Differential Nuclear Translocation and Transactivation Potential of β-Catenin and Plakoglobin

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Abstract. β-Catenin and plakoglobin are homologous proteins that function in cell adhesion by linking cadherins to the cytoskeleton and in signaling by transactivation together with lymphoid-enhancing binding/T cell (LEF/TCF) transcription factors. Here we compared the nuclear translocation and transactivation abilities of β-catenin and plakoglobin in mammalian cells. Overexpression of each of the two proteins in MDCK cells resulted in nuclear translocation and formation of nuclear aggregates. The β-catenin-containing nuclear structures also contained LEF-1 and vinculin, while plakoglobin was inefficient in recruiting these molecules, suggesting that its interaction with LEF-1 and vinculin is significantly weaker. Moreover, transfection of LEF-1 translocated endogenous β-catenin, but not plakoglobin to the nucleus. Chimeras consisting of Gal4 DNA-binding domain and the transactivation domains of either plakoglobin or β-catenin were equally potent in transactivating a Gal4-responsive reporter, whereas activation of LEF-1–responsive transcription was significantly higher with β-catenin. Overexpression of wild-type plakoglobin or mutant β-catenin lacking the transactivation domain induced accumulation of the endogenous β-catenin in the nucleus and LEF-1–responsive transactivation. It is further shown that the constitutive β-catenin–dependent transactivation in SW480 colon carcinoma cells and its nuclear localization can be inhibited by overexpressing N-cadherin or α-catenin. The results indicate that (*a*) plakoglobin and β-catenin differ in their nuclear translocation and complexing with LEF-1 and vinculin; (*b*) LEF-1–dependent transactivation is preferentially driven by β-catenin; and (*c*) the cytoplasmic partners of β-catenin, cadherin and α-catenin, can sequester it to the cytoplasm and inhibit its transcriptional activity.

Cell–cell adhesion plays an important role in tissue morphogenesis and homeostasis (Gumbiner, 1996; Huber et al., 1996*a*), and is commonly mediated by cadherins, a family of Ca^{2+}-dependent transmembrane adhesion receptors (for review see Geiger and Ayalon, 1992; Kemler, 1992; Takeichi, 1991, 1995). Cadherins were shown to form homophilic interactions with similar receptors on neighboring cells, whereas their cytoplasmic domains interact with the cytoskeleton. The latter interactions are essential for stable adhesion and are mediated via β-catenin, or its closely related homologue γ-catenin (plakoglobin), which interacts with microfilaments through α-catenin (Kemler, 1993; Geiger et al., 1995; Jou et al., 1995; Rimm et al., 1995) and α-actinin (Knudsen et al., 1995).

In addition to the direct role of β-catenin/plakoglobin in promoting adhesion, their homologue in *Drosophila* armadillo (Peifer and Weischaus, 1990), was also shown to be involved in the wingless (wg) signaling pathway that regulates cell fate during development (Peifer, 1995; Orsulic and Peifer, 1996). In *Xenopus*, β-catenin participates in the wnt-signaling pathway that determines body axis formation (for review see Miller and Moon, 1996; Gumbiner, 1995, 1996), and overexpression of β-catenin and plakoglobin in *Xenopus* embryos was shown to induce double axis formation (Funayama et al., 1995; Karnovsky and Klymkowsky, 1995; Merriam et al., 1997; Miller and Moon 1997; Rubenstein et al., 1997). In cultured cells, wnt overexpression elicits adhesion-related responses and increased levels of β-catenin and plakoglobin (Bradley et al., 1993; Hinck et al., 1994; Papkoff et al., 1996). β-Catenin levels are regulated by glycogen synthase kinase-3β and adenomatous polyposis coli (APC)[1] tumor suppressor protein (Papkoff et al., 1996; Rubinfeld et al., 1996; Yost et al., 1996) which are thought to target β-catenin for degradation by the ubiquitin–proteasome system (Aberle et al.,

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1. *Abbreviations used in this paper*: aa, amino acid(s); APC, adenomatous polyposis coli; DBD, DNA-binding domain; HA, hemagglutinin; LEF, lymphoid-enhancing binding factor; TCF, T cell factor; VSV, vesicular stomatitis virus; wt, wild-type.

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The Journal of Cell Biology, Volume 141, Number 6, June 15, 1998 1433–1448
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1997; Orford et al., 1997; Salomon et al., 1997). When β-catenin levels are high, it can associate with architectural transcription factors of the lymphoid-enhancing binding factor/T cell factor (LEF/TCF) family and translocate into the nucleus (Behrens et al., 1996; Huber et al., 1996*b*; Molenaar et al., 1996). In the nucleus, the β-catenin–LEF/TCF complex activates transcription of LEF/TCF-responsive genes that are not yet known in mammalian cells (Morin et al., 1997), but have partially been characterized in *Xenopus* (Brannon et al., 1997) and *Drosophila* (Riese et al., 1997; van de Wetering et al., 1997).

Elevation of β-catenin in colon carcinoma cells that express a mutant APC molecule (Powell et al., 1992; Polakis, 1997), or in melanoma where mutations in the NH_2-terminal domain of β-catenin were detected (both inhibiting β-catenin degradation) is oncogenic, most probably due to constitutive activation of target genes which contributes to tumor progression (Korinek et al., 1997; Morin et al., 1997; Peifer, 1997; Rubinfeld et al., 1997). Interestingly, plakoglobin was shown to suppress tumorigenicity when overexpressed in various cells (Simcha et al., 1996; Ben-Ze'ev, 1997), and displays loss of heterozigosity in sporadic ovarian and breast carcinoma (Aberle et al., 1995). Moreover, upon induction of plakoglobin expression in human fibrosarcoma and SV-40–transformed 3T3 cells β-catenin is displaced from its complex with cadherin and directed to degradation (Salomon et al., 1997).

In the present study we characterized the mechanisms underlying nuclear accumulation of β-catenin and/or plakoglobin and identified some of the partners associated with both proteins in the nucleus. Furthermore, we compared the nuclear translocation and transactivation abilities of wild-type (wt) and mutant β-catenin and plakoglobin constructs and found that these two proteins differ considerably in these properties, and demonstrated that N-cadherin, as well as α-catenin, can drive β-catenin from the nucleus to the cytoplasm and consequently block activation of LEF-1–responsive transcription. We propose that the deregulated transactivation associated with elevated β-catenin in certain tumors can be suppressed by cadherins and α-catenin.

Materials and Methods

Cell Culture and Transfections

Canine kidney epithelial cells MDCK, human fibrosarcoma HT1080, 293-T human embryonic kidney cells, Balb/C mouse 3T3, and human colon carcinoma SW480 cell lines were cultured in DME plus 10% calf serum (Gibco Laboratories, Grand Island, NY) at 37°C in the presence of 7% CO_2. The human renal carcinoma cell line KTCTL60 (Simcha et al., 1996) was grown in RPMI medium and 10% calf serum. Cells were transiently transfected with the cDNA constructs described below, using Ca^{2+}-phosphate or lipofectamine (Gibco Laboratories) and the expression of the transgene was assessed between 24 and 48 h after transfection. In some experiments, the expression of the stably transfected NH_2 terminus-deleted β-catenin (*Δ57*) (Salomon et al., 1997) was enhanced in HT1080 cells by overnight treatment with 2 mM sodium butyrate.

Construction of Plasmids

The β-catenin, plakoglobin, and α-catenin constructs were cloned in frame into the pCGN expression vector containing a 16-amino acid (aa) hemaglutinin (HA) tag at the NH_2 terminus (see Fig. 1; Tanaka and Herr, 1990). HA–β-catenin was obtained by PCR amplification of the 5′ end of mouse β-catenin (Butz et al., 1994) using oligonucleotides 5′-ACCTTCTAGAATGGCTACTCAAGCTGACCTG-3′ and 5′-ATGAGCAGCGTCAAACTGCG-3′. The XbaI/SphI fragment of the PCR product and the SphI/BamHI fragment of mouse β-catenin were joined and cloned into the pCGN vector. A β-catenin mutant containing armadillo repeats 1–10 (see Fig. 1, *HA β-catenin 1-ins*) was obtained using oligonucleotides 5′-ACCTTCTAGATTGAAACATGCAGTTGTCAATTTG-3′ and 5′-ACCTGGATCCAGCTCCAGTACACCCTTCG-3′. The amplified fragment was cloned into pCGN as an XbaI/BamHI fragment. Vesicular stomatitis virus (VSV)-tagged β-catenin at the COOH terminus was obtained by PCR amplification of the 3′ end of *Xenopus* β-catenin (McCrea et al., 1991) using 5′-AACTGCTCCTCTTACTGA-3′ and 5′-TATCCCGGGTCAAGTCAGTGTCAAACCA-3′. An XbaI/SmaI fragment of the amplified product was joined to an XbaI/EcoRI digest of *Xenopus* β-catenin from Bluescript and then cloned into the pSY-1 plasmid containing an 11-aa VSV glycoprotein tag (Kreis, 1986) at the COOH terminus. The product was subcloned into pCI-neo (Promega Corp., Madison, WI).

HA-tagged human plakoglobin (see Fig. 1, *HA plakoglobin*) was obtained by PCR amplification of the 5′ end of human plakoglobin cDNA (pHPG 5.1) (Franke et al., 1989) using 5′-ACCTTCTAGAATGGAGGTGATGAACCTGATGG-3′ and 5′-AGCTGAGCATGCGGACCAGAGC-3′ oligonucleotide primers. The XbaI/SphI fragment of the PCR product and the SphI/BamHI fragment of human plakoglobin cDNA were cloned into the pCGN vector. A plakoglobin mutant containing armadillo repeats 1–10 (see Fig. 1, *HA plakoglobin 1-ins*) was amplified using 5′-ACCTTCTAGACTCAAGTCGGCCATTGTGC-3′ and 5′-ACCTGGATCCTGCTCCGGTGCAGCCCTCC-3′ oligonucleotides. The amplified fragment was cloned into the XbaI/BamHI site of pCGN. A COOH terminus VSV-tagged plakoglobin (see Fig. 1, *plakoglobin-VSV*) was obtained by amplifying the 3′ terminus of plakoglobin cDNA using 5′-AGGCCGCCCGGGCAGCATG-3′ and 5′-CGCATGGAGATCTTCCGGCTC-3′ oligonucleotide primers. The EcoRI/BglII fragment of the plakoglobin cDNA and the BglII/SmaI fragment of the amplified product were cloned in frame with the COOH terminus VSV tag into the pSY-1 plasmid. VSV-tagged plakoglobin was recloned into the pCI neo-expression vector (Promega Corp.) as an EcoRI/NotI fragment.

HA-tagged α-catenin (see Fig. 1, *HA α-catenin*) was constructed by amplification of the 5′ end of chicken α-catenin (Johnson et al., 1993) using 5′-ACCTTCTAGAATGACGGCTGTTACTGCAGG-3′ and 5′-GCCTTCTTAGAGCGCCCTCG-3′ oligonucleotide primers. The XbaI/ApaI fragment of the PCR product and the ApaI/KpnI fragment of chicken α-catenin from pLK–α-catenin were cloned into the pCGN vector. An HA-tagged mutant α-catenin (see Fig. 1, *HA α-catenin Δβ*) lacking the β-catenin binding site (aa 118–166) was obtained by PCR-based mutagenesis using two fragments from the 5′ end of α-catenin corresponding to aa 1–117 and 167–303, and oligonucleotides 5′-ACCTTCTAGAATGACGGCTGTTACTGCAGG-3′, 5′-TGCCAGCGGAGCAGGGGTCATCAGCAAAC-3′, 5′-CTGCTCCGCTGGCACCGAGCAGGATCTG-3′ and 5′-ACGTTGCTCACTGAAGGTCG-3′. The two fragments were joined, and the product was amplified using 5′-ACCTTCTAGAATGACGGCTGTTACTGCAGG-3′ and 5′-ACGTTGCTCACTGAAGGTCG-3′ primers. The XbaI/BamHI fragment of the mutant construct and the BamHI/KpnI fragment of chicken α-catenin from HA-α-catenin were cloned into the pCGN vector. HA-tagged LEF-1 (Huber et al., 1996*b*) at the COOH terminus was a gift of R. Kemler (Max Planck Institute, Freiburg, Germany).

The DNA-binding domain of Gal4 (Gal4DBD) was obtained by PCR using a 5′-ACCTTCTAGAATGAAGCTACTGTCTTCTATC-3′ oligonucleotide with an XbaI site, and 5′-ACCTGAGCTCCGATACAGTCAACTGTCTTTG-3′ with a SacI site (see Fig. 1, *Gal4DBD β-catenin* and *Gal4DBD plakoglobin*), and with the antisense primer 5′-ACCTGGATCCTACGATACAGTCAACTGTCTTTG-3′ creating a BamHI site (see Fig. 1, *Gal4DBD*). Fragments corresponding to the COOH terminus of β-catenin (aa 682–781) and plakoglobin (aa 672–745) were obtained by PCR using sense primers containing a SacI site and antisense primers containing a BamHI site. The XbaI/SacI fragment of Gal4DBD and the SacI/BamHI fragments of β-catenin and plakoglobin were joined and cloned into pCGN (see Fig. 1). The XbaI/BamHI fragment of Gal4DBD was cloned into pCGN and used as control in transactivation assays. A cytomegalovirus promoter-driven N-cadherin cDNA was used (Salomon et al., 1992). The validity of the constructs shown in Fig. 1 was verified by sequencing and then the size of the proteins were determined after transfection into 293-T and MDCK cells by Western blotting with anti-HA and anti-VSV antibodies.

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Transactivation Assays

Transactivation assays were conducted with SW480 and 293-T cells grown in 35-mm-diam dishes that were transfected with 0.5 μg of a plasmid containing a multimeric LEF-1 consensus–binding sequence driving the luciferase reporter gene (TOPFLASH), or a mutant inactive form (FOPFLASH, provided by H. Clevers and M. van de Wetering, University of Utrecht, Utrecht, The Netherlands). A plasmid encoding β-galactosidase (0.5 μg) was cotransfected to enable normalization for transfection efficiency. The relevant plasmid expressing catenin constructs (4.5 μg) was cotransfected with the reporter, or an empty expression vector was included. After 24–48 h, expression of the reporter (luciferase) and the control (β-galactosidase) genes were determined using enzyme assay systems from Promega Corp.

The Gal4RE reporter plasmid for determining transactivation by Gal4DBD chimeras was constructed as follows: oligonucleotides comprising a dimer of 17 nucleotides of the Gal4 binding sequences (Webster et al., 1988) containing a SacI site at the 5′ end and a BglII site at the 3′ end, were obtained by PCR amplification using 5′-GGAAGACTCTCCTCCGGATCCGGAAGACTCTCCTCC-3′ and 5′-GATC-GGAGGAGAGTCTTCCGGATCCGGAGGAGAGTCTTCCAGCT-3′ and subcloned as a SacI/BglII fragment into the pGL3-promoter plasmid (Promega Corp.) driving luciferase expression.

Northern Blot Hybridization

Total RNA was extracted from cells by the guanidinium thiocyanate method. Northern blots containing 20 μg per lane of total RNA were stained with methylene blue to determine the positions of 18S and 28S rRNA markers, and then hybridized with plakoglobin (Franke et al., 1989) and β-catenin (Butz et al., 1992) cDNAs, which were labeled with ^{32}P-dCTP by the random priming technique, as described in Salomon et al. (1997).

Protease Inhibitors

The calpain inhibitor *N*-acetyl-leu-leu-norleucinal (ALLN, used at 25 μM) and the inactive analogue *N*-acetyl-leu-leu-normethional (ALLM, used at 10 μg/ml) were purchased from Sigma Chemical Co. (St. Louis, MO). Lactacystin A (dissolved in water at 0.4 mg/ml was used at a final concentration of 4 μg/ml) and MG-132 (used at 10 or 20 μM) were purchased from Calbiochem-Novabiochem (La Jolla, CA).

Immunofluorescence Microscopy

Cells cultured on glass coverslips were fixed with 3.7% paraformaldehyde in PBS and permeabilized with 0.5% Triton X-100 (Sigma Chemical Co.). Monoclonal antibodies against human plakoglobin (11E4), β-catenin (5H10), the COOH terminus of β-catenin (6F9), and α-catenin (1G5) were described (Johnson et al., 1993; Sacco et al., 1995), and provided by M. Wheelock and K. Johnson (University of Toledo, Toledo, OH). The secondary antibody was FITC- or Cy3-labeled goat anti–mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA). Polyclonal antiserum against β-catenin, monoclonal antibody against pan cadherin (CH-19), vinculin (h-VIN 1), and α-actinin (BM75.2) were from Sigma Chemical Co. (Holon, Israel). Monoclonal antibodies against the splicing factor SC35 and the HA epitope were provided by D. Helfman (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY). Polyclonal rabbit antibody against the VSV-G epitope was a gift of J.C. Perriard (Swiss Federal Institute of Technology, Zurich, Switzerland). FITC-labeled goat anti–rabbit IgG antibody was from Cappel/ICN (High Wycombe, UK). Monoclonal antibody against LEF-1 was provided by R. Grosschedl (University of California, San Francisco, CA). Polyclonal anti-HA tag antibody was a gift of M. Oren (Weizmann Institute, Rehovot, Israel). Antibodies to actin were provided by J. Lessard (Childrens Hospital Research Foundation, Cincinnati, OH) and I. Herman (Tufts University, Boston, MA). The cells were examined by epifluorescence with an Axiophot microscope (Carl Zeiss, Inc., Thornwood, NY). To determine the level of overexpression relative to the endogenous protein, digitized immunofluorescent microscopy was used. Images of the fluorescent cells were recorded with a cooled, scientific grade charge-coupled device camera (Photometrics, Tucson, AZ) as described (Levenberg et al., 1998). Integrated fluorescent intensities in transfected and nontransfected cells were determined after the background was substracted.

Electron Microscopy

Cells were processed for conventional electronmicroscopy by fixation with 2% glutaraldehyde followed by 1% OsO_4. The samples were dehydrated and embedded in Epon (Polysciences, Inc., Warington, PA), sectioned, and then examined in an electron microscope (model EM410; Philips Electron Optics, Eindhoven, The Netherlands). Samples processed for immunoelectronmicroscopy were fixed with 3% paraformaldehyde and 0.1% glutaraldehyde in 100 mM cacodylate buffer, pH 7.4, containing 5 mM $CaCl_2$, embedded in 10% gelatin and refixed as above, and then incubated with sucrose, frozen, and then cryosectioned as previously described (Sabanay et al., 1991). The sections were incubated with monoclonal anti-vinculin (h-VIN-1), monoclonal anti–β-catenin (5H10), or polyclonal anti-VSV tag (to recognize the VSV-tagged β-catenin), followed by secondary antibody conjugated to 10 nm gold particles (Zymed Labs, Inc., South San Francisco, CA). The sections were embedded in methyl cellulose and examined in an electron microscope (model CM12; Philips Electron Optics).

PAGE and Immunoblotting

Equal amounts of total cell protein were separated by SDS PAGE, electrotransferred to nitrocellulose, and then incubated with monoclonal antibodies. The antigens were visualized by enhanced chemiluminescence (Amersham Int., Little Chalfont, UK). In some experiments, cells were fractionated into Triton X-100–soluble and –insoluble fractions as described (Rodríguez Fernández et al., 1992). Briefly, cells cultured on 35-mm dishes were incubated in 0.5 ml buffer containing 50 mM MES, pH 6.8, 2.5 mM EGTA, 5 mM $MgCl_2$ and 0.5% Triton-X-100 at room temperature for

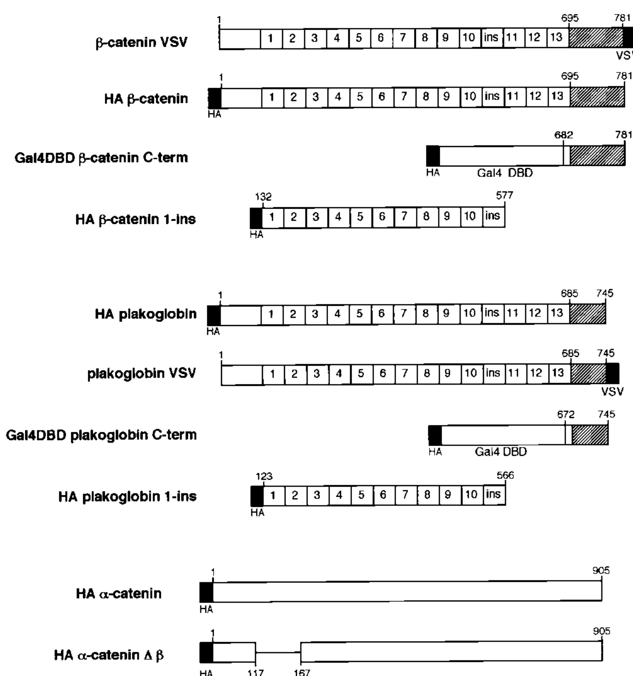

Figure 1. Schematic representation of catenin constructs used in this study. The molecules were tagged either with the hemagglutinin tag (*HA*) at the NH_2 terminus, or with the VSV-G protein tag (*VSV*) at the COOH terminus. Numbers 1–13 represent armadillo repeats in β-catenin and plakoglobin with a nonrepeat region (*ins*) between repeats 10 and 11. Mutant plakoglobin and β-catenin lacking the COOH transactivation domain (*HA plakoglobin 1-ins; HA β-catenin 1-ins*) were also constructed. An HA-tagged α-catenin that lacks the β-catenin–binding domain was also prepared (*HA α-catenin Δβ*). The COOH-terminal (*C-term*) transactivation domains of β-catenin and plakoglobin were fused to the DNA binding domain of Gal4 (*Gal4DBD*) to allow assessment of their transactivation potential.

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3 min. The Triton X-100–soluble fraction was removed, and the insoluble fraction was scraped into 0.5 ml of the same buffer. Equal volumes of the two fractions were analyzed by SDS-PAGE and followed by immunoblotting with the various antibodies.

Results

Overexpression of β-Catenin and Plakoglobin in MDCK Cells Results in Their Nuclear Accumulation and Nuclear Translocation of Vinculin

To determine the localization of overexpressed β-catenin and plakoglobin, MDCK cells that normally display these molecules at cell–cell junctions were transiently transfected with VSV-tagged β-catenin or plakoglobin cDNA constructs (Fig. 1) and immunostained with either anti-VSV, anti–β-catenin, or antiplakoglobin antibodies. The results in Fig. 2 show that when expressed at very low level, β-catenin was detected at cell–cell junctions (Fig. 2 *F*), but in cells expressing higher levels (approximately fivefold over the endogenous protein level, as determined by digital immunofluorescent microscopy), most of the transfected molecules were localized in the nuclei of cells, either in a diffuse form, or in aggregates of various shapes (Fig. 2, *A–C*, *speckles* and *rods*). These β-catenin–containing aggregates were organized in discernible structures that could be identified by phase microscopy (Fig. 3, *E* and *F*). Transmission electron microscopy of Epon-embedded cells revealed within the nucleus highly ordered bodies consisting of laterally aligned filamentous structures, with a filament diameter of ~10 nm and packing density of ~50 filaments/μm (Fig. 3 *A*). Immunogold labeling of ultrathin frozen sections indicated that these intranuclear bodies contained high levels of β-catenin (Fig. 3, *B* and *C*).

Transfection of plakoglobin also resulted in nuclear accumulation of the molecule and, in addition, showed diffuse cytoplasmic (Fig. 2 *D*) and junctional staining (see below, Fig. 7 *H*). Whereas nuclear aggregates were observed in plakoglobin-transfected cells (Fig. 2 *E*), large rods were not detected in the nuclei of these cells. Similar structures were observed with HA-tagged and untagged β-catenin or plakoglobin (data not shown), suggesting that these structures in the nucleus assembled due to high levels of these proteins and are not attributable to tagging.

The unique assembly of β-catenin into discrete nuclear structures is, most probably nonphysiological, yet it enabled us to examine the association of other molecules with β-catenin (Fig. 4). Interestingly, in addition to the transcription factor LEF-1 (Fig. 4 *B*) that was shown to complex with β-catenin in the nucleus (Behrens et al.,

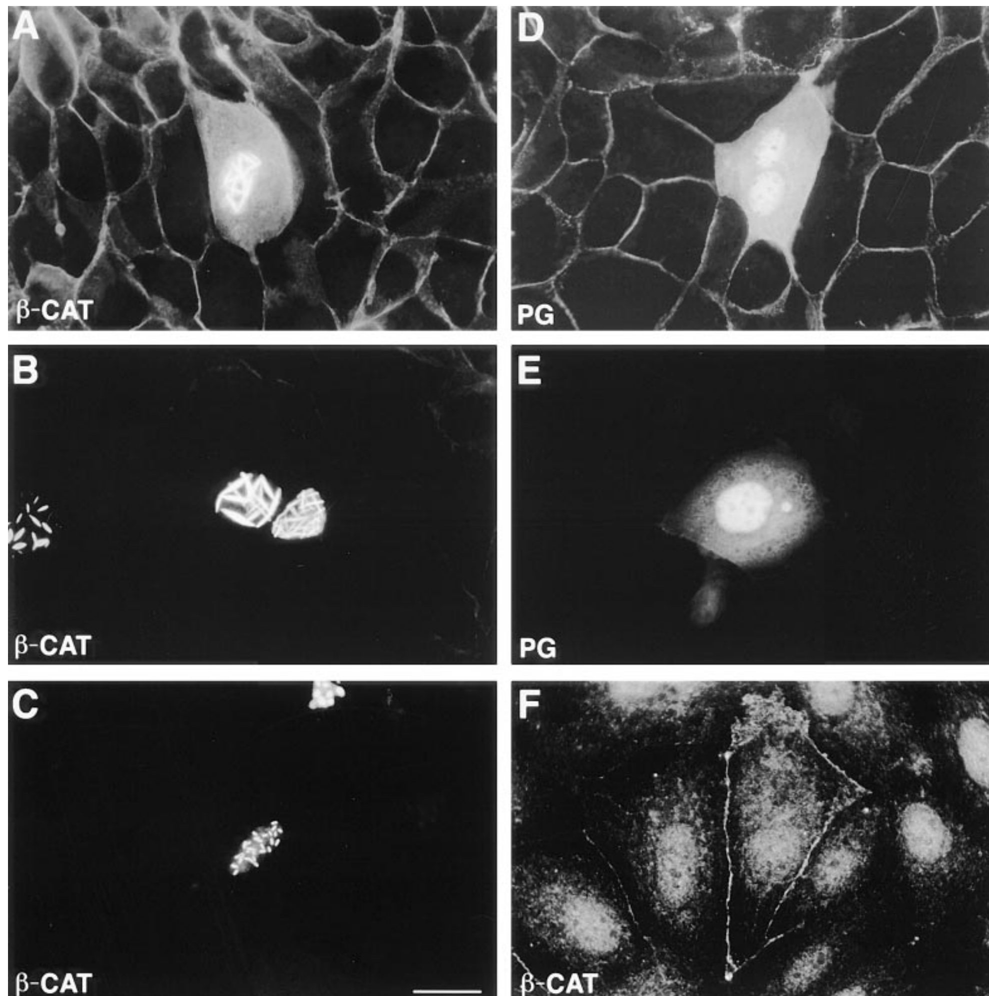

Figure 2. Nuclear localization of β-catenin and plakoglobin transiently transfected into MDCK cells. MDCK cells transfected with VSV-tagged β-catenin (*A–C* and *F*, *β-CAT*) or VSV-tagged plakoglobin (*D* and *E*, *PG*) were immunostained with either monoclonal anti–β-catenin antibody (*A*), anti-plakoglobin antibody (*D*), or anti–VSV-tag antibody (*B*, *C*, *E*, and *F*) and Cy3-labeled secondary antibody, 36 h after transfection. Note the nuclear localization of β-catenin and plakoglobin when overexpressed at high levels in MDCK cells (*A–E*), and of β-catenin at junctions when expressed at low level (*F*). Bar, 10 μm.

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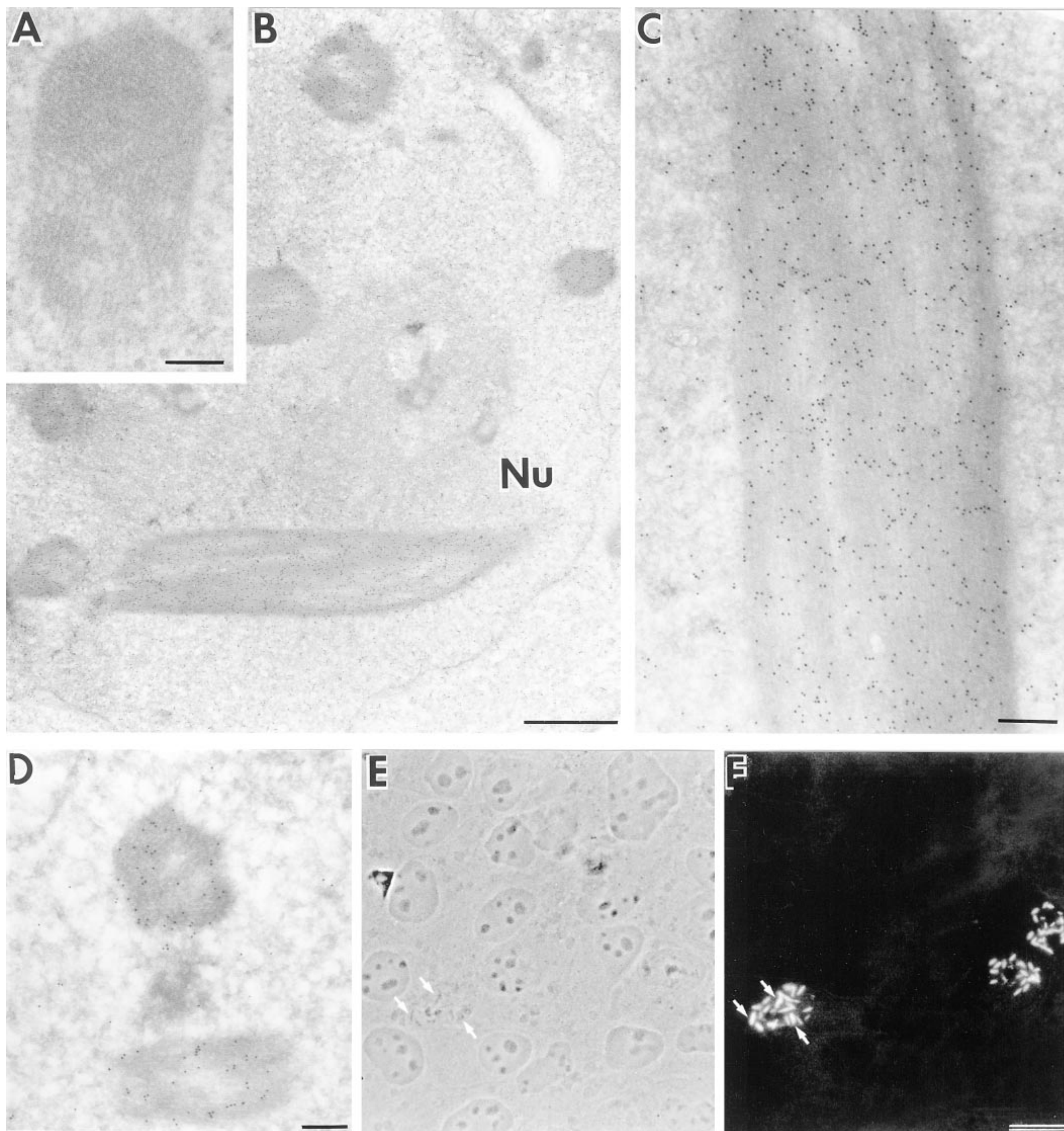

Figure 3. Electronmicroscopy characterization of β-catenin and vinculin-containing nuclear structures in β-catenin–transfected cells. 293-T cells transfected with β-catenin were fixed and processed for (*A*) conventional TE microscopy, or the β-catenin–induced nuclear structures were identified by cryo-EM with antibodies to β-catenin (*B* and *C*) or vinculin (*D*) using secondary antibodies bound to 10-nm gold particles. The nuclear structures were also visualized by phase (*E*) and immunofluorescence with anti–β-catenin antibodies (*F*). *Arrows* in *E* point to nuclear structures decorated by anti–β-catenin antibody (*F*). *Nu*, nucleus. Bars: (*A, C,* and *D*) 0.2 μm; (*B*) 1 μm; (*F*) 10 μm.

1996; Huber et al., 1996*b*; Molenaar et al., 1996), vinculin also strongly associated with the β-catenin–containing speckles and rods in the nucleus (Fig. 4, *C* and *D*). This was also confirmed by immunogold labeling of ultrathin frozen sections with anti-vinculin antibodies showing that the labeling was distributed throughout the entire nuclear aggregate (Fig. 3 *D*). In contrast, other endogenous proteins known to be involved in linking cadherins to actin at adherens junctions such as α-catenin (Fig. 4 *F*), α-actinin (Fig. 4 *J*), and plakoglobin (Fig. 4 *H*) were not associated

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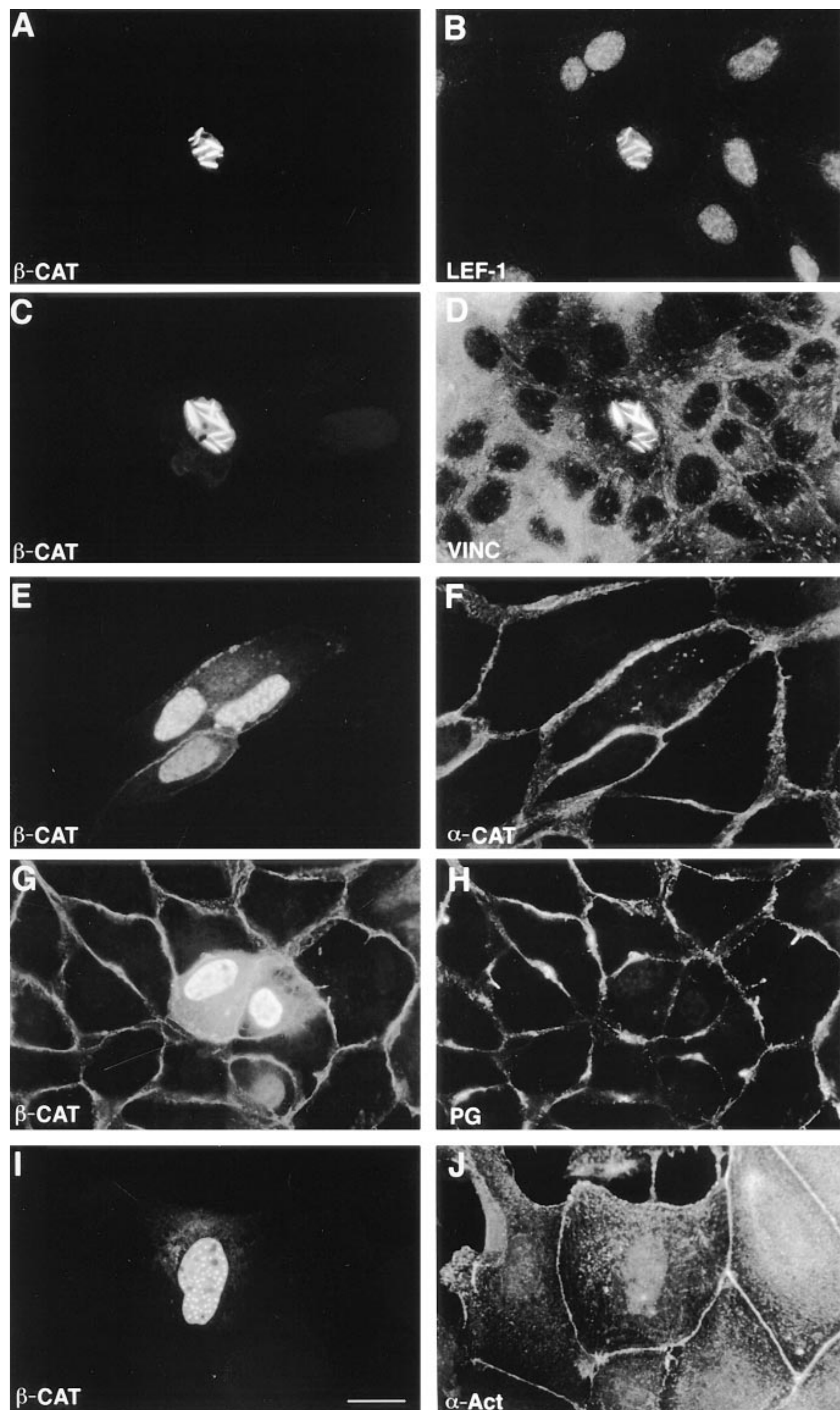

Figure 4. Nuclear translocation of vinculin in β-catenin–transfected cells. MDCK cells were transfected with VSV-tagged β-catenin and doubly stained with antibodies to the VSV tag (*A, C, E,* and *I*) or to β-catenin (*G*), and with antibodies to LEF-1 (*B*), vinculin (*D*), α-catenin (*F*), plakoglobin (*H*), or α-actinin (*J*). Note the strong costaining of LEF-1 and vinculin with β-catenin–containing nuclear rods, but not of plakoglobin, α-actinin, or α-catenin. Bar, 10 μm.

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with the β-catenin–containing nuclear aggregates. Actin was also missing from these nuclear aggregates (results not shown). Furthermore, the β-catenin–containing rods and speckles were clearly distinct from other nuclear structures such as the splicing component SC35 (data not shown), which also displays a speckled nuclear organization in many cells (Cáceres et al., 1997).

The molecular interactions of plakoglobin were distinctly different from those formed by β-catenin. Although nuclear speckles in MDCK cells overexpressing plakoglobin displayed some faint staining for LEF-1 (Fig. 5, compare *B* with *A*), this was less pronounced than that seen with β-catenin (Fig. 4 *B*), and essentially no nuclear costaining for vinculin, α-catenin (data not shown), or α-actinin (Fig. 5 *F*) was observed. Interestingly, plakoglobin-containing nuclear speckles (Fig. 5 *C*) were also stained with anti–β-catenin, and the cytoplasm of these cells was essentially devoid of the diffuse β-catenin staining seen in nontransfected cells (Fig. 5 *D*). This may be explained by the capacity of plakoglobin to compete and release β-cate-

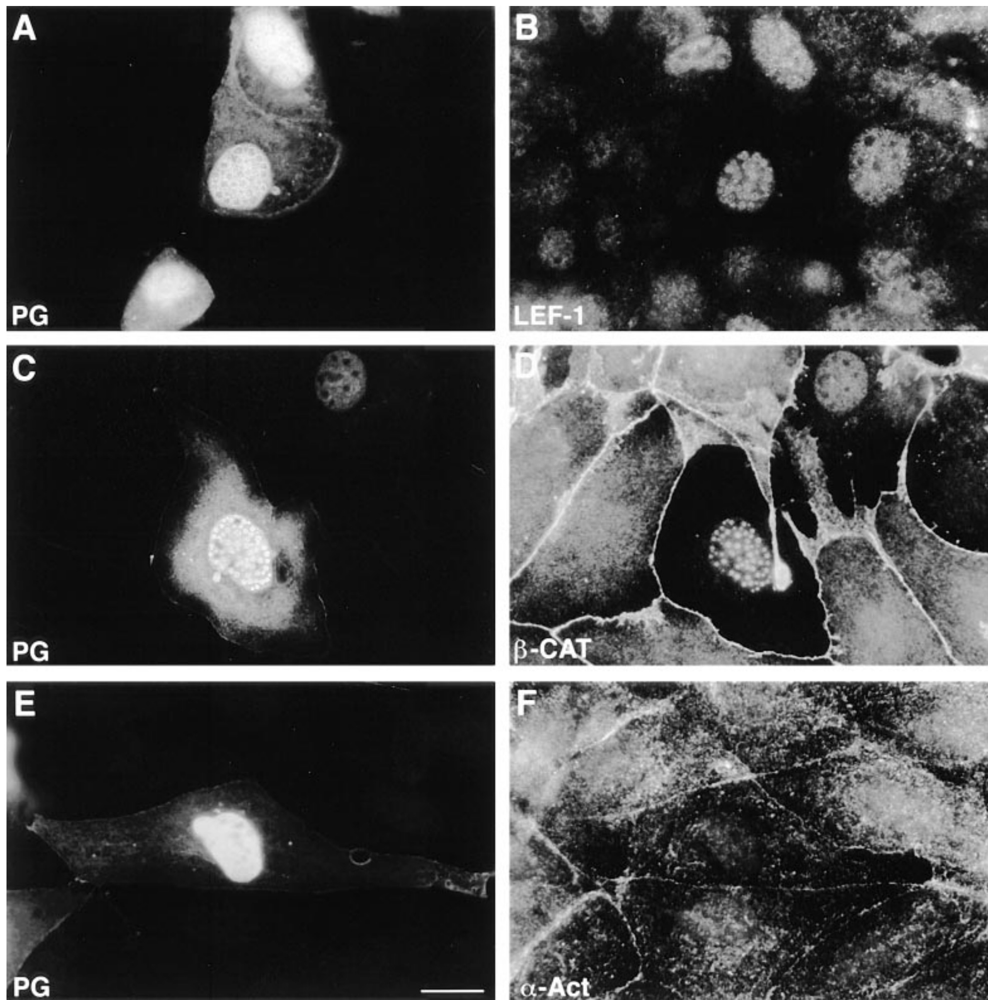

Figure 5. Plakoglobin overexpression causes nuclear accumulation of β-catenin. Cells transfected with plakoglobin were doubly stained for plakoglobin (*A, C,* and *E*), LEF-1 (*B*), β-catenin (*D*), or α-actinin (*F*). Note that in plakoglobin-transfected cells, β-catenin is translocated into the nucleus. *α-Act*, α-actinin; *β-CAT*, β-catenin; *PG*, plakoglobin. Bar, 10 μm.

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nin from its other partners (i.e., cadherin or APC) leading to its nuclear translocation.

Nuclear Accumulation of β-Catenin after Induced Overexpression

Transient transfection usually results in very high and nonphysiological levels of expression (and organization) of the transfected molecules. To obtain information on β-catenin that is more physiologically relevant, we have isolated HT1080 cells stably expressing a mutant β-catenin molecule lacking the NH_2-terminal 57 aa (*ΔN57*, Salomon et al., 1997) that is considerably more stable than the wt protein (Munemitsu et al., 1996; Papkoff et al., 1996; Rubinfeld et al., 1996; Yost et al., 1996). In such stably transfected cells, the level of expression was low and only faint nuclear β-catenin staining was detected, with the majority of β-catenin localized at cell–cell junctions (Fig. 6 *C*). However, when the cells were treated with butyrate, an approximate twofold increase in the expression of the transgene was observed by Western blot analysis (data not shown), and a dramatic translocation of β-catenin into the nucleus occurred (Fig. 6 *D*). This translocation was not observed in butyrate-treated control neo^r HT1080 cells (Fig. 6 *B*). These results suggest that an increase in β-catenin over certain threshold levels of either transiently or inducibly expressed β-catenin results in its nuclear translocation and accumulation.

LEF-1 Overexpression Induces Translocation of Endogenous β-Catenin but Not Plakoglobin into the Nuclei of MDCK Cells

Nuclear translocation of β-catenin was shown to be promoted by elevated LEF-1 expression. We have thus compared the ability of transfected LEF-1 to induce the translocation of endogenous β-catenin and plakoglobin into the nuclei of MDCK cells. Cells were transfected with an HA-tagged LEF-1 and after 36 h were doubly immunostained for LEF-1 and β-catenin, or for LEF-1 and plakoglobin. As shown in Fig. 7, whereas endogenous β-catenin was efficiently translocated into the nucleus in LEF-1–transfected cells and colocalized with LEF-1 (Fig. 7, *A* and *B*), plakoglobin was not similarly translocated into the nucleus (Fig. 7, compare *D* with *C*). This difference between β-catenin and plakoglobin could result from the larger pool of diffuse β-catenin (Fig. 8 *A*) that is available for complexing and translocation into the nucleus with LEF-1. In contrast, plakoglobin was almost exclusively found in the Triton X-100–insoluble fraction (Fig. 8 *A*), in association with adherens junctions and desmosomes. Vinculin and α-catenin that also display a large Triton X-100–soluble fraction (Fig. 8 *A*), in

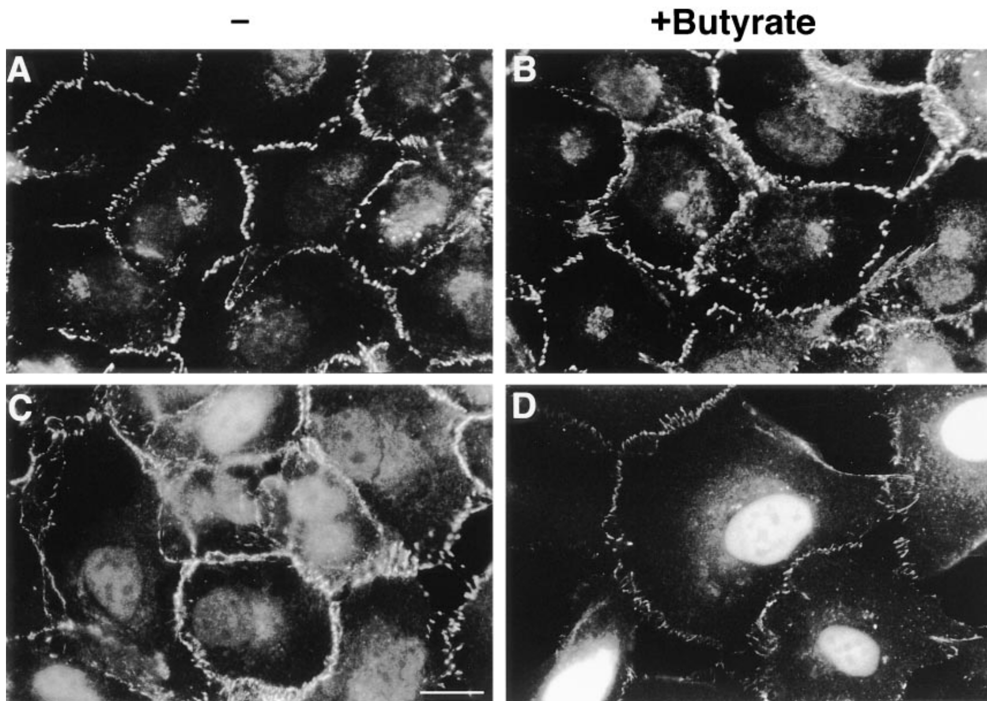

Figure 6. Induction of nuclear translocation of β-catenin in stably transfected cells. Control neo^r HT1080 cells (*A* and *B*), and HT1080 cells stably transfected with an NH_2-terminal–deleted β-catenin mutant (*C* and *D*, *ΔN57*) were either left untreated (*A* and *C*), or treated overnight with sodium butyrate (*B* and *D*) to enhance the expression of the transgene. Note the elevation in β-catenin content and its nuclear accumulation in butyrate-treated cells stably expressing ΔN57 β-catenin. Bar, 10 μm.

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contrast to α-actinin and plakoglobin, were not translocated into the nucleus after LEF-1 transfection (data not shown).

When LEF-1 was overexpressed in MDCK cells together with plakoglobin, both proteins were localized in the nucleus and displayed diffuse staining (Fig. 7, *G* and *H*), similar to β-catenin (Fig. 7, *E* and *F*). In cells doubly transfected with β-catenin and LEF-1, vinculin was also translocated into the nucleus displaying diffuse staining (Fig. 8 *B, panels a* and *b*), whereas plakoglobin was not detected in the nuclei of such cells (Fig. 8 *B, panels c* and *d*). Since LEF-1 transfection did not result in nuclear localization of vinculin (data not shown), this implies that vinculin translocation into the nucleus is only related to that of β-catenin.

Induction of β-Catenin Accumulation and Its Nuclear Localization by Inhibition of the Ubiquitin–Proteasome System

Another treatment through which β-catenin and plakoglobin content could be elevated in cells is the inhibition of degradation by the ubiquitin–proteasome pathway that apparently controls β-catenin and plakoglobin turnover (Aberle et al., 1997; Orford et al., 1997; Salomon et al., 1997). We have used two cell lines for this study: 3T3 cells that express β-catenin, and KTCTL60 renal carcinoma cells that do not express detectable levels of cadherin, α-catenin, β-catenin, or plakoglobin (Simcha et al., 1996), and treated them with various inhibitors of the ubiquitin–proteasome system (Fig. 9). In 3T3 cells, such treatment resulted in the appearance of higher molecular weight β-catenin forms representing most likely ubiquitinated derivatives of the molecule (Fig. 9 *A*). This was accompanied by the accumulation of β-catenin in the nuclei of the cells (Fig. 9 *D,* compare *panels a* and *b*). In KTCTL60 cells that contain minute levels of β-catenin, inhibitors of the ubiquitin–proteasome pathway induced a dramatic increase in the level of β-catenin (Fig. 9 *B*), and its translocation to the nucleus (Fig. 9 *D*, compare *panels c* and *d*). Since these cells express no cadherins (Simcha et al., 1996), it is conceivable that free β-catenin is very unstable and rapidly degraded by the proteasome pathway in these cells. In contrast, no plakoglobin was detected in KTCTL60 cells either before or after treating the cells with the proteasome inhibitors (Fig. 9 *B*) due to lack of plakoglobin RNA in these cells (Fig. 9 *C*). When plakoglobin was stably overexpressed in KTCTL60-PG cells (Fig. 9 *C*), its level was further increased by inhibitors of the ubiquitin–proteasome system (Fig. 9 *B*), and it accumulated in the nuclei of the cells (Fig. 9 *D*, *panels e* and *f*). These results demonstrate that in some cells the level of β-catenin can be dramatically enhanced by inhibiting its degradation by the ubiquitin–proteasome pathway. Plakoglobin levels were also enhanced by MG-132 treatment, but to a considerably lower extent. Under conditions of excess, both proteins accumulated in the nuclei of cells.

Transcriptional Coactivation by Plakoglobin and β-Catenin of Gal4- and LEF-1–driven Transcription

β-Catenin and its homologue in *Drosophila* armadillo were shown to be able to activate transcription of LEF/TCF-responsive consensus sequences by their COOH termini (Korinek et al., 1997; Morin et al., 1997; Riese et al., 1997; van de Wetering et al., 1997). To compare the ability of β-catenin to that of plakoglobin in transactivation, the COOH terminus of β-catenin and the corresponding domain in plakoglobin were fused to the Gal4DBD (refer to Fig. 1). Both constructs, when cotransfected with a reporter gene (luciferase) whose transcription was driven by a Gal4-responsive element, showed a similar ability to activate the expression of the reporter gene (Fig. 10 *A*). This implies that the COOH-terminal domain of plakoglobin,

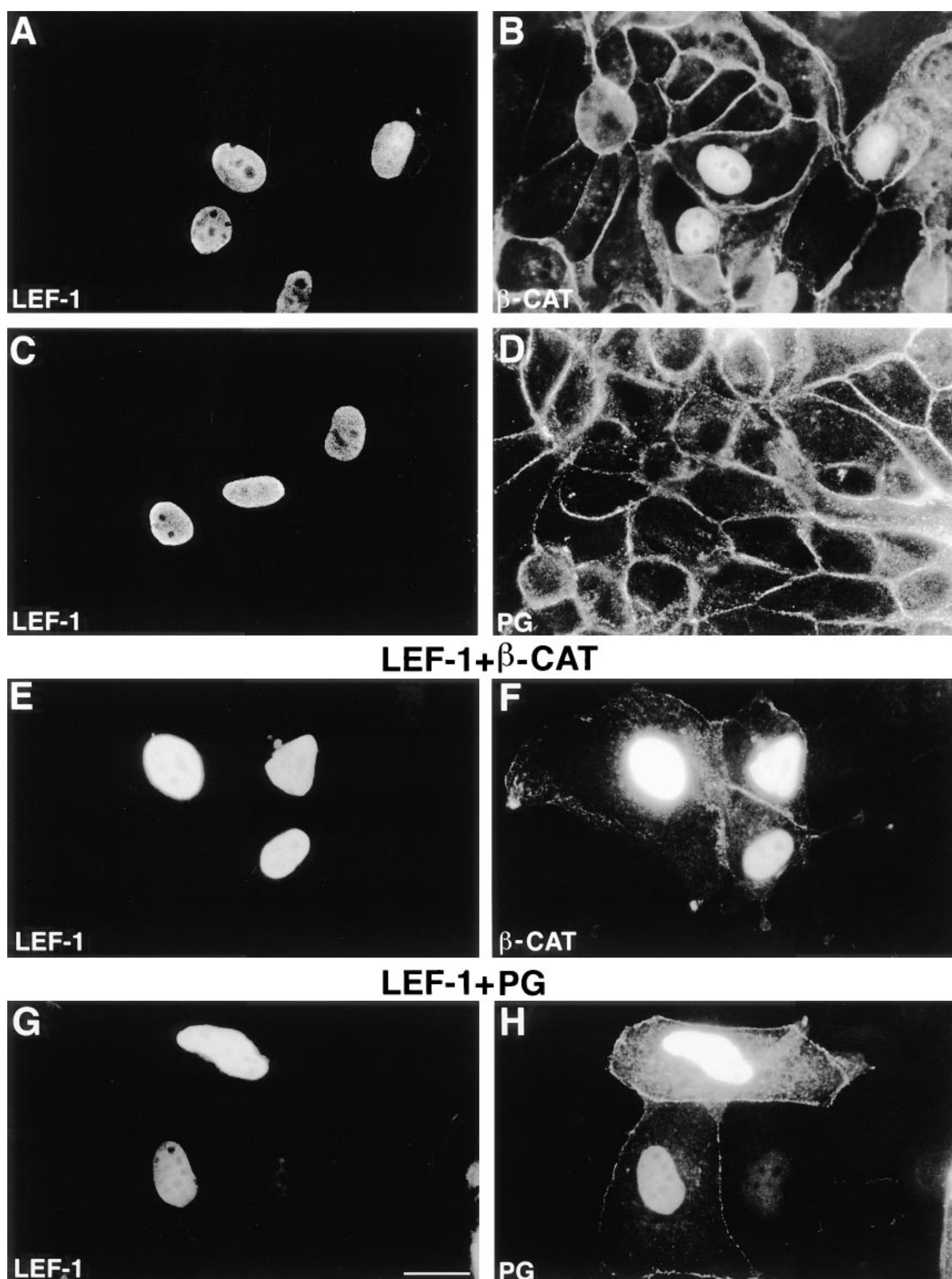

Figure 7. Nuclear translocation of β-catenin but not plakoglobin by LEF-1 overexpression. MDCK cells were transfected with either LEF-1 (*A–D*), with LEF-1 together with β-catenin (*E* and *F*), or with LEF-1 and plakoglobin (*G* and *H*). The cells were doubly stained with antibodies against LEF-1 (*A, C, E,* and *G*) and antibodies to β-catenin (*B*) or plakoglobin (*D*). In doubly-transfected cells (*E–H*), the transfected β-catenin (*F*) and plakoglobin (*H*) were detected by anti-VSV tag antibody. Note that LEF-1 efficiently translocated endogenous β-catenin into the nucleus, but not plakoglobin, whereas in cells transfected with both LEF-1 and plakoglobin or β-catenin, both transfected molecules were localized in the nucleus. Bar, 10 μm.

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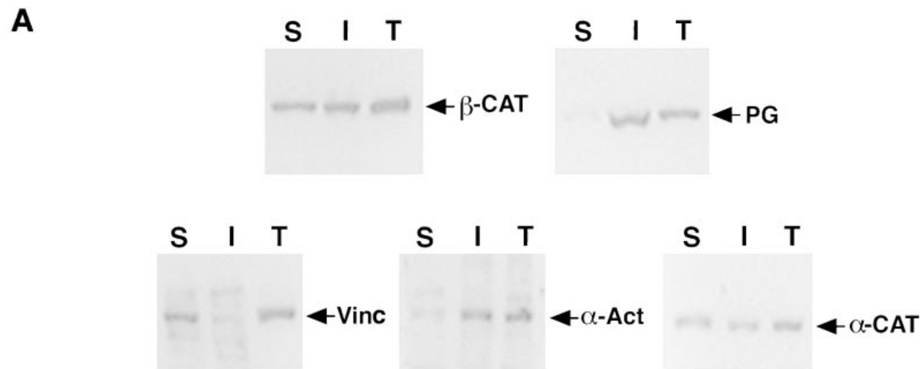
like that of armadillo and β-catenin, has the ability to activate transcription.

Next, we compared the capacity of β-catenin and plakoglobin to activate transcription of a reporter gene driven by a multimeric LEF-1–binding consensus sequence in 293 cells. The results summarized in Fig. 10 *B* demonstrate that β-catenin is a potent transcriptional coactivator of the multimeric LEF-1–responsive sequence. Interestingly, a mutant β-catenin lacking the COOH transactivation domain (refer to Fig. 1, *HA β-catenin 1-ins*) was also active in promoting LEF-1–driven transcription (Fig. 10 *B*). We examined whether this resulted from the substitution for endogenous β-catenin in its complexes with cadherin and APC by the mutant β-catenin as seen in *Xenopus* embryos injected with mutant β-catenin (Miller and Moon, 1997). This could release endogenous β-catenin from cytoplasmic complexes, resulting in its translocation into the nucleus and transcriptional activation of the LEF-1–responsive reporter. Double immunofluorescence using an antibody against the HA-tag linked to the mutant β-catenin (Fig. 10 *C, panel c*) and an anti–β-catenin antibody recognizing the COOH terminus of endogenous β-catenin (but not the mutant HA β-catenin 1-ins that lacks this domain), demonstrated that the level of endogenous β-catenin was elevated, and part of the endogenous protein translocated into the nucleus in cells expressing mutant β-catenin (Fig. 10 *C, panel d*).

Plakoglobin could also activate LEF-1–driven transcription, albeit at a three- to fourfold lower extent than β-catenin (Fig. 10 *B*). A mutant plakoglobin lacking the COOH transactivation domain (refer to Fig. 1, *HA plakoglobin*



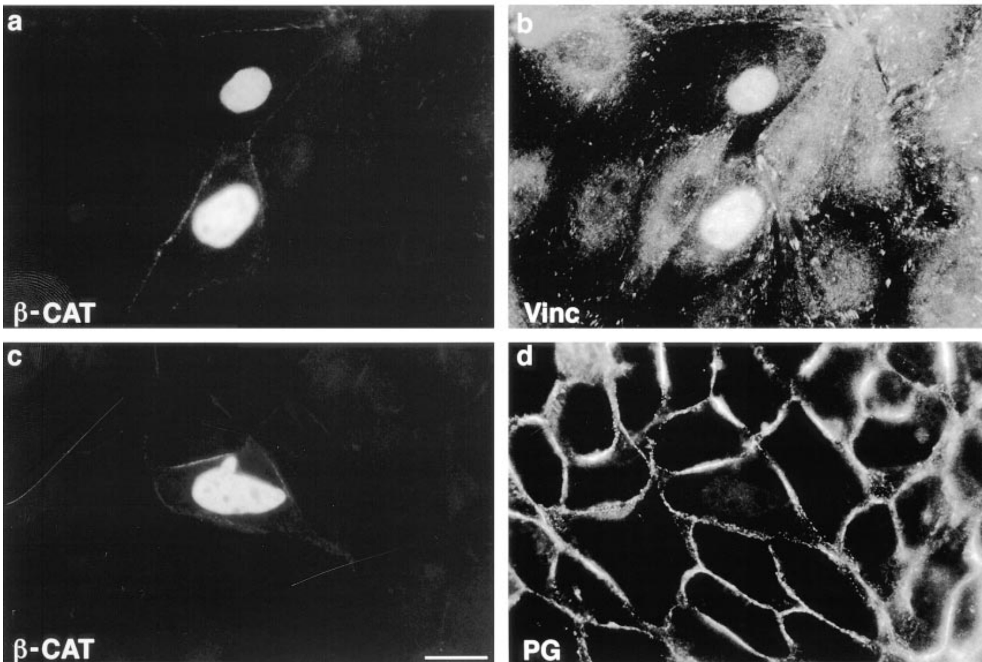


Figure 8. Differential Triton X-100 solubility of various junctional plaque proteins and nuclear translocation of vinculin in cells overexpressing β-catenin together with LEF-1. (*A*) Equal volumes of total MDCK cell proteins (*T*), and Triton X-100–soluble (*S*) and –insoluble (*I*) cell fractions, were analyzed by gel electrophoresis and Western blotting with antibodies to β-catenin (*β-CAT*), plakoglobin (*PG*), vinculin (*vinc*), α-actinin (*α-Act*), and α-catenin (α-*cat*). Note that whereas β-catenin, vinculin, and α-catenin display a large pool of a detergent-soluble fraction, plakoglobin and α-actinin are almost entirely insoluble in Triton X-100. (*B*) MDCK cells were cotransfected with LEF-1 and β-catenin and doubly stained for β-catenin (*a*) and vinculin (*b*), and β-catenin (*c*) and plakoglobin (*d*). Note that in cells doubly transfected with β-catenin and LEF-1 vinculin, translocated into the nucleus but plakoglobin remained junctional. Bar, 10 μm.

1-ins), was unable to enhance LEF-1–driven transcription (Fig. 10 *B*). To examine if full-length or mutant plakoglobin overexpression resulted in nuclear accumulation of endogenous β-catenin, cells transfected with HA-tagged plakoglobin were doubly stained for HA (Fig. 10 *C, panel a*), and β-catenin (Fig. 10 *C, panel b*). The results demonstrated that plakoglobin overexpression resulted in nuclear translocation of endogenous β-catenin (Fig. 10 *C, compare panel a with b*). In contrast, the COOH deletion mutant plakoglobin that was abundantly expressed in the transfected cells (Fig. 10 *C, panel e*), was unable to cause translocation of endogenous β-catenin into the nucleus (Fig. 10 *C, panel f*).

Taken together, these results strongly suggest that although both plakoglobin and β-catenin have a COOH-terminal domain that can act as cotranscriptional activator when fused to the Gal4DBD, LEF-1–driven transcriptional activation by mutant β-catenin and wild-type plakoglobin mostly resulted from the release of endogenous β-catenin from its cytoplasmic partners, nuclear translocation, and induction of LEF-1–responsive transcription. Thus, elevated plakoglobin expression can influence β-catenin–driven transactivation.

Inhibition of β-Catenin Nuclear Localization and Transactivation Capacity by N-Cadherin and α-Catenin

Constitutive transactivation by high levels of β-catenin was suggested to be involved in tumor progression in colon carcinoma (Korinek et al., 1997; Morin et al., 1997). In addition, the signaling activity of β-catenin in *Xenopus* development could be blocked by its junctional partners (i.e., C-cadherin and the NH_2-terminal of α-catenin [Funayama et al., 1996; Sehgal et al., 1997]). We have investigated the localization and transcriptional activation capacity of β-catenin in SW480 colon carcinoma cells that overexpress β-catenin due to lack of APC (Munemitsu et al., 1995), before and after transfection with N-cadherin and α-catenin. In these cells, β-catenin is abundant in the nucleus (Fig. 11 *B*, *panels b*, *d*, and *f*) and a high level of constitutive LEF-1–driven transcription was detected (Fig. 11 *A*), in agreement with Korinek et al. (1997). This activity of β-catenin was effectively blocked by the cotransfection of N-cadherin or α-catenin (Fig. 11 *A*). Deletion of the β-catenin binding site on α-catenin (refer to Fig. 1, *α-catenin Δβ*) abolished the transactivation inhibition capacity of this molecule (Fig. 11 *A*). Double immunofluorescence

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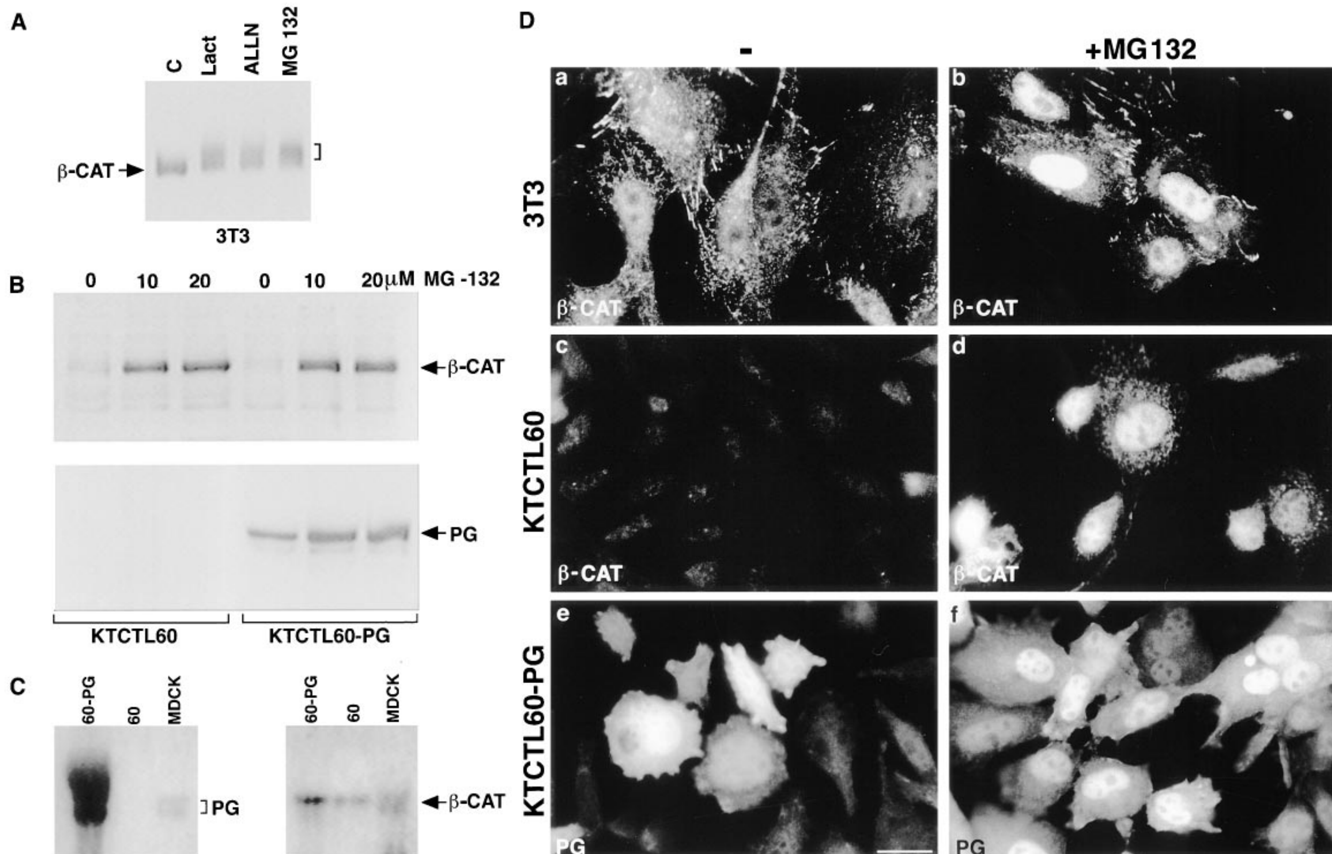

Figure 9. Elevation of β-catenin and plakoglobin content and nuclear localization after treatment with inhibitors of the ubiquitin–proteasome pathway. (*A*) Balb/C 3T3 cells were treated for 4 h with inhibitors of the ubiquitin–proteasome system: lactacystin (*Lact*), ALLN, or MG-132, and equal amounts of protein were analyzed by Western blotting with anti–β-catenin antibody. (*B*) KTCTL60 and KTCTL60-PG cells (stably overexpressing plakoglobin) were treated with 10 and 20 μM MG-132 and probed with anti–β-catenin and plakoglobin antibodies. (*C*) Northern blot hybridization for β-catenin and plakoglobin in KTCTL60, KTCTL60-PG, and MDCK cells. (*D*) Balb/C 3T3 and KTCTL60 cells (*panels a, c, and e*), were treated for 4 h with MG-132 (*panels b, d, and f*) and stained with antibodies to β-catenin (*panels a–d*) or plakoglobin (*panels e* and *f*). Note the appearance of higher mol wt β-catenin forms in 3T3 cells (*bracket* in *A*), the dramatic elevation in β-catenin content of KTCTL60 cells, and the moderate increase in plakoglobin after treatment of KTCTL60-PG with the proteasome inhibitors. Bar, 10 μm.

microscopy indicated that both molecules can drive β-catenin out of the nucleus in transfected SW480 cells (Fig. 11 *B*). In these cells, β-catenin was sequestered to the cytoplasm by α-catenin (Fig. 11 *B, panels c* and *d*) or to cell–cell junctions by the transfected N-cadherin (Fig. 11 *B, panels a* and *b*). The α-catenin mutant lacking the β-catenin binding site (Fig. 11 *B, panels e* and *f*) did not show this activity. The results suggest that the partners of β-catenin that are active in cell adhesion, are effective antagonists of the nuclear localization of β-catenin and its function in transcriptional regulation.

Discussion

In mammalian cells, β-catenin and the closely related molecule plakoglobin (Butz et al., 1992; Knudsen and Wheelock, 1992; Peifer et al., 1992) have been shown to complex independently with similar partners, and are both involved in the formation of adherens type junctions (Butz and Kemler, 1994; Hülsken et al., 1994; Nathke et al., 1994; Rubinfeld et al., 1995). Plakoglobin, in addition, can associate with various desmosomal components (Cowin et al., 1986; Schmidt et al., 1994; Chitaev et al., 1996; Kowalczyk et al., 1997), whereas β-catenin does not normally associate with desmosomes, except in plakoglobin-null mouse embryos where the segregation between adherens junctions and desmosomes collapses (Bierkamp et al., 1996; Ruiz et al., 1996). Plakoglobin is also unable to substitute for β-catenin during development, since β-catenin–null mouse embryos die early in development (Haegel et al., 1995). In this study we highlight some common features of β-catenin and plakoglobin, as well as considerable differences in their nuclear translocation under various conditions, and their capacity to function in transcriptional activation. For both proteins, the increase in free protein levels induces nuclear translocation. This translocation can be blocked by junctional proteins that bind to β-catenin and sequester it to the plasma membrane or the cytoplasm.

Nuclear Translocation

Under the various conditions that resulted in increased levels of β-catenin and plakoglobin, both proteins translocated into the nucleus independently of, or in complex with

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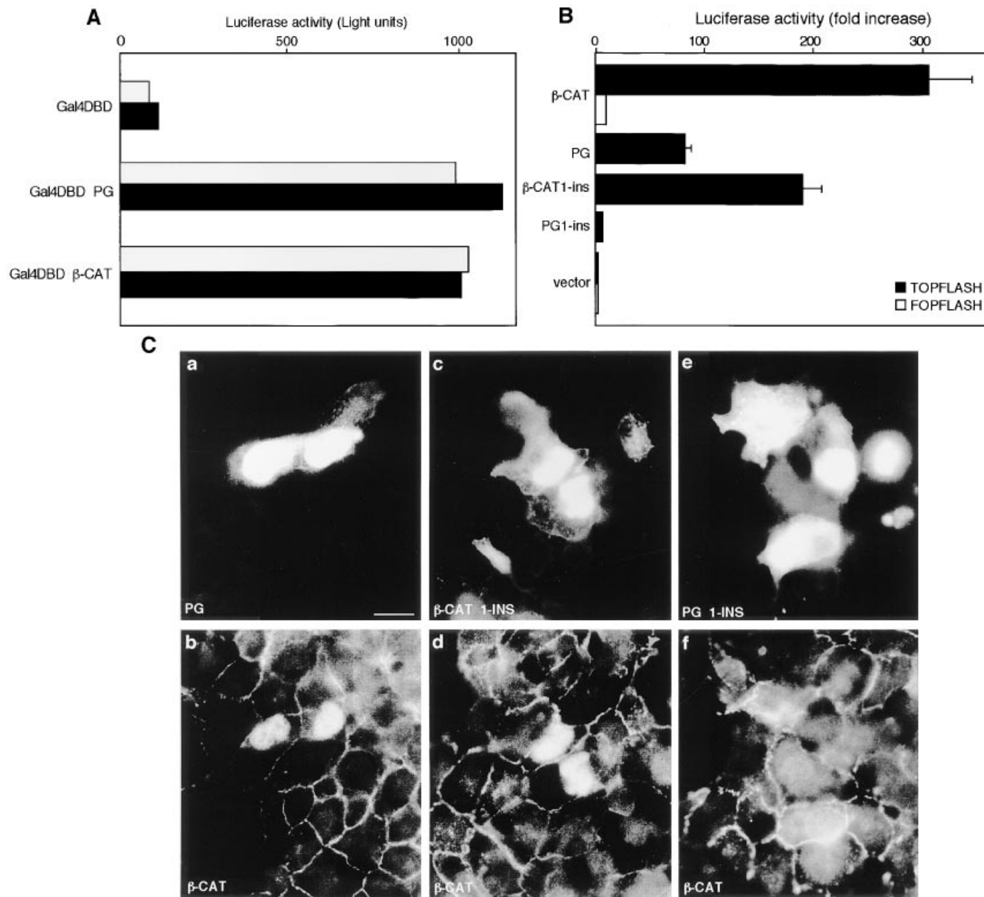

Figure 10. Activation of Gal4- and LEF-1–driven transcription by β-catenin and plakoglobin. (*A*) Constructs consisting of the DNA-binding domain of Gal4 (*Gal4DBD*) fused to the COOH-terminal transactivation domains of β-catenin and plakoglobin were cotransfected with a reporter gene (luciferase) driven by Gal4-responsive sequences into 3T3 cells, and the levels of luciferase activity determined from duplicate transfections (*black* and *white bars*). (*B*) Transactivation of LEF-1 consensus sequence (*TOPFLASH*)-driven transcription by full-length and truncated β-catenin and plakoglobin in 293 cells. The values (*fold increase*) were normalized for transfection efficiency by analyzing β-galactosidase activity of cotransfected lacZ, and for LEF-1 specificity with an inactive mutant LEF-1 sequence (*FOPFLASH*, *white bars*). (*C*) Double immunofluorescence for β-catenin (*panels b, d,* and *f*) in cells transfected with plakoglobin (*a*), β-catenin 1-ins (*c*), and plakoglobin 1-ins (*e*). Note that chimeras consisting of β-catenin and plakoglobin fused to Gal4DBD were both active in transcription stimulation, but LEF-1–responsive transactivation by β-catenin (and a β-catenin mutant) was much more potent than by full-length plakoglobin, and a COOH-deletion mutant of plakoglobin was inactive in LEF-1–driven transactivation. Full-length plakoglobin and the β-catenin mutant (*β-cat 1-ins*) were effective in translocating endogenous β-catenin into the nucleus, whereas the plakoglobin mutant (*PG 1-ins*) was not. Bar, 10 μm.

LEF-1. Whereas in β-catenin–overexpressing cells the nuclear complexes that were formed by excess β-catenin also contained vinculin in addition to LEF-1, plakoglobin overexpression did not result in the recruitment of these molecules into the nuclear speckles. Interestingly, α-catenin and α-actinin, both of which bind β-catenin and plakoglobin-containing complexes (Knudsen et al., 1995; Huber et al., 1997; Nieset et al., 1997), were not cotranslocated into the nucleus by β-catenin or plakoglobin probably due to their stronger binding to actin filaments, resulting in a limited soluble pool in cells, in contrast to vinculin that is mostly in the detergent-soluble fraction. Our study is the first demonstration that β-catenin can associate with and recruit into the nucleus vinculin, but not other components of the cadherin–catenin system (i.e., α-catenin, α-actinin, or cadherin) in a complex that also contains LEF-1. The association between vinculin and β-catenin was recently demonstrated by coimmunoprecipitation of these proteins together with E-cadherin, but was most pronounced in cells lacking α-catenin (Hazan et al., 1997). Taken together, these findings reveal a new interaction of β-catenin with vinculin, that under certain conditions may lead to their colocalization in the nucleus where such a complex may play an important physiological role yet to be determined.

Although both plakoglobin and β-catenin exhibited a largely similar nuclear translocation, they were distinct in their ability to colocalize with LEF-1 in the nucleus. Endogenous β-catenin was readily translocated into the nucleus after transfection with LEF-1, in agreement with previous studies (Behrens et al., 1996; Huber et al., 1996*b*; Molenaar et al., 1996), whereas the endogenous plakoglobin remained junctional. This difference may be attributed to the availability of a larger pool of soluble β-catenin in MDCK cells, or to an intrinsic difference between the two molecules in their binding to LEF-1.

Plakoglobin and β-catenin also differed in their ability to influence the localization of endogenous LEF-1 when individually overexpressed. Plakoglobin overexpression could drive part of the endogenous β-catenin into the nucleus, most probably by displacing it from cadherin or other cytoplasmic partners, in agreement with results obtained with HT1080 cells (Salomon et al., 1997) and with *Xenopus* embryos (Miller and Moon, 1997). This implies that plakoglobin may have a regulatory role in the control of the extrajunctional function of β-catenin. In contrast, β-catenin was inefficient in altering plakoglobin's localization in MDCK, 293, and SK-BR-3 cells (all expressing desmosomes, our unpublished results). This was partly expected since β-cate-

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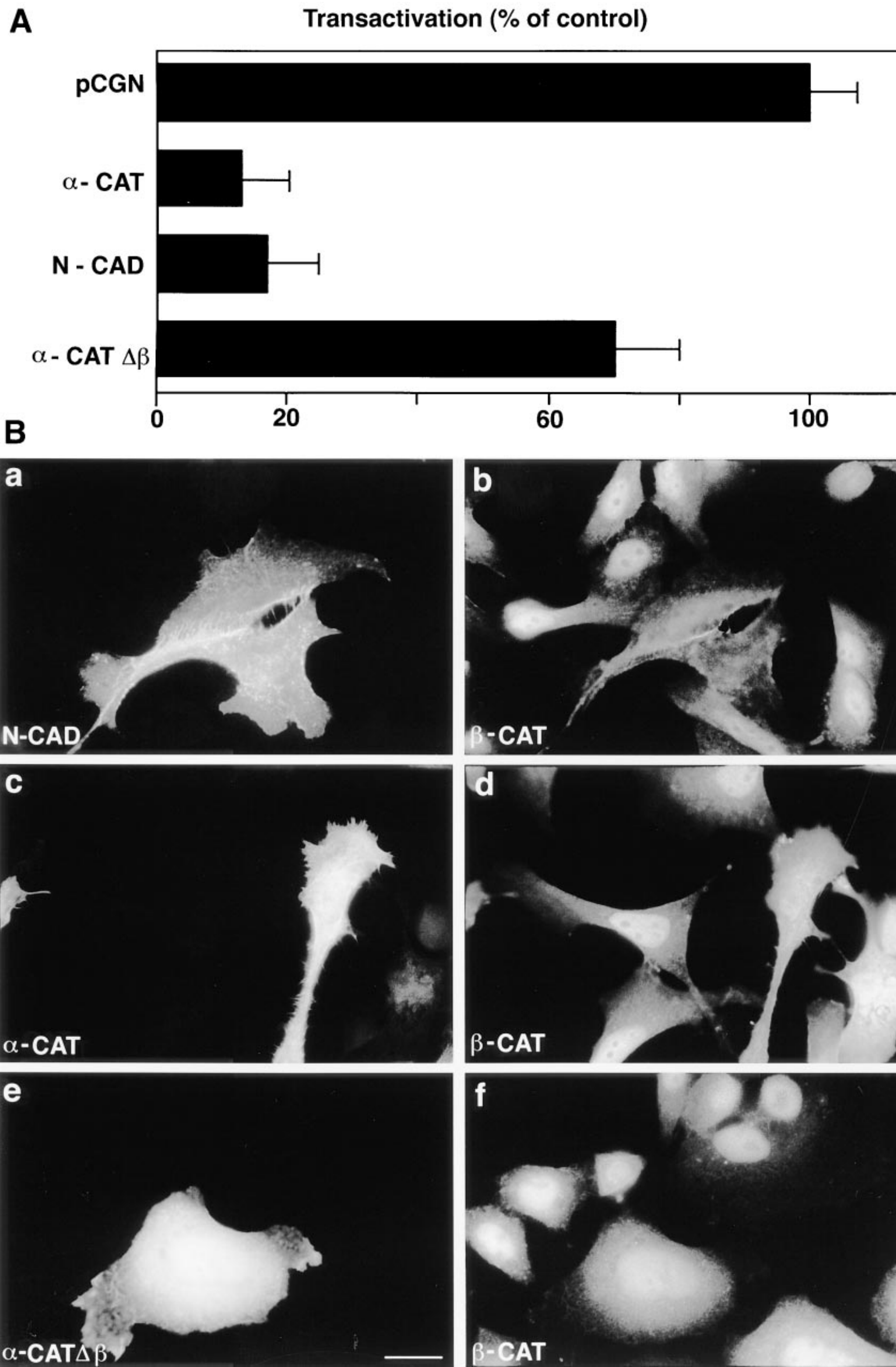

Figure 11. Inhibition of transactivation and nuclear accumulation of β-catenin in SW480 colon carcinoma cells after transfection with N-cadherin or α-catenin. (*A*) SW480 cells were transfected with either empty vector (*pCGN*), N-cadherin, α-catenin, or a mutant α-catenin lacking the β-catenin binding site (refer to Fig. 1, *HA* α*-catenin* Δβ) together with a multimeric LEF-1–binding consensus sequence driving the expression of luciferase. The values of luciferase expression were corrected for transactivation specificity with a mutant LEF-1 consensus sequence, and with β-galactosidase activity for transfection efficiency. (*B*) Cells were transfected with N-cadherin (*panels a* and *b*), α-catenin (*panels c* and *d*), or mutant α-catenin (α-*CAT*Δβ) (*panels e* and *f*) and doubly stained for β-catenin (*panels b*, *d,* and *f*) and N-cadherin (*panel a*), α-catenin (*panel c*) and mutant α-catenin (*panel e*). Note the inhibition of transactivation and cytoplasmic retention of β-catenin in cells transfected with N-cadherin or α-catenin, but not with mutant α-catenin. Bar, 10 μm.

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nin is not normally associated with desmosomes and the soluble pool of plakoglobin in these cells is very low.

Plakoglobin and β-catenin also responded differently to the inhibition of the ubiquitin–proteasome pathway, in particular in the renal carcinoma cell line KTCTL60 that does not express detectable levels of proteins of the cadherin–catenin system (Simcha et al., 1996). The level of β-catenin could be dramatically induced in these cells with proteasome inhibitors, suggesting that efficient degradation of β-catenin is responsible for the very low level of β-catenin in these cells. Plakoglobin was absent from these cells since there was no plakoglobin RNA, but when it was stably expressed (Simcha et al., 1996), its level was only moderately enhanced by inhibitors of the ubiquitin–proteasome system. It is interesting to note that this stable expression of plakoglobin did not result in the elevation of β-catenin content in KTCTL60 cells, implying that plakoglobin cannot, by itself, effectively protect β-catenin from degradation in cells lacking cadherins. Only when the level of plakoglobin was further increased in these cells by bu-

tyrate treatment could some accumulation of β-catenin be detected (our unpublished results).

Transactivation Capacity

Comparison between the presumptive transactivating domains of β-catenin and plakoglobin, fused to the DNA-binding domain of Gal4, indicated comparable transcriptional activation by the two molecules. This demonstrated that plakoglobin, like β-catenin and armadillo (van de Wetering et al., 1997), has a potent transactivation domain. However, the specific transcriptional activation of LEF-1–driven reporter gene by plakoglobin was severalfold less efficient than that of β-catenin. Interestingly, a deletion mutant of β-catenin that lacked the transactivating domain but retained the cadherin-binding domain, was also capable of inducing transcription by the LEF-1 consensus construct. This can be attributed to competition and displacement of endogenous β-catenin from a complex with its cytoplasmic partners, nuclear translocation of the endogenous β-catenin, and consequently, LEF-1–driven transactivation. This finding is in agreement with recent studies by Miller and Moon (1997) who found that a variety of membrane-anchored mutant forms of β-catenin can act in signaling for axis duplication in *Xenopus* embryos by releasing endogenous β-catenin from cell–cell junctions or from a complex with APC, thus enabling its translocation into the nucleus.

Overexpression of full-length plakoglobin was capable of inducing nuclear translocation of the endogenous β-catenin in MDCK and 293 cells, whereas a COOH terminus mutant plakoglobin, previously shown to be inefficient in displacing β-catenin from its complex with cadherin (Sacco et al., 1995; Salomon et al., 1997), was also unable to induce nuclear localization of the endogenous β-catenin, or transcriptional activation of the LEF-1–driven reporter. Since plakoglobin overexpression was inefficient in driving LEF-1 to complex with the nuclear speckles formed by plakoglobin overexpression, it is conceivable that the majority of the transcriptional stimulation of LEF-1–driven transcription in plakoglobin-overexpressing cells was due to the endogenous β-catenin that relocated to the nucleus under these conditions.

Another recent study examining mammalian β-catenin and plakoglobin's embryonic signaling abilities in *Drosophila* (rescue of the segment polarity phenotype of *armadillo*) suggested that although both proteins can rescue *armadillo* mutants in adhesion properties, β-catenin had only a weak and plakoglobin had no detectable signaling activity (White et al., 1998). Nevertheless, since we found that the COOH terminus of plakoglobin is potent in transcriptional activation in the Gal4-fusion chimera and deletion mutants at the COOH terminus were inefficient in transactivation and most of the overexpressed plakoglobin was localized in the nuclei of transfected cells, one cannot exclude the possibility that plakoglobin can also play a direct role in the transcriptional regulation of specific genes that are yet to be identified (Rubenstein et al., 1997). This possibility is being currently examined by analyzing the transactivation capacities of plakoglobin and β-catenin in cells that lack such endogenous proteins.

In the human colon carcinoma SW480 cells that lack APC and therefore accumulate abnormally high levels of β-catenin in the nuclei, transcriptional activation of the LEF-1–driven reporter could be inhibited by members of the cadherin–catenin complex that sequestered β-catenin to the cytoplasm. The role of cadherin in regulating β-catenin levels is complex. On one hand, elevation in the content of cadherin can protect β-catenin from degradation and increase its levels. On the other hand, a strong binding of β-catenin to cadherin, rather than to LEF-1, may result in its cytoplasmic sequestration and the inhibition of transactivation by it. Furthermore, the cytoplasmic tail of cadherin that contains the binding site for β-catenin can also inhibit transactivation by β-catenin even when bound to it in the nucleus of the transfected cells (Sadot, E., M. Shtutman, I. Simcha, A. Ben-Ze'ev, and B. Geiger, unpublished results). The association of β-catenin with overexpressed α-catenin in SW480 cells also resulted in the cytoplasmic retention of nuclear β-catenin by binding of α-catenin to the actin cytoskeleton. These results are in agreement with those obtained for β-catenin signaling in axis specification of developing *Xenopus* that is antagonized by overexpression of cadherin (Heasman et al., 1994; Fagotto et al., 1996), or by the NH_2 terminus of α-catenin (Sehgal et al., 1997).

These results may have important implications for the possible role of β-catenin in the regulation of tumorigenesis, since E-cadherin (Navarro et al., 1991; Vleminckx et al., 1991) and α-catenin (Bullions et al., 1997) were suggested to have tumor suppressive effects when reexpressed in cells deficient in these proteins and were shown to affect the organization of cell–cell adhesion. In addition, modulation of vinculin and α-actinin levels in certain tumor cells was shown to influence the tumorigenic ability of these cells (Rodríguez Fernández et al., 1992; Glück et al., 1993) and to affect anchorage independence and tumorigenicity in 3T3 cells (Rodríguez Fernández et al., 1993; Glück and Ben-Ze'ev, 1994; Ben-Ze'ev, 1997). It is possible that such effects are attributable to the capacity of vinculin and α-actinin to bind β-catenin, thus affecting both its localization and its role in regulating transcription.

Since the β-catenin–binding domains on E-cadherin (Ozawa et al., 1990; Stappert and Kemler, 1994) and α-catenin (Bullions et al., 1997; Huber et al., 1997; Nieset et al., 1997; Obama and Ozawa, 1997) are well characterized, it will be possible to test in the future, whether shorter peptides comprising these domains are effective in blocking the oncogenic action conferred by constitutive transactivation of LEF/TCF-responsive genes by β-catenin in colon cancer (Korinek et al., 1997; Morin et al., 1997) or melanoma (Rubinfeld et al., 1997).

We are grateful to our colleagues for sending reagents: R. Kemler, R. Grosschedl, M. Wheelock, K. Johnson, D. Helfman, J.C. Perriard, M. Waterman (University of California, Irvine, CA), K. Jones (The Salk Institute, La Jolla, CA), H. Clevers, and M. van de Wetering; to I. Sabanay (The Weizmann Institute of Science) for electron microscopy and Z. Kam (The Weizmann Institute of Science) for helping with digital immunofluorescent microscopy.

These studies were supported by grants from the USA–Israel Binational Foundation, the German–Israeli Foundation for Scientific Research and Development, by the Forchheimer Center for Molecular Genetics, and by the Cooperation Program in Cancer Research between the German Cancer Research Center (DKFZ) and the Israeli Ministry of Sciences and Arts (IMOSA).

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Received for publication 19 February 1998 and in revised form 7 April 1998.

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