Apoptosis-induced Cleavage of β-Catenin by Caspase-3 Results in Proteolytic Fragments with Reduced Transactivation Potential*

Ulrike Steinhusen§, Volker Badock§, Andreas Bauer†, Jürgen Behrens, Brigitte Wittman-Liebold§, Bernd Dörken**, and Kurt Bommert§§

From the ¶Max-Debrück-Center for Molecular Medicine, Department of Medical Oncology and Tumorimmunology, the ¶Department of Protein Chemistry, the ¶Department of Epithelial Differentiation, Invasion, and Metastasis, Robert-Rössle-Strasse 10, D-3092 Berlin, Germany, the ¶Max-Planck Institute of Immunobiology, Department of Molecular Embryology, Stabeweg 51, D-79108 Freiburg, Germany, and the §§Humboldt University of Berlin, University Medical Center Charité, Robert-Rössle-Klinik, Department of Hematology, Oncology and Tumorimmunology, D-3092 Berlin, Germany

β-Catenin is a member of the Armadillo repeat protein family with a dual cellular function as a component of both the adherens junction complex and the Wnt/wingless signaling pathway. Here we show that β-catenin is proteolytically cleaved during anoikis and staurosporine-induced apoptosis. Cleavage of β-catenin was found to be caspase-dependent. Five cleavage products of β-catenin were identified in vivo and after in vitro cleavage by caspase-3. Amino acid sequencing and mass spectrometry analysis indicated two caspase-3 cleavage sites at the C terminus and three further sites at the N terminus, whereas the central Armadillo repeat region remained unaffected. All β-catenin cleavage products were still able to associate with E-cadherin and α-catenin and were found to be enriched in the cytoplasm. Functional analysis revealed that β-catenin deletion constructs resembling the observed proteolytic fragments show a strongly reduced transcription activation potential when analyzed in gene reporter assays. We therefore conclude that an important role of the β-catenin cleavage during apoptosis is the removal of its transcription activation domains to prevent its transcription activation potential.

Growth of epithelial and endothelial cells is strictly anchorage-dependent, with strong cell-matrix and cell-cell contacts (1–3). When cultured in suspension, the cells rapidly undergo apoptosis a process termed anoikis (1). It has been suggested that the biological role of anoikis is to restrict inappropriate cell growth after the loss of matrix attachment (4). Different signaling pathways are involved in this process. Thus, it was shown that integrin signaling is necessary for survival and growth of epithelial cells and prevents anoikis (3, 5, 6). Furthermore, overexpression of onecogenes like v-Ha-ras, v-src, and bcl-2 prevents apoptosis after detachment (1, 7), whereas activation of the Jun-N-terminal kinase pathway appears to be involved in the induction of anoikis (8–10).

The protein family of caspases plays a major role during the execution phase of apoptosis (11). Up to now more than 10 different human caspases have been cloned (12–14), and these can be subdivided into two groups: initiator caspases whose main function is to activate downstream caspases and executor caspases, which are responsible for dismantling cellular proteins (14). Caspases recognize tetrapeptide motifs and cleave their substrates behind aspartate. A growing number of caspase-3 substrates has been identified, including the focal adhesion kinase, which is involved in cell matrix adhesion via integrins (15–19) and mitogen-activated protein kinase/extracellular signal-regulated kinase kinase-1, which plays a role in the Jun-N-terminal kinase pathway (10). Other proteins cleaved by caspases are involved in structural changes in epithelial cells during apoptosis, like keratin 18 and 19 (20, 21), p21-activated kinase 2 (22), growth arrest-specific gene 2 (23), or Gelsolin (24). Disruption of cell-cell contacts may also play a role in anoikis, as expression of a dominant negative N-cadherin in the mouse intestinal epithelium was shown to disturb cell-cell adhesion and subsequently led to an increased number of apoptotic cells (25, 26). Cell-cell adhesion is predominantly mediated by the cadherintype of transmembrane proteins, generating a homophilic interaction within two cells. During recent years further cytoplasmic proteins were isolated as part of the cadherin cell-cell adhesion complex linking the cadherins to the actin cytoskeleton, thus termed catenins. Three major catenins are known, α-catenin, β-catenin, and γ-catenin (plakoglobin). β-Catenin and γ-catenin directly bind to the cadherin in a mutually exclusive manner, whereas α-catenin binds to β-catenin or γ-catenin and additionally makes a direct or indirect contact to the actin cytoskeleton (27, 28). β-Catenin belongs to the Armadillo repeat protein family, containing 13 Armadillo repeat motifs that seem to be involved mainly in protein-protein interaction. β-Catenin shows dual cellular function, being involved in cell-cell adhesion and Wnt signaling. Upon Wnt signaling β-catenin becomes stabilized in the cytoplasm and subsequently translocates into the nucleus where it is involved in the expression of specific target genes such as Tlx2, Xnr3, siamois, c-myc, cyclin D1, c-jun, and fra-1 (29–34) together with transcription factors of the lymphocyte enhancer-binding factor 1/T-cell factor (TCF) family (35–38).

In this study we could show that β-catenin is proteolytically cleaved during apoptosis. The cleavage was inhibited by the

Received for publication, February 22, 2000
Published, JBC Papers in Press, March 9, 2000, DOI 10.1074/jbc.M001458200

1 The abbreviations used are: TCF, T-cell factor; MDCK, Madine-Darby canine kidney cells; PAGE, polyacrylamide gel electrophoresis; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonic acid; CAPS, 3-(cyclohexylamino)propanesulfonic acid; HPLC, high pressure liquid chromatography; MS, mass spectrometry; GST, glutathione S-transferase; bp, base pairs; FL, full-length; STS, staurosporine. Z-DEVD-fmk, benzyloxycarbonyl-Asp(OMe)-Glu(OMe)-Val-Asp(OMe)fluoromethyl ketone.

* This work was supported by Deutsche Forschungsgemeinschaft Grant SFB 386. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§§ To whom correspondence should be addressed. Tel.: 49-30-9406-3817; Fax: 49-30-9406-3124; E-mail: bommert@mdc-berlin.de.
Caspase-3 family specific peptide inhibitor Z-DEVD-fmk demonstrating that caspase-3 is the major protease involved in this process. The determination of the exact cleavage sites revealed that β-catenin is exclusively cleaved in the N- and C-terminal regions at multiple positions, whereas the central core region was not affected. Consistently, an interaction of all cleavage products was detected in the E-cadherin-α-catenin complex but additionally to a high extent in the cytoplasm not associated with E-cadherin. β-catenin depletion constructs resembling the apoptotic cleavage products of β-catenin showed a strongly reduced transactivation potential in reporter gene assays.

**MATERIALS AND METHODS**

**Cell Culture and Induction of Apoptosis**—The human breast epithelial cell line H184A1 was cultured in Dulbecco’s modified Eagle’s medium Ham’s F12 (Biochrom, Berlin, Germany) supplemented with 5% fetal calf serum, 10 μg/ml transferrin (Life Technologies, Inc.), 10 μg/ml insulin (Biochrom), and 1.8 μg/ml hydrocortisol (Sigma). Madine-Darby canine kidney cells (MDCK), the human breast carcinoma cell line MCF-7.3.28 (stably transfected with a caspase-3 expressing plasmid), and the human HEK293 kidney epithelial cell line were cultured in Dulbecco’s modified Eagle’s medium supplemented with 5% fetal calf serum.

Apoptosis was induced by maintaining cells in suspension as follows. Cells were grown to confluency and harvested by trypsinization. Approximately 1 x 10⁷ cells were transferred to 175-cm² culture flasks coated with 1% poly(2-hydroxyethylmethacrylate) (Sigma) to keep them in suspension as described (1). After different times suspended cells were harvested and washed twice with PBS. Cell pellets were frozen in liquid nitrogen and stored at −80 °C for further analysis. Alternatively, apoptosis was induced by incubating 50% confluent cells in medium containing 1 μM staurosporine for up to 20 h. The percentage of apoptotic cells was determined with APO-BRDU™ kit (Pharmingen, Hamburg, Germany) by nick-end 5-bromo-2-deoxyuridine triphosphate labeling of single- and double-strand DNA breaks with 5-bromo-2-deoxyuridine triphosphate by terminal transferase. Incorporation of the nucleotide analog was detected with FITC-conjugated antibodies and measured by fluorescence-activated cell sorter analysis (FACSort Becton Dickinson, Heidelberg, Germany).

**Antibodies and Reagents**—Monoclonal anti-β-catenin (clone 571–781), anti-α-catenin and anti-E-cadherin antibodies were purchased from Transduction Laboratories (Dianova, Hamburg, Germany). Monoclonal anti-cytokeratin antibody was purchased from His (Freiburg, Germany), monoclonal anti-caspase-6 and -7 antibodies from Santa Cruz (Heidelberg, Germany). Recombinant caspases-3 and -6 were purchased from Pharmingen, recombinant caspase-7 was kindly provided by R. Beyaert (40). Z-DEVD-fmk was purchased from Calbiochem (Bad Soden, Germany) and staurosporine and poly(2-hydroxyethylmethacrylate) were purchased from Sigma. Tropix Dual-Light Chemiluminescence kit was purchased from Perkin Elmer (Weiterstadt, Germany).

**Immunoblotting and Immunoprecipitation**—Immunoblotting was essentially done as described previously (41). In short, cells were lysed by boiling in 10 mM Tris-HCl, pH 7.5, 2 mM EDTA, 1% SDS, and 30–50 μg protein was separated by SDS-PAGE and transferred to nitrocellulose membrane (Schleicher & Schuell). Primary antibodies were diluted 1:1000, anti-β-catenin (clone 571–781) was diluted 1:3000, and horse-radish peroxidase-conjugated secondary anti-mouse or anti-rabbit antibody (Promega, Mannheim, Germany) was diluted 1:10,000. Proteins were visualized using the ECL-system (Amersham Pharmacia Biotech). Immunoprecipitation was done as described (42). In short, 3 x 10⁶ cells were harvested, and proteins were extracted in lysis buffer (140 mM NaCl, 4.7 mM KCl, 0.7 mM MgSO₄, 1.2 mM CaCl₂, 10 mM Tris, pH 8.0, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 5 μg/ml leupeptin) on ice. After centrifugation at 12,000 × g, the supernatant was collected as the detergent soluble fraction. The detergent insoluble fraction was washed twice with lysis buffer and solubilized by boiling in SDS sample buffer. Appropriate antibodies and protein A-Sepharose were added to the sample. Precipitates were washed with 500 μl NaCl, 5 mM EDTA, 50 mM Tris–HCl, pH 8.0, 1% Triton X-100. Western blot analysis was performed with monoclonal anti-β-catenin antibody (clone 571–781), anti-α-catenin, and anti-E-cadherin antibody. Western blots were quantified by using the NIH Image program version 1.59 (National Institutes of Health, Bethesda, MD).

**Purification of 6His β-Catenin**—His-tagged β-catenin was expressed in Escherichia coli and purified to homogeneity by nickel-chelate chromatography (38).

**In Vitro Cleavage of β-Catenin with Recombinant Caspases**—Cleavage reactions with recombinant caspase-3, -6, or -7 were performed in 20 μl of caspase-buffer (50 mM HEPES, pH 7.4, 0.1% CHAPS, 5 mM dithiothreitol, 1 mM β-mercaptoethanol, 200 μg/ml aprotinin) at 37 °C for 2–4 h using 0.5 μg of affinity-purified recombinant 6His-β-catenin as a substrate. Identification of Cleavage Sites of β-Catenin—To identify caspase-3 cleavage sites within β-catenin, 5 μg of recombinant β-catenin was digested with 100 ng of recombinant caspase-3. Cleavage products were separated by SDS-PAGE and transferred to a polyvinylidene difluoride membrane (ProBlot, Applied Biosystems, Foster City, CA) using a semi-dry blotting apparatus (Bio-Rad) and 100 mM CAPS, 10% methanol as blotting buffer. Proteins transferred to the membrane were stained with Coomasie Blue R-250 (Serva, Heidelberg, Germany) for 1 h. Coomasie Blue-stained protein bands were excised, soaked in 10% acetonitrile for destaining, and analyzed by Edman sequencing. Edman sequencing was performed on a Procise sequencer (Applied Biosystems).

The two C-terminal β-catenin fragments were identified by chromatography of in vitro cleavage products using high-pressure liquid chromatography (HPLC) (Smart System, Amersham Pharmacia Biotech) on a C₅ reversed-phase column (2.1 mm inner diameter, 300 Å, 5 μm) obtained from Vydac (Hesperia, CA). Collected fractions were screened for specific C-terminal fragment by matrix-assisted laser desorption ionization–mass spectrometry (VG Tof Spec, Fisons, Manchester, UK). The sequences of the C-terminal fragments were obtained by electrospray ionization-MS/MS using a Q-Tof (Micromass, Manchester, UK) equipped with a nanoelectrospray ion source.

**Glutathione S-transferase (GST)-E-Cadherin Affinity Precipitation Assay**—A recombinant glutathione S-transferase-tagged cytoplasmic domain of mouse E-cadherin (kindly provided by O. Huber, Berlin) was adsorbed on glutathione S-transferase-agarose beads. Approximately 5 μg of GST-E-cadherin was used for the affinity precipitation of β-catenin as described (43).

**β-Catenin Expression Constructs**—β-Catenin FL (bp 215–2557), ΔC1 (bp 215–2506), ΔC2 (bp 215–2467), ΔN (bp 560–2557), ΔNAC1 (bp 215–2467) and ΔNAC2 (bp 215–2467) were obtained from Invitrogen. Reporter Assays—Approximately 2 x 10⁶ HEK293 or H184A1 cells were transiently transfected by the calcium phosphate precipitation method using 1 μg of luciferase reporter pS01234 or its negative control containing mutated TCF sites (qsP3) (31), 0.25 μg of hTGF-β vector (44), 0.5 μg of β-catenin constructs, and 1 μg of pCH10 β-galactosidase expression vector as internal control. 48 h after transfection cells were harvested and resuspended in 100 mM potassium phosphate buffer, pH 7.2. Cell lysis was carried out by three cycles of freezing and thawing. The lysate was cleared by centrifugation, and luciferase and β-galactosidase activities were measured with a Tropix Dual Light Chemiluminescence kit according to the manufacturer’s instructions. Luciferase activity was normalized to β-galactosidase activity.

**RESULTS**

Anoikis-induced Cleavage of β-Catenin in the Human Mammary Epithelial Cell Line H184A1 Is Mediated by Caspase-3-like Proteases—To investigate if components of the cadherin-mediated cell-cell adhesion complex are impaired in apoptotic cells, two antibodies were used: protein level of E-cadherin, α-catenin, and β-catenin in the human mammary epithelial cell line H184A1. Anoikis was induced as described under “Materials and Methods.” Western blot analysis revealed lower molecular mass cleavage products of β-catenin, whereas E-cadherin and α-catenin remained apparently unaffected. The different β-catenin cleavage products appeared in a time-dependent manner. Already 4 h after induction of anoikis, truncated β-catenin fragments were detectable at ~90 (fragment A), 76 (fragment B), and 72 kDa (fragment C). A fourth 70-kDa cleav-
age product (fragment D) became visible after 8 h of induction (Fig. 1A). An 85-kDa fragment (fragment E*) was already observed in uninduced cells, thus representing an apoptosis-unspecific β-catenin degradation product. The amounts of the three lower molecular mass polypeptides (fragments B, C, and D) increased during the time course of apoptosis in correlation with the increasing proportion of apoptotic cells as determined by terminal dUTP nick-end labeling assay. 24 h after induction 96% of the cells were found apoptotic (Fig. 1B). The total amount of the β-catenin cleavage products was comparable to the amount of full-length (FL) β-catenin as determined by densitometric measurement of the band intensities (data not shown).
shown). Low levels of E-cadherin at time points 0 and 4 h were because of the trypsinization process of anoikis induction (Fig. 1A).

As caspase-3 family members have recently turned out to be important effector molecules in apoptosis, we tested whether β-catenin cleavage could be inhibited by incubating H184A1 cells with the irreversible caspase-3 family-specific inhibitor Z-DEVD-fmk. Cells incubated with 100 μM Z-DEVD-fmk and harvested 24 h after induction of anoikis were analyzed by Western blotting for β-catenin cleavage products except C3. The rate of apoptosis was determined by terminal dUTP nick-end labeling assay (data not shown).

In Vitro Cleavage of β-Catenin with Recombinant Caspase-3, -6, and -7—To identify the caspase that is directly involved in the cleavage of β-catenin, we digested recombinant His-β-catenin with recombinant caspase-3, -6, and -7 in vitro. β-Catenin was cleaved by all caspases, resulting in different cleavage products, and this could be blocked in each case by the addition of the caspase-specific inhibitor Z-DEVD-fmk (Fig. 3A). The rate of apoptosis was determined by terminal dUTP nick-end labeling assay (data not shown).

Mapping of the β-Catenin Cleavage Sites—Two consensus motifs corresponding to a potential caspase-3/7 cleavage site (DXXD) are found at positions Asp-251 and Asp-257 in the β-catenin amino acid sequence. Three further putative caspase-6 cleavage sites (EXED) are present in the N-terminal region at positions Asp-11, Asp-17, and Asp-58 (46). However, the calculated molecular masses of such putatively truncated β-catenin molecules did not correlate with the apparent molecular masses of the observed β-catenin cleavage products except for a potential cleavage at Asp-251. We analyzed apoptosis as determined by terminal dUTP nick-end labeling assay (data not shown).
induced cleavage products of β-catenin by Western blotting with antibodies directed against the N- and C-terminal domains of β-catenin, respectively. Both antibodies detected only full-length β-catenin but none of the degradation fragments (data not shown). Therefore, we presume that the N- and C-terminal regions of β-catenin were cleaved off and that the cleavage products were too small (<7.5 kDa) to be detected in our assay system.

For identification of the cleavage sites we digested recombinant His-β-catenin with recombinant caspase-3 and sequenced the resulting β-catenin fragments A, B, C, D, and E by N-terminal Edman degradation (Fig. 4A). Sequencing of fragment A revealed a complete, nontruncated N-terminal region of β-catenin indicating that cleavage had occurred at the C terminus. We identified the potential corresponding 1.8-kDa β-catenin cleavage product by HPLC separation. Analysis of this peptide by electrospray ionization-MS/MS revealed the sequence GLPPGDSNQLAWFDTDL, which corresponds to the amino acids 765–781 in the C terminus of β-catenin. The cleavage therefore had occurred at position Asp-764, a potential caspase-3 consensus sequence DLM

The time course of β-catenin cleavage showed that, in vitro, β-catenin was first cleaved at the C terminus generating fragment A as the main cleavage product after 10 min followed by cleavage at the N terminus (fragments E, B, and C). Finally, β-catenin was further truncated at the second C-terminal cleavage site, resulting in the appearance of fragment D after 1 h (Fig. 4A). A β-catenin cleavage product of similar size as fragment E at about 85 kDa was already detectable under in vivo conditions. However, this fragment (E*) was already observed in uninduced cells and was found to be apoptosis-unspecific (Fig. 1A, 0 h). The appearance of this fragment was not blocked by caspase-specific inhibitors, revealing that it is not created in a caspase-dependent manner (Fig. 1C). We thus conclude that fragments E and E* might be independent of each other and that the generation of fragment E under in vivo conditions is too small to be detected in relation to the unspecific product (E*).

Binding of N-terminal Truncated β-Catenin to α-Catenin and E-Cadherin—We then determined whether truncation of β-catenin impairs its association with α-catenin or E-cadherin, because the identified caspase-3 cleavage sites in the N terminus of β-catenin are in close proximity to the α-catenin-binding domain located to amino acids 129–143 of β-catenin. After staurosporine-induced apoptosis, cell lysates from MDCK cells...
were subjected to immunoprecipitation with monoclonal antibodies to E-cadherin and β-catenin and a polyclonal antibody to α-catenin. Western blot analysis of the E-cadherin, α-catenin, and β-catenin immunocomplexes with anti-β-catenin antibodies showed association of the β-catenin cleavage products B, C, and D with E-cadherin (Fig. 5A, STS/E-cad) as well as with α-catenin (Fig. 5A, STS/α-cat). Subsequent reprobing with anti-α-catenin and anti-E-cadherin antibodies showed that both proteins were detectable in all immunoprecipitates (Fig. 5A).

The overall amount of all components of the cadherin-catenin complex is significantly lower in the detergent-insoluble, cytoskeleton-associated protein fraction (P) than in the soluble (S) protein fraction. After induction of apoptosis, a reduction in the overall amount of β-catenin was observed in both protein fractions but not for α-catenin or E-cadherin. The β-catenin cleavage products were, however, mainly detectable in the detergent-soluble protein fraction (Fig. 5B, S/STS). Just small amounts were visible in the insoluble fraction after longer exposure (data not shown). This suggests that the β-catenin proteolytic fragments might still be able to interact with E-cadherin and α-catenin but might not be able to compensate for full-length β-catenin in the functional cadherin-catenin cell-adhesion complex.

We next determined if the amount of the uncomplexed signaling competent pool of β-catenin was changed in apoptotic cells. Soluble β-catenin was precipitated with a recombinant GST-E-cadherin fusion protein containing the cytoplasmic tail of E-cadherin. Just small amounts of cytoplasmic, uncomplexed β-catenin were observed in uninduced cells. A significant, 5-fold increase in the amount was observed 2 h after induction of apoptosis as determined by densitometric measurement (Fig. 5C). 8 h after induction no full-length β-catenin was detectable anymore, whereas the cytoplasmic β-catenin cleavage products were enriched to a significant extent with a 7-fold excess compared with the initial full-length β-catenin at 0 h. The overall

FIG. 5. Binding of truncated β-catenin to α-catenin and E-cadherin. MDCK cells were grown in medium containing 0.2% Me2SO (Control) or 1 μM STS for 6 h. Cells were harvested, and protein extracts were divided into the soluble and insoluble protein fraction. A, the soluble fractions were immediately prepared for immunoprecipitation with monoclonal antibodies to E-cadherin (E-cad) and β-catenin (β-cat) and a polyclonal antibody to α-catenin (α-cat). Immunoprecipitates and (B) aliquots of the detergent soluble (S) and insoluble (P) protein fraction were resolved by SDS-PAGE and analyzed by Western blotting with monoclonal antibodies to α-catenin, β-catenin, and E-cadherin. C, affinity precipitation of the uncomplexed pool of β-catenin. MDCK cells were treated with STS for the indicated time. The detergent-soluble fractions of the cell lysates were incubated with 5 μg of GST-E-cadherin fusion protein coupled to GST-agarose beads. Precipitates and whole cell lysates were separated by SDS-PAGE and analyzed for β-catenin by Western blotting.
amount of β-catenin did not change detectably as shown by Western blot analysis of whole cell lysates (Fig. 5C).

Deletion Constructs of β-Catenin Show Reduced Transactivation Potential in Gene Reporter Assays—To investigate the consequence of β-catenin cleavage for its signaling function, we designed different β-catenin deletion constructs corresponding to the identified caspase-3 cleavage sites (Fig. 6A). All constructs showed comparable expression levels after transfection into HEK293 and H184A1 cells (Fig. 6B). Subsequently, we analyzed their transactivation potential in a gene reporter assay. The pS01234 reporter plasmid containing the TCF/β-catenin-dependent siamois promoter in front of the luciferase gene was co-transfected with a hTCF-4 expression plasmid together with the different β-catenin constructs. The activation of the luciferase reporter gene by the various β-catenin constructs in HEK293 and H184A1 cells is shown in Fig. 6C. Very similar results were obtained for both cell lines. FL β-catenin showed an over 12-fold induction of the luciferase reporter in HEK293 and H184A1 cells when co-transfected with hTCF-4. Truncation of the C terminus by 17 amino acids (Fig. 6B, ΔC1), which corresponds to fragment A of the apoptotic cleavage products, already showed a slightly reduced luciferase activity of 8.5- and 10-fold activation in the two cell types (Fig. 6C). The deletion construct ΔNΔC1, which resembles fragment C of apoptotic cleavage products, showed only 3–4-fold increased luciferase activity and this was even more reduced when the C terminus was further truncated as shown for ΔNΔC2. This deletion construct, resembling the smallest apoptotic cleavage product fragment D, only showed basal reporter gene activity when compared with luciferase activity of hTCF-4 alone (Fig. 6C). We also wanted to know to what extent the reduction in the transactivation potential of β-catenin deletion constructs depended on truncation of either the N or C terminus alone. Analysis of constructs deleted at the N or C terminus (Fig. 6A, ΔN or ΔC2) revealed that removal of either one of these domains reduced the luciferase activity to 50% of the FL level.
Transfection of a reporter plasmid with mutated TCF binding sites (pS) instead of pS01234 did not show reporter gene activity (data not shown).

**DISCUSSION**

Apoptosis is an important feature of many epithelial tissues with a high turnover and serves to balance accurately the rate of new cell production. Death by apoptosis eliminates specific cells without extensive tissue damage, playing a pivotal role during tissue regeneration and the elimination of tumor cells. Survival factors required to prevent cells from undergoing apoptosis may involve cytokine signaling from neighboring cells, extracellular matrix as well as cell-cell contacts.

Here we could demonstrate that the cell-cell adhesion molecule β-catenin is proteolytically cleaved during anoikis or staurosporine-induced apoptosis. Four apoptosis-specific cleavage products of β-catenin with apparent molecular masses of $\sim 90$ (fragment A), 76 (fragment B), 72 (fragment C), and 70 kDa (fragment D) were observed after induction of apoptosis in the human breast epithelial cell line H184A1 and the canine kidney cell line MDCK. The β-catenin cleavage was almost entirely inhibited by the caspase-3-specific tetrapeptide inhibitor Z-DEVD-fmk indicating that caspase-3 family proteases might play a dominant role during the cleavage process. This notion was further supported by cleavage of recombinant β-catenin with recombinant caspase-3 under *in vitro* conditions. The resulting cleavage pattern was found to be identical to the already observed cleavage pattern *in vivo*. Also consistent with this idea, no apoptosis-specific β-catenin cleavage products were observed in the caspase-3-deficient MCF-7 human breast carcinoma cell line after STS-induced apoptosis, but β-catenin cleavage could be restored by re-introduction of a caspase-3-expressing plasmid.

β-Catenin is a 92-kDa protein containing a central core region of 13 incomplete conserved Armadillo repeat motifs and unique N- and C-terminal regions. The core region acts as a surface for the association of several interacting proteins, e.g., cadherins, α-catenin, the adenosomatous polyposis coli protein, Axin/Conductin, Pontin52, or lymphocyte enhancer-binding factor TCF (35, 47–52) and is able to mediate cell-cell adhesion on its own. Both the core region and the C-terminal domain of β-catenin are involved in its signaling activity (44, 53–56). By identification of the proteolytic cleavage sites in the β-catenin sequence, we observed that the C terminus as well as the N terminus of β-catenin are deleted during apoptosis, but the integrity of the core region was not affected. Consistently we found that all proteolytic fragments of β-catenin were able to associate with E-cadherin and α-catenin under *in vivo* conditions. This demonstrates that the truncated β-catenin fragments can substitute for the full-length molecule at least in the detergent-soluble cadherin-catenin complex. Just small amounts of the cleavage products were detectable in the insoluble, cytoskeleton-associated cadherin-catenin protein complex. The cytoskeleton-associated fraction, however, is believed to participate solely in active cell-cell adhesion, whereas the soluble cadherin-catenin complex is not directly involved in the cell to cell interaction. Moreover, we found that the amount of full-length β-catenin was greatly diminished in both subcellular fractions in staurosporine-induced apoptotic cells. This reduction of the protein level was found to be specific for β-catenin and was not observed for E-cadherin or α-catenin. We conclude that the proteolytic cleavage of β-catenin during apoptosis might have a direct influence on cell-cell adhesion. A reduction of cell-cell contacts during apoptosis was already described for NIH3T3 cells when grown in serum-free media (57). However, the cleavage pattern of β-catenin was found to be remarkably different from those that we have found in H184A1 and MDCK cells. Additionally, no association of the β-catenin proteolytic fragments with α-catenin was observed in this study. In contrast, the interaction of β-catenin cleavage products with α-catenin was described in apoptotic endothelial cells (58). In those cells a similar β-catenin cleavage pattern was identified, as we have observed, but just some of the cleavage products were able to interact with α-catenin. Because of the missing interaction with α-catenin an influence on the cell-cell adhesion was speculated. However, the interaction of the β-catenin truncated forms with E-cadherin has not been demonstrated so far. From our biochemical analysis and the previous results it is hard to conclude if the β-catenin cleavage has a strong influence on cell-cell adhesion. This needs to be analyzed in more detail with more physiological assays to determine cell-cell adhesion. We recently obtained some first evidence that besides β-catenin E-cadherin also is proteolytically cleaved during apoptosis under specific conditions, which indicates rather a reduced cell-cell contact during apoptosis.

Beside its involvement in cell-cell adhesion, β-catenin is also involved in cell signaling as a component of the Wnt/wingless signaling pathway. As a major step of Wnt signal transduction, β-catenin becomes stabilized in the cytoplasm because of the inhibition of protein degradation (59). It is believed that the amount of the cytoplasmic, uncomplexed β-catenin is the critical parameter for Wnt signal transduction. Cells with a high amount of free β-catenin, e.g., Wnt-induced cells or cells from colon carcinoma cell lines, show a strong activation potential for endogenous Wnt target genes. Here we found that the cytoplasmic, uncomplexed pool of β-catenin is significantly enriched 2 h after induction of apoptosis. A similar increase in the free pool of β-catenin was already described in Wnt-induced cells (59). At later stages no full-length β-catenin could be observed, although the cleavage products were found enriched to a high extent in the cytoplasmic fraction. According to our results, the integrity of the core region of β-catenin is not affected by proteolytic cleavage during apoptosis, but the N- and C-terminal regions are removed. Both regions contain transcription activation domains that mediate the transactivation of Wnt-target genes if β-catenin is in the complex together with lymphocyte enhancer-binding factor TCF transcription factors. Concordantly, in gene reporter assays we were able to demonstrate that β-catenin deletion constructs resembling the observed *in vivo* cleavage products had a reduced transcription activation potential. Deletion of the first 115 N-terminal amino acids of β-catenin (fragment B) already showed an over 50% reduced reporter activation compared with the full-length β-catenin. Additional removal of the C terminus reduced the activity down to 30%, and the shortest deletion construct ΔNΔC2 had no significant activity anymore compared with the activation by hTCF-4 alone. This observation is consistent with previous reports demonstrating that deletions of the N- or C terminus both lead to a decreased transactivation potential (53, 60, 61). We therefore assume that an important role for the β-catenin cleavage during apoptosis might be the removal of the β-catenin transcription activation domains to prevent its transactivation potential. Already 8 h after induction of apoptosis the full-length, transactivation-competent β-catenin was entirely cleaved in the cytoplasmic fraction, whereas the transactivation-incompetent cleavage products were enriched to a high extent. Increased levels of β-catenin were found in several melanoma and colon carcinoma cell lines because of mutations in the adenosomatous polyposis coli gene or the β-catenin gene leading to a stabilized protein (62). Furthermore, wild-type β-catenin was found to contain a neoplastic transformation

---

2 O. Huber, unpublished results.
potential in NIH3T3 fibroblasts (63). The same result was obtained for a stabilized mutant form of β-catenin with a point mutation or deletion of the N-terminal region when transfected into RK3E cells (64). The transformation potential of the mutant forms of β-catenin was found to be greatly diminished in deletion constructs with a missing lymphocyte enhancer-binding factor 1/TCF binding site or a deletion of the C-terminal transactivation domain, indicating that the β-catenin transactivation potential is important for its transformation capability. In this respect it was recently found that the TCF-β-catenin complex directly regulates the transcription of both c-myc and the cyclinD1 gene (32, 33). Both gene products are involved in cell proliferation by controlling cell cycle progression and thus might be critical target genes for the neoplastic transformation potential of β-catenin. Removal of the β-catenin transactivation domains by proteolytic cleavage might thus prevent the cell from overcoming the apoptotic program by a deregulation of cell proliferation because of the activation of critical β-catenin target genes like c-Myc or CyclinD1. During recent years β-catenin has become further implicated in the regulation of apoptosis. Consistent with our observations it was reported that the overexpression of a β-catenin deletion construct with truncated N- and C-terminal regions leads to an increased rate of apoptosis in rat hippocampal neurons (65). The overexpression of a dominant negative TCF had the same effect, indicating that the inhibition of TCF/β-catenin signaling induces apoptosis. Proteolytic cleavage of β-catenin thus might not be simply an effect of apoptosis rather than inducing the apoptotic program. The versatile functions of β-catenin within the interplay of cell growth and cell death will be an interesting aspect of apoptosis in rat hippocampal neurons (65). The overexpression of a β-catenin deletion construct with truncated N- and C-terminal regions leads to an increased rate of apoptosis in rat hippocampal neurons (65). The overexpression of a dominant negative TCF had the same effect, indicating that the inhibition of TCF/β-catenin signaling induces apoptosis. Proteolytic cleavage of β-catenin thus might not be simply an effect of apoptosis rather than inducing the apoptotic program. The versatile functions of β-catenin within the interplay of cell growth and cell death will be an interesting aspect of apoptosis in rat hippocampal neurons (65). The overexpression of a β-catenin deletion construct with truncated N- and C-terminal regions leads to an increased rate of apoptosis in rat hippocampal neurons (65). The overexpression of a dominant negative TCF had the same effect, indicating that the inhibition of TCF/β-catenin signaling induces apoptosis. Proteolytic cleavage of β-catenin thus might not be simply an effect of apoptosis rather than inducing the apoptotic program. The versatile functions of β-catenin within the interplay of cell growth and cell death will be an interesting aspect of apoptosis in rat hippocampal neurons (65).