E-Cadherin and APC Compete for the Interaction with β-Catenin and the Cytoskeleton

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Abstract. β-Catenin is involved in the formation of adherens junctions of mammalian epithelia. It interacts with the cell adhesion molecule E-cadherin and also with the tumor suppressor gene product APC, and the Drosophila homologue of β-catenin, *armadillo*, mediates morphogenetic signals. We demonstrate here that E-cadherin and APC directly compete for binding to the internal, *armadillo*-like repeats of β-catenin; the NH_2-terminal domain of β-catenin mediates the interaction of the alternative E-cadherin and APC complexes to the cytoskeleton by binding to α-catenin. Plakoglobin (γ-catenin), which is structurally related to β-catenin, mediates identical interactions. We thus show that the APC tumor suppressor gene product forms strikingly similar associations as found in cell junctions and suggest that β-catenin and plakoglobin are central regulators of cell adhesion, cytoskeletal interaction, and tumor suppression.

It recently became evident that the proper formation of intercellular junctions is critical for the maintenance of epithelial differentiation, and that destabilization of junctions allows invasiveness of epithelial cells and the progression of carcinomas (cf. for review Tsukita et al., 1993; Birchmeier et al., 1993). Interestingly, various structural components of intercellular junctions have been found to be related to products of tumor suppressor genes. For instance, the adherens junction molecule E-cadherin suppresses invasiveness of carcinoma cells, and the E-cadherin gene is mutated in 50% of the diffuse-type gastric carcinomas (Behrens et al., 1989; Frixen et al., 1991; Vleminckx et al., 1991; Becker et al., 1994). The tumor suppressor gene product APC binds to β-catenin, which is cytoplasmically associated to E-cadherin (Rubinfeld et al., 1993; Su et al., 1993; Nagafuchi and Takeichi, 1988; Ozawa et al., 1989). The tight junction–associated protein ZO-1 is related to the *dlg* tumor suppressor gene product of *Drosophila*, and the neurofibromatosis-2 tumor suppressor gene product merlin/schwannomin is related to the ezrin/radixin/moesin family of junctional proteins (Woods and Bryant, 1991; Rouleau et al., 1993; Trofatter et al., 1993). Conversely, products of oncogenes have been demonstrated to destabilize intercellular junctions, such as *src*, *ras*, *fos*, or *met* (Behrens et al., 1993; Hamaguchi et al., 1993; Behrens et al., 1989; Shibamoto et al., 1994; Reichmann et al., 1992; Weidner et al., 1990). *Src*, the EGF receptor, and *met* phosphorylate β-catenin on tyrosine residues, which may modulate the adhesive properties of cells.

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Adherens junctions of epithelial cells are specialized regions of the plasma membrane where transmembrane E-cadherin molecules on opposing cells make contact with each other (Boller et al., 1985; Tsukita et al., 1992; for a discussion of the related desmosomes see Garrod, 1993). The cytoplasmic domain of E-cadherin forms complexes with α-, β-, and γ-catenin/plakoglobin, which are essential for the adhesive function (Nagafuchi and Takeichi, 1988; Ozawa et al., 1989; Ozawa et al., 1990; Kintner, 1992). α-Catenin shows sequence similarity to vinculin and interacts with the cytoskeleton (Nagafuchi et al., 1991; Herrenknecht et al., 1991; Hirano et al., 1992). β-Catenin is the vertebrate homologue of the segment polarity gene *armadillo* of *Drosophila* and is related to plakoglobin (McCrea et al., 1991; Peifer et al., 1992; Butz et al., 1992). γ-Catenin is identical to plakoglobin, which is also found in desmosomal junctions (Cowin et al., 1986; Franke et al., 1989; Knudsen and Wheelock, 1992; Peifer et al., 1992; Hinck et al., 1994*a*,*b*; Näthke et al., 1994). Recent experimental evidence suggests that any significant change in expression or structure of one of these components leads to adherens junction disassembly and consequently, to non-adhesive and invasive cells. Molecular aberrations which result in such defects have been studied: an E-cadherin deletion mutant lacking its catenin binding region was not functional in cell–cell adhesion when expressed in fibroblasts (Nagafuchi and Takeichi, 1988; Ozawa et al., 1990). Cadherin mutants without the extracellular domain acted in a dominant-negative fashion, i.e., disturbed epithelial cell adhesion (Kintner, 1992). Apparently, these truncated molecules collect catenins, which are thus not available to form proper complexes with intact E-cadherin. The adhesive properties of PC9 lung cancer cells, which harbor a null mutation in the α-catenin gene, could be restored by transfection with α-catenin cDNA (Hirano et al., 1992).

Mutations of the E-cadherin gene have recently been discovered in 50% of diffuse-type gastric carcinomas, which lead to shortened mRNAs in which exons 8 or 9 are skipped (Becker et al., 1994). Mutations of the E-cadherin gene have also been identified in human ovarian carcinomas and in two non-adhesive cell lines from gastric carcinomas (Risinger et al., 1994; Oda et al., 1994).

Recently, the tumor suppressor gene product APC has been found to associate with the adherens junction proteins α- and β-catenin (Su et al., 1993; Rubinfeld et al., 1993). The APC gene codes for a cytoplasmic 300 kDa protein and is mutated in a high percentage of colon carcinomas, which represent 15% of all cancers in the United States (Fearon and Vogelstein, 1990). Various genetic changes during colon cancer progression have been found, such as in the genes of APC, *ras*, *p53*, and DCC. Mutations of the tumor suppressor gene APC occur early during progression of the disease. Furthermore, an inherited disorder exists, *Familial Adenomatous Polyposis*, also caused by APC mutations, which is characterized by an early onset of numerous polyps in the colon which progress to malignancy (Groden et al., 1991; Kinzler et al., 1991). Little is known, however, about the functional role of APC in colonic epithelia, nor is it known why mutation of the APC gene product promotes colon cancer.

It was previously unclear how the interaction between membrane-bound E-cadherin, the cytoskeleton, and the tumor suppressor gene product APC is organized on a molecular level. There was indication that β-catenin associates earlier than α-catenin to E-cadherin during biogenesis of the adherens junction complex, and that β-catenin is also more strongly bound to E-cadherin (Ozawa et al., 1990; Hinck et al., 1994*b*). It has furthermore been shown that catenin/APC immunoprecipitates do not contain E-cadherin (Su et al., 1993), indicating that APC and E-cadherin might not be bound to β-catenin at the same time. We demonstrate here that APC and E-cadherin compete for binding to β-catenin, and that β-catenin and plakoglobin are direct but independent linkers between E-cadherin or APC and the cytoskeleton-associated component α-catenin. We also show that this link is mediated by different domains of β-catenin, i.e., the *armadillo*-like repeats bind to E-cadherin and APC whereas the NH_2-terminal domain is required for the interaction to α-catenin.

Materials and Methods

cDNA Constructs and Deletion Mutants

The human β-catenin cDNA was isolated from a cDNA library of placenta (HL1075B; Clontech, Palo Alto, CA) using the *Xenopus* cDNA (McCrea et al., 1991) as a probe. The 2,585-bp β-catenin cDNA contains the complete coding region as well as 200- and 42-bp of 5′- and 3′-noncoding sequence, respectively (cf. GenBank accession no. Z19054). The deduced amino acid sequence is highly similar to the *Xenopus* sequence (97% identity), and identical to the mouse sequence except for one amino acid difference at position 706 (cf. also McCrea et al., 1991; Butz et al., 1992). 13 internal repeats of about 40 amino acids in length, as originally described for *armadillo* (Peifer and Wieschaus, 1990; Peifer et al., 1994), are flanked by unique NH_2- and COOH-terminal sequences of 131 and 86 amino acids, respectively. Sequence comparisons were performed using the TFASTA, MOTIF, +TREE, MULTalign, and CLUSTAL program packages of the HUSAR-system (European Molecular Biology Laboratory, Heidelberg, Germany).

The human cDNA of β-catenin was used for the generation of deletion mutants (Fig. 1 *A*). To detect β-catenin and the mutants by immunological methods, a tag epitope (EQKLISEEDL, derived from human *c-myc*; Evan et al., 1985) was added to either the NH_2- or COOH-terminal side. Oligonucleotides coding for either the NH_2-terminal or COOH-terminal tag plus respective start or stop codons were first inserted into the pBluescript $SKII^+$ vector (Fig. 1 *B*). Deletions of β-catenin containing appropriate restriction sites for cloning were then generated by PCR-mediated site-directed mutagenesis (shown for the ΔN/C-term mutant in Fig. 1 *C*) and were inserted into the tag-containing vectors. The resulting cDNA constructs were cloned into the pBAT expression vector (Nagafuchi and Takeichi, 1988). The cDNAs for mouse E-cadherin (Nagafuchi et al., 1987), mouse α-catenin (Nagafuchi et al., 1991), and human plakoglobin (Franke et al., 1989) were also used in the pBAT vector.

The fragment of APC encoding the β-catenin binding site (Su et al., 1993) was isolated from genomic DNA of human MCF7 cells by PCR using the primer pair 5′-CCATCCAGCAACAGAAAATCCA-3′ and 5′-ACTTTTGGAGGGAGATTTCGCT-3′. A NH_2-terminal FLAG-epitope (MDYKDDDDK; IBI, New Haven, CT) including a translation initiation site (Kozak, 1986) and a stop codon were introduced at amino acid positions 957 and 1211, respectively, by PCR-mediated site-directed mutagenesis using the primer pair 5′-GCGCCTCTAGACCGCCATGGACTACAAGGACGACGACGACAAGGGGCCCGGGAGATCTTCAAATGATAGTTTAAATAGTGTCAGT-3′ and 5′-GCGCCGTCGACTATTAATGTTCGGTTTTACTGCTTTGT-3′. The resulting PCR product was introduced into the pBAT vector using XbaI/SalI sites.

Cells and Immunological Methods

The different cDNA constructs (15 μg each) were transiently transfected into Neuro2A cells using the calcium phosphate method (Hartmann et al., 1992). Neuro2A cells do not express E-cadherin, APC, or the catenins endogenously. After transfection with E-cadherin cDNA, some α- and β-catenin protein expression was induced (cf. Nagafuchi et al., 1991), which represents a minor amount in comparison to the exogenously expressed proteins. Furthermore, endogenous presence of catenins would not affect our results, since α- and β-catenins do not form homodimers (cf. below). Immunodetection of the expressed proteins was carried out after two days of culture. Immunofluorescence staining was performed as described (Behrens et al., 1989) after fixation of the cells with methanol for 5 min. For immunoprecipitation, Neuro2A cells were labeled overnight with 20 μCi/ml [^{35}S]methionine one day after transfection, and cells were then extracted with 140 mM NaCl, 4.7 mM KCl, 0.7 mM $MgSO_4$, 1.2 mM $CaCl_2$, 10 mM Hepes, pH 7.4, containing 1% Triton X-100, and 1 mM phenylmethyl sulfonylfluorid. After ultracentrifugation at 100,000 *g*, the appropriate antibodies and protein A–Sepharose were added, and precipitates were washed with 100 mM NaCl, 5 mM EDTA, 50 mM Tris/HCl, pH 8.0, containing 1% Triton X-100, and 1 mg/ml ovalbumin (Behrens et al., 1989). Western-blot analysis was performed using the Western-Light protocol (Tropix Inc., Bedford, MA). The mouse mAb MycI-9E10 (Evan et al., 1985) was used to detect the tag-epitope of the β-catenin mutants, the mouse mAb anti-FLAG M2 (IBI) to detect the FLAG-epitope of the APC fragment. E-cadherin was detected with DECMA-1 antibody (Vestweber and Kemler, 1985) and plakoglobin was with mouse mAb PG5.1 (Cowin et al., 1986). The anti-α-catenin antibody was produced in rabbits against a synthetic peptide (HVNPVQALSEFK) corresponding to 12-amino acid residues near the COOH terminus. The anti-β-catenin antibody was raised in rabbits against a synthetic peptide (PGDSNQLAWFDTDL) corresponding to the last 14-amino acid residues of the COOH terminus.

Results

Interaction between β-Catenin, E-Cadherin, and APC

We constructed deletion mutants of the human β-catenin cDNA that encode proteins which lack either COOH- or NH_2-terminal domains or both, or which have truncations within the region of the 13 *armadillo*-like repeats (Fig. 1 *A*). In all constructs, a sequence encoding an epitope of human *c-myc* was ligated to the 5′- or 3′-termini, which served as a tag to identify full size or mutant β-catenin immunologically (Fig. 1, *B* and *C*). The location of the tag-epitope did

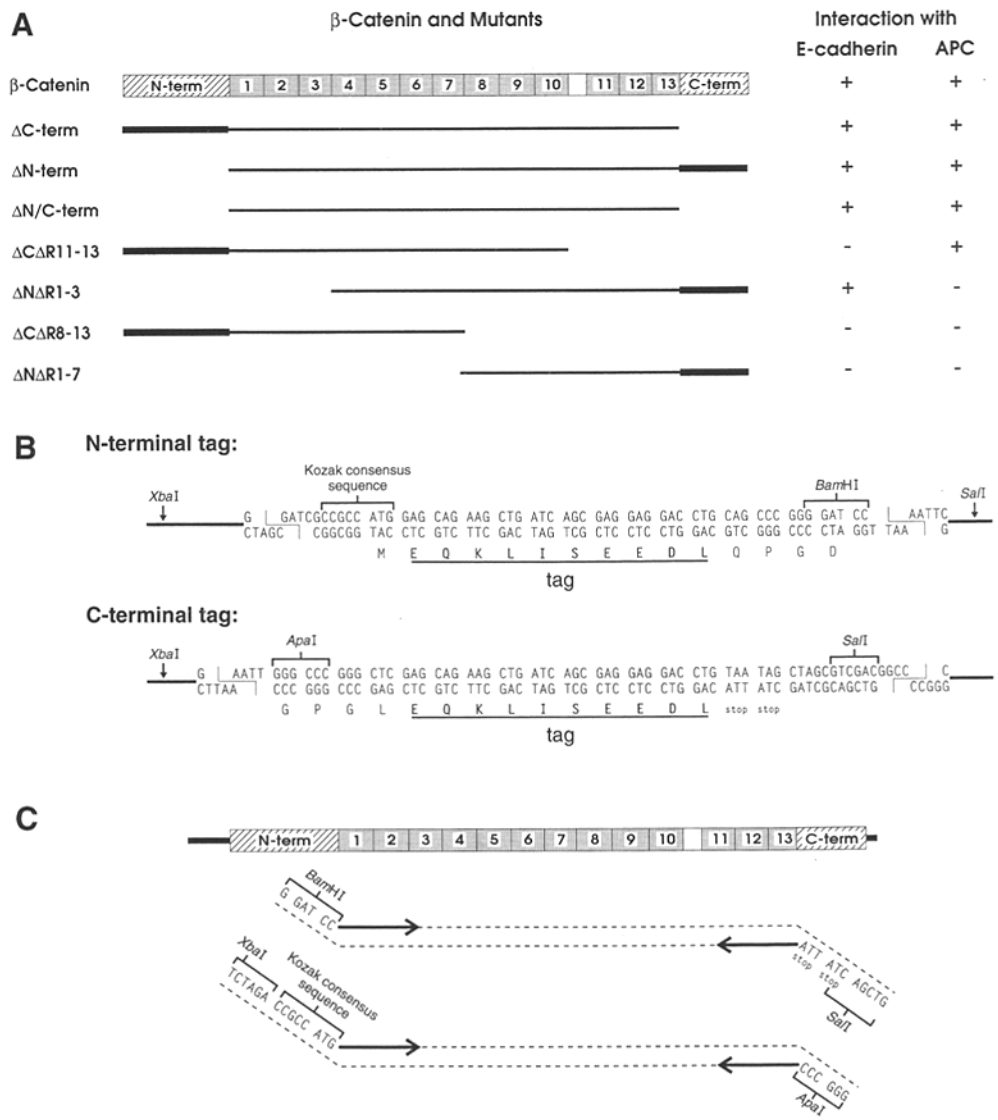

Figure 1. (*A*) Schematic representation of full-size and mutant β-catenin and summary of their ability to interact with E-cadherin or APC. On the left, the NH_2- and COOH-terminal domains and the 13 internal *armadillo*-like repeats of β-catenin are indicated by striped and shadowed boxes, respectively. The empty box between repeat 10 and 11 indicates a stretch of unrepeated amino acids. The following amino acid residues were deleted in the mutated proteins: ΔC-term, 696-781; ΔN-term, 1-131; ΔN/C-term, 1-131 and 696-781; ΔCΔR11-13, 555-781; ΔNΔR1-3, 1-258; ΔCΔR8-13, 424-781; ΔNΔR1-7, 1-422. On the right, a summary of the binding studies between the β-catenin mutants and E-cadherin or APC is presented. (*B*) Sequence of oligonucleotides encoding NH_2- or COOH-terminal tag epitope (*underlined*) in the corresponding vectors are shown. A Kozak consensus sequence of translational initiation (Kozak, 1986), stop codons, and restriction sites used for cloning are indicated. (*C*) Generation of the β-catenin deletions exemplified for the ΔN/C-term mutant. PCR products of the β-catenin cDNA and 5′ add-on primers containing start and stop codons as well as suitable restriction sites for the insertion into the two tag-containing vectors (*B*) are shown.

not alter the interactions observed. The cDNA constructs were transiently transfected into Neuro2A cells (mouse neuroblastoma; cf. Hartmann et al., 1992), which neither express E-cadherin nor catenin proteins, as revealed by immunofluorescence and Western blot experiments; the absence of APC expression was tested by Northern blotting (data not shown). When the E-cadherin and β-catenin cDNAs were separately expressed, E-cadherin was located at the cell membrane while β-catenin was diffusely distributed throughout the cytoplasm, as revealed by immunofluorescence staining (Fig. 2, *a* and *b*). When the two cDNAs were cotransfected, both proteins were membrane-associated, reflecting the direct interaction of β-catenin with E-cadherin. Likewise, β-catenin lacking both the NH_2- and COOH-terminal domains was membrane-associated in the presence of E-cadherin (Fig. 2 *c*). However, β-catenin lacking the NH_2-terminal domain plus repeat units 1 to 7 did not associate with E-cadherin at the cell membrane (Fig. 2 *d*).

Immunoprecipitations of β-catenin from [^{35}S]methionine-labeled Neuro2A cells with anti-tag antibodies corroborated these results: E-cadherin was associated with full-size β-catenin as well as with mutants lacking either the COOH- or NH_2-terminal domain or both (Fig. 3, *A* and *B*, lanes *2–5*). However, deletion of repeat units 1 to 3 diminished binding to E-cadherin, deletion of repeats 11 to 13 abolished binding (Fig. 3 *B*, lanes *6* and *7*); β-catenin with larger truncations of repeat units did not bind (Fig. 3 *B*, lanes *8* and *9*; cf. also Fig. 1 *A* for a summary). Interestingly, certain mutants of β-catenin which alone did not bind (e.g., those lacking repeats 1–7 and 11–13), were able to interact with E-cadherin when expressed in combination (Fig. 3 *B*, lanes *10* and *11*). These mutant proteins directly interacted with each other even in the absence of E-cadherin (Fig. 3 *C*, lane *1*). The interaction was mediated by overlapping repeat regions but not the terminal domains, since exact halves of β-catenin did not interact (Fig. 3 *C*, lane *2*). Interestingly, we could not de-

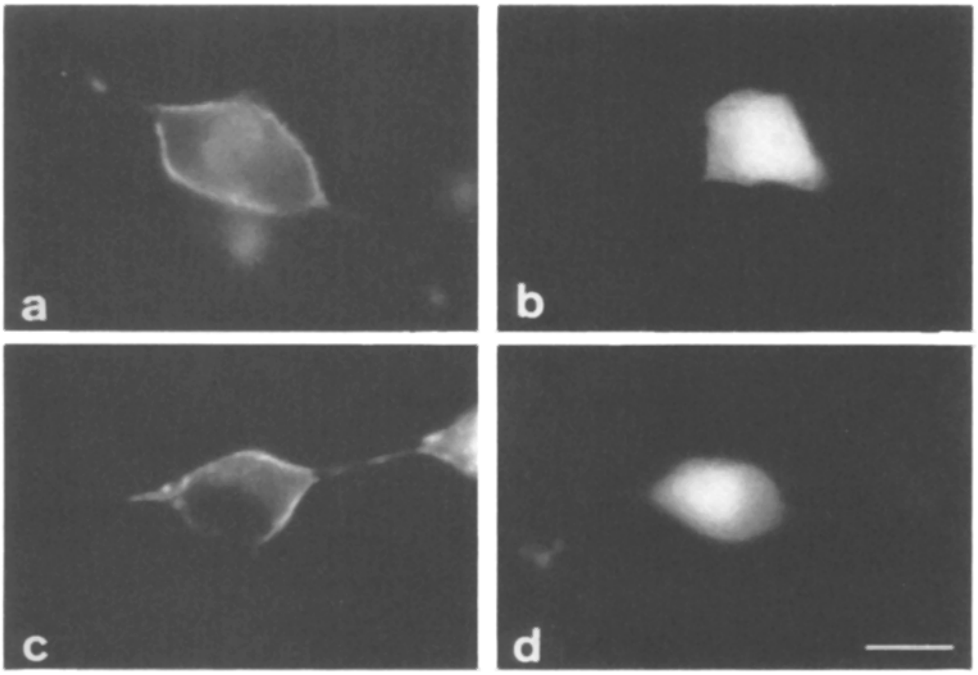

Figure 2. Immunofluorescence localization of recombinant E-cadherin and β-catenin in mouse neuroblastoma (Neuro2A) cells after cotransfection with expression constructs for (*a*) E-cadherin, (*b*) β-catenin, (*c*) E-cadherin plus β-catenin lacking the NH_2- and COOH-terminal domains, and (*d*) E-cadherin plus β-catenin lacking the NH_2-terminal domain and repeats 1 to 7. Staining was performed with (*a*) anti-E-cadherin antibody or (*b–d*) anti-tag antibody (MycI-9E10; Evan et al., 1985) to detect β-catenin. Bar, 20 μm.

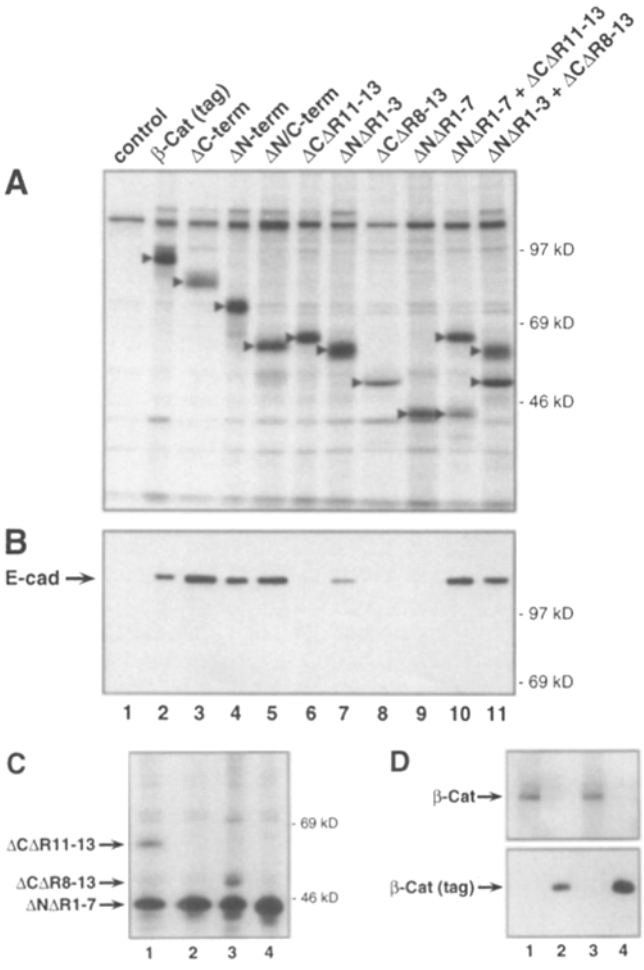

Figure 3. (*A* and *B*) Association of (tagged) wild-type and mutant β-catenin with E-cadherin, as revealed by immunoprecipitation of [^{35}S]methionine-labeled cell extracts using anti-tag antibody (*A*, an autoradiograph is shown) followed by Western blot analysis with anti–E-cadherin antibody (*B*). Neuro2A cells were cotransfected with expression constructs for E-cadherin and the various β-catenin mutants as indicated above the figure. Control, E-cadherin expression construct alone. The positions of the β-catenin mutants in *A* are marked by arrowheads, the location of E-cadherin in *B* by an arrow. Western blotting (*B*) has been performed since contaminating bands mask E-cadherin in *A*. Molecular weight markers are indicated. (*C*) Association of β-catenin mutants with each other in the absence of E-cadherin. Neuro2A cells were transfected with the β-catenin mutant ΔNΔR1-7 alone (lane *4*) or in combination with the mutants ΔCΔR11-13 (lane *1*) or ΔCΔR8-13 (lanes *2* and *3*). [^{35}S]Methionine-labeled cell extracts were immunoprecipitated with anti–β-catenin antiserum (lanes *1*, *2*, and *4*) or anti-tag antibody (lane *3*, to reveal expression of both mutants). Note that the anti–β-catenin antiserum does not recognize mutants lacking the COOH-terminal domain. (*D*) Lack of homodimerization of full-size β-catenin. Neuro2A cells were transfected with β-catenin without a tag (lane *3*), β-catenin with a COOH-terminal tag (lane *4*), or a combination of both (lanes *1* and *2*). Immunoprecipitation was carried out using the anti–β-catenin antiserum (lanes *1* and *3*) or the anti-tag antibody (lanes *2* and *4*), and precipitates were analyzed by Western blotting with anti–β-catenin antiserum (*top*) or anti-tag antibody (*bottom*). Specific recognition of tagged and wild-type β-catenin was possible, since the anti–β-catenin antiserum does not detect its epitope in the presence of a COOH-terminal tag.

tect dimerization of full-size β-catenin (Fig. 3 *D*). The combined results show: (*a*) that interaction of β-catenin with E-cadherin is mediated by the *armadillo*-like repeats and not by the terminal domains; (*b*) that multiple rather than single repeat units are involved in the binding; and (*c*) that internal repeat units can interact with each other and thus cooperate in the binding to E-cadherin (cf. Fig. 7).

We also identified the sequences of β-catenin that bind to APC and examined, how this binding is influenced by the formation of the E-cadherin/β-catenin complex. A fragment

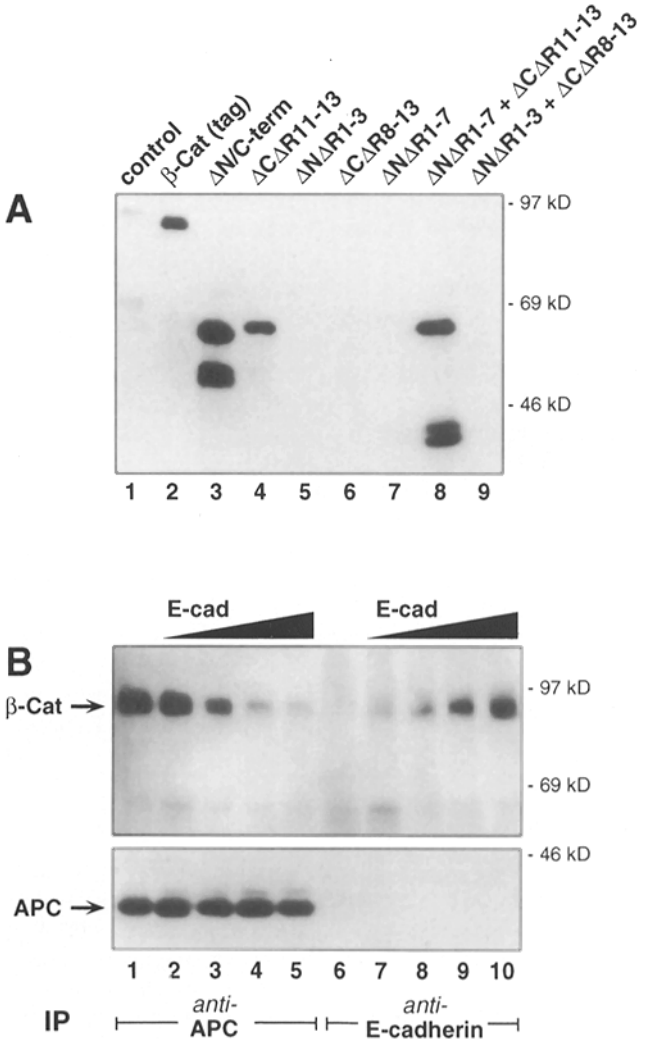

Figure 4. (*A*) Association of the recombinant APC fragment with β-catenin mutants in cotransfected Neuro2A cells, as revealed by immunoprecipitation of APC using an anti-FLAG antibody followed by Western blot analysis of (tagged) β-catenin with anti-tag antibody. The β-catenin mutants used are indicated above. Control, APC expression construct alone. The lower band in lane *3* is a degradation product of the ΔN/C-term mutant which was observed in some experiments. (*B*) Competition between E-cadherin and the APC fragment for binding to β-catenin. Neuro2A cells were transfected with 15 μg expression constructs for each APC and β-catenin plus increasing amounts of expression construct for E-cadherin (lanes *1* and *6*, 0 μg; lanes *2* and *7*, 1 μg; lanes *3* and *8*, 2 μg; lanes *4* and *9*, 5 μg and lanes *5* and *10*, 10 μg). APC (lanes *1* to *5*) and E-cadherin (lanes *6* to *10*) were immunoprecipitated (*IP*) followed by Western blotting using anti-β-catenin antiserum (*top*) or anti-FLAG antibody against APC (*bottom*).

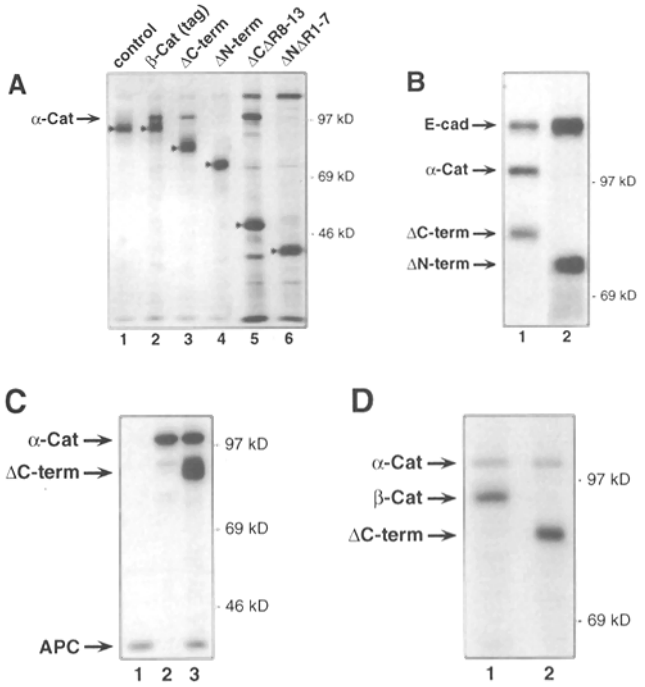

Figure 5. (*A*) Association of recombinant α-catenin with (tagged) β-catenin mutants in cotransfected Neuro2A cells, as revealed by immunoprecipitation of [^{35}S]methionine-labeled cell extracts using anti-tag antibody (an autoradiograph is shown). The β-catenin mutants used are indicated above and their positions are marked by arrowheads. Control, transfection of β-catenin expression construct alone. The position of co-immunoprecipitated α-catenin is marked on the left side. The anti-tag antibody also recognizes an unspecific 120-kD protein which is unrelated to E-cadherin and is also present in untransfected cells. Lanes *5* and *6* were exposed three times longer than lanes *1* to *4*. (*B*) Association of α-catenin with the E-cadherin/catenin complex after expression of E-cadherin, α-catenin, and (tagged) β-catenin mutants lacking either the COOH-terminal domain (lane *1*) or the NH_2-terminal domain (lane *2*). The complexes were co-immunoprecipitated from [^{35}S]methionine-labeled extracts using anti-tag antibody. (*C*) Association of APC to α-catenin through linkage by β-catenin. Neuro2A cells were cotransfected with expression constructs for APC and α-catenin (lanes *1* and *2*) or APC, α-catenin, and β-catenin without the COOH-terminal domain (lane *3*). Immunoprecipitation from [^{35}S]methionine-labeled cell extracts was carried out using anti-APC (FLAG) (lane *1* and *3*) or anti-α-catenin (lane *2*) antibody. (*D*) Lack of homodimerization of α-catenin. Neuro2A cells were cotransfected with expression constructs for α-catenin, wild type β-catenin and the tagged β-catenin mutant lacking the COOH-terminal domain. Immunoprecipitation was performed from [^{35}S]methionine-labeled cell extracts with anti-β-catenin antiserum (lane *1*) or anti-tag antibody (lane *2*). Note that the anti-β-catenin antiserum is directed against an epitope in the COOH-terminal domain of β-catenin.

of the human APC gene encoding the β-catenin–binding domain (amino acid residues 958-1210; Su et al., 1993) was isolated, and an oligonucleotide encoding a synthetic epitope (FLAG) was ligated to the 5′ end in order to detect the protein immunologically. We found that the APC fragment also binds to the repeat units of β-catenin and not the terminal domains (Fig. 4 *A*, lanes *2* and *3*), and binding was again not confined to an individual repeat but rather to a region covering multiple repeat units (Fig. 4 *A*, lanes *3–7*). In contrast to E-cadherin, however, the NH_2-terminal part of the repeat region (repeat units 1 to 10) is sufficient for binding to APC (Fig. 4 *A*, lane *4*; see also Figs. 1 *A* and 7, for a summary). Interestingly, the APC-binding site of β-catenin could not be reconstituted by any combination of non-binding mutants, as it was found for the interaction of the β-catenin mutants with E-cadherin (Fig. 4 *A*, lane *9*; cf. Fig. 3, *A* and *B*; and Hülsken, J., unpublished results).

The fact that both E-cadherin and APC bind to the repeat

units of β-catenin raises the question whether the adhesion molecule and the tumor suppressor gene product compete for binding to β-catenin. Thus, Neuro2A cells were cotransfected with constant amounts of expression constructs for APC and β-catenin as well as with increasing amounts of the E-cadherin expression construct. Gradual increase of E-cadherin expression indeed induced a redistribution of β-catenin from APC to E-cadherin (Fig. 4 *B*).

β-Catenin Links α-Catenin to E-Cadherin and APC

We have found that β-catenin directly interacts with the cytoskeleton-associated component α-catenin, as revealed by co-immunoprecipitation after transfection of Neuro2A cells with expression constructs for both components (Fig. 5 *A*, lane *2*). α-Catenin was also co-immunoprecipitated with mutants of β-catenin lacking repeat units 8 to 13 and/or the COOH-terminal domain (Fig. 5 *A*, lanes *3* and *5*), but clearly not with mutants lacking the NH_2-terminal domain (Fig. 5 *A*, lanes *4* and *6*). When a combination of E-cadherin, α-catenin, and the (tagged) β-catenin mutant lacking the COOH-terminal domain was expressed, anti-tag antibody immunoprecipitated a complex of all three components (Fig. 5 *B*, lane *1*); however, when the mutant of β-catenin lacking the NH_2-terminal domain was used, a complex without α-catenin was seen (Fig. 5 *B*, lane *2*). This indicates that α-catenin does not directly interact with E-cadherin but indirectly through its interaction with β-catenin (cf. also Fig. 7). Accordingly, α-catenin was only located at the cell membrane when coexpressed with E-cadherin and β-catenin, as revealed by immunofluorescence experiments (data not shown). We also examined whether β-catenin mediates a similar linkage between α-catenin and APC. In fact, APC is only indirectly linked to α-catenin via β-catenin (Fig. 5 *C*).

It has been suggested that α-catenin forms homodimers through its domain with sequence similarity to the vinculin homodimerization motif (Nagafuchi et al., 1991; Herrenknecht et al., 1991). We analyzed this by expressing a combination of α-catenin, wild type β-catenin (without a tag), and the tagged mutant of β-catenin lacking the COOH-terminal domain. Since our anti-β-catenin antibody recognizes an epitope in the COOH-terminus, we could distinguish the complexes α-catenin/wild type β-catenin and α-catenin/β-catenin with COOH-terminal deletion (Fig. 5 *D*). Apparently, α-catenin does not homodimerize, since no quadruple complex (βα–αβmutant) was seen.

Plakoglobin and β-Catenin Form Similar Complexes

β-Catenin and plakoglobin (γ-catenin) are structurally related consisting of central *armadillo*-like repeats flanked by NH_2- and COOH-terminal domains (Franke et al., 1989; McCrea et al., 1991; Knudsen and Wheelock, 1992). We therefore investigated whether plakoglobin may similarly form a complex with E-cadherin or APC. When a quadruple combination of E-cadherin, α-catenin, β-catenin, and plakoglobin was expressed, anti-plakoglobin antibody immunoprecipitated a complex including E-cadherin and α-catenin, but not β-catenin (Fig. 6 *A*, lane *1*); conversely, anti-β-catenin antibody identified a complex containing E-cadherin and α-catenin, but not plakoglobin (Fig. 6 *A*, lane *3*). Plakoglobin also binds to α-catenin in the absence of E-cadherin (Fig. 6 *B*, lane *2*), but there was no direct association with β-catenin (Fig. 6 *B*, lane *3*). Furthermore, plakoglobin can also substitute for β-catenin in the APC complex (Fig. 6 *C*). These results suggest that: (*a*) the structurally related molecules plakoglobin (γ-catenin) and β-catenin mediate binding between E-cadherin or APC and α-catenin in two mutually exclusive complexes; and that (*b*) similar molecular interactions occur in plakoglobin- or β-catenin–containing complexes.

Discussion

We have here expressed the cDNAs of E-cadherin, APC, and three catenins (including various deletion mutants of β-catenin) in the suitable expression system of Neuro2A cells. We could reconstruct the molecular interactions under conditions which closely resemble the in vivo situation. With antibodies against all the expressed proteins (the β-catenin mutants and APC carried an added epitope), we were able to monitor the respective associations between these components by specific co-immunoprecipitation. Our data allow us to model the multiple interactions of β-catenin which may occur within adherens junctions and the APC complex (Fig. 7): β-catenin or plakoglobin directly interact with either the cell adhesion molecule E-cadherin or the tumor suppressor

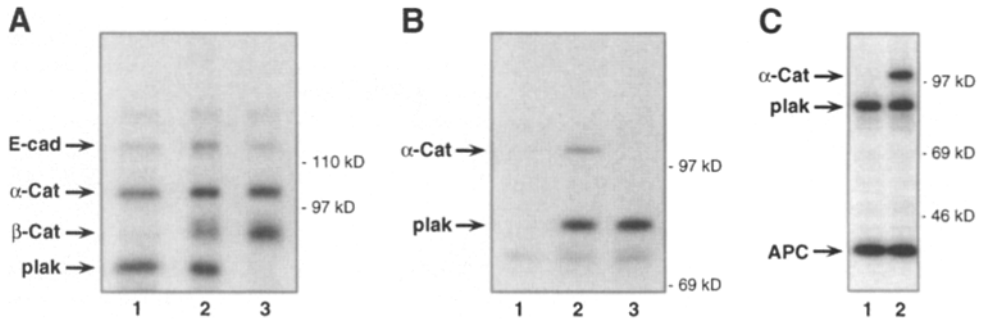

Figure 6. (*A*) β-Catenin and plakoglobin form distinct complexes with E-cadherin. Immunoprecipitations of [^{35}S]-methionine-labeled Neuro2A cells transfected with a combination of expression constructs for E-cadherin, α-catenin, β-catenin, and plakoglobin were carried out with antibodies against plakoglobin (lane *1*), E-cadherin (lane *2*), or β-catenin (lane *3*). The location of immunoprecipitated proteins is marked on the left. (*B*) Plakoglobin directly associates with α-catenin. Immunoprecipitations with anti-plakoglobin antibody were performed from [^{35}S]methionine-labeled cells transfected with expression constructs for α-catenin and β-catenin (control, lane *1*), α-catenin and plakoglobin (lane *2*), or β-catenin and plakoglobin (lane *3*). (*C*) Association of APC with plakoglobin and linkage of α-catenin to APC through plakoglobin. Neuro2A cells were cotransfected with expression constructs for APC and plakoglobin (lane *1*), or APC, α-catenin, and plakoglobin (lane *2*). Immunoprecipitation from [^{35}S]methionine-labeled cell extracts was carried out using anti-APC (anti-FLAG) antibody.

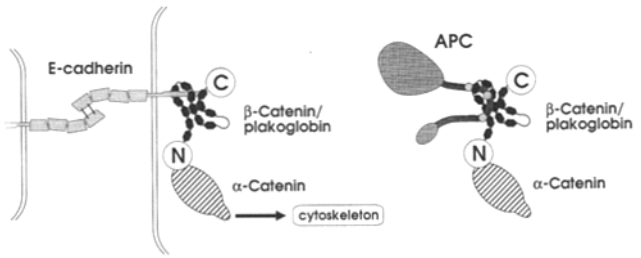

Figure 7. Schematic representation of the E-cadherin and APC complexes with β-catenin or plakoglobin. β-Catenin/plakoglobin interact with the cytoplasmic region of the cell adhesion molecule E-cadherin via their internal *armadillo*-like repeats (drawn as links of a chain). α-Catenin, which mediates the connection to the cytoskeleton, binds to the NH_2-terminal domain of β-catenin. No function has yet been assigned to the COOH-terminal domain of β-catenin. β-Catenin/plakoglobin also interact with APC through the *armadillo*-like repeats. The three β-catenin–binding repeats of APC (Su et al., 1993) are shown. We also indicate that the repeat units of β-catenin may form an intramolecular loop and that the repeats located more NH_2- or COOH-terminally interact preferentially with APC or E-cadherin, respectively.

gene product APC, and APC and E-cadherin actually compete for binding to the region of the internal, *armadillo*-like repeats of β-catenin. Furthermore, β-Catenin and plakoglobin are direct linkers of both E-cadherin and APC to the cytoskeleton-associated protein α-catenin. This link requires a distinct region of β-catenin, the NH_2-terminal domain. β-Catenin and plakoglobin are thus centrally located in both the cell adhesion and the APC complexes, and it is also evident that the overall structural arrangement of the E-cadherin/catenin and the APC/catenin complexes is similar (Fig. 7). These findings indicate that β-catenin/plakoglobin represent important links in the communication between cell adhesion, cytoskeletal interaction, and tumor progression.

There exist distinct differences, however, between the E-cadherin/catenin and APC/catenin complexes. No similarity in the primary structure between the catenin-binding region of E-cadherin or APC can be recognized (Su et al., 1993). Furthermore, we observed differences in the binding of β-catenin mutants to the cell adhesion molecule and the tumor suppressor gene product, i.e., deletion of NH_2- or COOH-terminal *armadillo* repeat units affected binding differently. Apparently, there exist two subregions within the repeat sequence of β-catenin, to which E-cadherin and APC bind preferentially. Nevertheless, E-cadherin clearly competes with APC for the binding to β-catenin. Although we have not thoroughly studied binding affinities, it seems that E-cadherin binds more strongly, i.e., at comparable expression levels of APC and E-cadherin, β-catenin is linked primarily to E-cadherin. Our finding that E-cadherin and APC bind to catenins in separate complexes is in line with previous observations, i.e., the two components could not be coimmunoprecipitated (Su et al., 1993).

APC and E-cadherin are both involved in the suppression of neoplastic transformation and invasiveness (Fearon and Vogelstein, 1990; Birchmeier et al., 1993; Behrens et al., 1989; Frixen et al., 1991; Vleminckx et al., 1991); we might therefore speculate that both components cooperate in this function via the competitive interaction with β-catenin. β-Catenin has been suggested to modulate E-cadherin–mediated cell adhesion during processes which require reduced cell adhesion, such as cytokinesis and cell movement, and tyrosine phosphorylation of β-catenin interferes with E-cadherin function (Behrens et al., 1993; Hamaguchi et al., 1993; Shibamoto et al., 1994). APC could thus sequester negative regulatory variants of β-catenin and prevent their interaction with E-cadherin. Alternatively, APC might directly interfere with E-cadherin–mediated cell adhesion, e.g., promote shedding of epithelial cells into the colonic lumen. As a correlate, APC expression has been found to increase from the base of the colonic crypts to the luminal surface (Smith et al., 1993). Absence or mutation of APC might thus promote progression of colon carcinomas by retention of proliferating cells, which then could collect further mutations known to contribute to the progression of colon cancer (Fearon and Vogelstein, 1990). It is generally accepted that E-cadherin regulates cytoskeletal organization through its interaction with the catenins (Ozawa et al., 1990; McNeill et al., 1990). The structural similarity of the E-cadherin and APC complexes could also indicate that APC exerts its tumor suppressor function through modulation of the cytoskeleton. APC has been detected in the 100,000 *g* insoluble membrane fraction and was recently found to be associated with microtubules (Smith et al., 1994; Munemitsu et al., 1994).

We have further demonstrated that plakoglobin can interact with either the cell adhesion molecule E-cadherin or the tumor suppressor gene product APC in the same manner as β-catenin. β-Catenin and plakoglobin might thus be functionally redundant; this question can now be examined in systems where the gene for one or the other is deleted. Alternatively, β-catenin and plakoglobin might have functionally different roles and may be the subject of separate regulation. It is known that both proteins differ in their association with cell junctions in vivo, i.e., β-catenin is largely associated with adherens junctions whereas plakoglobin is also part of desmosomes (Cowin et al., 1986). That β-catenin and plakoglobin form mutually exclusive complexes with E-cadherin has recently also been suggested by Hinck et al. (1994*a*).

Recent evidence suggests that β-catenin, besides functioning in adhesion complex formation and in the interaction with APC, is also involved in signal transduction: its homologue *armadillo* is part of a signal cascade from *wingless* (the vertebrate homologue is *wnt*-1) to *engrailed* in determining segment polarity in Drosophila (Bejsovec and Wieschaus, 1993; van Leeuwen et al., 1994). Interestingly, overexpression of *wnt*-1 in *Xenopus* embryos results in a duplication of the embryonic axis, and the same phenotype occurs after microinjection of anti–β-catenin antibody into early embryos (Sokol et al., 1991; McCrea et al., 1993), which suggests similar signalling events also during vertebrate development. In mammalian cells, it has been shown that *wnt*-1 expression results in a strong accumulation of β-catenin and plakoglobin at the cell membrane and in the strengthening of Ca^{2+}-dependent adhesion (Bradley et al., 1993; Hinck et al., 1994*a*). It remains to be determined whether these signalling processes make use of β-catenin in a membrane-associated complex or whether independent interactions occur.

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