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Wnt-1 Regulates Free Pools of Catenins and Stabilizes APC-Catenin Complexes

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The *Wnt-1* proto-oncogene induces the accumulation of β-catenin and plakoglobin, two related proteins that associate with and functionally modulate the cadherin cell adhesion proteins. Here we have investigated the effects of *Wnt-1* expression on the tumor suppressor protein APC, which also associates with catenins. Expression of *Wnt-1* in two different cell lines greatly increased the stability of APC-catenin complexes. The steady-state levels of both catenins and APC were elevated by Wnt-1, and the half-lives of both β-catenin and plakoglobin associated with APC were also markedly increased. The stabilization of catenins by Wnt-1 was primarily the result of a selective increase in the amount of uncomplexed, monomeric β-catenin and plakoglobin, detected both by affinity precipitation and size-exclusion chromatography of cell extracts. Exogenous expression of β-catenin was possible in cells already responding to Wnt-1 but not in the parental cells, suggesting that Wnt-1 inhibits an essential regulatory mechanism for β-catenin turnover. APC has the capacity to oppose this Wnt-1 effect in experiments in which overexpression of the central region of APC significantly reduced the size of the monomeric pool of β-catenin induced by Wnt-1. Thus, the Wnt-1 signal transduction pathway leads to the accumulation of monomeric catenins and stabilization of catenin complex formation with both APC and cadherins.

The *Wnt-1* gene is required for normal development of the mammalian central nervous system and can contribute to mammary oncogenesis when inappropriately expressed in the mammary gland or mammary-derived cell lines in culture (17). The specific mechanism by which *Wnt-1* functions in growth, differentiation, and malignant transformation is not clearly defined, but it is presumed to involve a signal transduction pathway initiated by the interaction of the secreted Wnt-1 glycoprotein with an as yet unidentified cell surface receptor (3, 20). It is clear that at least some of the biochemical consequences of Wnt-1 protein expression are analogous to those utilized by its *Drosophila* counterpart, wingless. For example, wingless expression in *Drosophila* embryos promotes the posttranscriptional accumulation of armadillo protein (23). Rapid accumulation and stabilization of armadillo protein have also been demonstrated in cultured *Drosophila* cells incubated with soluble wingless ligand (30). Similarly, in mammalian cells, *Wnt-1* expression leads to increased steady-state levels of both of the armadillo homologs β-catenin and plakoglobin (γ-catenin) (4, 9). The stabilization of catenins in mammalian cells is also posttranscriptional and results from a specific decrease in the rate of catenin protein degradation (9).

The catenins were first described as proteins that function through binding to the cytoplasmic domain of the cadherin family of transmembrane cell-to-cell adhesion proteins (13, 16, 18). Three predominant catenins have been identified in association with cadherin: the armadillo family members β-catenin and plakoglobin (γ-catenin) and α-catenin, which is related to the cytoskeletal protein vinculin. While β-catenin and plakoglobin bind directly to cadherins, α-catenin binds indirectly through either β-catenin or plakoglobin (1, 11, 27) and also interacts with the cell cytoskeleton to strengthen adhesive contacts (19). *Wnt-1* expression, however, does not lead to increased accumulation of α-catenin (9). With the mammalian cell system, it was also possible to show that the increased steady-state level of β-catenin led to an increased stabilization of the cadherin β-catenin complex and to increased cell-to-cell adhesion (4, 9).

A function for β-catenin independent of cadherin-mediated cell adhesion has been suggested from data obtained with *Drosophila* and *Xenopus* model systems. For *Drosophila* cells, it has been proposed that the accumulation of armadillo protein, particularly in the cytoplasm, fulfills a signaling requirement leading to the expression of specific genes essential for proper segmental pattern formation during development (22). The signals leading to armadillo accumulation include the inhibition of a serine/threonine kinase, shaggy (sgg)/zeste-white 3 (ZW-3) (23), but the downstream targets or effectors of armadillo remain to be identified. Overexpression of β-catenin or plakoglobin but not cadherin in fertilized *Xenopus* oocytes results in duplication of the dorsal embryonic axis during subsequent development (7, 12). β-Catenin mutants that are incapable of binding α-catenin are still active in this process, further suggesting that β-catenin can function independently of cell adhesion events (7). Notably, this phenotype is similar to that obtained by overexpression of XWnt-1 or XWnt-8 in fertilized *Xenopus* oocytes (14). Similarly, inhibition of the activity of the ZW3 homolog glycogen synthase kinase 3 (GSK3) by using a dominant negative strategy also leads to embryonic axis duplication during *Xenopus* development (8).

In mammalian cells, it is not yet known if the catenins that accumulate in response to Wnt-1 also participate in other signaling pathways or function solely by strengthening cell-cell adhesion contacts. However, a mutant β-catenin lacking α-catenin binding sites was reported to transform NIH 3T3 cells when overexpressed (31). Additional functions for catenins have also been inferred from their association with the APC tumor suppressor protein (26, 29). This has led to the proposal that APC may serve as a cytoplasmic effector of catenins,

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thereby controlling some aspect of cell growth (21). It is also conceivable that APC is involved directly in regulating the stability of uncomplexed β-catenin. Accordingly, we have found that the expression of wild-type APC in a cancer cell line expressing only mutant APC protein promotes the downregulation of cytoplasmic β-catenin (15). This activity was mapped to the central region of the APC protein, which is typically deleted from the mutant APC proteins identified in cell lines derived from colonic tumors (24). By contrast, these mutant APC proteins retain the ability to associate with β-catenin (26, 27, 29). Therefore, the absence of wild-type APC may lead to unscheduled cell growth as a result of an inability of the mutant form to downregulate β-catenin levels. An extension of this hypothesis is that both wild-type and mutant APCs also serve as effectors for β-catenin signaling but only wild-type APC retains the inherent capacity to terminate this signal.

The finding that both Wnt-1 and APC influence the stability of catenins suggests that these two proteins may function in a common growth-regulatory pathway involving the modulation of catenin levels. We therefore examined the effects of *Wnt-1* expression on the interaction of wild-type APC with catenins and further investigated the influence of *Wnt-1* expression on the distribution of β-catenin and plakoglobin into complexed and free pools. Based on these findings, a model in which APC and β-catenin function in concert to fulfill Wnt-1 signaling is presented.

MATERIALS AND METHODS

Cell lines, antisera, and vectors. The control and *Wnt-1*-expressing cell lines C57MG and AtT20, respectively, have been described previously (2, 9, 20). C57MG is a normal mouse mammary epithelial cell line that responds to the effects of *Wnt* genes with morphological alterations and changes in growth properties (2, 3). AtT20 is a mouse pituitary cell line that has been used to study the biochemistry of Wnt protein processing and secretion (20, 28). Both of these cell lines were transfected with an expression vector for murine *Wnt-1* cDNA.

Rabbit polyclonal antibodies against catenins were previously described (9). Rabbit polyclonal antibodies directed against the purified APC2 and APC3 fragments and affinity purified against these same proteins were described previously (26).

Expression vectors for the mutant APC proteins, APC1 (consisting of amino acids 1 to 1210) and APC25 (consisting of amino acids 1342 to 2075), were generated as outlined previously (15). A mammalian expression vector was constructed for full-length β-catenin, modified by the addition of an epitope tag, Glu-Glu, as described before (25). The encoded protein was detected with a previously characterized antibody, anti-Glu-Glu, directed against the epitope tag (25). A mammalian expression vector for Jak2 protein modified by the addition of a hemagglutinin (HA) epitope tag was generously provided by Mikhail Gishizsky (SUGEN, Inc.).

Transfections. For the transient-transfection experiment shown in Fig. 6, control and *Wnt-1*-expressing cells were seeded in 60-mm dishes, and 5 μg of the indicated plasmid DNA was introduced per dish in 20 μl (C57MG) or 40 μl (AtT20) of lipofectamine reagent (GIBCO/BRL) as directed by the manufacturer. After 48 h, the cells were harvested into immunoprecipitation buffer for analysis. For the transient transfection of C57MG cells shown in Fig. 5, human adenovirus 5 strain DI343 was included with a DEAE-dextran transfection protocol as described before (6) to introduce 5 μg of either the APC1 or APC25 expression vector into these cells. After 48 h, the cells were harvested and analyzed by size-exclusion chromatography.

Immunoprecipitations and Western immunoblots. Cells were harvested into immunoprecipitation buffer consisting of 10 mM sodium phosphate (pH 7.0), 0.15 M NaCl, 1% Nonidet-P-40, and a cocktail of protease and phosphatase inhibitors (26). After incubation on ice for 20 min, extracts were clarified by centrifugation for 10 min in a microcentrifuge and subjected to immunoprecipitation with either 1 μg of anti-APC3, 5 μl of anti-β-catenin, or 10 μl of partially purified anti-Glu-Glu monoclonal antibody (0.5 mg/ml) as indicated. Immune complexes were collected by using protein A-Sepharose, washed three times with immunoprecipitation buffer, solubilized in Laemmli sample buffer, and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Immunoblots were incubated in Tris-buffered saline containing 0.1% Tween-20, including 5% nonfat dried milk during the blocking and antibody incubation steps. All antibodies were used at a 1:1,000 dilution, followed by detection with horseradish peroxidase (HRP)-conjugated protein A and the ECL system (Amersham).

Size-exclusion chromatography. Size-exclusion chromatography was performed with a Waters model 650E fast protein liquid chromatography (FPLC) system interfaced with a Superose 12 HR 10/30 column (Pharmacia) equilibrated in 25 mM Tris (pH 8.0)–0.25 M NaCl–0.1% Nonidet P-40–1 mM dithiothreitol–10 μg of pepstatin per ml–10 μg of leupeptin per ml. For sample preparation, a single 10-cm dish of confluent cells was harvested by scraping and washed once with cold phosphate-buffered saline (PBS), and the cell pellet was solubilized in a fivefold volume of Triton X-100 lysis buffer (20 mM Tris buffer [pH 8.0], 1.0% Triton X-100, 140 mM NaCl, 10% glycerol, 1 mM EGTA [ethylene glycol tetraacetic acid], 1 mM dithiothreitol, 1 mM sodium orthovanadate, 50 mM NaF, 1 mM Pefabloc, 10 μg of pepstatin per ml, 10 μg of leupeptin per ml). Following centrifugation for 10 min in a microcentrifuge, the supernatants were adjusted to 2.5 mg of total protein per ml with lysis buffer, and 300 μl was injected onto the column. Chromatography was performed at 4°C at a flow rate of 0.5 ml/min, and fractions of 0.5 ml were collected. Twenty microliters of each fraction was analyzed by SDS-PAGE and immunoblotting. Immunoblots were developed with ^{125}I-protein A-Sepharose and then quantitated with an Ambis model 4000 beta scanner.

Pulse-chase analysis. For the pulse-chase analysis, replicate cultures of control and *Wnt-1*-expressing cells were incubated in the absence of methionine for 20 min, pulse labeled for 30 min with 250 μCi of [^{35}S]methionine per ml, washed twice, and incubated in medium containing excess unlabeled methionine for 0, 0.5, 1.0, 2.0, or 4.0 h. At each indicated time, cells were extracted with immunoprecipitation buffer, and aliquots of each extract containing equivalent amounts of protein were immunoprecipitated with an antibody directed against either APC or β-catenin as described above.

Affinity precipitation. For the experiment shown in Fig. 3, control and *Wnt-1*-expressing cells were solubilized in immunoprecipitation buffer. Aliquots of each clarified extract containing equivalent amounts of protein were incubated with Sepharose beads to which either a control glutathione *S*-transferase (GST) protein or a GST-cadherin fusion protein was bound. After incubation at 4°C for 1 h, the Sepharose beads were collected by brief centrifugation in a microcentrifuge and washed three times with immunoprecipitation buffer. Laemmli sample buffer was added to the pellet of washed beads, and the samples were analyzed by SDS-PAGE followed by Western immunoblotting with either β-catenin or plakoglobin antibodies as described above. The GST fusion protein for E-cadherin was constructed by using the pGEX vector system (Promega). The protein consists of bacterial GST fused in frame to the entire cytoplasmic domain of E-cadherin. The fusion protein was purified from bacterial lysates, bound to glutathione-Sepharose beads, washed in immunoprecipitation buffer, and used for affinity precipitation of catenins from cell extracts.

RESULTS

***Wnt-1* expression increases APC levels and stabilizes complex formation with catenins.** A previous study demonstrated that *Wnt-1* expression in two different cell lines, AtT20 and C57MG, leads to increased accumulation of both β-catenin and plakoglobin and increased stabilization of the β-catenin/cadherin complex (9). Since APC protein also associates with catenins independently of cadherin, it was of interest to examine the effects of Wnt-1 on these complexes as well. Both the parental AtT20 and C57MG cell lines and their *Wnt-1*-expressing counterparts contain full-length, wild-type APC protein, as determined by immunoprecipitation and Western blotting (Fig. 1). Expression of *Wnt-1* resulted in an increased steady-state level of APC protein as well as an increase in the amount of β-catenin coimmunoprecipitated by antibodies specific to the APC protein (Fig. 1).

Previous results with AtT20 cells showed that the β-catenin in complex with N-cadherin exhibits a greatly increased half-life in response to Wnt-1 (9). We therefore examined whether the β-catenin and plakoglobin associated with APC in *Wnt-1*-expressing AtT20 and C57MG cells also has an extended half-life. Pulse-chase analysis was performed on the two sets of cell lines, and the levels of [^{35}S]methionine-labeled catenins immunoprecipitated with antibodies specific for either APC or β-catenin were visualized following fluorography of SDS-PAGE gels. The β-catenin associated with APC from the parental cells exhibited an extremely short half-life, less than 30 min, whereas that associated with APC from both of the *Wnt-1*-expressing cell types was considerably more stable (Fig. 2). Similarly, an increased half-life was observed for plakoglobin complexed with APC from AtT20 cells (Fig. 2). The level of plakoglobin expression in C57MG cells was too low for detection by pulse-chase analysis. As previously described in a study

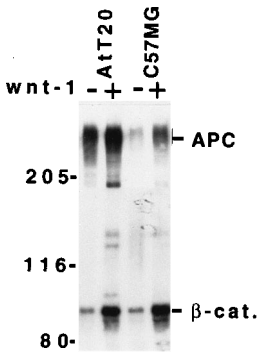

FIG. 1. APC/β-catenin complexes in control and *Wnt-1*-expressing cell lines. Extracts of control (lanes −) and *Wnt-1*-expressing (lanes +) AtT20 and C57MG cells were incubated with 1 μg each of anti-APC3 antibody, and the immunoprecipitates were analyzed by SDS-PAGE followed by immunoblotting. The Western blots were cut horizontally; the top half was developed with anti-APC2 antibody, and the bottom half was developed with anti-β-catenin antibody. Sizes are shown in kilodaltons.

using antibodies specific to β-catenin, the overall pool of β-catenin shows a greatly extended half-life in both cell types under the influence of *Wnt-1* (Fig. 2). We have also noted that the electrophoretic mobility of APC is reproducibly slower in the C57MG cells expressing *Wnt-1* than in the control cells (Fig. 2), perhaps suggesting differences in phosphorylation state. The significance of this is unclear and is presently under investigation. Overall, these results indicate that *Wnt-1* expression increases the steady-state levels of APC, plakoglobin, and β-catenin and stabilizes the APC-catenin complexes.

***Wnt-1* expression increases uncomplexed pools of catenins.** The stabilization of catenins observed in cells expressing *Wnt-1* may result in the accumulation of catenins to levels which exceed the binding capacity of cadherin and APC. This is also suggested from the cytoplasmic staining of plakoglobin in *Wnt-1*-expressing cells (4) and by the appearance of armadillo protein in the cytoplasm of *Drosophila* embryo cells in response to wingless expression (23). Therefore, one consequence of Wnt-1 signaling may be the generation of uncomplexed catenins that are available for association with other protein targets. To test this, a purified GST fusion protein containing the cytoplasmic domain of E-cadherin was incubated with detergent extracts of cells and recovered by precipitation with glutathione-Sepharose, and the precipitates were analyzed for the presence of β-catenin or plakoglobin. Since the catenin molecules that are already in complex with APC or cadherin are unavailable to bind to the cadherin-GST fusion protein, this method offers a useful way to define free pools of catenins which are available for association with the exogenously added binding site. The cadherin-GST fusion protein bound significant quantities of β-catenin in extracts from *Wnt-1*-expressing AtT20 and C57MG cells, whereas little or no β-catenin is available for binding in the parental cell extracts (Fig. 3). Similarly, plakoglobin protein can be selectively isolated from the extracts of *Wnt-1*-expressing AtT20 cells with the cadherin-GST protein, while no free pool of plakoglobin was available in the parental extracts (Fig. 3).

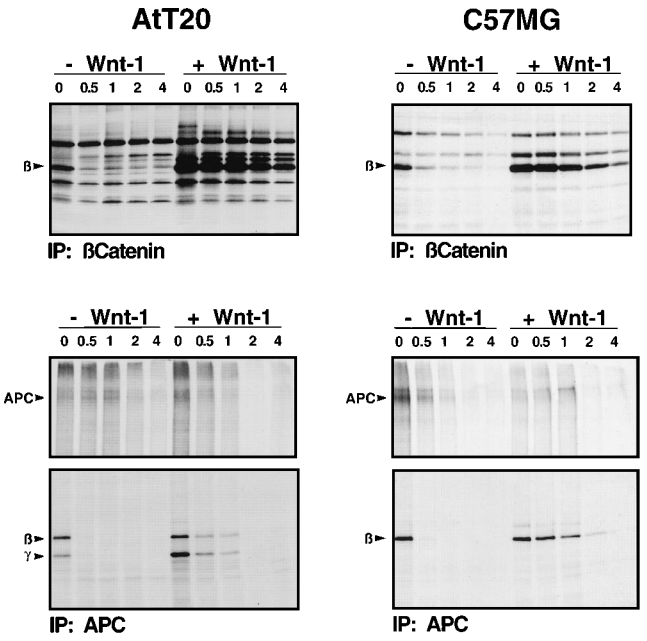

FIG. 2. Wnt-1 stabilizes β-catenin and the catenin complex with APC. Replicate cultures of control (− Wnt-1) and *Wnt-1*-expressing (+ Wnt-1) cells were pulse labeled (0 h of chase) with [^{35}S]methionine for 20 min and chased in the absence of label for the indicated times (0.5, 1, 2, or 4 h). At each time point, cell extracts were prepared, and aliquots were immunoprecipitated with specific antibodies directed against either β-catenin (IP: βCatenin) or APC (IP: APC). The immunoprecipitates were analyzed by SDS-PAGE followed by fluorography. The positions of APC, β-catenin (β), and plakoglobin (γ) are indicated.

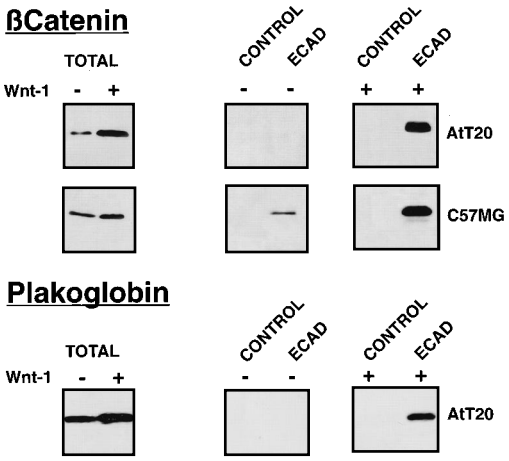

FIG. 3. Wnt-1 increases uncomplexed pools of β-catenin and plakoglobin. Extracts of either control (lanes −) or *Wnt-1*-expressing (lanes +) cells were incubated either with a GST fusion protein containing the cytoplasmic domain of E-cadherin (ECAD) or with a control GST protein alone (Control). The GST proteins were recovered from the cell extracts by binding to glutathione-Sepharose, washed, and analyzed by SDS-PAGE followed by immunoblotting with antibodies specific for either β-catenin or plakoglobin.

The ability of cadherin protein to bind to uncomplexed catenins from *Wnt-1*-expressing cell lysates is similar to our previous finding that APC protein can bind to β-catenin in lysates prepared from cells expressing mutant APC but not from cells expressing wild-type APC (15). Furthermore, examination of these lysates by size fractionation revealed a substantial pool of monomeric β-catenin in cells with mutant APC protein, whereas β-catenin from cells expressing wild-type APC migrated exclusively as a high-molecular-weight complex. We therefore examined the oligomeric status of catenins in AtT20 and C57MG cells by size-exclusion chromatography. Detergent extracts of cells were fractionated, and the amount of catenin in each fraction was estimated by quantitative immunoblotting. As expected for cells expressing wild-type APC, both of the parental cell lines contained β-catenin which eluted

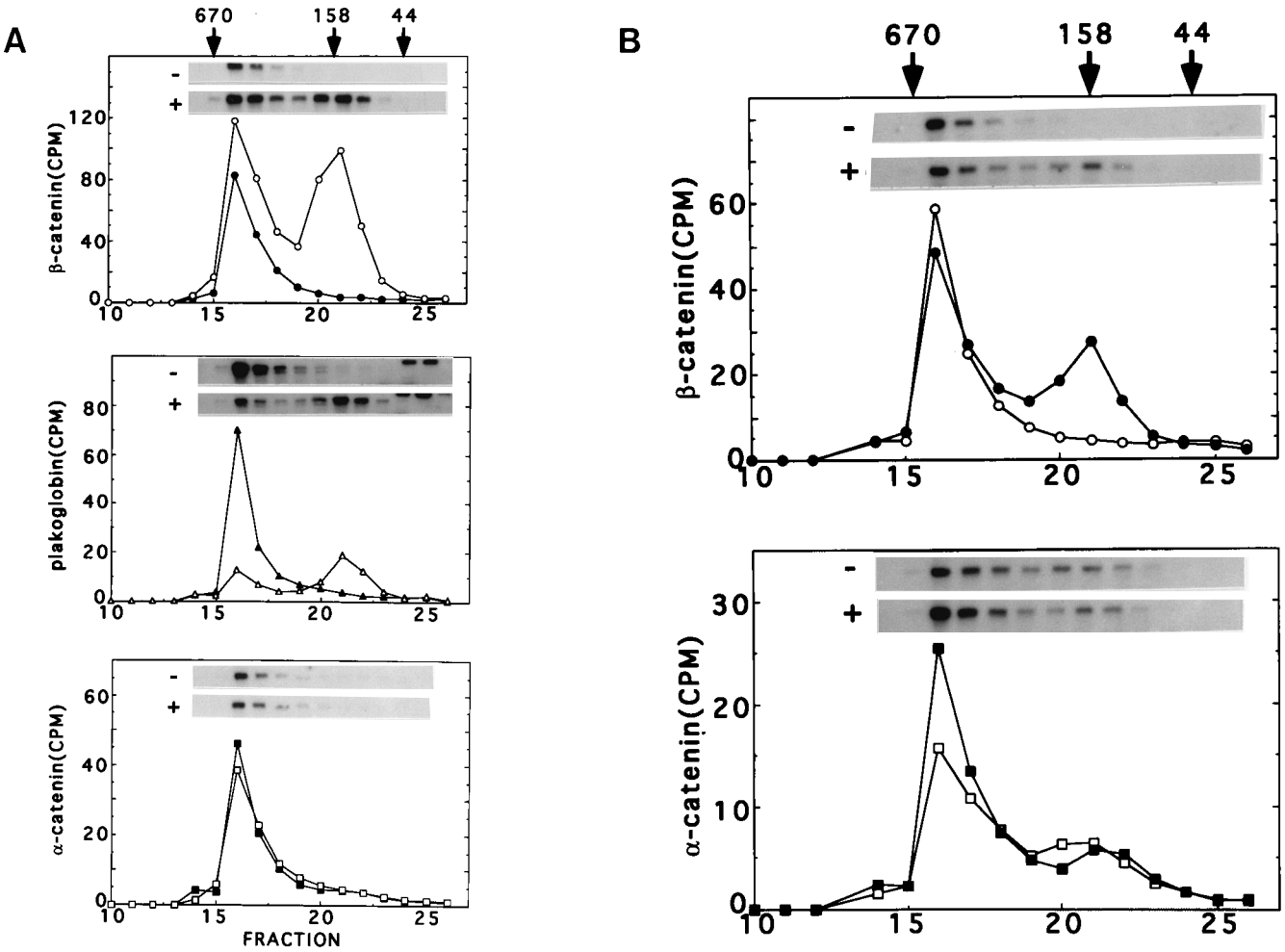

FIG. 4. Wnt-1 increases monomeric pools of catenins. Extracts of either control (lanes −) or *Wnt-1*-expressing (lanes +) cells were subjected to size-exclusion chromatography on a Superose 12 column, and fractions were analyzed by SDS-PAGE followed by immunoblotting with antibodies specific to α-catenin, β-catenin, or plakoglobin, as indicated. Proteins were detected by using ^{125}I-protein A, and the chromatograms were generated with a beta scanner. Autoradiograms are presented in the insets, with each band aligned above its corresponding fraction on the chromatogram. Values at the top indicate positions and molecular masses (in kilodaltons) of the column calibration standards: thyroglobin (670 kDa), aldolase (158 kDa), and ovalbumin (44 kDa). (A) AtT20 cells without (solid symbols) or with (open symbols) Wnt-1. (B) C57MG cells without (open symbols) or with (solid symbols) Wnt-1.

only as a high-molecular-weight complex (Fig. 4). However, expression of *Wnt-1* resulted in a dramatic increase in the pool of monomeric β-catenin (Fig. 4). We have shown previously that this elution position corresponds to that of highly purified recombinant β-catenin (15). A marginal increase in the high-molecular-weight form of β-catenin was also observed in response to Wnt-1. No obvious change in the elution position of α-catenin was noted (Fig. 4). Wnt-1 also promoted a substantial increase in the monomeric pool of plakoglobin in AtT20 cells. However, in this case *Wnt-1*-expressing cells also exhibited a reduced amount of the high-molecular-weight plakoglobin population relative to control cells (Fig. 4). The plakoglobin levels in C57MG cells were too low for this analysis.

The presence of a pool of monomeric β-catenin in response to Wnt-1 is interesting, as we have previously observed this phenomenon only in cells expressing mutant APC (15). In that study, we found that overexpression of wild-type APC or a fragment containing the central region of the protein eliminated the pool of monomeric β-catenin. We therefore tested the effects of APC overexpression on the accumulation of monomeric β-catenin induced by Wnt-1. C57MG cells stably expressing *Wnt-1* were transiently transfected with cDNAs coding for either the central region of APC (APC25, amino acids 1342 to 2075) or, as a control, the amino-terminal fragment (APC1, amino acids 1 to 1210), which resembles the mutant forms of APC found in colorectal cancer cells. Expression of the central region of APC resulted in a reduction in the complexed as well as the monomeric pool of β-catenin induced by Wnt-1, whereas expression of the amino-terminal fragment did not (Fig. 5). The decreased level of β-catenin was consistent with the transfection efficiency, which ranged from 25 to 50%, as judged by transfection with an expression vector encoding β-galactosidase under identical conditions (data not shown).

Wnt-1 stabilizes exogenously expressed β-catenin. The abilities of Wnt-1 and APC to regulate the levels of monomeric, free pools of catenins suggest that catenin accumulation in an uncomplexed form may be a critical step in growth control or differentiation. Accordingly, we sought to study the biological consequences to the cell of ectopic overexpression of β-catenin in parental cells but were unable to obtain stable lines expressing appreciable levels of recombinant β-catenin. Thus, the cell possesses a regulatory mechanism to ensure that β-catenin

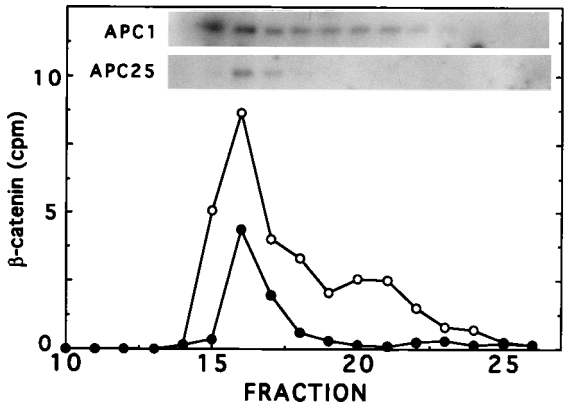

FIG. 5. APC expression reverses Wnt-1-induced accumulation of β-catenin. C57MG cells expressing *Wnt-1* were transfected with expression vectors encoding either APC1 (amino acids 1 to 1210) or APC25 (amino acids 1342 to 2075) and harvested 48 h following transfection. Aliquots of cell lysates were analyzed by size-exclusion chromatography followed by SDS-PAGE and Western blotting with an antiserum against β-catenin as described in the legend to Fig. 4. The graph below the Western blots summarizes the data. Open circles, β-catenin expression in cells transfected with APC1; solid circles, β-catenin expression in cells transfected with APC25.

levels are sufficient to support complex formation with proteins such as cadherin but that free pools are rapidly degraded. The Wnt-1-induced accumulation of free pools of β-catenin could be achieved through suppression of the mechanism that normally degrades uncomplexed pools of β-catenin. Expression levels of exogenous β-catenin may therefore be regulated by a maximal setpoint established for endogenous β-catenin, and this setpoint may be elevated in cells expressing *Wnt-1*. To test this, we transiently expressed a cDNA encoding an epitope-tagged β-catenin in parental AtT20 and C57MG cells and their *Wnt-1*-expressing counterparts. The recombinant β-catenin was recovered by immunoprecipitation with antibody specific to the epitope tag. Significantly higher yields of epitope-tagged β-catenin were recovered from the cells expressing *Wnt-1* than from the parental cell lines, similar to the relative expression levels of endogenous β-catenin (Fig. 6A).

A control experiment was performed to ensure that the transfection efficiency was equivalent between the *Wnt-1*-expressing and parental cells. An expression vector for an epitope-tagged Jak2 protein was transfected, under identical conditions, into parental AtT20 and C57MG cells and their *Wnt-1*-expressing counterparts. The transient expression of this protein was detected by immunoprecipitation from equivalent aliquots of each cell extract with an epitope tag-specific antibody, followed by Western immunoblotting of the immunoprecipitates with the same antibody. The level of tagged Jak2 expression was similar in each of the cell types, indicating a similar transfection efficiency (Fig. 6B). These and other data presented above support a mechanism for downregulation of uncomplexed pools of β-catenin through the action of wild-type APC and imply that Wnt-1 acts to inhibit this APC function, leading to a stable increase in the population of uncomplexed catenins.

DISCUSSION

Previous studies have shown that *Wnt-1* expression in several different cell lines leads to an increase in the steady-state levels of both β-catenin and plakoglobin and a stabilization of cadherin-catenin complexes, thus facilitating cell-to-cell adhesion (4, 9). Here we have shown that a second catenin-binding protein, the tumor suppressor gene product APC, is regulated by Wnt-1 in a similar fashion. Wnt-1 induced an increase in the steady-state level of APC protein and stabilized the APC-catenin complexes. The increased steady-state levels of the APC-catenin complexes are probably a consequence of an increased pool of free β-catenin and plakoglobin which become available for recruitment into the complex. Indeed, we observed that the vast majority of additional catenin detected in *Wnt-1*-expressing cells can be localized to an uncomplexed, monomeric pool. Thus, the effect of Wnt-1 is twofold, leading first to an overall increase in catenin protein stability and consequently to increased recruitment of catenins into complexes with cadherin and APC (Fig. 7). Additional targets of catenin, such as the epidermal growth factor receptor (10) as well as yet to be defined targets, would also be predicted to form more stable complexes with β-catenin in response to Wnt-1 (Fig. 7). These cadherin-independent targets may exhibit a lower affinity for β-catenin than cadherin or APC proteins and thus be highly sensitive to an increasing uncomplexed catenin pool. It is also conceivable that abnormally high levels of β-catenin may lead to its interaction with inappropriate targets. In this manner, Wnt-1 could modulate both cell adhesion and other signal transduction pathways that would provide combinatorial functions in growth, differentiation, and transformation.

The increased population of uncomplexed, monomeric β-catenin and plakoglobin in response to Wnt-1 signaling is similar to the biochemical phenotype of carcinoma cells harboring mutant APC protein (15, 26). Furthermore, it has been observed that these mutant APC proteins lack a function, mapped to the central portion of APC protein, that can lead to downmodulation of free pools of catenins. These findings suggest a model in which APC normally plays a central role in the downregulation of uncomplexed, monomeric pools of catenins and Wnt-1 functions to affect catenin levels through a signal transduction pathway that modulates the activity of APC protein. The Wnt-1 effect could be accomplished through a post-translational modification of APC protein or by subcellular

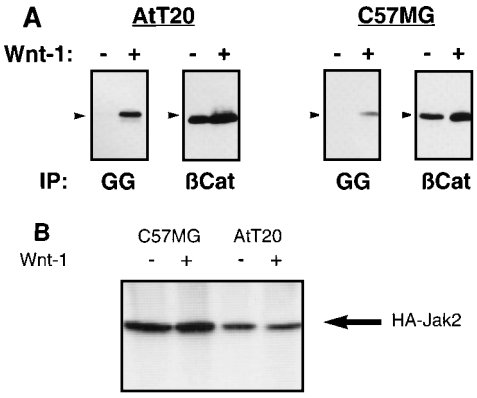

FIG. 6. Wnt-1 increases the setpoint for β-catenin levels. (A) Control (lanes −) and *Wnt-1*-expressing (lanes +) AtT20 and C57MG cells were transfected with an expression vector for an epitope-tagged β-catenin protein. At 48 h following transfection, the cells were solubilized, and aliquots of extracts were immunoprecipitated (IP) with either an antibody against β-catenin (βCat) or an antibody against the protein tag (GG). Immunoprecipitates were analyzed by SDS-PAGE followed by Western immunoblotting with an antibody against β-catenin. (B) Control (lanes −) and *Wnt-1*-expressing (lanes +) AtT20 and C57MG cells were transfected with an expression vector for an epitope-tagged Jak2 protein. At 48 h following transfection, the cells were solubilized, and aliquots of extracts were immunoprecipitated with an antibody against the HA protein tag. Immunoprecipitates were analyzed by SDS-PAGE followed by Western immunoblotting with an antibody against the HA protein tag.

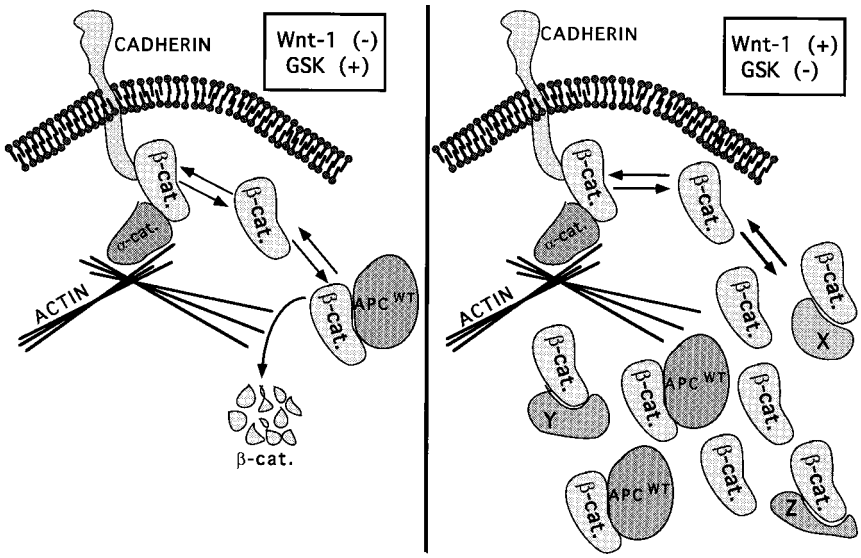

FIG. 7. Model for regulation of β-catenin pool and complex formation. In the absence of Wnt-1 (left), GSK is active, the turnover of β-catenin (β-cat.) is rapid, and low steady-state levels of free β-catenin are present. In the presence of Wnt-1 (right), GSK activity is reduced, high steady-state levels of free β-catenin are attained, and complex formation is facilitated. The proteins designated X, Y, and Z are potential undefined targets for β-catenin. In this model, APC is considered a potential effector of β-catenin. WT, wild type.

redistribution, thereby limiting the access to free pools of catenins. Alternatively, Wnt-1 may simply regulate the susceptibility of β-catenin to downregulation by APC without changing the intrinsic ability of APC to induce catenin degradation.

An additional component of the catenin regulatory pathway is the serine/threonine kinase ZW-3/GSK3. Evidence from studies with both *Drosophila* (23) and *Xenopus* (8) models suggests that ZW-3/GSK3 is constitutively active and is inhibited by wingless/Wnt-1 signaling. These studies further suggest that ZW-3/GSK3 functions upstream of armadillo/β-catenin and that its inactivation may promote downstream signaling events mediated by β-catenin. Deletional analysis of the β-catenin and armadillo proteins suggests that these signaling events are independent of the role of catenins in cell adhesion (7, 22). Thus, it appears that the uncomplexed population of β-catenin is tightly regulated in normal cells, and a selective increase in free catenin levels may result from modification of the activities of several proteins. One means of increasing β-catenin levels involves the inactivation of GSK3 (Fig. 7). By analogy to the wingless/armadillo system, this likely occurs in response to Wnt-1 or, as demonstrated recently, by growth factors (5). A second mechanism to increase uncomplexed catenin levels requires a loss of APC function due to mutations that truncate the protein. The pattern of APC mutations in tumors suggests there is a selective pressure to eliminate the region of APC protein that downregulates catenin levels (24). Alternatively, wild-type APC may be posttranslationally modified in a fashion that renders it incapable of regulating catenin levels. Finally, posttranslational modification of catenins may occur directly, so that they are no longer susceptible to regulation. This has been proposed as a mechanism for armadillo regulation in *Drosophila* cells (22). It is possible, though, that changes in posttranslational modification of catenins or APC may be a direct outcome of GSK3 inactivation.

The proposed signaling functions for β-catenin appear to require the so-called armadillo repeat region (7), which is required for interaction with cadherin, APC, and the epidermal growth factor receptor, but specific downstream effectors have yet to be demonstrated. One candidate is the APC protein itself, which could serve both as an effector for the β-catenin signal and as a means to turn off this signal through downmodulation of free β-catenin. It is expected that additional targets of β-catenin will be identified. These proteins should provide important clues as to how β-catenin can both regulate cell adhesion through its association with cadherins and contribute to other aspects of growth control and differentiation through interaction with additional downstream targets.

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