β-Catenin Mediates the Interaction of the Cadherin–Catenin Complex with Epidermal Growth Factor Receptor

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Abstract. Catenins mediate the linkage of classical cadherins with actin microfilaments and are part of a higher order protein structure by which cadherins are connected to other cytoplasmic and transmembrane proteins. The ratio of actin-bound to free cadherin–catenin complex, which varies depending on the type and growth rate of cells, is thought to be altered by cellular signals, such as those associated with mitosis, polarization of cells and growth factors during development. EGF induces an immediate tyrosine phosphorylation of β-catenin and γ-catenin (plakoglobin). We show here an association of the EGF-receptor with the cadherin–catenin complex. Using recombinant proteins we demonstrate the interaction of EGF-receptor and β-catenin in in vitro kinase assays. This interaction is mediated by the evolutionarily conserved central "core" region of β-catenin. These results suggest that catenins represent an important link between EGF-induced signal transduction and cadherin function.

CADHERINS comprise a group of structurally highly homologous transmembrane proteins which mediate cell–cell interaction of different cell types in various invertebrate and vertebrate species. Besides the by now classical cadherins L-CAM, E- (uvomorulin), N-, and P-cadherin for which an involvement in cell adhesion has unambiguously been demonstrated, a growing number of new proteins have been identified by their structural homologies (Kemler, 1992).

Classical cadherins exhibit the highest degree of homology in their cytoplasmic domain, and the search for the biological function associated with this structural conservation led to the identification of catenins as cytoplasmic anchorage proteins (Ozawa et al., 1989; Nagafuchi and Takeichi, 1989). Molecular cloning and primary structure analysis of catenins revealed homologies to other peripheral cytoplasmic proteins. α-Catenin is homologous to vinculin, a protein found localized in adherens junctions and in focal contacts, where it is involved in the cytoplasmic anchorage of receptors for extracellular matrix proteins. α-Catenin has been cloned in mouse (Nagafuchi et al., 1991; Herrenknecht et al., 1991), human (Claverie et al., 1993), chicken (Hirano et al., 1992), and in *Drosophila* (Oda et al., 1993). Sequence analysis revealed the existence of at least two isoforms (αE and αN) for α-catenin (Hirano et al., 1992). Biochemical evidence indicated that α-catenin does not bind directly to the cytoplasmic domain of cadherins, but rather mediates the connection of the cadherin–catenin complex with actin filaments (Ozawa et al., 1990). The importance of α-catenin for cadherin function has been demonstrated by transfection experiments (Hirano et al., 1992). β-Catenin exhibits homology to human plakoglobin, a component of desmosomal plaques and adherens junctions (Cowin et al., 1986; Franke et al., 1989), and to the product of the *Drosophila* segment polarity gene *armadillo* (McCrea et al., 1991; Butz et al., 1992). Pulse–chase experiments and the analysis of different non-ionic detergent cell lysates indicated that β-catenin binds directly to the cytoplasmic domain of E-cadherin (Ozawa and Kemler, 1992). The molecular identity of γ-catenin has remained less well understood, since the relative amount of γ-catenin in the cadherin–catenin complex varied depending on cell types and because γ-catenin was not always found in the complex in biochemical analyses of different cell lines. Mainly for these reasons γ-catenin was placed in the periphery of the cadherin–catenin complex (Kemler, 1992). Peptide pattern analysis (Ozawa et al., 1989) and immunochemical analysis (Peifer et al., 1992) suggested that γ-catenin might be closely related or identical to plakoglobin and this was further substantiated since plakoglobin is a component of the cadherin–catenin complex (Knudsen and Wheelock, 1992; Piepenhagen and Nelson, 1993).

Catenins play a central role in cadherin function. They mediate the connection of cadherins to the actin filament network and are thought to regulate thereby the strength of cadherin-mediated adhesiveness (Ozawa et al., 1990). They are also part of a higher order sub-membranous protein network by which cadherins are connected to other integral membrane proteins and peripheral cytoplasmic proteins (McNeill et al., 1990). The amount of actin-bound complexes varies depending on cell type and differentiation state of cells and is believed to be modulated during mitosis or

during changes of the developmental state of cells (McNeill et al., 1990; Näthke et al., 1994). It has been reported that α- and β-catenin become phosphorylated at tyrosine residues in cells expressing v-src and that this posttranslational modification correlates with changes of the epithelial phenotype (Matsuyoshi et al., 1991; Behrens et al., 1993; Kamaguchi et al., 1993). In addition, tyrosine phosphorylation of catenins was also observed in cells treated with hepatocyte growth factor and EGF (Shibamoto et al., 1994). This opened up the possibility that tyrosine phosphorylation of catenins might represent one mechanism which modulates the function of catenins. We have been particularly interested in the action of EGF on the cadherin–catenin complex. It is well established that EGF induces cell rounding and membrane ruffling and that the EGF-receptor (EGF-R)[1] molecules are co-localized with cadherins on the basolateral membrane of epithelial cells (Fukuyama and Shimiza, 1991). We show here an association of the EGF-R with the cadherin–catenin complex and an EGF-induced tyrosine phosphorylation of β-catenin and plakoglobin. We provide evidence that β-catenin binds to the EGF-R and that this interaction is mediated by the conserved central "core" region of β-catenin.

Materials and Methods

Cell Lines

Human epidermoid carcinoma cells A431 (ATCC, CRL1555; American Type Culture Collection, Rockville, MD) and TR146 (Rupniak et al., 1985), and porcine kidney epithelial cell line LLC-PK₁ (ATCC, CL101) were grown in DMEM supplemented with 10% (vol/vol) heat-inactivated FCS, at 37°C in a 10% CO_2 atmosphere.

Antibodies

Affinity-purified antibodies against E-cadherin, α- and β-catenin, and plakoglobin have been described previously (Ozawa et al., 1989; Herrenknecht et al., 1991; Butz et al., 1992). The phosphotyrosine (p-tyr) specific monoclonal antibody 4G10 and the polyclonal anti-EGF-receptor (EGF-R) antibodies were obtained from Upstate Biotechnology Inc. (via Biomol, Hamburg, Germany). Peroxidase-labeled secondary antibodies were from Dianova GmbH (Hamburg, Germany).

Cell Extract Preparation

Cells were seeded at a density of 10^6 cells per 90 mm dish, grown 24 h in DMEM, 10% FCS, and stimulated with 50 ng/ml EGF (human recombinant) (Sigma, Munich, Germany) for the indicated times. For metabolic labeling, cells were grown 4 h in methionine-free DME/FCS and then grown overnight in the presence of 50 μCi/ml [³⁵S]methionine (3,000 Ci/mmol). Cells were washed twice with PBS and lysed at a cell density of 10^7 cells/ml in 20 mM imidazole-HCl, pH 6.8, 100 mM KCl, 2 mM $MgCl_2$, 20 mM EGTA, 300 mM sucrose, 1 mM NaF, 1 mM Na-vanadate, 1 mM Na-molybdate, 0.2% (vol/vol) Triton X-100, 10 μg/ml leupeptin, 10 μg/ml soybean trypsin inhibitor, 10 μg/ml PMSF, and 0.1 U/ml α₂-macroglobulin. Crude cell extracts were clarified by centrifugation (14,000 g, 10 min), and the supernatant (soluble fraction) and pellet (insoluble fraction) were separated. The insoluble, cytoskeletal fraction was washed with lysis buffer without Triton X-100 and solubilized in 0.1% SDS. After dilution to 0.02% SDS, immunoprecipitates were collected with anti-β-catenin antibodies.

Immunoprecipitation

Immunoprecipitations were carried out at 4°C. Immunoprecipitations of the detergent-insoluble fraction were carried out after solubilization of the pellet with 0.1% SDS and further dilution to 0.02% SDS with lysis buffer as described above. Supernatants and the solubilized cytoskeleton fraction were precleared by incubation with 10% (wt/vol) protein A-Sepharose beads (Pharmacia, Freiburg, Germany) preabsorbed with ovalbumin (1 mg/ml). Unspecifically bound proteins were removed by centrifugation at 1,000 g for 5 min. 250 μl precleared cell lysates (2.5 × 10⁶ cells) were incubated with 10 μg specific antibodies for 1 h. Antigen–antibody complexes were recovered with 50 μl protein A-Sepharose slurry for 1 h. Beads were washed five times with lysis buffer. Bound proteins were eluted with 2% SDS and separated by SDS-PAGE as described (Ozawa et al., 1989).

Construction and Expression of Fusion Proteins

Two EcoRI fragments covering the entire β-catenin coding region (Butz et al., 1992) were subcloned into the pSKII vector (Stratagene, Heidelberg, Germany). The two plasmids were termed pSK8E2 (NH₂-terminal coding 1.3-kb fragment, amino acids (aa) 1-422, 5'-3' orientation), and pSK8E1 (COOH-terminal coding 2.1-kb fragment, aa 422-781, 3'-5' orientation).

To generate a β-catenin full-length expression construct the cDNA sequence coding for the β-catenin NH₂ terminus (aa 1-119) was amplified with the primer pairs MKNDEI (5'-CATATGGCTACTCAAGCTGACC) and MKNTERM (5'-CTATTAGGGATGAGCAGCGTCAAAC) on the pSK8E2 template. The PCR product was blunt-ended in both orientations into the EcoRV site of pSKII and subcloned. The corresponding vectors were termed pSKNTERM5' and 3'. The entire β-catenin coding sequence (pSKβtot) was assembled by combining the BamHI/SphI fragment of pSKNTERM5', the SphI/EcoRI fragment of pSK8E2, the EcoRI/SstI fragment of pSK8E1 and the SstI/BamHI fragment of pSKβEH2(HH)I. pSKβEH2(HH)I was obtained by digesting pSK8E2 with HindIII and ligating the resulting COOH-terminal coding HindIII fragment (bp 1362-2460 of cDNA) in the corresponding site of pSKII (3'-5' orientation). To transfer the β-catenin cDNA into a prokaryotic expression vector, pSkβtot was BamHI digested and subcloned into the corresponding site of pGEX4T1 (Pharmacia).

With a similar strategy a GST fusion protein expressing the β-catenin core region was generated, which will be described in detail (Aberle et al., 1994). GST fusion proteins were expressed in Epicurian Coli^R XL-1 Blue MRF (Stratagene). The bacteria were grown in LB medium supplemented with 200 mg/ml ampicillin and 2% (wt/vol) glucose. Expression of recombinant proteins was induced with 1 mM IPTG for 60 min at 30°C at an OD_{600} of 0.5. The bacteria were pelleted and resuspended in 10 volumes of PBS containing 1% (vol/vol) Triton X-100, 1 mM $MgCl_2$, 10 μg/ml DNAse 1, 10 μg/ml RNAse, 10 μg/ml leupeptin, 10 μg/ml PMSF, 10 μg/ml soybean trypsin inhibitor and 0.1 U/ml α₂-macroglobulin. The bacteria were lysed by two passes though a french pressure cell (18,000 psi). Cell debris was removed by centrifugation (10 min, 14,000 g). GST fusion proteins were isolated by affinity chomatography on glutathione-agarose (Sigma), eluted with 10 mM glutathione in 100 mM Tris-HCl, pH 8, 10 μg/ml leupeptin, and dialyzed against 50 mM Hepes-NaOH, pH 7.4. The protein solutions were adjusted to 50% glycerol (vol/vol) and stored at −20°C.

Preparation of Recombinant EGF Receptor

The human EGF-R cloned into a baculovirus was provided by Dr. M. Waterfield (Ludwig Institute for Cancer Research, London). Propagation of virus and infection of insect cells were essentially done as described (Waterfield and Greenfield, 1991). Infected cells were washed twice with serum-free TC100 and lysed at a cell density of 10^7 cells/ml in 10 mM Hepes-NaOH, pH 7.4, 200 mM KCl, 1% (vol/vol) Triton X-100, 10 μg/ml leupeptin, 10 μg/ml PMSF, 10 μg/ml soybean trypsin inhibitor, and 0.1 U/ml α₂-macroglobulin. After centrifugation (10 min, 14,000 g) low molecular weight components were removed by gel filtration on a PD10-column (Pharmacia; elution buffer: 10 mM Hepes-NaOH, pH 7.4, 200 mM KCl, 0.1% (vol/vol) Triton X-100), and aliquots of the extracts were stored at −80°C.

In Vitro Protein Kinase Assays

For in vitro kinase assays 20 μg total protein from insect cell lysates were incubated with 20 μg fusion proteins for 30 min at 37°C in 500 μl kinase buffer (20 mM Hepes-NaOH, pH 7.4, 100 mM KCl, 2 mM $MgCl_2$, 10 mM $MnCl_2$, 0.1% (vol/vol) Triton X-100, 0.1 mM Na-vanadate, 0.1 mM ATP). The GST-fusion proteins were isolated with glutathione-agarose, specifically bound proteins were eluted with 2% SDS and separated by SDS-PAGE, and immunoblots were developed with p-tyr and EGF-R-specific antibodies.

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1. *Abbreviations used in this paper*: aa, amino acids; EGF- and PDGF-R, EGF- and PDGF-receptor; p-tyr, phosphotyrosine.

Other Methods

Results

In an initial series of experiments the effect of EGF on the cadherin–catenin complex in human epidermoid carcinoma cells A431 was analyzed. A431 cells have been studied extensively as a model system for the EGF/EGF-R–induced signal transduction, and an association of the EGF-R with actin filaments has been reported in these cells (den Hartigh et al., 1992).

Immunoprecipitations from whole cell lysates of [^{35}S]methionine labeled A431 cells with anti-E-cadherin antibodies detected a comparable relative ratio of the cadherin–catenin complex with or without EGF treatment (Fig. 1, lanes *1* and *2*). Semiconfluent A431 cells were incubated with 50 ng/ml EGF, and at different times cadherin–catenin complexes were collected with antibodies against anti E-cadherin and probed with anti-p-tyr antibodies in immunoblots. As can be seen in Fig. 1 (lane *3*) already at time 0 a trace amount of γ-catenin (plakoglobin) was tyrosine phosphorylated. By 3–5 min after EGF treatment, an increasing amount of tyrosine phosphorylation was already detected for both β- and γ-catenin (Fig. 1, lanes *4–6*). These results clearly demonstrate that EGF treatment results in tyrosine phosphorylation of β- and γ-catenin. They differ from those obtained with v-*src* transformed cells where α- and β-catenin were the primary targets for tyrosine phosphorylation (Hamaguchi et al., 1993). This suggests that the EGF-induced and v-*src*-mediated phosphorylation are each specific for different catenins. Tyrosine phosphorylation of β- and γ-catenin seems to be specific for the EGF signal transduction pathway. In transfected Ltk⁻ cells expressing both the platelet-derived growth factor receptor (PDGF-R) and the E-cad-

herin–catenin complex, PDGF had no detectable effect on the phosphorylation of catenins (not shown).

Cadherin–catenin complexes partition into both the non-ionic detergent-soluble and -insoluble cytoskeletal fractions (Ozawa et al., 1989). It was therefore of interest to examine whether tyrosine-phosphorylated catenins separate equally in both fractions. EGF-treated A431 cells (50 ng/ml EGF, 45 min) were solubilized and both the Triton X-100–soluble and -insoluble fractions (supernatant and pellet from 14,000 g centrifugation) were immunoprecipitated with anti-β-catenin antibodies. As can be seen in Fig. 2 (lanes *1* and *2*) β-catenin was immunoprecipitated from the detergent-soluble (S) and -insoluble (P) fraction (to obtain comparable amounts of β-catenin 5–10 times more of the immunoprecipitate of the insoluble fraction was loaded on the gel). When these blots were stained with anti-p-tyr antibodies, tyrosine-phosphorylated β-catenin was exclusively found in the detergent-soluble fraction (Fig. 2, lanes *3* and *4*). Anti-p-tyr antibodies also detected a phosphorylated protein of 180 kD in the cadherin–catenin complex immunoprecipitates (Fig. 1, lanes *3–6*). As subsequent immunoblots with anti EGF-R antibodies revealed, this represents EGF-R (Fig. 1, lane *10*). An association of EGF-R with the cadherin–catenin complex could also be demonstrated in A431 cells before treatment with EGF (Fig. 1, lane *8*) and the receptor is weakly tyrosine-phosphorylated (Fig. 1, lane *3*). Since the EGF-stimulation experiments were carried out on semi-confluent cell layers 24 h after plating, it is likely that cells received mitotic stimuli due to fetal calf serum components, which led to some activation of EGF-R. This would explain the weak tyrosine phosphorylation of plakoglobin and EGF-R already at time 0 of EGF stimulation and an association of EGF-R with the complex (Fig. 1, lane *3*). However, an association of non-activated EGF-R with the cadherin–catenin complex can not be excluded from these experiments. Comparison of the entire EGF-R pool to EGF-R associated with the cadherin–catenin complex indicated that less than 10% of total EGF-R is associated with the complex (not shown). Since A431 cells express a high amount of EGF-R, it was of interest to see if tyrosine phosphorylation of catenins and an
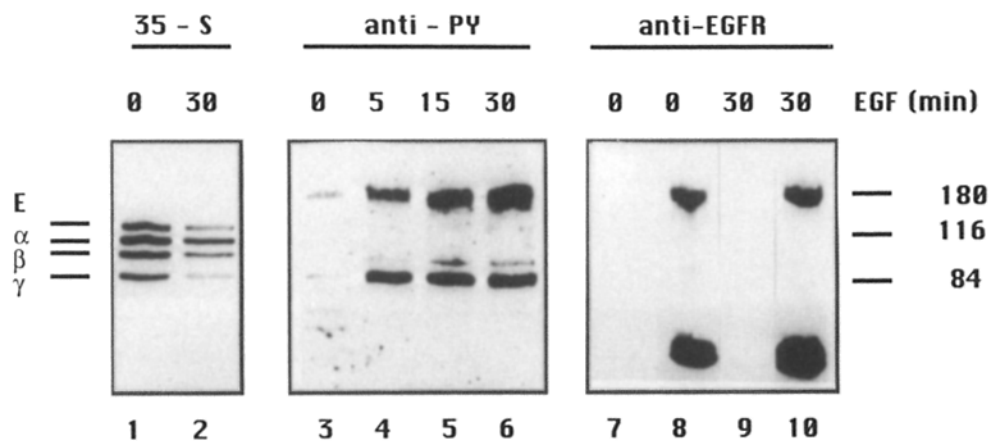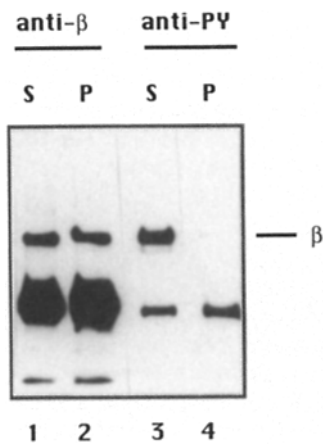

Figure 1. EGF-dependent phosphorylation of β- and γ-catenin in A431 cells and association of the EGF-R with the cadherin–catenin complex. Proteins were immunoprecipitated with an E-cadherin-specific antibody from lysates of metabolically labeled A431 cells treated with 50 ng/ml EGF (lane *2*) or untreated (lane *1*). The cadherin–catenin complex was collected from A431 cells treated with EGF for the indicated times, proteins were separated by SDS-PAGE and immunoblots were developed with a phosphotyrosine-specific (anti-p-tyr) antibody (lanes *3* to *6*). A431 cells without (lanes *7* and *8*) and with EGF stimulation (50 ng/ml, lanes *9* and *10*) were immunoprecipitated with anti E-cadherin antibodies (lanes *7* and *9* are controls). Immunoprecipitates were blotted with anti-EGF-R antibodies, revealing the presence of EGF-R in the E-cadherin–catenin complex independent of EGF-treatment. Positions of cadherin and catenins are indicated on the left; positions of prestained molecular weight markers are indicated on the right. The low molecular weight proteins in lanes *8* and *10* correspond to heavy chains of the precipitating antibody.

anti-β   anti-PY
S   P   S   P

1   2   3   4

Figure 2. Tyrosine phosphorylated β-catenin after EGF stimulation of A431 cells (50 ng/ml, 30 min) is detected in the non-ionic detergent-soluble fraction. β-catenin was immunoprecipitated from the detergent-soluble (S) and SDS-solubilized insoluble fraction (P) and stained with anti-β-catenin antibodies in immunoblots (lanes *1* and *2*). To obtain comparable amounts of β-catenin 5–10 times more of the immunoprecipitates from the insoluble fraction was loaded on the gel. When these blots were stained with anti-p-tyr antibodies, tyrosine phosphorylated β-catenin was detected in the detergent-soluble fraction (lanes *3* and *4*). The position of β-catenin is indicated on the right. The low molecular weight proteins in lanes *1* to *4* correspond to the heavy chains of the precipitating antibody.

association of the EGF-R with the cadherin–catenin complex could also be observed in cells which express a more physiological amount of EGF-R. For this purpose we examined polarized proximal kidney epithelial cells, LLC-PK₁, grown as transfilter cultures (Costar Corp., Cambridge, MA), and the human squamous epithelial carcinoma cell line TR146. The cadherin–catenin complex was immunoprecipitated with anti-β-catenin antibodies from cell lysates of metabolically labeled LLC-PK₁ and TR146 cells (Fig. 3, lanes *1* and *3*, respectively). Under these experimental conditions γ-cat-

LLC – PK1   TR 146

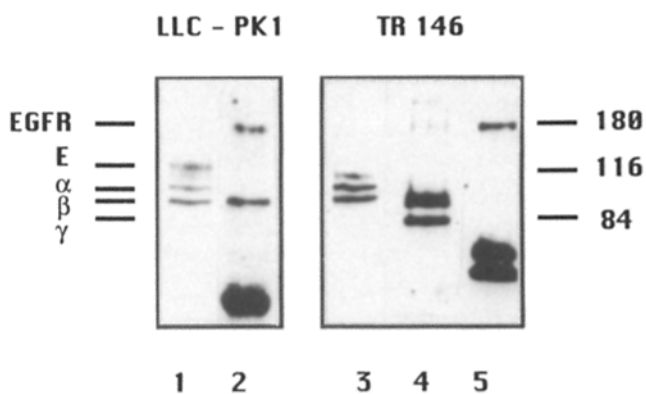EGFR —
E —
α
β
γ

1   2   3   4   5

— 180
— 116
— 84

Figure 3. EGF-induced tyrosine phosphorylation of β- and γ-catenin in LLC-PK₁ and TR146 cells. Proteins were immunoprecipitated with a β-catenin-specific antibody from lysates of metabolically labeled LLC-PK₁ (lane *1*) or TR146 cells (lane *3*). The cadherin–catenin complex was collected from lysates of EGF-treated cells, proteins were separated by SDS-PAGE and immunoblots were developed with phosphotyrosine- (lanes *2* and *4*) or EGF-R-receptor-specific antibody (lane *5*). Although very little γ-catenin (plakoglobin) is present in the complex collected with anti-β-catenin antibodies, it appears to be a major target for tyrosine phosphorylation in TR146 cells (lane *4*). Positions of cadherin and catenins are indicated on the left; positions of prestained molecular weight markers are indicated on the right. The low molecular weight bands in lanes *2* and *5* correspond to the heavy chain of the precipitating antibody.

enin (plakoglobin) appears to be under-represented in the E-cadherin–catenin complex (Näthke et al., 1994). EGF, exposed basolaterally (50 ng/ml), also induces in both these cell types tyrosine phosphorylation of β-catenin and, interestingly, only in TR146 cells in addition tyrosine phosphorylation of γ-catenin (plakoglobin). These results suggest that β-catenin and plakoglobin are taken differently as substrates in LLC-PK₁ and TR146 cells after EGF-stimulation. Immunoblots with anti p-tyr antibodies revealed a tyrosine phosphorylated high molecular weight protein (Fig. 3, lanes *2* and *4*), as for A431 cells. Subsequent immunoblots of immunoprecipitates from TR146 cells with anti-EGF-R antibodies revealed, as for A431 cells, the presence of EGF-R in the complex (Fig. 3, lane *5*). A possible association of the EGF-R with the cadherin–catenin complex could not be demonstrated for LLC-PK₁ cells since anti-EGF-R antibodies did not cross-react with the porcine EGF-R. Taken together, all these results demonstrate in three different epithelial cell types an EGF-induced tyrosine phosphorylation of β-catenin and plakoglobin and an association of the EGF-R with the cadherin–catenin complex.

To further elucidate the interaction of catenins with the EGF-R, β-catenin, and a conserved central β-catenin region (amino acid position 120-683, designated β-catenin core region) were expressed as glutathione-S-transferase fusion proteins in *Escherichia coli*. The core region of β-catenin is largely composed of hydrophobic repeats and exhibits the highest degree of homology to plakoglobin and the armadillo protein (Peifer et al., 1992; Rosenthal, 1993). Fusion proteins were purified by glutathione–agarose affinity chomatography (Fig. 4, lanes *1* and *2*) and reacted with anti peptide antibodies in immunoblots (not shown). β-catenin fusion proteins and recombinant EGF-R, expressed in the baculovirus system, were subjected to in vitro kinase assays. Cell lysates from insect cells expressing wild-type baculovirus were included as controls. Glutathione–agarose purified proteins were subjected to immunoblot analysis with anti-p-tyr antibodies (Fig. 4). β-catenin, but not the β-catenin core region or the GST fusion partner, was phosphorylated in these assays (Fig. 4, lanes *4* and *5*), while no tyrosine phosphorylated protein was detected with control cell lysates expressing wild-type baculovirus (not shown). These results indicate that the phosphorylated tyrosine residues are located either in the NH₂- or in the COOH-terminal domain of β-catenin. More importantly, β-catenin and the β-catenin core region were associated with a tyrosine phosphorylated protein which reacted with anti-EGF-R antibodies (Fig. 4, lane *6*). Since no other tyrosine phosphorylated proteins were detected in these assays, these results suggest that β-catenin binds directly to EGF-R and that this interaction is mediated by the β-catenin core region.

Discussion

EGF acts on cells by binding to its receptor (EGF-R), thereby initiating a variety of cellular changes including rapid alteration in cell surface morphology, cytoskeletal reorganization and redistribution of the EGF-R (den Hartigh et al., 1992). On epithelial cells EGF counteracts the adhesive function of E-cadherin. While E-cadherin is of crucial importance for the biogenesis of an epithelium and mediates a tight association of these cells (Larue et al., 1994), EGF
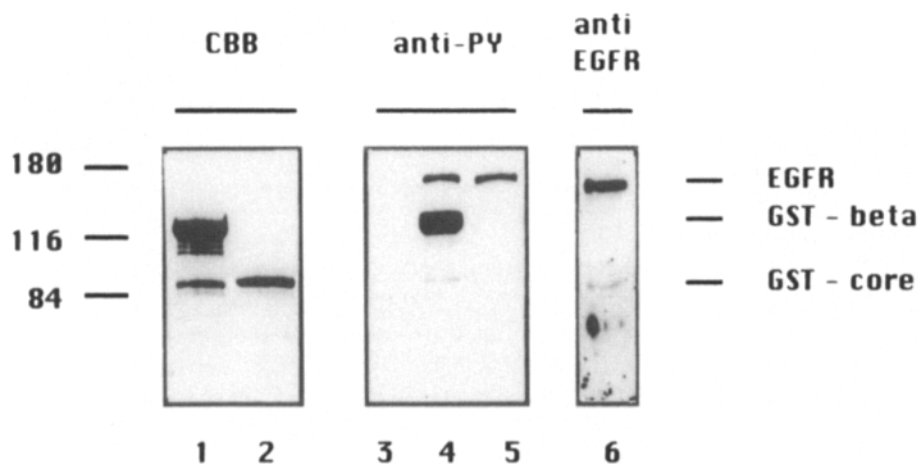
initiates cell rounding and membrane ruffling. Furthermore, EGF-R can be found co-localized with the cadherin–catenin complex at the lateral membrane of epithelial cells, and an association of EGF-R with the cytoskeleton has been reported (den Hartigh et al., 1992; van Bergen en Henegouwen et al., 1992). All this stimulated us to investigate a possible molecular interaction between EGF/EGF-R and the cadherin–catenin complex. We show here that EGF induces an immediate tyrosine phosphorylation of β- and γ-catenin (plakoglobin). Depending on cell type, either β- or γ-catenin was the primary target for EGF-dependent tyrosine phosphorylation, while α-catenin was never found phosphorylated in these experiments. γ-catenin was more extensively phosphorylated in A431 cells, whereas β- and γ-catenin were equally phosphorylated in TR146 cells, and only phosphorylated β-catenin was detected in LLC-PK₁ cells (compare Fig. 1, lane *6*, with Fig. 3, lanes *2* and *4*). Similar results on the tyrosine phosphorylation of catenins induced by hepatocyte growth factor and by EGF in human carcinoma cells have been reported (Shibamoto et al., 1994). We have found no effect on tyrosine phosphorylation with PDGF; acidic fibroblast growth factor (αFGF) also appears not to phosphorylate the cadherin–catenin complex on tyrosine residues (Boyer et al., 1992). These results suggest a selective interaction of distinct growth factor receptors with the cadherin–catenin complex and might indicate that this interaction depends on the correct spatial arrangement of the respective tyrosine kinase receptor and the cadherin–catenin complex. The biological consequence of tyrosine phosphorylation of catenins is currently not known. The fact that phosphorylated β-catenin is exclusively found in the detergent-soluble fraction is suggestive that tyrosine phosphorylation might induce a disassembly of the cadherin–catenin complex from the actin filament network, but other explanations are possible.

Our most important finding would appear to be that the autophosphorylated EGF-R becomes associated with the cadherin–catenin complex. We demonstrated this associa-

tion in different cell lines after EGF treatment, but these experiments did not allow us to distinguish whether the association was direct or indirect. To further clarify this point we then performed in vitro phosphorylation assays with recombinant proteins. Recombinant β-catenin has several structural features in common with native β-catenin, e.g., it is recognized by epitope-specific antibodies and associates with native α-catenin (Aberle et al., 1994). In in vitro kinase assays with recombinant β-catenin and EGF-R, tyrosine phosphorylation of β-catenin, and an association between β-catenin and EGF-R were demonstrated. It is generally accepted that proteins which interact with tyrosine kinase receptors become phosphorylated (Mustelin and Burn, 1993). If so, our results would indicate a direct interaction between EGF-R and β-catenin which appears to be mediated by the β-catenin core region. The core region exhibits, with about 80% of amino acid identity, the highest degree of homology to plakoglobin (γ-catenin) and the armadillo protein, which suggests that these proteins might also have EGF-R–binding properties. Our results indicate that tyrosine phosphorylation occurs at the amino- and/or carboxy-terminal regions of β-catenin. The in vitro kinase assays should allow us to determine more precisely which tyrosine residues are substrates for EGF-R or other kinases. Finally, β-catenin exhibits no obvious homology to SH2 domains, which are known to bind directly to tyrosine kinase receptors. It will be extremely important to identify the amino acid sequences on both EGF-R and β-catenin which mediate this interaction. β-catenin is also complexed to other members of the cadherin protein family and might also link these cell adhesion molecules to other members of the tyrosine kinase receptor family. If so, β-catenin may prove to be an important regulatory protein between receptor-mediated signaling and cadherin function.

References

Aberle, H., S. Butz, J. Stappert, H. Weissig, R. Kemler, and H. Hoschuetzky. 1994. Assembly of the cadherin-catenin complex in vitro with recombinant proteins. *J. Cell Sci.* In press.

Behrens, J., L. Vakaet, R. Friis, E. Winterhager, F. VanRoy, M. M. Mareel, and W. Birchmeier. 1993. Loss of epithelial differentiation and gain of invasiveness correlates with tyrosine phosphorylation of the E-cadherin/β-catenin complex in cells transformed with a temperature-sensitive v-src gene. *J. Cell Biol.* 120:757-766.

Boyer, B., S. Dufour, and J. P. Thiery. 1992. E-cadherin expression during the acidic FGF-induced dispersion of rat bladder carcinoma cell line. *Exp. Cell Res.* 201:345-357.

Butz, S., J. Stappert, H. Weissig, and R. Kemler. 1992. Plakoglobin and β-catenin: distinct but closely related [letter]. *Science (Wash. DC).* 257:1142-1144.

Claverie, J. M., J. P. Hardelin, R. Legouis, J. Levilliers, L. Bougueleret, M. G. Mattei, and C. Petit. 1993. Characterization and chomosomal assignment of a human cDNA encoding a protein related to the murine 102-kDa cadherin-associated protein (α-catenin). *Genomics.* 15:13-20.

Cowin, P., H.-P. Kapprell, W. W. Franke, J. Tamkun, and R. O. Hynes. 1986. Plakoglobin: a protein common to differnt kinds of intercellular adhering junctions. *Cell.* 46:1063-1073.

den Hartigh, J. C., P. M. van Bergen en Henegouwen, A. J. Verkleij, and J. Boonstra. 1992. The EGF-receptor is an actin binding protein. *J. Cell Biol.* 119:349-355.

Franke, W. W., M. D. Goldschmidt, R. Zimbelmann, H. M. Mueller, D. L. Schiller, and P. Cowin. 1989. Molecular cloning and amino acid sequence of human plakoglobin, the common junctional plaque protein. *Biochemistry.* 86:4027-4031.

Fukuyama, R., and N. Shimizu. 1991. Detection of epidermal growth factor receptors and E-cadherins in the basolateral membrane of A431 cells by laser scanning fluorescence microscopy. *Jpn. J. Cancer Res.* 82:8-11.

Hamaguchi, M., N. Matsuyoshi, Y. Ohnishi, B. Gotoh, M. Takeichi, and Y. Nagai. 1993. p60^v-src causes tyrosine phosphorylation and inactivation of the N-cadherin-catenin cell adhesion system. *EMBO (Eur. Mol. Biol. Organ.) J.* 12:307-314.

Herrenknecht, K., M. Ozawa, C. Eckerskorn, F. Lottspeich, M. Lenter, and R. Kemler. 1991. The uvomorulin-anchorage protein α-catenin is a vinculin homologue. *Proc. Natl. Acad. Sci. USA.* 88:9156-9160.

Hinck, L., I. S. Näthke, J. Papkoff, and W. J. Nelson. 1994. Dynamics of cadherin/catenin complex formation: novel protein interactions and pathways of complex assembly. *J. Cell Biol.* 125:1327-1340.

Hirano, S., N. Kimoto, Y. Shimoyama, S. Hirohashi, and M. Takeichi. 1992. Identification of a neural α-catenin as a key regulator of cadherin function and multicellular organization. *Cell.* 70:293-301.

Kemler, R. 1992. Classical cadherins. *Semin. Cell Biol.* 3:149-155.

Kemler, R. 1993. From cadherins to catenins: cytoplasmic protein interactions and regulation of cell adhesion. *Trends Genet.* 9:317-321.

Knudsen, K. A., and M. J. Wheelock. 1992. Plakoglobin, or an 83-kD homologue distinct from β-catenin, interacts with E-cadherin and N-cadherin. *J. Cell Biol.* 118:671-679.

Larue, L., M. Oshugi, J. Hirchenhain, and R. Kemler. 1994. E-cadherin null mutants fail to form trophectoderm epithelium. *Proc. Natl. Acad. Sci. USA.* 91:8263-8267.

Matsuyoshi, N., M. Hamaguchi, S. Taniguchi, A. Nagafuchi, S. Tsukita, and M. Takeichi. 1992. Cadherin-mediated cell-cell adhesion is perturbed by v-src tyrosine phosphorylation in metastatic fibroblasts. *J. Cell Biol.* 118:703-714.

McCrea, P. D., C. W. Turck, and B. Gumbiner. 1991. A homolog of the armadillo protein in *Drosophila* (plakoglobin) associated with E-cadherin. *Science (Wash. DC).* 254:1359-1361.

McNeill, H., M. Ozawa, R. Kemler, and W. J. Nelson. 1990. Novel function of the cell adhesion molecule E-cadherin as an inducer of cell surface polarity. *Cell.* 62:309-316.

Mullin, J. M., and M. T. McGinn. 1988. Epidermal growth factor induced mitogenesis in kidney epithelial cells (LLC-PK1). *Cancer Res.* 48:4886-4891.

Mustelin, T., and P. Burn. 1993. Regulation of src family tyrosine kinases in lymphocytes. *Trends Biochem. Sci.* 18:215-220.

Nagafuchi, A., and M. Takeichi. 1989. Transmembrane control of cadherin-mediated cell adhesion: a 94 kDa protein functionally associated with a specific region of the cytoplasmic domain of E-cadherin. *Cell Regul.* 1:37-44.

Nagafuchi, A., M. Takeichi, and S. Tsukita. 1991. The 102 kD cadherin-associated protein: similarity to vinculin and posttranscriptional regulation of expression. *Cell.* 65:849-857.

Näthke, S. I., L. Hinck, J. R. Swedlow, J. Papkoff, and W. J. Nelson. 1994. Defining interactions and distributions of cadherin and catenin complexes in polarized cells. *J. Cell Biol.* 125:1341-1352.

Oda, T., Y. Kanai, Y. Shimoyama, A. Nagafuchi, S. Tsukita, and S. Hirohashi. 1993. Cloning of the human α-catenin cDNA and its aberrant mRNA in a human cancer cell line. *Biochem. Biophys. Res. Commun.* 193:897-904.

Ozawa, M., and R. Kemler. 1992. Molecular organization of the uvomorulin-catenin complex. *J. Cell Biol.* 116:989-996.

Ozawa, M., H. Baribault, and R. Kemler. 1989. The cytoplasmic domain of the cell adhesion molecule uvomorulin associates with three independent proteins structurally related in different species. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:1711-1717.

Ozawa, M., M. Ringwald, and R. Kemler. 1990. Uvomorulin-catenin complex formation is regulated by a specific domain in the cytoplasmic region of the cell adhesion molecule. *Proc. Natl. Acad. Sci. USA.* 87:4246-4250.

Peifer, M., and E. Wieschaus. 1990. The segment polarity gene *armadillo* encodes a functionally modular protein that is the *Drosophila* homolog of human plakoglobin. *Cell.* 63:1167-1178.

Piepenhagen, P. A., and W. J. Nelson. 1993. Defining E-cadherin-associated protein complexes in epithelial cells: plakoglobin, β-catenin and γ-catenin are distinct components. *J. Cell Sci.* 104:751-762.

Rosenthal, E. 1993. Identification of homologues to β-catenin/plakoglobin/armadillo in two invertebrates, *Urechis caupo* and *Tripneuste gratilla*. *Biochim. Biophys. Acta.* 1173:337-341.

Rupniak, H. T., C. Rowlatt, E. B. Lane, J. G. Steele, L. K. Trejdosiewicz, B. Laskievicz, S. Povey, and B. T. Hill. 1985. Characteristics of four new human cell lines derived from squamous cell carcinomas of the head and neck. *J. Natl. Cancer Inst.* 75:621-635.

Shibamoto, S., M. Hayakawa, K. Takeuchi, T. Hori, N. Oku, K. Miyazawa, N. Kitamura, M. Takeichi, and F. Ito. 1994. Tyrosine phosphorylation of β-catenin and plakoglobin enhanced by hepatocyte growth factor and epidermal growth factor in human carcinoma cells. *Cell Adhesion Communi.* 1:295-305.

van Bergen en Henegouwen, P. M. J. I. den Hartigh, P. Romegu, A. J. Verkleij, and J. Boonstra. 1992. The epidermal growth factor receptor is associated with actin filaments. *Exp. Cell Res.* 199:90-97.

Waterfield, M. D., and C. Greenfield. 1991. Expression and properties of epidermal growth factor receptor expressed from baculovirus vectors. *Methods Enzymol.* 200:627-645.