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Effect of Disrupting Cell Contact on the Nuclear Accumulation of β-Catenin and Subsequent Apoptosis of Rat Ovarian Surface Epithelial Cells In Vitro

John J. Peluso, Anna Pappalardo, and Stefanie A. Hess

Departments of Physiology and Obstetrics and Gynecology, University of Connecticut Health Center, Farmington, CT

The present studies were designed to determine how disrupting cell contact induces rat ovarian surface epithelial cells (i.e., ROSE-179 cells) to undergo apoptosis. In the first series of studies, the effect of depleting serum and calcium on the levels of the adhesion proteins N-cadherin and β-catenin was examined. These studies revealed that the depletion of serum and calcium results in the degradation of N-cadherin but not β-catenin. However, the localization of β-catenin changed from principally the plasma membrane to the nucleus. The nuclear localization of β-catenin was demonstrated by Western blot and confocal microscopy. A second series of studies demonstrated that cells that lost contact in response to the depletion of serum and calcium showed enhanced β-catenin-dependent transcription. Finally, forced expression of a stable form of β-catenin resulted in an increase in β-catenin within the cytoplasm of transfected ROSE-179 cells. When these β-catenin transfected ROSE-179 cells were deprived of serum and calcium, β-catenin accumulated within the nucleus and accelerated the rate at which these cells became apoptotic. These data indicate that in viable cells, β-catenin is part of the adhesion complex that maintains cell contact. If calcium-dependent cell contacts are broken, β-catenin accumulates within the nucleus, where it promotes transcription and ultimately the apoptotic death of ROSE-179 cells.

Key Words: Rat; Ovarian surface epithelial Cells; Cell contact; β-catenin; N-cadherin; apoptosis.

Introduction

Ovulation is a complex process that involves three physiological events: oocyte maturation, luteinization of the granulosa cells, and follicular rupture *(1)*. As part of follicular rupture, the ovarian surface epithelial cells undergo apoptosis *(2)*. To define the mechanism through which ovarian surface epithelial cells undergo apoptosis, previous work has been done on a cell derived from rat ovarian surface epithelial cells (i.e., ROSE-179 cells) *(3–5)*. These cells are similar to ovarian surface epithelial cells in that they have similar morphological characteristics and form both cadherin-mediated adhesion-type and gap junctions *(6)*. Interestingly, it is the calcium-dependent cadherin-mediated junctions that play important roles in regulating ovarian surface epithelial cell viability *(3,7,8)*.

Cadherins are a family of adhesion proteins that are composed of three major domains. The extracellular domain has a series of calcium-binding sites. Calcium binds to these sites and promotes interaction between the extracellular cadherin domains of adjacent cells, thereby establishing in part an adhesion-type junction. Cadherins also possess a transmembrane and intracellular domain *(9)*. The intracellular domain binds β-catenin, which in turn associates with α-catenin, thereby forming an attachment point for the actin stress fibers *(10)*.

In addition, the N-cadherin-catenin complex is involved in preventing ROSE-179 cells from undergoing apoptosis *(3,7,8)*. The mechanism through which N-cadherin-catenin complex inhibits apoptosis is unknown. Previous studies have shown that hepatocyte growth factor disrupts N-cadherin-mediated cell contact, which leads to an increase in $[Ca^{2+}]_i$. Further disruption of homophilic N-cadherin binding with either an antibody to N-cadherin or a synthetic N-cadherin peptide results in an increase in $[Ca^{2+}]_i$ levels and apoptosis of ROSE-179 cells *(3,5)*. The sustained increase in $[Ca^{2+}]_i$ is the critical event that commits cells to undergo apoptosis. Interestingly, *de novo* RNA and protein synthesis is required in order for this increase in $[Ca^{2+}]_i$ levels to occur *(5)*.

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Author to whom all correspondences and reprint requests should be addressed: Dr. John J. Peluso, Department of Physiology, University of Connecticut Health Center, Farmington, CT 06030. E-mail: peluso@nso2.uchc.edu

It is difficult to explain how simply disrupting N-cadherin-mediated cell contact initiates the gene transcription that leads to an increase in $[Ca^{2+}]_i$. Recent studies have shown that β-catenin is detected within the nucleus of various cancer cells *(11–14)*. Furthermore, nuclear β-catenin promotes transcription by dimerizing with a member of the lymphoid enhancer factor-1 (LEF-1)/T-cell transcription factor (TCF) family *(15–17)*. Based on these observations, the present studies were designed to test the hypothesis that in healthy ROSE-179 cells, β-catenin functions as part of adhesion-type junctions. If the adhesion complex is disrupted, then β-catenin accumulates within the nucleus, where it induces transcription and ultimately apoptosis.

Results

Western blot analysis confirmed that N-cadherin was expressed by ROSE-179 cells. Removal of calcium from the culture medium resulted in N-cadherin being cleaved by 3 h, as indicated by the presence of a cleavage product (Fig. 1). By 6 h of calcium deprivation, neither intact nor cleaved N-cadherin was detected by Western blot (data not shown). Calcium supplementation prevented the degradation of N-cadherin (Fig. 1). By contrast, the total amount of β-catenin increased by approx 50% within 3 h of calcium depletion (Fig. 2, top). Furthermore, β-catenin was associated with N-cadherin when ROSE-179 cells were cultured in the presence of either serum or calcium. As would be predicted from the N-cadherin levels, very little β-catenin was associated with N-cadherin by 6 h of calcium deprivation (Fig. 2, bottom).

Although β-catenin was not degraded, its cellular distribution was altered by calcium depletion. Confocal microscopic analysis revealed that with calcium supplements, most of the β-catenin was localized to the plasma membrane (Fig. 3A). These cells also possessed nuclei with normal heterochromatin (Fig. 3C). By 3 h after calcium depletion, very little of the β-catenin was localized to the plasma membrane (Fig. 3B). Rather, β-catenin was observed within the nucleus of 40 ± 2% of the cells by 3 h after calcium withdrawal (Fig. 3B). In several of these cells, the heterochromatin was starting to condense and reorganize into apoptotic bodies as assessed by an increase in the intensity of DAPI staining (Fig. 3D). The relatively high levels of nuclear β-catenin were confirmed by Western blots (Fig. 4).

Because β-catenin accumulated within the nucleus after calcium depletion, it is possible that it was stimulating transcription. To test this hypothesis, ROSE-179 cells were transfected with the expression vector with two LEF-1/TCF sites (TOP-GFP), the vector with two mutated LEF-1/TCF sites (FOP-GFP), or pEGFP-C1. The fluorescent intensity of cells transfected with either FOP-GFP or TOP-GFP was minimal as long as the cells were maintained in serum-supplemented medium (Fig. 5A,B). By 5 h after serum and calcium removal, β-catenin-dependent transcription (i.e., specific TOP-GFP fluorescence) was observed in 7 ± 1% of the cells. β-Catenin-dependent transcription was observed only in cells that had lost cell contact (Fig. 5C,D). The transfection efficiency for these studies averaged 26 ± 3%. By correcting for transfection efficiency, it was estimated that β-catenin stimulated transcription in approx 30% of the cells after serum and calcium withdrawal.

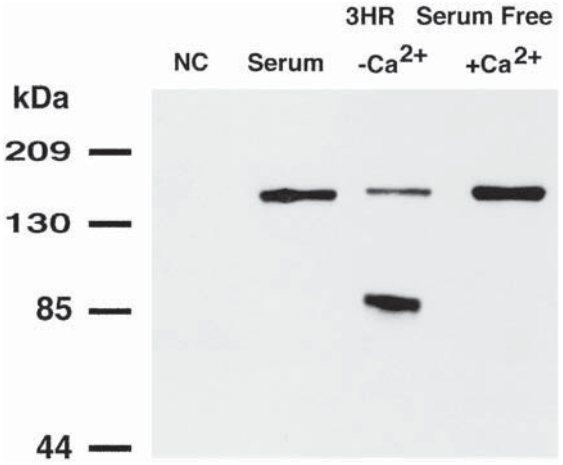

Fig. 1. Effect of serum and calcium depletion on N-cadherin levels. In this and subsequent Western blots, NC denotes a negative control in which the primary antibody was omitted from the Western blot protocol, and all samples were run on the sample gel. The order of each lane in the original gel was often rearranged in the final figure to coincide with its presentation in the text.

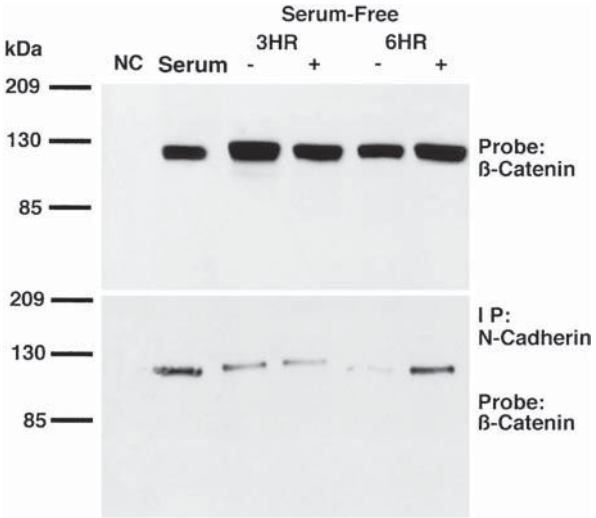

Fig. 2. The effect of serum and calcium depletion on total and N-cadherin-associated β-catenin levels. In this study, an aliquot of each cell lysate was used to assess the total amount of β-catenin (top). The remainder of the sample was immunoprecipitated using an N- cadherin antibody. The immunoprecipitate was then probed using an antibody to β-catenin (bottom). Lanes marked with a minus sign identify samples that were collected after culture without serum and calcium supplements. The plus sign denotes samples that were collected after culture in serum-free media supplemented with calcium.

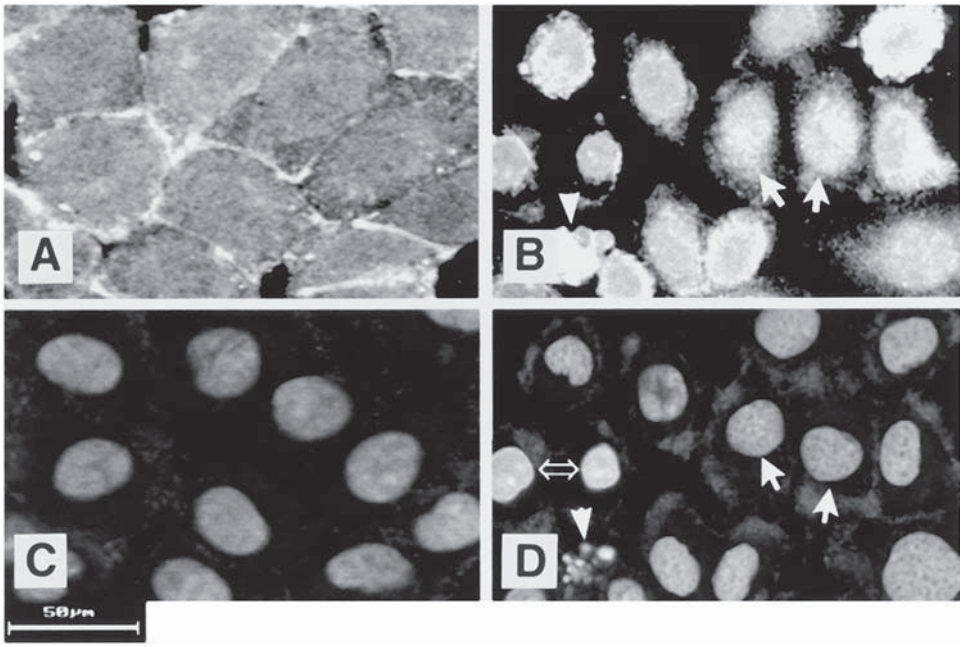

Fig. 3. Effect of serum and calcium depletion on cellular localization of β-catenin and nuclear morphology as revealed by confocal microscopy. Cells were collected in serum-free media in the presence **(A,C)** or absence **(B,D)** of calcium. The cells were stained with DAPI to visualize the nucleus (C,D). Confocal images were collected through the optical plane that best imaged the nuclear morphology. Confocal images of the distribution of pattern of β-catenin were collected at the same optical plane as the nuclear images *(A,B)*. The white arrows in (B) and (D) identify cells in which β-catenin is localized to both the perinuclear and nuclear region of the cell. Note that these cells have relatively normal nuclei. The double-headed arrow identifies cells in which β-catenin is localized almost exclusively to the nucleus. These cells show signs of DNA condensation as indicated by the more intense DAPI fluorescence. A cell in which only apoptotic bodies are detected is marked by an arrowhead. This cell is intensely stained for β-catenin. Cells, stained without the primary antibody (i.e., negative controls), did not stain and could not be photographed under the lighting and optical conditions used to photograph the cells shown.

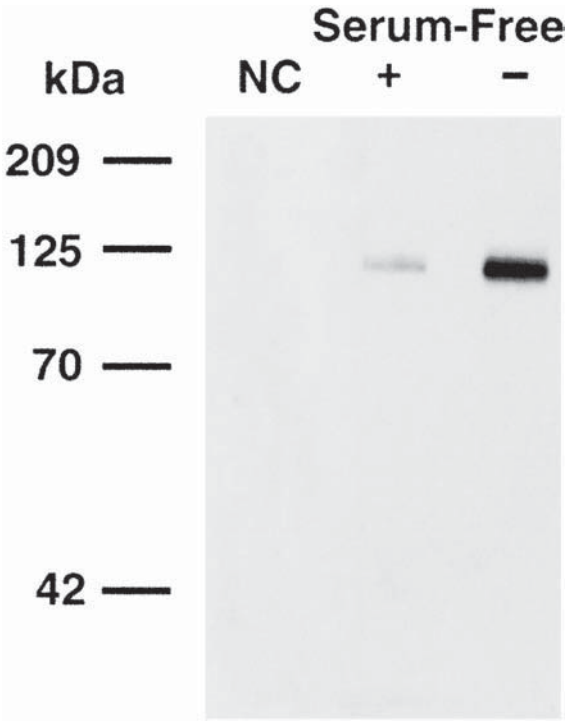

Fig. 4. Effect of serum and calcium depletion on the nuclear levels of β-catenin. Note that depletion of serum and calcium results in an increase in nuclear β-catenin.

To determine whether an increase in nuclear β-catenin was responsible for inducing apoptosis, ROSE-179 cells were transfected with a GFP expression vector and either a β-catenin or control expression vector. Transfection with the GFP expression vector and the control vector neither altered the nuclear morphology nor the localization of β-catenin levels compared to nontransfected cells (data not shown). After transfection with the GFP expression vector and the β-catenin expression vector, the cells possessed normal nuclear morphology but had relatively high levels of β-catenin distributed throughout their cytoplasm. Both normal nuclear morphology and cytoplasm localization of β-catenin were maintained as long as the cells were cultured in serum-supplemented medium (Fig. 6A–C). However, within 3 h after serum and calcium removal, β-catenin was observed within the nucleus of most of the transfected cells (Fig. 6D–F). In addition, the nuclear morphology was altered such that the heterochromatin was condensed (Fig. 6D). As predicted from previous studies (*see* Fig. 3), depletion of serum and calcium also resulted in the accumulation of β-catenin within the perinuclear and nuclear regions of nontransfected cells. However, the nuclei of these nontransfected cells (*see* arrows in Fig. 6E) appeared normal. Finally, forced expression of β-catenin resulted in nearly 60% of the cells undergoing apoptosis within

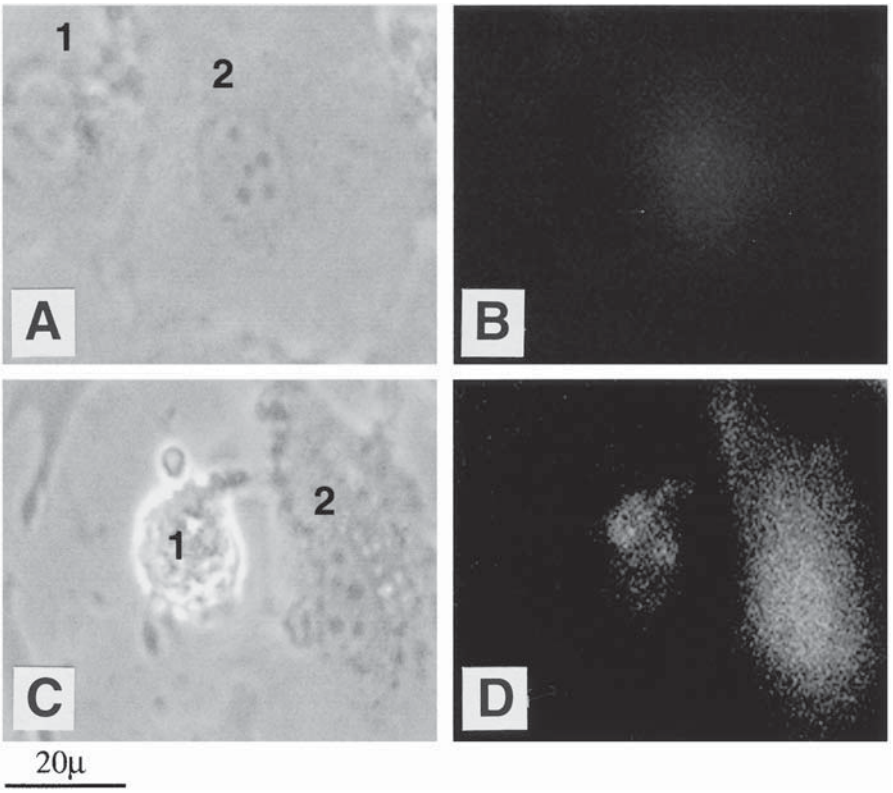

Fig. 5. Detection of β-catenin-dependent transcription within single cells. Two cells that were transfected with TOP-GFP and cultured for 24 h in serum-supplemented medium are shown **(A)**. GFP fluorescence was not detected in either of these cells **(B)**. By 5 h after serum/calcium depletion, the cells had lost contact with each other. Cell number 1 was collapsed, whereas cell number 2 was relatively intact **(C)**. Both of these cells show GFP fluorescence, indicating that they have undergone β-catenin-dependent transcription **(D)**.

3 h of serum and calcium removal. This was significantly greater than the 25% of the apoptotic cells transfected with control vector ($p < 0.05$; Fig. 7). These observations indicate that forced expression of β-catenin accelerated the rate at which ROSE-179 cells underwent apoptosis, because apoptotic nuclei were not normally increased until 6 h after serum and calcium depletion.

Discussion

The present study demonstrates that in ROSE-179 cells, depleting extracellular calcium results in the degradation of the adhesion protein N-cadherin. N-cadherin degraded rapidly with about 50% of the N-cadherin being cleaved by 3 h. Virtually all of the N-cadherin was cleaved and subsequently degraded by 6 h after calcium removal. While the degradation of N-cadherin is likely to account for loss of cell contact, the mechanism that induces N-cadherin degradation in ROSE-179 cells is unknown. Previous studies in astrocytes have shown that the levels of intracellular cyclic adenosine monophosphate (cAMP) increase when extracellular calcium levels are reduced *(18)*. Moreover, in human granulosa cells, cAMP increases metalloproteinase activity, which in turn cleaves N-cadherin *(19)*. In addition, 1-10 phenanthroline, a metalloproteinase inhibitor, not only prevents the cleavage of N-cadherin but also the subsequent apoptosis of human granulosa cells *(19)*. It is possible, then, that an increase in cAMP occurs in ROSE-179 cells once cell contacts are broken. This could activate a metalloproteinase that specifically cleaves N-cadherin. This concept is presently being assessed.

Unlike N-cadherin, β-catenin levels were maintained throughout the 6-h culture period. In 3T3 fibroblasts *(20)* and endothelial cells *(21)*, β-catenin was cleaved by 6 h after exposure to an apoptotic stimulus. This cleavage is mediated by caspase-3 *(20)*. Although caspase-3 is involved in ovarian cell apoptosis *(22–24)*, it does not appear to be activated during the first 6 h of ROSE-179 cell apoptosis, because β-catenin remains intact.

Although not degraded, the cellular distribution of β-catenin was dramatically altered during apoptosis. In the presence of serum or calcium, most of the ROSE-179 cells maintained cell contact and β-catenin remained localized to the plasma membrane. After calcium withdrawal, β-catenin was detected within the nucleus of about 40% of the cells and β-catenin-dependent transcription was estimated to be ongoing in 30% of the cells. Furthermore, β-catenin-dependent transcription was observed only in cells that had lost cell contact. Taken together, these observations are consistent with the hypothesis that nuclear β-catenin triggers a gene cascade that promotes apoptosis.

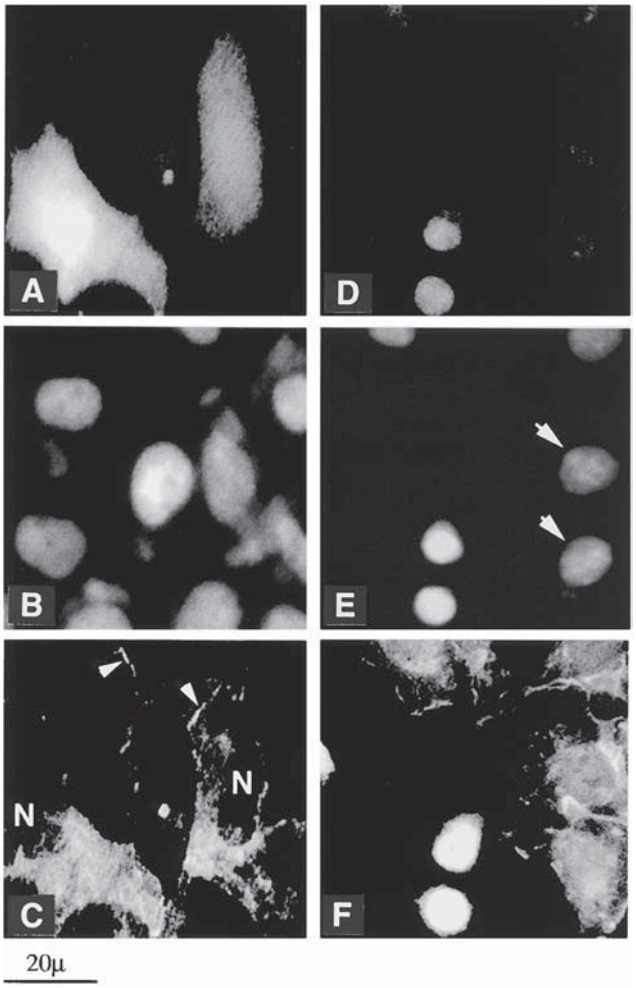

Fig. 6. Effect of serum and calcium depletion and forced expression of β-catenin on cellular localization of β-catenin and nuclear morphology. In this study, cells were cotransfected with β-catenin expression vector and pEGFP-C1 vector. After 24 h, the cells were either maintained in serum-supplemented media **(A–C)** or cultured in serum-free, calcium-free media for 3 h **(D–F)**. Transfected cells as indicated by GFP fluorescence are shown in (A) and (D). Their corresponding nuclear morphology and β-catenin distribution are shown in (B,E) and (C,F), respectively. When transfected cells were maintained in serum (A), nuclear morphology remained normal (B), although high levels of β-catenin were detected throughout the cytoplasm (C). β-Catenin was not detected within the nucleus of transfected cells (*see* N in [C]). Note that in the nontransfected cells, β-catenin was localized only to the plasma membrane (arrowheads in [C]). By contrast, transfected cells cultured in serum-free, calcium-free media (D) had condensed nuclei (E), and β-catenin was localized within their nucleus (F). Nontransfected (arrows in [E]) cells possessed normal-looking nuclei with β-catenin detected at the plasma membrane as well as the perinuclear and nuclear regions.

Additional support for this hypothesis is found in the observations that β-catenin induces the expression of several genes, including c-*myc (9,25,26)*, c-j*un (16)*. Both of these gene products are involved in the induction of apoptosis and also function as transcription factors *(27–29)*.

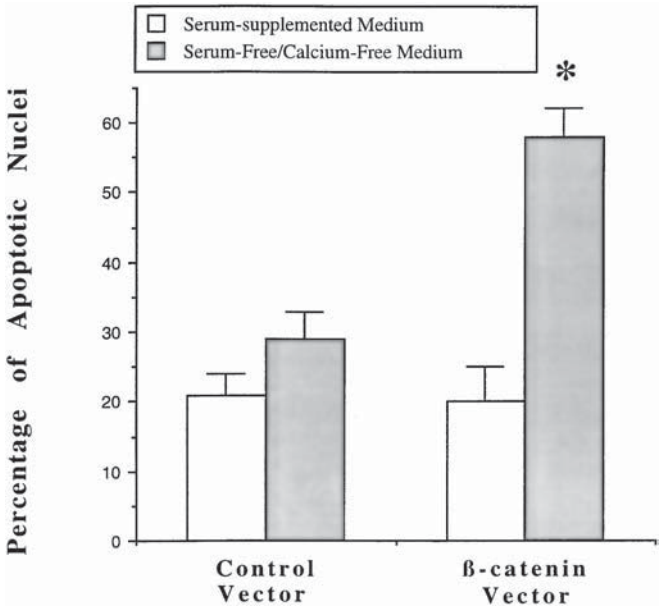

Fig. 7. Effect of force of β-catenin on the percentage of ROSE-179 cells undergoing apoptosis in response to calcium depletion. Apoptotic nuclei were assessed 3 h after serum and calcium removal. The percentage of apoptotic nuclei was determined as described in Material and Methods. Values are presented as a mean percentage ± 1 SE ($n = 5$). * The value is different from all other groups as assessed by a one-way analysis of variance ($p < 0.05$).

Thus, they could promote the expression of other genes that mediate cellular events that ultimately induce ROSE-179 cell apoptosis. As indicated in Lail-Trecker et al.'s study *(4)*, the enhanced expression of type 3 inositol triphosphate (IP_3) receptor is required for ROSE-179 cell apoptosis. Whether β-catenin either directly or indirectly regulates the expression of type 3 IP_3 receptor needs to be assessed.

The β-catenin forced expression studies support the concept that nuclear β-catenin induces apoptosis. In these studies, a stable form of β-catenin was expressed resulting in the accumulation of β-catenin throughout the cytoplasm but not within the nucleus. This demonstrates that an increase in the overall level of β-catenin is not sufficient to induce apoptosis. Rather, it is only when calcium is depleted that β-catenin accumulates in the nucleus. This ultimately results in the cells undergoing apoptosis. These observations indicate that the accumulation of β-catenin within the nucleus plays a causative role in inducing apoptosis.

The mechanism that directs β-catenin into the nucleus is not known. Previous studies have shown that a mutation prevents β-catenin from interacting with glycogen synthase kinase-3β. This ultimately prevents the degradation of β-catenin and results in its accumulation within the nucleus of some cell types *(30)*. Although mass action could account for β-catenin's entry into the nucleus *(14)*, at least two sets of observations argue against this possibility. First, β-catenin does not have a nuclear localization sequence, and, therefore, it is not automatically directed to the nucleus

(31). Second, forced expression of β-catenin in ROSE-179 cells increase its levels within the cytoplasm but not in the nucleus. Similar findings were reported by Brabletz et al. *(32)*. These observations suggest that a mechanism other than mass action accounts for the accumulation of β-catenin in the nucleus. It has been reported that β-catenin can bind to members of the LEF-1/TCF family *(12,33)*. These proteins have a nuclear localization sequence, and thus binding to these proteins could allow β-catenin to "piggyback" its way into the nucleus *(31)*. A novel member of the frizzled gene family, FzE3, has also been shown to bind β-catenin *(34)*. FzE3 prevents degradation and promotes nuclear transport of β-catenin *(34)*. In addition, forced expression of LEF-1 enhances FzE3-mediated β-catenin transport into the nucleus *(34)*. If this mechanism is involved in mediating the accumulation of β-catenin into the nucleus, then FzE3 and at least one LEF-1/TCF family member must be induced early in the apoptotic cascade of ROSE-179 cells. Alternatively, others have suggested that β-catenin can bind directly to the nuclear pores *(31)*. This would also promote β-catenin's entry into the nucleus *(31)*. The transport protein, importin B, also binds to the nuclear pores such that it competes with β-catenin for the same binding site *(31)*. If importin B is involved, then it would have to decrease as the cells undergo apoptosis in order to allow β-catenin to enter the nucleus. Which, if any, of these mechanisms accounts for the accumulation of β-catenin within the nuclei of ROSE-179 cells remains to be determined.

In summary, the present studies show that the disruption of N-cadherin-mediated cell contact leads to an accumulation of β-catenin within the nucleus. It is likely that once in the nucleus, β-catenin activates a gene cascade that results in ROSE-179 cells undergoing apoptosis. Future studies are required to define the mechanism by which β-catenin accumulates in the nucleus and acquires its transcriptional activity.

Materials and Methods

Analysis of N-Cadherin and β-Catenin

ROSE-179 cells were generously provided by Dr. Robert Burghardt of Texas A&M University (College Station, TX) and cultured as previously described *(6)*. Briefly, ROSE-179 cells were cultured in serum-supplemented medium until they were approx 80–90% confluent. The cells were then washed once with Joklik-modified minimal essential medium (MEM) supplemented with 15 m*M* HEPES (pH 7.0), Pen-Strep, and 9.2% defined serum substitute with lipids as described by White and Bancroft *(35)*. This medium contained approx 0.01 m*M* Ca^{2+} *(36)*. Cells were then cultured in Joklik-modified MEM in the presence or absence of a 0.07 m*M* calcium chloride supplement. As a control, cells were also cultured in serum-supplemented medium.

After treatment, cell lysates were prepared for Western blot analysis and protein levels were determined as previously described *(37)*. N-Cadherin and β-catenin were detected using a 1:500 dilution of either a monoclonal cadherin antibody built against N-cadherin (Sigma, St. Louis, MO) or a monoclonal β-catenin antibody (Transduction, Lexington, KY). To determine changes in the N-cadherin/β-catenin complex, an antibody to N-cadherin (Santa Cruz Biotech, Santa Cruz, CA) was used in an immunoprecipitation assay using a protocol provided by Boehringer Mannheim. For all protocols, equal amounts of protein were used. Further, the efficiency of protein transfer was assessed by staining the blots with Ponceau S.

To monitor the nuclear localization of β-catenin, nuclear fractions were prepared from ROSE-179 cells according to the following published protocol of Lee et al. *(38)*. Briefly, ROSE-179 cells were harvested and resuspended in hypotonic buffer for 15 min. The cells were then lysed by drawing them through a 21 g needle five times. The cell lysate was then centrifuged at 3000*g* for 5 min in a microfuge. The pellet, which contained the nuclear fraction was resuspended, incubated in buffer for 30 min at 4°C, and then centrifuged at 12,000*g* for 5 min in a microfuge. The supernate contained the nuclear fraction and was assayed for β-catenin by Western blot.

Localization of β-Catenin

To localize β-catenin, the cells were incubated with a 1:50 dilution of the anti-β-catenin antibody (Transduction) for 2 h at room temperature, washed four times in phosphate-buffered saline (PBS), and incubated with a 1:100 dilution of Fluorescein Isothiocyanate (FITC)-labeled goat antimouse IgG for 1 h at room temperature. The β-catenin-stained slides were washed four times in PBS, stained with DAPI (0.2 μg/mL), coverglassed with DABCO, and observed under the confocal microscope. Specific staining was assessed by omitting the primary antibody from the protocol.

Assessment of β-Catenin-Dependent Expression in Single Cells

To monitor β-catenin-dependent transcription in single cells, the β-catenin binding site (i.e., the LEF-1/TCF TOP motif) was cloned into the pGFPemd-p vector (Packard, Meriden, CT) using the following oligonucleotides:

1. Antisense:5'PCCCTTTGGCCTTACCCCC TTTGGC CTTACCCCCTTTGGCCTT'3.
2. Sense: 5' PAAGGCCAAAGGGGGTAAGGCCAAA GGGGGTAAGGCCAAAGGG'3.

The same sequences with two base pair exchanges were used to provide a control vector.

1. Antisense:5'PCCCTTTGATCTTACCCCCT TTGATC TTACCCCCTTTGATCTT'3
2. Sense:5'PAAGATCAAAGGGGGTAAGATCAAAGGGGG AAGATCAAA GGG'3.

Oligonucleotides were annealed in 250 m*M* Tris, pH 7.7. and ligated into the *ECO*RV site of the pGFPemd-p vector.

Successful insertion and the number of oligonucleotides inserted was determined by restriction enzyme digestion. Clones containing two inserts were used in subsequent experiments.

ROSE-179 cells were plated on gridded cover glass within 35-mm culture dishes and cultured for 24 h in Dulbecco's modified Eagle's medium/F-12 medium that was supplemented with 5% fetal bovine serum. Transfections were performed with Lipofectamin (Life Technologies, Rockville, MD) according to the manufacturer's instructions. Cells were transfected with the TOP-GFP, FOP-GFP, or pEGFP-C1 DNA (Clontech, Palo Alto, CA). Two micrograms of DNA/culture dish was used in each transfection. The cells, transfected with pEGFP-C1, were used to monitor transfection efficiency.

Transfection efficiency was estimated by observing the cells under a fluorescent microscope using the FITC filter set 48 h after transfection and determining the percentage of GFP-positive cells. The percentage of cells showing β-catenin-dependent expression was determined 72 h after transfection using the following protocol. For both TOP-GFP and FOP-GFP-transfected cells, 20 phase and fluorescent images were captured. The precise location of these cells was recorded based on the grid markings. After the images were collected, serum and calcium was removed from half of the cultures, and all the cultures were incubated for an additional 5 h. Phase and fluorescent images of the exact same cells were then collected. The mean fluorescent intensity of cells transfected with either TOP-GFP or FOP-GFP was determined using IPLab Spectrum (Scanalytics, Vienna, VA). To ensure that the fluorescence associated with the TOP-GFP-transfected cells was a specific reflection of β-catenin-dependent transcription, the mean fluorescent intensity of cells transfected with FOP-GFP was subtracted from the fluorescent images of TOP-GFP-transfected cells. The percentage of cells showing β-catenin-dependent transfection (i.e., TOP-GFP cells) was determined. This experiment was repeated four times.

Forced Expression of β-Catenin and Apoptosis

ROSE-179 cells were transfected using Lipofectamine. In this experiment, cells were transfected with 1 μg of pEGFP-C1 DNA (Clontech) and either 1μg of the β-catenin expression vector DNA, ΔN57-β-catenin, or a control vector (pECE). The β-catenin vector was provided by Dr. Ben-Ze'ev (Weizmann Institute of Science, Rehovot, Israel) and expressed a mutated β-catenin in which the first 57 NH_2-terminal amino acids were deleted *(30)*. This mutation enhanced the stability of β-catenin *(30)*.

After transfection, the cells were cultured for 24 h in serum-supplemented medium. Half the cultures were depleted of serum and calcium, and all cultures continued for an additional 3 h. To assess the effect of forced expression of the cellular localization of β-catenin, cells were fixed in 10% formalin and then sequentially stained for β-catenin using a rhodamine-labeled IgG secondary antibody. The nuclei were stained with DAPI. The cells were observed under fluorescent microscopy using FITC filters to detect transfected cells (i.e., GFP-positive cells), a rhodamine filter set to detect β-catenin, and a DAPI filter set to observe the nuclei. Because the GFP fluorescence was not permanent, the slides were observed within 24 h of fixation.

A similar transfection study was conducted to assess the effect of forced β-catenin expression on the rate of ROSE-179 cell apoptosis. The ROSE-179 cells were cultured as previously described. After 3 h with or without serum supplementation, the cells were incubated with hydroethidine (Polysciences, Warrington, PA) for 5 min to stain their nuclei *(39)*. To identify transfected cells (i.e., GFP-positive cells), the cells were observed under fluorescent optics using the FITC filter set. The filter set was then changed to a rhodamine filter set to visualize the nuclear morphology of the transfected cells. Briefly, cells that possessed condensed and/or fragmented nuclei were considered to be apoptotic *(39)*. The percentage of apoptotic cells was determined. These experiments were conducted in duplicate and repeated three times.

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References

1. Tsafriri, A. and Reich. R. (1999). *Exp. Clin. Endocrinol. Diabetes* **107,** 1–11.
2. Murdoch, W. J. (1996). *Prostaglandins* **52,** 497–506.
3. Trolice, M. P., Pappalardo. A., and Peluso, J. J. (1997). *Endocrinology* **138,** 107–113.
4. Lail-Trecker, M., Peluso, C., and Peluso, J. (2000). *Endocrine* **12,** 303–314.
5. Gulati, R. and Peluso, J. J. (1997). *Endocrinology* **138,** 1847–1856.
6. Hoffman, A. G, Burghardt, R. C., Tilley, R., and Auersperg, N. (1993). *Int. J. Cancer* **54,** 828–838.
7. Peluso, J. J., Pappalardo, A., and Trolice, M. P. (1996). *Endocrinology* **137,** 1196–1203.
8. Peluso, J. J. (1997). *Biochem. Pharmacol.* **54,** 847–853.
9. Potter, E., Bergwitz, C., and Brabant, G. (1999). *Endocr. Rev.* **20,** 207–239.
10. Gumbiner, B. M. (1993). *Neuron* **11,** 551–564.
11. Behrens, J., Jerchow, B. A., Wurtele, M., Grimm, J., Asbrand, C., Wirtz, R., Kuhl, M., Wedlich, D., and Birchmeier. W. (1998). *Science* **280,** 596–599.
12. Huber, O., Korn, R., McLaughlin, J., Ohsugi, M., Herrmann, B. G., and Kemler, R. (1996). *Mech. Dev.* **59,** 3–10.
13. Rimm, D. L., Caca, K., Hu, G., Harrison, F. B., and Fearon, E. R. (1999). *Am. J. Pathol.* **154,** 325–329.

14. Takahashi, M., Fukuda, K., Sugimura, T., and Wakabayashi, K. (1998). *Cancer Res.* **58,** 42–46.
15. Christofori, G. and Semb, H. (1999). *Trends Biochem. Sci.* **24,** 73–76.
16. Mann, B., Gelos, M., Siedow, A., et al. (1999). *Proc. Natl. Acad. Sci. USA* **96,** 1603–1608.
17. Sadot, E., Simcha, I., Shtutman, M., Ben-Ze'ev, A., and Geiger, B. (1998). *Proc. Natl. Acad. Sci. USA* **95,** 15,339–15,344.
18. Chiesa, R., Angeretti, N., Del Bo, R., Lucca, E., Munna, E., and Forloni, G. (1998). *J. Neurochem.* **70,** 1474–1483.
19. Makrigiannakis, A., Coukos, G., Christofidou-Solomidou, M., Gour, B. J., Radice, G. L., Blaschuk, O., and Coutifaris, C. (1999). *Am. J. Pathol.* **154,** 1391–1406.
20. Brancolini, C., Lazarevic, D., Rodriguez, J., and Schneider, C. (1997). *J. Cell. Biol.* **139,** 759–771.
21. Herren, B., Levkau, B., Raines, E. W., and Ross, R. (1998). *Mol. Biol. Cell* **9,** 1589–1601.
22. Boone, D. L. and Tsang, B. K. (1998). *Biol. Reprod.* **58,** 1533–1539.
23. Izawa, M., Nguyen, P. H., Kim, H. H., and Yeh, J. (1998). *Fertil. Steril.* **70,** 549–552.
24. Rueda, B. R., Hendry, I. R., Tilly, J. L., and Hamernik, D. L. (1999). *Biol. Reprod.* **60,** 1087–1092.
25. He, T. C., Sparks, A. B., Rago, C., Hermeking, H., Zawel, L., da Costa, L.T., Morin, P. J., Vogelstein, B., and Kinzler, K. W. (1998). *Science* **281,** 1509–1512.
26. Omer, C. A., Miller, P. J., Diehl, R. E., and Kral, A. M. (1999). *Biochem. Biophys. Res. Commun.* **256,** 584–590.
27. King, K. L. and Cidlowski, J. A. (1998). *Annu. Rev. Physiol.* **60,** 601–617.
28. Murohashi, I., Yoshida, K., Handa, A., Murayoshi, M., Yoshida, S., Jinnai, I., Bessho, M., and Hirashima, K. (1997). *Exp. Hematol.* **25,** 1042–1050.
29. Zornig, M. and Evan, G. I. (1996). *Curr. Biol.* **6,** 1553–1556.
30. Salomon, D., Sacco, P. A., Roy, S. G., Simcha, I., Johnson, K. R., Wheelock, M. J., and Ben-Ze'ev, A. (1997). *J. Cell. Biol.* **139,** 1325–1335.
31. Fagotto, F., Gluck, U., and Gumbiner, B. M. (1998). *Curr. Biol.* **8,** 181–190.
32. Brabletz, T., Jung, A., Hermann, K., Gunther, K., Hohenberger, W., and Kirchner, T. (1998). *Pathol. Res. Pract.* **194,** 701–704.
33. Simcha, I., Shtutman, M., Salomon, D., Zhurinsky, J., Sadot, E., Geiger, B., and Ben-Ze'ev, A. (1998). *J. Cell. Biol.* **141,** 1433–1448.
34. Tanaka, S., Akiyoshi, T., Mori, M., Wands, J. R., and Sugimachi, K. (1998). *Proc. Natl. Acad. Sci. USA* **95,** 10,164–10,169.
35. White, B. A. and Bancroft, C. (1987). *Methods Enzymol.* **139,** 655–667.
36. Delidow, B. C., Lail-Trecker, M., and White, B. A. (1992). *Mol. Endocrinol.* **6,** 1268–1276.
37. Shapiro, D. B., Pappalardo, A., White, B. A., and Peluso, J. J. (1996). *Endocrinology* **137,** 1187–1195.
38. Lee, K. A., Bindereif, A., and Green, M. R. (1988). *Gene Anal. Tech.* **5,** 22–31.
39. Luciano, A. M., Pappalardo, A., Ray, C., and Peluso, J. J. (1994). *Biol. Reprod.* **51,** 646–654.