly hydrophobic amino acids in an α-helical structure in plakoglobin and β-catenin are necessary for efficient interaction with α-catenin, and a similar motif for the heterodimerizing peptide sequence within α-catenin was postulated (Aberle
Fig. 7. Summary of the mutational analysis. (A) An α-helical wheel model representing 20 amino acids of the β-catenin binding site in α-catenin (aa 124-143). All hydrophobic residues are located on one side of the α-helix comparable to the α-catenin binding domain in β-catenin and plakoglobin (see Aberle et al., 1996). (B) Single aa substitutions in the α-catenin binding site affect binding to β-catenin. The aa differing from the wild-type sequence model are indicated. Their corresponding positions in the α-helical wheel are given in brackets. Relative percentages of bound 35S-labeled β-catenin was quantified using a Phospholmager. Wild-type MBP-α-catenin 1-146 was set to 100%. The graph represents mean values of 4 experiments.

et al., 1996). Our alanine mapping approach supports this view and underlines the importance of hydrophobic amino acid residues for stable interactions between α-catenin and β-catenin/plakoglobin. Replacement of hydrophobic aa in α-catenin affected the protein interaction more moderately compared to the complementary substitutions in β-catenin or plakoglobin. As a possible explanation for this apparent discrepancy, we note that the hydrophobic region in α-catenin includes more hydrophobic amino acids. This may explain why a single mutation influences less dramatically the binding to β-
catenin. Whether α-catenin binds to β-catenin or plakoglobin via hydrophobic forces within parallel or antiparallel arranged α-helical stretches remains elusive. To obtain a final picture of this protein interaction, NMR or X-ray structural analysis will be required.

αN-catenin, a recently identified isoform of αE-catenin, is expressed predominantly in nervous tissue (Uchida et al., 1994). Transfection experiments in an α-catenin-deficient cell line showed that αE- and αN-catenin are functionally inter-changeable in developing mouse or chicken embryos despite their temporally and spatially regulated expression (Hirano et al., 1992). Comparison of the S27D peptide sequence with the corresponding aa sequences in the αN-catenins reveals a highly conserved β-catenin/plakoglobin binding site in α-catenin isoforms with only a single aa exchange at position 123, where ASP in mouse αE-catenin is exchanged to THR in mouse, human and chicken αN-catenin.

α-Catenin is homologous to the focal adhesion protein vinculin (Herrenknecht et al., 1991; Nagafuchi et al., 1991). Alignment of the 27 aa β-catenin/plakoglobin binding site in mouse α-catenin with the corresponding site in mouse vinculin reveals only 29.6% identity on the aa level, and computer analysis does not predict an α-helical secondary structure for this aa sequence in vinculin.

Decreased cell-cell adhesion is a phenomenon often seen in tumors. The interaction domains within the cadherin-catenin complex represent potential mutational hot spots in tumorigenesis. Mutations in the β-catenin/plakoglobin binding site of α-catenin could result in low affinity or lost interaction with the actin cytoskeleton, leading to reorganizations of the cytoskeleton. Such changes destabilize intercellular junctions and could lead to a switch from an adhesive to an invasive state during tumor progression.

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α-Catenin binding site for β-catenin and plakoglobin


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