Identification of Four Distinct Pools of Catenins in Mammalian Cells and Transformation-dependent Changes in Catenin Distributions among These Pools

Daniel B. Stewart and W. James Nelson‡
From the Department of Molecular and Cellular Physiology, Stanford University School of Medicine, Stanford, California 94305-5345

Catenins are cytoplasmic proteins that were initially identified in a complex with cadherins, a superfamily of transmembrane glycoproteins important for cell adhesion in normal and disease states. We have used gel filtration to identify four complexes of catenins in extracts from normal and transformed epithelial cells. In normal Madin-Darby canine kidney epithelial cells, a significant fraction of α- and β-catenin and plakoglobin co-elute with cadherin in a high molecular weight complex (complex I). A portion of α-catenin and the remainder of β-catenin and plakoglobin co-elute in a high molecular weight complex that does not contain cadherin (complex II). The remainder of α-catenin elutes in a low molecular weight fraction (complex III). In extracts from two colon carcinoma cell lines, HCT116 and SW480, β-catenin elutes in an additional low molecular weight pool (complex IV) not present in Madin-Darby canine kidney cell extracts. In two subclones derived from SW480 cells, SW-E8 and SW-R2, β-catenin is distributed evenly between high and low molecular weight pools in SW-E8 cells, whereas it elutes primarily in the low molecular weight pool (complex IV) in SW-R2 cells. These changes in β-catenin elution profiles correlate with an increase in transformed phenotype and decreased cell-cell adhesion in the SW-R2 cells.

Cadherins comprise a superfamily of Ca2+-dependent transmembrane glycoproteins that play essential roles in the initiation and stabilization of cell-cell contacts (1). Regulation of cadherin-mediated intercellular adhesion is critical for normal embryonic development and the maintenance of mature epithelial tissues (2, 3). Loss of this regulation has been implicated in disease processes such as invasion and metastasis (4–6). For example, mutations in APC protein that correlate with the tumor polyposis coli (APC)1 tumor suppressor gene (28, 29). In addition, modulation of APC/β-catenin association has been suggested to play a role in the regulation of both cadherin-based cell-cell adhesion and cell motility (30–33).

Studies in Drosophila and Xenopus show that accumulation of cytoplasmic armadillo/β-catenin, not bound to cadherin, correlates with changes in cell fate and transcriptional activation (34–36). Because of the role of armadillo in the wingless pathway and the ability of β-catenin to bind to transcription factor LEF-1/Tcf family members (37–39), alterations in β-catenin levels may affect intracellular signaling in addition to, or in combination with, effects on cadherin-mediated adhesion. For example, mutations in APC protein that correlate with the accumulation of β-catenin may result in excess transcriptional activation by β-catenin-Tcf complexes (40–42). In addition, Drosophila homologs of LEF-1/Tcf have been implicated as components of the wingless signaling pathway (43, 44).

Consistent with the multiple functions assigned to catenins, studies in Madin-Darby canine kidney (MDCK) cells indicate that at least 50% of the catenins within a cell are not associated with cadherin (45). Hinck et al. (14) suggested that catenins may exist as monomers, may dimerize with other catenins, or may associate with other proteins within the cell. Recently, several proteins, including ZO-1, APC protein, EGF receptor, and fascin, have been found to interact with β-catenin (46–49).

Although catenins have been implicated in a variety of cellular processes, the dynamics of cadherin/catenin assembly and the complexes in which they function are poorly understood, especially as they relate to changes in cell phenotype during differentiation or transformation. Therefore, we sought to frac-

* This work was supported by National Institutes of Health Grants GM 35527 and DK45573 (to W. J. N.) and a Howard Hughes Medical Institute predoctoral fellowship (to D. S.). 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: Dept. of Molecular and Cellular Physiology, Beckman Center, Room B-121, Stanford University Medical Center, Stanford, CA 94305-5345. Tel.: 415-725-7596; Fax: 415-498-5286.

α-Catenin, an actin-binding protein that shares 30% sequence identity with vinculin in conserved regions (17–19), is thought to stabilize the cadherin complex at the plasma membrane by linking it to the actin-based cytoskeleton (20, 21). Cadherins cannot mediate strong cell-cell adhesion in cell lines that lack α-catenin, and tumor cell lines show increased invasiveness following mutation and/or down-regulation of α-catenin (22).

Both β-catenin and plakoglobin are members of the armadillo gene family (23–25). armadillo is a segment polarity gene in the Drosophila wingless signaling pathway (26). Expression of truncated forms of β-catenin, which can bind cadherin but not α-catenin, correlates with loss of cadherin function in cancer cell lines (27). Other tumor lines express unusually high levels of β-catenin, possibly due to mutations in the adenomatous polyposis coli (APC)1 tumor suppressor gene (28, 29). In addition, modulation of APC/β-catenin association has been suggested to play a role in the regulation of both cadherin-based cell-cell adhesion and cell motility (30–33).

The extracellular domain of cadherins is responsible for regulating specific homotypic binding (10), and the conserved cytoplasmic domain facilitates adhesion through binding to intracellular proteins, termed catenins (11–13). Each cadherin molecule can bind to either β-catenin or γ-catenin (plakoglobin), which in turn binds α-catenin (14–16).

The abbreviations used are: APC, adenomatous polyposis coli; MDCK, Madin-Darby canine kidney; PAGE, polyacrylamide gel electrophoresis; Rs, Stokes radius; FPLC, fast protein liquid chromatography; EGF, epidermal growth factor.

This paper is available on line at http://www.jbc.org
Catenin Complexes in Epithelial Cells

EXPERIMENTAL PROCEDURES

Cell Lines and Antisera—Human colon carcinoma HCT116 and human colon adenocarcinoma SW480 cells were obtained from ATCC. SW-E8 and SW-R2 cells were kindly provided by Dr. I. Bernard Weinstein (Columbia University) (50). MDCK cells have been described previously (51). Cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. 3G mouse monoclonal antibody, specific for the extracellular domain of canine E-cadherin (52), and rabbit polyclonal antibodies against α-catenin, β-catenin, plakoglobin (53), APC protein (32), and the cytoplasmic domain of mouse E-cadherin (54) have been described previously. A mouse monoclonal antibody specific for fascin (49) was kindly provided by Dr. Pierre McCreas (University of Texas, Houston, TX).

SDS-PAGE and Immunoblotting—For total protein analysis, 3 × 105 cells were washed twice in ice-cold Tris-saline (20 mM Tris-HCl, pH 7.5, 154 mM NaCl) and then solubilized in hot SDS immunoprecipitation buffer (1% SDS, 10 mM Tris-HCl, pH 7.5, 2 mM EDTA). Protein concentrations were determined for each sample using the bicinchoninic acid protein assay reagent kit (BCA)/Pierce. Equal volumes of total protein were separated in 7.5% SDS-PAGE gels, electrophoretically transferred to Immobilon-P (Millipore Corp., Bedford, MA), and processed for immunoblotting with antibodies specific for cadherin, α-catenin, β-catenin, plakoglobin, APC protein, or fascin as described previously (14, 32, 49). Antibodies against cadherin, α-catenin, β-catenin, and plakoglobin were detected using 0.1 μCi/ml 125I-labeled goat anti-rabbit secondary antibody (ICN Pharmaceuticals, Irvine, CA), and then exposed to x-ray film (Hyperfilm, Amersham). Monoclonal antibodies for APC protein and fascin were processed for ECL according to the manufacturer’s protocol (Amersham Corp.). Monoclonal antibodies for APC protein and fascin were visualized with a sheep anti-mouse horseradish peroxidase-conjugated secondary antibody (Amersham). Immunoblots probed with 125I-labeled goat anti-rabbit secondary antibody were visualized using a Molecular Dynamics Storm 820 Phosphor-Imager system (Molecular Dynamics, Sunnyvale, CA).

To determine the distribution of total protein in 0.5% Nonidet P-40-soluble and -insoluble cell fractions, cells were washed twice with Tris-saline, extracted in 1 ml of MEBC buffer (0.5% Nonidet P-40, 50 mM Tris-HCI, pH 7.5, 100 mM NaCl) containing protease inhibitors (0.1 mM Na4VO4, 50 mM NaF, 1 mM Pefabloc (Boehringer Mannheim), and 10 μg/ml each of leupeptin, antipain, chymostatin, and pepstatin A) for 10 min at 4°C. Cell lysates were then sedimented in a Microfuge (Beckman Instruments, Inc., Fullerton, CA) for 10 min. Supernatants were removed, and pellets were solubilized in 200 μl of SDS immunoprecipitation buffer at 100°C for 30 min with occasional vortexing. 1 ml of MEBC was then added to the solubilized pellets, and 200 μl of SDS immunoprecipitation buffer was added to the supernatants to normalize the total volume of samples. Equal volumes of supernatant and pellet were analyzed by Western blotting and quantitated, as above.

Phase Contrast and Indirect Immunofluorescence Microscopy—For phase contrast microscopy, cells were grown 18 h on plastic tissue culture dishes and then photographed using a Zeiss Axiosvert 10 microscope (Carl Zeiss, Thornwood, NY) and Kodak Tri-X Pan 400 film (Kodak). For immunofluorescence, cells were grown on collagen-coated glass coverslips, fixed with 1.75% formaldehyde, permeabilized with 1% Triton X-100, and incubated with antibodies specific for cadherin (1:100 dilution), α-catenin (1:50), β-catenin (1:100), or plakoglobin (1:50). After washing, cells were incubated with rhodamine-conjugated sheep anti-rabbit secondary antibody (Jackson ImmunoResearch Laboratories, West Grove, PA) at a dilution of 1:200. Slides were viewed using an Axioplan microscope equipped for epifluorescence (Carl Zeiss) and photographed using Kodak Elite II Ektachrome slide film.

Size Exclusion Chromatography—Cells were extracted with MEBC buffer as above, incubated at 4°C for 10 min, and sedimented for 10 min in a Microfuge. The supernatant was then transferred to a clean tube, centrifuged in a 100.3 rotor (Beckman Instruments, Inc.) for 30 min at 100,000 × g, and then passed through a 0.22-μm syringe filter (Millipore). 200 μl of extract was loaded on a Superose 6 HR 10/30 column (10 mm × 30 cm; Pharmacia Biotech, Inc.), which had been equilibrated in MEBC buffer containing 1 mM dithiothreitol and 0.1 mM Pefabloc. Proteins were eluted at a flow rate of 0.3 ml/min at 17°C, and 0.5-ml fractions were collected. Protein concentration for each fraction was determined as above. Fractions 7–30 were separated in 1.5-mm 7.5% SDS-PAGE gels, and gels were stained with Coomassie Brilliant Blue R. 15 μg of recombinant α- and β-catenin produced in Escherichia coli (recombinant proteins were kindly provided by Dr. Bill Weis, Stanford University, Stanford, CA) were diluted in MEBC buffer, centrifuged at 100,000 × g, passed through a 0.22-μm syringe filter, and analyzed by gel filtration and Western blotting, as above. For experiments using a mixture of recombinant proteins, 15 μg of α- and β-catenin were incubated on ice for 8 h, diluted in MEBC buffer, and processed as above.

Immunoprecipitations—Following gel filtration, fractions 13–24 were preincubated with 5 μl of preimmune serum and 35 μl of pan-sorbin cells (Calbiochem) and then centrifuged for 10 min at 14,900 × g. 10 μl of antibody specific for cadherin or β-catenin was added to each fraction for 30 min followed by the addition of 65 μl of a 50% slurry of protein A-Sepharose (Pharmacia), rotated for 2 h at 4°C, and washed as described previously (14). Immunoprecipitates were boiled in SDS sample buffer and then divided between replicate 7.5% SDS-PAGE gels, which were processed further for immunoblotting as above. For cadherin immunodepletion, antibody specific for cadherin was used to complete four consecutive immunoprecipitations from MDCK cell extracts, which removed >99% of cadherin from the samples as determined by Western blot analysis. Control immunoprecipitations were done in parallel using nonimmune rabbit sera. Immunodepleted and control samples were then fractionated and processed for immunoblotting as above.

FIG. 1. Morphology of MDCK, HCT116, and SW480 cells. Cells were plated at 85% confluency and grown for 18 h. Phase contrast images were obtained using a Zeiss Axiostar 10 microscope with a × 10 objective. MDCK cells form flat, well defined colonies with compacted cells. HCT116 cells also form a monolayer, but the cell periphery is much less compacted than MDCK cells. SW480 cells both spread out and exhibit loss of contact inhibition as cells begin to grow on top of each other. Bar, 30 μm.
Characterization of Cadherin and Catenin Expression in MDCK, HCT116, and SW480 Cell Lines—Cells were plated at 85% confluence (~$2.5 \times 10^6$ cells/35), grown for 18 h at 37 °C, and examined using phase contrast microscopy. MDCK cells grew in a tight monolayer in which colonies had well defined edges (Fig. 1). Cells were uniform in shape, which generated a characteristic cobblestone appearance of the monolayer. A few rounded cells were found, but they mostly represented dividing cells. HCT116 cells also grew as a monolayer, although the cells did not pack together as tightly as MDCK cells; translucent spaces between cells are evident even within the center of colonies. SW480 cells appeared highly transformed as they were rounded up and grew on top of each other, even before a confluent density and grown for 36 h. Cells were rounded up and grew on top of each other, even before a confluent density and grown for 36 h. Cells were solubilized in buffer containing 1% SDS, and protein concentration was determined for each sample. Equal amounts of total protein were separated by SDS-PAGE. Gels were transferred to Immobilon-P and immunoblotted with antibodies specific for cadherin, α-catenin, β-catenin, or plakoglobin. Secondary 125I-labeled goat anti-rabbit IgG was used to detect primary antibodies, and signals were quantitated using a Phosphor-Imager. Blots shown are representative of one experiment. Protein levels in HCT116 and SW480 cells are plotted relative to levels in MDCK cells; bar graphs show mean results ± S.D. from three independent experiments. B, to determine solubility characteristics of cadherin and catenins in buffer containing 0.5% Nonidet P-40, cells were plated as grown as in A and scraped from plates in MEBC buffer. Samples were sedimented in a Microfuge for 10 min, and the supernatant (Supnt) was removed to a clean tube. Pellets were solubilized by boiling in buffer containing 1% SDS. After normalizing sample volumes, identical volumes from each sample were separated by SDS-PAGE and processed as in A. Blots shown are representative of one experiment. Relative levels of protein in 0.5% Nonidet P-40-soluble and -insoluble fractions are plotted as the mean ± S.D. from three independent experiments. M, MDCK; H, HCT116; S, SW480; E, SW-E8; R, SW-R2.

RESULTS

Identification of Cadherin-dependent and -independent Pools
of Catenins—To examine and compare cadherin- and catenin-containing complexes in different cell lines, we used FPLC Superose 6 gel filtration to separate protein complexes in the molecular weight range of 5–5000 kDa. Lysates from confluent cell lines were centrifuged and filtered and then loaded on a sizing column pre-equilibrated in extraction buffer (for details, see “Experimental Procedures”).

Protein assays on column fractions from FPLC fractionation of MDCK cell extracts revealed that the void volume eluted in fraction 7, that all proteins eluted between fractions 7 and 35, and that approximately half of the proteins eluted between fractions 23 and 29 (Fig. 3A). The peak elution profiles of globular proteins with known molecular weights and Stokes radii are indicated. Coomassie Brilliant Blue staining of an SDS-PAGE, and proteins were visualized using Coomassie Brilliant Blue-R. The relative electrophoretic mobilities of molecular weight markers are shown in the corresponding graphs shown in Fig. 3A. Immunoblots and corresponding graphs shown in Fig. 3A are representative of at least three independent experiments. Values plotted are the mean ± S.D. from three independent experiments. The arrows refer to elution volumes of standard proteins with known molecular weights and Stokes radii: thyroglobulin (\(M_r = 669,000\); \(R_S = 85.0 \text{ Å}\)) (a); apoferritin (\(M_r = 443,000\); \(R_S = 61.0 \text{ Å}\)) (b); catalase (\(M_r = 232,000\); \(R_S = 52.2 \text{ Å}\)) (c); bovine serum albumin (\(M_r = 66,000\); \(R_S = 35.5 \text{ Å}\)) (d); and cytochrome c (\(M_r = 12,400\); \(R_S = 16.3 \text{ Å}\)) (e). B, samples from fractions 7–30 were separated on two 7.5% gels using SDS-PAGE, and proteins were visualized using Coomassie Brilliant Blue-R.

After FPLC Superose 6 gel filtration of an HCT116 cell extract, a single high molecular weight peak of cadherin eluted at fraction 16. Approximately 85% of \(\alpha\)-catenin eluted at fraction 16, and a second peak of \(\alpha\)-catenin eluted at fraction 23/24. In HCT116 cells, \(\beta\)-catenin eluted in two discrete peaks. 85% of \(\beta\)-catenin eluted at a peak at fraction 16, and the remainder eluted at fraction 23. Plakoglobin eluted with peaks at fractions 16 and 23, similar to those of \(\alpha\)- and \(\beta\)-catenin.

Following fractionation of SW480 cell extracts, cadherin eluted as a single peak at fraction 15/16, similar to that of cadherin in MDCK and HCT116 cells. However, the elution profiles of catenins extracted from SW480 cells were very dif-
different from those from either MDCK or HCT116 cells. 30% of α-catenin from SW480 cells eluted in a peak at fraction 15/16. The remainder of α-catenin eluted as a broad peak at fraction 21 with a slight shoulder at fraction 23. 10% of β-catenin eluted as a high molecular weight peak at fraction 15/16; the majority of β-catenin eluted with a broad peak at fraction 23/24. Plakoglobin eluted as three peaks at fractions 16, 21, and 24, respectively. The peaks at fractions 16 and 21 each accounted for approximately 20% of soluble plakoglobin, and the remaining 60% eluted at fraction 24.

Together, these results show that cadherins extracted from different cell types fractionate with very similar profiles with a peak elution in fraction 15/16. This indicates the existence of a single, uniform population of cadherin in these cells (complex I). The elution profiles of each of the catenins were different from each other and different among the cell lines examined. Three separate populations of catenins were detected: a major population of α- and β-catenin and plakoglobin eluted with cadherin in a peak at fraction 16/17 (complexes I/II; see below) in MDCK and HCT116 cells; a second population of α- and β-catenin and plakoglobin eluted in a peak at fraction 23/24 (complexes III/IV, see below) in extracts from some cell lines; a third population of α- and β-catenin and plakoglobin eluted in a peak at fraction 21 in extracts from some cell lines. The relative amounts of catenin in each of these separate populations varied between cell lines.

Characterization of Cadherin and Catenin Expression Levels in SW480 Clonal Derivatives—We noted by light microscopy...
that SW480 cells have a heterogeneous morphology (Fig. 1). SW480 cells have been subcloned into two lines, designated SW-E8 and SW-R2, that have flat epithelial-like and rounded appearances, respectively (Fig. 5) (50). We examined whether there were changes in cadherin and catenin complexes associated with differences in the transformed phenotype and apparent degree of cell-cell adhesion between these subclones. Cells from each clone had similar total levels of cadherin, β-catenin, and plakoglobin as MDCK and HCT116 cells and had the same 3–4 fold increase in total β-catenin present in SW480 cells compared with levels in MDCK and HCT116 cells (Fig. 2). Also, the solubility characteristics of cadherin and catenins were similar for each clone, and were similar to those in the other cell lines examined (Fig. 2).

The FPLC elution profiles of cadherin and each catenin from cell extracts of each subclone are shown in Fig. 6. Fractionation of SW-E8 cell extracts revealed a single peak of cadherin at fraction 15/16. α-Catenin from these cells had a complex elution profile with three separate peaks at fractions 16, 20/21, and 23. 50% of the α-catenin eluted at fraction 16, 30% at fraction 20, and 20% at fraction 23. β-Catenin from SW-E8 extracts was evenly divided between two peaks that eluted at fractions 16 and 23. The elution profile for plakoglobin showed two peaks at fractions 16 and 23/24.

Fractionation of SW-R2 cell extracts revealed that cadherin eluted as a single population with a peak at fraction 16 (Fig. 6). α-Catenin eluted with peaks at fractions 16, 20/21, and 23/24. Approximately 35% of the α-catenin eluted at fraction 16, 55% at fraction 20/21, and 10% at fraction 23/24. Over 95% of the β-catenin eluted as a single peak at fraction 23; however, a small peak of β-catenin consistently eluted at fraction 16. The elution profiles of plakoglobin from SW-R2 and SW-E8 cell extracts were similar: two peaks at fractions 16 and 23/24.

Because of the proportionately low amount of β-catenin co-eluting with cadherin in the SW-R2 cell extracts, we were interested to test whether cadherin from these cells was still complexed with β-catenin. Immunoprecipitations with antibodies specific for cadherin were performed from fractions 13–20, and the immunoprecipitated complexes were analyzed by immunoblotting with antibodies specific for cadherin and β-catenin (Fig. 7). Cadherin immunoprecipitated from SW-R2 cell extracts eluted with a peak in fraction 16. A distinct peak of β-catenin co-immunoprecipitated with cadherin at fraction 16.

We compared the subcellular distributions of cadherin and catenins in SW-E8 and SW-R2 clones by immunofluorescence microscopy. In SW-E8 cells, cadherin, α- and β-catenin appeared primarily at areas of cell-cell contacts with some intracellular staining (Fig. 8). Plakoglobin was diffusely distributed in these cells, but some staining in regions of cell-cell contact was present. In SW-R2 cells, cadherin staining appeared primarily at the periphery of cells, potential regions of cell-cell contact, while the catenins displayed abundant intracellular staining. While some cell borders stained for catenins in SW-R2 cells, this staining was due primarily to the rounded shape of individual cells rather than to specific accumulation of protein at regions of cell-cell contact. These results suggest that the protein pool containing both cadherin and β-catenin is large in SW-E8 cells but small in SW-R2 cells and are consistent with our FPLC data.

**MDCK Cells Have Two High Molecular Weight Catenin Pools**—Elution profiles of cadherins and catenins from MDCK cell extracts revealed that the peak of the high molecular weight catenin pool (fraction 17) eluted one fraction later than the peak of cadherin (Fig. 4). It is possible that the elution profile of catenins included two high molecular weight complexes of which one contains cadherin and one does not. To first confirm the presence of high molecular weight cadherin-catenin complex, we used an antibody against cadherin to immunoprecipitate complexes containing cadherin from fractions 13–24. Immunoblotting these cadherin immunoprecipitates with antibodies against cadherin and β-catenin revealed a complex containing cadherin and β-catenin, which eluted with a peak at fraction 16 (Fig. 9, A and B). Note that the fractions containing the cadherin-catenin complex are coincident with the elution profile of cadherin but not with that of total β-catenin (see Fig. 4).

Next, we immunoprecipitated protein complexes from the same fractions with an antibody against β-catenin. Immunoblots of the co-immunoprecipitated protein complexes with cadherin antibody demonstrated the elution of a protein complex containing cadherin and β-catenin with a peak at fraction 16 (Fig. 9C). However, β-catenin Western blots from these β-catenin immunoprecipitates showed a peak of β-catenin at fraction 17, indicating the presence of a high molecular weight, cadherin-independent pool of β-catenin (Fig. 9D).

To confirm the presence of this cadherin-independent pool, we examined the elution profile of catenins from a MDCK cell extract that had been immunodepleted of cadherin with an antibody against cadherin prior to fractionation by FPLC. Western blots of the FPLC fractions confirmed that cadherin had been removed from the extract (Fig. 10). Despite the absence of the cadherin-catenin complex, a population of α- and β-catenin and plakoglobin co-eluted at fraction 17 in a high molecular weight complex.

The presence of a high molecular weight cadherin-independent pool of catenins suggested that this complex might contain other catenin-binding proteins. To initially analyze this complex, we immunobotted FPLC fractions from MDCK cell extracts with antibodies specific for APC protein and fascin, two proteins shown previously to bind catenins (see Introduction) (Fig. 11). APC protein eluted with a peak in fraction 9, separate from the peak of β-catenin at fraction 17 (Figs. 4 and 11). Fascin eluted with a peak in fraction 28 (Fig. 11); however, upon overexposure of the blot, a small amount of fascin could be detected in fraction 18, partially overlapping the peak of β-catenin. While both APC protein and fascin fractionated separately from the major peak of β-catenin, these proteins bind
only a small portion of total cellular β-catenin, and levels of fascin-β-catenin complexes have been demonstrated to be relatively low in MDCK cell extracts in comparison with those from A-431 and HeLa cells.3

Recombinant Catennins Co-fractionate with the Low Molecular Weight Catenin Pool—Our analysis of the elution profiles of catennins showed that both α- and β-catenin elute in a low molecular weight peak at fraction 23. We sought to determine the molecular organization of catennins in this fraction by comparing the elution profiles of pure, recombinant α- and β-catenin; recombinant proteins were fractionated under buffer conditions identical to those used for extraction and gel filtration of proteins from MDCK, HCT116, and SW480 cells. Recombinant α-catenin purified from E. coli extracts lacked the 50 N-terminal amino acids due to proteolytic degradation during purification. Recombinant α-catenin eluted in fraction 23 (Fig. 12A), similar to that of α-catenin found in the low molecular weight fractions from whole cell extracts. The major peak of recombinant β-catenin eluted at fraction 23, and this peak co-fractionated with the β-catenin in the low molecular weight fractions in the HCT116 and SW480 lines. Small amounts of higher molecular weight aggregates of β-catenin occasionally appeared in fractions 12–20. After incubation at 4 °C for 8 h, a mixture of recombinant α- and β-catenin eluted identically to recombinant proteins fractionated individually, with peaks at fraction 23 (Fig. 12A). Western blots of immunoprecipitations specific for β-catenin from fractionated samples of recombinant α- and β-catenin mixtures revealed that heterodimers of α- and β-catenin eluted at fraction 23 (Fig. 12B).

3 P. McCrea, personal communication.
DISCUSSION

Cadherin and catenins play important roles in cell-cell adhesion, migration, development of cellular polarity, and intracellular signaling (1, 35, 55–57). Incorrect regulation of cadherin-catenin complex formation and functions is frequently associated with disruption of cell-cell adhesion, changes in cell morphology, and the progression of cell transformation (5, 58, 59). This range of cellular functions indicates that these proteins comprise multiple, distinct complexes within cells. In the present study we have examined cadherin- and catenin-containing complexes from cells representing normal (MDCK), moderately transformed (HCT116, SW-E8), and highly transformed (SW-R2) phenotypes.

All cells examined had similar levels of cadherin, which fractionated as a single, high molecular weight complex. We found that catenins co-fractionated and co-immunoprecipitated with this pool of cadherin. However, the relative amount of catenins complexed with this cadherin pool differed between these cell lines. In MDCK cells, which form tight cell-cell contacts, a substantial portion of both α- and β-catenin co-fractionated with cadherin and could be co-immunoprecipitated in a complex with cadherin. However, in SW-R2 cells, which exhibit poor cell-cell adhesion, relatively little β-catenin co-eluted with the high molecular weight cadherin complex, although the total amount of β-catenin in SW-R2 cells was 2–3 times greater than that in MDCK cells. In SW-R2 cells, >95% of β-catenin fractionated in a low molecular weight complex. Since all cells were treated identically, it is unlikely that β-catenin simply dissociated from cadherins during protein extraction and fractionation. Also, some β-catenin co-immunoprecipitated with cadherin in fractionated SW-R2 extracts. The mechanism that causes β-catenin to accumulate in the low molecular weight complex may involve mutations in APC protein that do not target β-catenin for degradation (28). We note that our previous studies have shown that this low molecular weight pool of β-catenin is competent to bind the cytoplasmic domain of cadherin (16).

The estimated molecular mass for the cadherin complex (~1400 kDa) is much higher than that calculated for a monomeric cadherin-β-catenin-α-catenin complex based upon globular protein standards (~300 kDa). This apparent high molecular weight may have been due to the presence of multimers, the presence of additional proteins in the complex, or aberrant migration during gel filtration due to unusual shape. It is noteworthy that the cadherin complex extracted from SW-R2 cells eluted in the same fraction as that of cadherin from adherent MDCK cells, although the SW-R2 cadherin complex

**FIG. 7. β-Catenin from SW-R2 cells co-immunoprecipitates with cadherin.** Protein complexes from fractions 13–20 were immunoprecipitated with antibodies specific for the cytoplasmic domain of cadherin. Immunoprecipitated complexes were washed stringently and then separated by SDS-PAGE. Proteins were transferred to Immobilon-P membranes, which were subsequently immunoblotted with antibodies specific for cadherin and β-catenin.

**FIG. 8.** Subcellular localization of cadherin and catenins in SW-E8 and SW-R2 cells. Cells were grown on glass coverslips, washed thoroughly, fixed with 1.75% formaldehyde, and permeabilized in buffer containing 1% Triton X-100. Samples were individually incubated with antibodies specific for cadherin, α-catenin, β-catenin, or plakoglobin, followed by rhodamine-conjugated sheep anti-rabbit secondary antibody. Note the large intracellular pools of catenins in SW-R2 cells in comparison with SW-E8 cells. Bar, 15 μm.

**FIG. 9.** β-Catenin exists in cadherin- and non-cadherin-containing complexes in MDCK cell extracts. Following Superose 6 gel filtration chromatography of proteins extracted from MDCK cells extracted with MBEC buffer, fractions 13–24 were processed for immunoprecipitation with antibodies specific for either cadherin (A and B) or β-catenin (C and D) under non-denaturing conditions. Immunoprecipitated complexes were separated by SDS-PAGE, transferred to Immobilon-P, and immunoblotted with antibodies specific for either cadherin (A and C) or β-catenin (B and D). In cadherin immunoprecipitates, both cadherin and β-catenin eluted with a peak at fraction 16. In β-catenin immunoprecipitates, however, cadherin eluted with a peak at fraction 16, while β-catenin eluted with a peak at fraction 17. The relative electrophoretic mobilities of molecular weight markers are shown to the left of each immunoblot.
had relatively little β-catenin (see above). This result indicates that the apparent high molecular weight of the cadherin-catenin complex might be due to multimers of cadherin rather than due to the association of catenins, per se. In support of this notion, it has been reported recently that cadherins may form dimers and higher ordered structures (60).

In addition to the pool of cadherin-associated catenins, we found evidence for multiple pools of catenins that did not co-elute with cadherin. One population of catenins had an elution profile similar to that of the cadherin-catenin complex but did not contain cadherin (complex II). The apparent molecular mass of this complex was high (~1100 kDa) relative to the elution patterns of pure, recombinant α- and β-catenin. It is possible that other proteins associate with this population of catenins. However, the elution profiles of APC protein and fascin, which have been shown previously to bind β-catenin independently of cadherin (46, 49), did not overlap with this population of catenins. The investigation of this high molecular weight catenin complex will be the subject of future studies.

The other major peak of catenins eluted at fraction 23/24 (complex III/IV). It is noteworthy that pure, recombinant α- and β-catenin eluted individually at fraction 23. Therefore, cellular catenins that co-elute in these fractions could be monomers, heterodimers, or homodimers. To attempt to resolve the organization of catenins in these fractions, we separated a mixture of pure, recombinant α- and β-catenin. We found that these proteins co-eluted in fraction 23/24 and could be co-immunoprecipitated as an α/β-catenin heterodimer.

Fig. 10. Cadherin-independent catenins from MDCK cell extracts fractionate in both high and low molecular weight complexes. Cadherin was immunodepleted from MDCK cell extracts prior to fractionation by Superose 6 gel filtration. Fractions 7–30 were separated by SDS-PAGE, transferred to Immobilon-P, and immunoblotted with antibodies specific for E-cadherin, α- and β-catenin, and plakoglobin. Primary antibodies were detected using 125I-labeled goat anti-rabbit secondary antibody. α-Catenin eluted with peaks at fractions 17 and 23/24. Both β-catenin and plakoglobin elute as a single population with a peak at fraction 17.

Fig. 11. APC protein and fascin do not co-fractionate with the high molecular weight peak of catenins. MDCK cell extracts were fractionated by Superose 6 gel filtration. Fractions 7–30 were separated by SDS-PAGE, transferred to Immobilon-P, and immunoblotted with antibodies specific for APC protein and fascin. Primary antibodies were detected using horseradish peroxidase-conjugated sheep anti-mouse secondary antibody. APC protein elutes with a peak at fraction 9, and fascin elutes with a peak at fraction 28.

Fig. 12. A, Superose 6 gel filtration chromatography of pure, recombinant α- and β-catenin. Purified, bacterially expressed recombinant α- and β-catenin were fractionated separately (rows 1 and 2) or as a mixture (rows 3 and 4) under conditions identical to those used to fractionate cell extracts. Fractions 7–30 were immunoblotted with antibodies specific for either α-catenin (rows 1 and 3) or β-catenin (rows 2 and 4). Recombinant α- and β-catenin eluted with a peak at fraction 23, when fractionated either separately or as a preincubated mixture. B, immunoprecipitation of α-catenin-β-catenin heterodimers. A mixture of recombinant α- and β-catenin were fractionated by gel filtration. Fraction 23 was processed for immunoprecipitation with antibodies specific for β-catenin. Immunoprecipitated complexes were washed stringently and then processed for immunoblotting with antibodies against α-catenin (a) and β-catenin (b). The relative electrophoretic mobilities of molecular weight markers are shown to the left of the immunoblots.
ultracentrifugation of pure, recombinant α-catenin indicates that α-catenin can also form homodimers. A large pool of β-catenin in MDCK cells in the absence of corresponding pools of β-catenin or plakoglobin indicates that this low molecular weight pool comprises either monomers or homodimers. That cellular α-catenin may be present as homodimers in vivo is supported by our observation that α-catenin from MDCK cells eluted with a peak at the same fraction as α/β heterodimers.

The amount of catenins in complexes I–IV varies relative to the state of cellular transformation, as assessed by cell organiza-

Catenin Complexes in Epithelial Cells

4 B. Weiss, personal communication.

In summary, we have identified four distinct pools of catenins in extracts of epithelial cells. Significantly, we have shown that the extent of cell-cell adhesion and transformed cell phenotype correlates with the relative amounts of β-catenin in high and low molecular weight pools. Furthermore, we have identified a high molecular weight complex of catenins that is not bound directly to cadherins. The identification of these pools of catenins provides a foundation for future studies on mechanisms that determine the distribution of catenins among these different pools. An understanding of these mechanisms may provide new insights into metabolic pathways involved in cellular differentiation and transformation.

Acknowledgments—We thank Dr. I. Bernard Weinstein (Columbia University) for providing the SW-E8 and SW-R2 cells and Dr. Bill Weiss (Stanford University) for pure, recombinant α- and β-catenin. We thank members of the Nelson laboratory, particularly Inke Naitke and Peter Piepenhagen, for helpful discussions and for critically reading the manuscript.

REFERENCES

1. Takeichi, M. (1991) Science 251, 1451–1455
2. Huber, O., Bierkamp, C., and Kemler, R. (1996) Curr. Opin. Cell Biol. 8, 685–691
3. Larue, L., Antos, C., Butz, S., Huber, O., Delmas, V., Dominis, M., and Kemler, R. (1996) Development 122, 3185–3194
4. Nelson, W. J., Wilson, R., Wellner, D. A., Mays, R., McNeill, H., Siemens, K. A. (1992) Cold Spring Harbor Symp. Quant. Biol. 57, 621–630
5. Oka, H., Shiozaki, H., Kobayashi, K., Inoue, M., Tahara, H., Kobayashi, T., Takatatsu, Y., Matsuosy, N., Hirano, S., Takeichi, M., and Mori, T. (1993) Cancer Res. 53, 1069–1071
6. Takeichi, M. (1993) Curr. Op. Cell Biol. 5, 806–811
7. Dorudi, S., Sheffield, J. P., Poulsom, R., Northover, J. M. A., and Hart, I. R. (1993) Am. J. Pathol. 142, 981–986
8. Birchmeier, W., Hulsken, J., and Behrens, J. (1995) Cancer Surveys 24, 129–140
9. Gamallo, C., Palacios, J., Suarez, A., Pizarro, A., Navarro, P., Quintanilla, M., and Cano, A. (1993) Am. J. Pathol. 142, 887–903
10. Nose, A., Tanji, K., and Takeichi, M. (1996) Cell 81, 147–155
11. Nagafuchi, A., and Takeichi, M. (1988) EMBO J. 7, 3679–3684
12. Ozawa, M., Baribault, H., and Kemler, R. (1989) EMBO J. 8, 1711–1717
13. Gunther, B. M., and McCreA, P. D. (1990) J. Cell Sci. Suppl. 17, 155–158
14. Hinck, L., Nathke, I. S., Papkoff, J., and Nelson, W. J. (1994) J. Cell Biol. 125, 1327–1340
15. Aberle, H., Butz, S., Stappert, J., Weissig, H., Kemler, R., and Hoschuetzky, H. (1994) J. Cell Sci. 105, 3655–3663
16. Jou, T. S., Stewart, D. B., Stappert, J., Nelson, W. J., and Marris, J. A. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 5067–5071
17. Herrenknecht, K., Ozawa, M., Eckerskorn, C., Lottspeich, F., Lenter, M., and Kemler, R. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 9156–9160
18. Nagafuchi, A., Takeichi, M., and Tsukita, S. (1991) Cell 65, 849–857
19. Rimm, D. L., Koshov, E., Kebriaei, P., Bianco, I., and Morrow, J. S. (1995) Nature 375, 8813–8817
20. Hirano, S., Nose, A., Hatta, K., Kawakami, A., and Takeichi, M. (1997) J. Cell Biol. 125, 2391–2398
21. Ozawa, M., Ringwald, M., and Kemler, R. (1996) Proc. Natl. Acad. Sci. U. S. A. 87, 4246–4250
22. Vermeulen, S. J., Bruyneel, E. A., Bracke, M. E., and Gumbiner, B. (1991) Science 255, 396–414
23. Oyama, T., Kanai, Y., Ochiai, A., Akimoto, S., Oda, T., Yanagihara, K., Nagafuchi, A., Tsukita, S., Shibamoto, S., Ito, F., Takeichi, M., Matsuda, H., and Hirohoshi, S. (1994) Cancer Res. 54, 6282–6287
24. Munemitsu, S., Albert, I., Souza, B., Rubinfeld, B., and Polakis, P. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 3046–3050
25. Rubinfeld, B., Albert, I., Porfiri, E., Fiol, C., Munemitsu, S., and Polakis, P. (1996) Science 272, 1023–1026
26. Herrnstein, M. L., and Gordon, J. J. (1995) Science 270, 1203–1207
27. Herrnstein, M. L., and Gordon, J. J. (1995) J. Cell Biol. 129, 489–506
28. Nakahze, I. S., Adams, C. L., Polakis, P., Sellin, J. H., and Nelson, W. J. (1996) J. Cell Biol. 134, 165–179
29. Barth, A. I. M., Pollack, A. L., Autschbach, Y., Mostov, K. E., and Nelson, W. J. (1997) J. Cell Biol. 136, 693–706
30. McCrea, P. D., Turck, C. W., and Gumbiner, B. (1991) Science 254, 1359–1361
31. Butz, S., Stappert, J., Weissig, H., and Kemler, R. (1992) Science 257, 1142–1144
32. Klingensmith, J., and Nussle, R. (1994) Dev. Biol. 166, 396–414
33. Herrnstein, M. L., and Gordon, J. J. (1995) Science 270, 1203–1207
34. Herrnstein, M. L., and Gordon, J. J. (1995) J. Cell Biol. 129, 489–506
35. Nakahze, I. S., Adams, C. L., Polakis, P., Sellin, J. H., and Nelson, W. J. (1996) J. Cell Biol. 134, 165–179
36. Barth, A. I. M., Pollack, A. L., Autschbach, Y., Mostov, K. E., and Nelson, W. J. (1997) J. Cell Biol. 136, 693–706
37. McCrea, P. D., Breier, W. M., and Gumbiner, B. M. (1993) J. Cell Biol. 123, 477–484
38. Peifer, M., Orsulic, S., Pai, L. M., and Loureiro, J. (1995) Dev. Suppl. 993, 163–176
39. van Leeuwen, F., Sams, C. H., and Nussle, R. (1994) Nature 368, 342–344
40. Behrens, J., von Kries, J. P., Kuhl, M., Bruhn, L., Wedlich, D., Grosschedl, R., and Birchmeier, W. (1996) Science 275, 638–642
41. Huber, O., Kern, R., McLaughlin, J., Ohsugi, M., Herrmann, B. G., and Kemler, R. (1996) Mech. Dev. 59, 3–10
42. Molenar, M., van de Wetering, M., Oosterwegel, M., Petersen-Maduro, J., Godsave, S., Korinek, V., Rosse, J., Destree, O., and Clevers, H. (1996) Cell 86, 391–399
43. Korinek, V., Barker, N., Morin, P. J., van Wichen, D., de Weger, R., Kinzler, K. W., Vogelstein, B., and Clevers, H. (1997) Science 275, 1784–1787
44. Morin, P. J., Sparks, A. B., Korinek, V., Barker, N., Clevers, H., Vogelstein, B.,
42. Rubinfeld, B., Robbins, P., El-Gamil, M., Albert, I., Porfiri, E., and Polakis, P. (1997) *Science* **275**, 1787–1790
43. Brunner, E., O., P., Schweizer, L., and Basler, K. (1997) *Nature* **385**, 829–833
44. van de Wetering, M., Cavallo, R., Dooijes, D., van Beest, M., van Es, J., Leuener, J., Ypma, A., Hursl, D., Jones, T., Bejsovec, A., Peifer, M., Martin, M., and Clevers, H. (1997) *Cell* **88**, 789–799
45. Nathke, I. S., Hinck, L., Swedlow, J. R., Papkoff, J., and Nelson, W. J. (1994) *J. Cell Biol.* **125**, 1341–1352
46. Rubinfeld, B., Souza, B., Albert, I., Muller, O., Chamberlain, S. H., Masiarz, F. R., Munemitsu, S., and Polakis, P. (1993) *Science* **262**, 1731–1734
47. Hoschuetzky, H., Aberle, H., and Kemler, R. (1994) *J. Cell Biol.* **127**, 1375–1380
48. Rajasekaran, A. K., Hojo, M., Huima, T., and Rodriguez-Boulan, E. (1996) *J. Cell Biol.* **132**, 451–463
49. Tao, Y. S., Edwards, R. A., Tubb, B., Wang, S., Bryan, J., and McCrea, P. D. (1996) *J. Cell Biol.* **134**, 1271–1281
50. Tomita, N., Jiang, W., Hibshoosh, H., Warburton, D., Kahn, S. M., and Weinstein, I. B. (1992) *Cancer Res.* **52**, 6840–6847
51. Nelson, W. J., and Veshnock, P. J. (1986) *J. Cell Biol.* **103**, 1751–1765
52. Shore, E. M., and Nelson, W. J. (1991) *J. Biol. Chem.* **266**, 19672–19680
53. Hinck, L., Nelson, W. J., and Papkoff, J. (1994) *J. Cell Biol.* **124**, 729–742
54. Marrs, J. A., Napolitano, E. W., Murphy, E. C., Mays, R. W., Reichardt, L. F., and Nelson, W. J. (1993) *J. Cell Biol.* **123**, 149–164
55. Rodriguez-Boulan, E., and Nelson, W. J. (1989) *Science* **245**, 718–725
56. Nagafuchi, A., Ishibara, S., and Tsukita, S. (1994) *J. Cell Biol.* **127**, 235–245
57. Miller, J. R., and Moon, R. T. (1996) *Genes Dev.* **10**, 2527–2539
58. Breen, E., Steele, G., and Mercurio, A. M. (1996) *Ann. Surg. Oncol.* **2**, 378–385
59. Vermeulen, S., van Marck, V., van Heerde, L., van Roy, F., Bracke, M., and Marcel, M. (1996) *Pathol. Res. Pract.* **192**, 694–707
60. Brieher, W. M., Yap, A. S., and Gumbiner, B. M. (1996) *J. Cell Biol.* **135**, 487–496
61. Behrens, J., Vakaet, L., Friis, R., Winterhager, E., van Roy, F., Marcel, M., and Birchmeier, W. (1993) *J. Cell Biol.* **120**, 757–766
62. Hamaguchi, M., Matsuyoshi, N., Ohsaki, Y., Gotoh, B., Takeichi, M., and Nagai, Y. (1993) *EMBO J.* **12**, 307–314
63. Warren, S. L., Handel, L. M., and Nelson, W. J. (1988) *Mol. Cell Biol.* **8**, 632–646

Catenin Complexes in Epithelial Cells