Axin Directly Interacts with Plakoglobin and Regulates Its Stability*

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Plakoglobin is homologous to β-catenin. Axin, a Wnt signal negative regulator, enhances glycogen synthase kinase (GSK)-3β-dependent phosphorylation of β-catenin and stimulates the degradation of β-catenin. Therefore, we examined the effect of Axin on plakoglobin stability. Axin formed a complex with plakoglobin in COS cells and SW480 cells. Axin directly bound to plakoglobin, and this binding was inhibited by β-catenin. Axin promoted GSK-3β-dependent phosphorylation of plakoglobin. Furthermore, overexpression of Axin down-regulated the level of plakoglobin in SW480 cells. These results suggest that Axin regulates the stability of plakoglobin by enhancing its phosphorylation by GSK-3β and that Axin may act on β-catenin and plakoglobin in similar manners.

Cell adhesion plays a central role in various biological processes, including motility, growth, and differentiation. The most direct effect of adhesion is on morphogenesis, i.e., the assembly of the individual cells into highly ordered tissues and organs through cell-cell junctions (1–3). There are two types of cell-cell junctions: desmosomes and adherens junctions (4, 5). Desmosomes have two transmembrane components, desmoglein and desmocollin, which are members of the cadherin family (6). Plakoglobin has been shown to interact with the cytoplasmic regions of desmoglein and desmocollin (7–11). The transmembrane components of adherens junctions are members of the classical cadherin family (6). Plakoglobin is homologous to β-catenin, with desmoglein and desmocollin. Thus, plakoglobin, with desmoglein and desmocollin (7–11). The transmembrane components of adherens junctions are members of the classical cadherin family. The cytoplasmic region of cadherin forms a complex with three proteins, α-, β-, and γ-catenins (4, 12); γ-catenin is identical to plakoglobin (13, 14). The cytoplasmic components of adherens junctions and desmosomes are necessary for linking to actin filaments and intermediate filaments, respectively (4, 12). β-Catenin and plakoglobin are closely related and are part of the gene family that includes the Drosophila homolog armadillo (15). It has been shown that β-catenin and plakoglobin bind directly to cadherin and α-catenin (16–19). The N-terminal regions of β-catenin and plakoglobin contain their α-catenin-binding sites. The central region, which has multiple copies of the armadillo motif, is involved in the association with cadherin, and in the case of plakoglobin, with desmoglein and desmocollin. Thus, plakoglobin is a common plaque component of both types of cell-cell junctions and is essential for sorting out of desmosomes and adherens junctions. Disruption of plakoglobin in mice causes embryonic death at embryonic days 12–16 because of severe defects in heart structure, resulting in ventricle burst and blood flooding the pericardium (20, 21). β-Catenin knockout mutations are lethal in early mouse embryos, causing specific defects in the embryonic ectoderm cell layer (22). Therefore, β-catenin and plakoglobin do not appear to compensate for each other in embryos.

In addition to their roles in cell-cell adhesion, β-catenin and plakoglobin are components in the Wnt signaling pathway (19, 23–25). Wnt genes encode secreted glycoproteins required for a large number of developmental processes (26). In the fly, the Wnt-1 homolog, wingless, is required for the patterning of each segment, and for pattern formation and cell proliferation in imaginal discs (27). Multiple components of the wingless signaling pathway have been identified and characterized genetically (23–25). They include Dfrizzled2, dishevelled, shaggy, armadillo, and pangolin. Vertebrate homologs of these genes have also been identified (23–25). Armadillo is homologous to both β-catenin and plakoglobin. Wingless expression in Drosophila embryos promotes the posttranslational accumulation of armadillo (28). Rapid accumulation and stabilization of armadillo have also been demonstrated in cultured Drosophila cells incubated with soluble wingless (29). Similarly, in mammalian cells, Wnt-1 expression leads to increasing steady state level of β-catenin and plakoglobin (30–32). In Xenopus laevis, overexpression of β-catenin or plakoglobin leads to ectopic axis formation, and this effect is also obtained with overexpression of Xenopus Wnt (33–35). These results suggest that β-catenin and plakoglobin may have similar functions in the Wnt signaling pathway.

Recent biochemical studies in mammals have revealed the molecular mechanism by which Wnt regulates the stabilization of β-catenin (23–25). In the absence of Wnt, GSK-3β, a shaggy homolog, phosphorylates β-catenin, and the phosphorylated β-catenin is ubiquitinated, resulting in the degradation of β-catenin by proteasomes (36–38). Wnt binds to its receptor Frizzled, a Dfrizzled2 homolog, and inactivates GSK-3β, probably through Dvl, a dishevelled homolog, although the mechanism is not clear (39). This leads to the stabilization of β-catenin, accumulated β-catenin translocates into the nucleus where it binds to and activates the transcriptional factor Tef/Lef, a pangolin homolog (40, 41), resulting in the expression of target genes including c-myc, c-jun, fra, and cyclin D1 (42–44).

We have identified rat Axin (rAxin) and its homolog, Axil (for Axin-like), as GSK-3β-interacting proteins (45, 46). Conductin
has been identified as a β-catenin-binding protein (47) and is identical to Axil. Both Axin and Axil bind not only to GSK-3β but also to β-catenin and APC (45–49) and promote GSK-3β-dependent phosphorylation of β-catenin and APC (45, 46, 49). Axin enhances a complex formation of phosphorylated β-catenin with β-TrCP/PWD (50), resulting in stimulating the degradation of β-catenin (47, 49, 51, 52). Furthermore, Dvl also binds to Axin (53, 54) and inhibits GSK-3β-dependent phosphorylation of β-catenin, APC, and Axin (53, 55). Thus, Axin functions as a scaffold protein and regulates the stability of β-catenin.

Although it has been shown that the expression of Wnt-1 also stabilizes plakoglobin in several cell lines (30, 32), how the stability of plakoglobin is regulated is not fully understood. These results prompted us to examine whether Axin is involved in the regulation of the stability of plakoglobin. Here we demonstrate that Axin directly binds to plakoglobin and enhances GSK-3β-dependent phosphorylation of plakoglobin. Further, we show that Axin degrades plakoglobin. These results suggest that Axin regulates the stability of plakoglobin via a mechanism like that by which Wnt regulates β-catenin.

EXPERIMENTAL PROCEDURES

Materials and Chemicals—Plakoglobin cDNA, SW480 cells, and the anti-HA and anti-MBP antibodies were kindly supplied by Drs. W. W. Franke (German Cancer Research Center, Heidelberg, Germany), E. Tahara (Hiroshima University, Hiroshima, Japan), Q. Hu (Chiron Corp., Emeryville, CA), and M. Nakata (Sumitomo Electronics, Yokohama, Japan), respectively. The anti-Myc antibody was generated from Neche-richia coli according to the manufacturer's instructions. SW480 cells stably expressing Myc-rAxin were made as described (53). The anti-plakoglobin and anti-β-catenin antibodies were purchased from Amersham Pharmacia Biotech. Other materials were from commercial sources.

Plasmid Constructions—pBJ-Myc/rAxin (full length), pMAL-c2/rAxin (full length), pBSKS/rAxin (full length), pMAL-c2/rAxin (298–832), pUC19/rAxin (298–832), pEF-BOS-Myc/rAxin (298–506), pMAL-c2/rAxin (298–506), pEF-BOS-Myc/rAxin (508–832), pBJ-Myc/rAxin (713–832), pMAL-c2/rAxin (713–832), pGEX-2T/β-catenin, pGEX-2T/GSK-3β, and pGEX-2T/Axin were excised by BamHI and SacI and blunted with Klenow fragment and then digested with BamHI. To construct pGAD10/plakoglobin, the fragment encoding plakoglobin (full length) was synthesized by polymerase chain reaction and then digested with BamHI. The fragment encoding rAxin (1–229) was inserted into pMAL-c2 that had been digested with BamHI and blunted with HindIII and then blunted with Klenow fragment and then digested with BamHI. The fragment encoding rAxin (1–229) was inserted into pBSKs/rAxin (full length) that had been digested with BamHI and then blunted with XhoI and EcoRI, and the appropriate fragment was inserted into pGEX-2T that had been digested with BamHI and EcoRI and then blunted with Klenow fragment and then digested with BamHI. The fragment encoding rAxin (1–229) was inserted into pMAL-c2 that had been digested with HindIII and blunted with Klenow fragment and then digested with BamHI. To construct pBJ-Myc/rAxin (1–353), pBSKs/rAxin (full length) was digested with Smal and BamHI and then blunted with Klenow fragment. The fragment encoding rAxin (1–353) was inserted into pBJ-Myc that had been digested with XhoI and blunted with Klenow fragment. To construct pEGFP-c3/rAxin (298–832), pUC19/rAxin (298–832) was digested with SacI, and the appropriate fragment was inserted into pEGFP-c3 that had been digested with SacI.

Interaction of Plakoglobin with rAxin in Intact Cells—COS cells transiently transfected with pBj- and pEF-BOS-derived plasmids or SW480 cells stably expressing Myc-rAxin were lysed in the lysis buffer (20 mM Tris/HCl, pH 8.0, 1% Nonidet P-40, 137 mM NaCl, 1% glycerol, 1 mM phenylmethylsulfonyl fluoride, 2 μg/ml aprotinin, 2 μg/ml leupeptin, 40 mM β-glycerophosphate, and 1 μM sodium orthovanadate). rAxin and GST-plakoglobin were tagged with Myc epitope at the N termini. Plakoglobin was tagged with HA epitope at its N terminus. The lysates (200–800 μg of protein) were immunoprecipitated with the anti-Myc antibody, and the precipitates were probed with the anti-Myc, anti-HA, and anti-plakoglobin antibodies.

Interaction of Plakoglobin with rAxin in Vitro—For determination of the region of plakoglobin that binds to rAxin, various deletion mutants of GST-plakoglobin (each at 250 nM) were incubated with 500 nM MBP-rAxin (full length) for 1 h at 4 °C in 50 μl of reaction mixture (20 mM Tris/HCl, pH 7.5, and 1 mM dithiothreitol). GST-plakoglobin deletion mutants were precipitated with glutathione-Sepharose 4B, and then the precipitates were probed with the anti-MBP antibody. To examine the region of rAxin that binds to plakoglobin, various deletion mutants of rAxin (7.5 pmol of each) immobilized on amyllose resin were incubated with 500 nM GST-plakoglobin (full length) for 1 h at 4 °C in 50 μl of reaction mixture. MBP-rAxin deletion mutants were precipitated by centrifugation, and then the precipitates were probed with the anti-plakoglobin antibody.

Separation and Purification of Axin and Its Fragments—Axin and its deletion mutants were tagged with Myc epitope at their N termini. Plakoglobin was tagged with HA epitope at its N terminus. Therefore, we first examined whether Axin forms a complex with GST-plakoglobin and 180 nM GST-plakoglobin were expressed in COS cells (Fig. 1A). When the lysates of cells expressing Myc-rAxin and HA-plakoglobin were immunoprecipitated with the anti-Myc antibody, HA-plakoglobin was com-

Kinetics of the Binding of rAxin, β-Catenin, and Plakoglobin—To determine the Kd value of the binding of plakoglobin to rAxin, MBP-rAxin (full length) (5 pmol) immobilized on amyllose resin was incubated with various concentrations of GST-plakoglobin (full length) for 1 h at 4 °C in 40 μl of reaction mixture. To show the inhibition of the binding of plakoglobin to rAxin by β-catenin, MBP-rAxin (full length) (3 pmol) immobilized on amyllose resin was incubated with 500 nM GST-plakoglobin (full length) and various concentrations of GST-β-catenin (full length) for 1 h at 4 °C in 40 μl of reaction mixture. MBP-rAxin was precipitated by centrifugation, and then the precipitates were probed with the anti-β-catenin and anti-plakoglobin antibodies. The relative intensities of precipitated GST-plakoglobin and GST-β-catenin were quantified by densitometric tracing of the stained immunoblots using the NIH image program.

Phosphorylation of Plakoglobin by GSK-3β—After 1.2 μM GST-plakoglobin (full length) (180 nM GST-plakoglobin) was incubated with 100 nM MBP-rAxin in 30 μl of kinase reaction mixture (50 mM Tris/HCl, pH 7.5, 10 mM MgCl2, 1 μM dithiothreitol, and 50 μM [γ-32P]ATP (500–2000 cpm/ pmol)) for 30 min at 30 °C, the samples were subjected to SDS-polyacrylamide gel electrophoresis followed by autoradiography. Where specified, the radioactivities of the phosphorylated plakoglobin were counted.

Degradation of Plakoglobin by rAxin in SW480 Cells—SW480 cells were grown on glass coverslips and transfected with pEGFP-c3/rAxin (298–832) by TransFast Reagent (Promega Corp., Madison, WI). At 48 h after the transfection, the cells were fixed for 20 min in PBS containing 4% paraformaldehyde. After washing with PBS three times, the cells were permeabilized with PBS containing 0.2% Triton X-100 and 2 mg/ml bovine serum albumin for 4 h. The cells were washed and incubated for 1 h with the anti-plakoglobin or anti-β-catenin antibody. After washing with PBS, they were further incubated for 1 h with Alexa™ 594 goat anti-mouse IgG (Molecular Probes, Eugene, OR). Coverslips were washed with PBS, mounted on glass slides, and viewed with a confocal laser-scanning microscope (TCS-NT®, Leica-laser-technik GmbH, Heidelberg, Germany).

RESULTS

Formation of Complex between Axin and Plakoglobin—Plakoglobin shares 66% overall identity with β-catenin. Both proteins have armadillo repeats in the central region, and the percentage of the armadillo repeats is 77%. We first hypothesize that Axin directly binds to the armadillo repeats of β-catenin (45, 51). Therefore, we first examined whether Axin forms a complex with plakoglobin in intact cells. Myc-rAxin and HA-plakoglobin were expressed in COS cells (Fig. 1A). When the lysates of cells expressing Myc-rAxin and HA-plakoglobin were immunoprecipitated with the anti-Myc antibody, HA-plakoglobin was coprecipitated with the anti-Myc antibody, HA-plakoglobin was coprecipitated.
was coprecipitated with Myc-rAxin (Fig. 1A). When the lysates expressing HA-plakoglobin alone were immunoprecipitated with the anti-Myc antibody, HA-plakoglobin was not observed in the immune complex (Fig. 1A). We next examined whether Axin forms a complex with endogenous plakoglobin in SW480 cells, because this cell line contains increased cytoplasmic β-catenin and plakoglobin because of the truncation of APC (57). Plakoglobin in SW480 cells was observed as two bands (Fig. 1B). Although we do not know the reason for this, other reports also showed similar results with the antibody that we used (38, 58). When the lysates of SW480 cells stably expressing Myc-rAxin were immunoprecipitated with the anti-Myc antibody and the immunoprecipitates were probed with the anti-HA antibody, HA-plakoglobin was not observed (lanes 4 and 5). The same lysates (200 µg of protein) expressing HA-plakoglobin alone (lane 2) or both Myc-rAxin and HA-plakoglobin (lane 3) were probed with the anti-Myc and anti-HA antibodies. A lysate of COS cells transfected with vectors only was used as a control (lane 1). The same lysates (200 µg of protein) were immunoprecipitated with the anti-Myc antibody (lanes 2 and 5). The immunoprecipitates were probed with the anti-Myc and anti-HA antibodies. A lysate of COS cells transfected with vectors only was used as a control (lane 1). The same lysates (200 µg of protein) were immunoprecipitated with the anti-Myc antibody (lanes 2 and 5). The immunoprecipitates were probed with the anti-Myc and anti-HA antibodies. A lysate of COS cells transfected with vectors only was used as a control (lane 1).

**Fig. 1.** Formation of complex between Axin and plakoglobin in intact cells. A, interaction of plakoglobin with rAxin in COS cells. The lysates (20 µg of protein) of cells expressing HA-plakoglobin alone (lane 2) or both Myc-rAxin and HA-plakoglobin (lane 3) were probed with the anti-Myc and anti-HA antibodies. A lysate of COS cells transfected with vectors only was used as a control (lane 1). The same lysates (200 µg of protein) were immunoprecipitated with the anti-Myc antibody (lanes 2 and 5). The immunoprecipitates were probed with the anti-Myc and anti-HA antibodies. B, interaction of rAxin with endogenous plakoglobin in SW480 cells. Lysates (60 µg of protein) of wild type SW480 cells (lane 1) or SW480 cells stably expressing Myc-rAxin (full length) (lane 2) were probed with the anti-Myc and anti-plakoglobin antibodies. The same lysates (800 µg of protein) were immunoprecipitated with the anti-Myc antibody, and the immunoprecipitates were probed with the anti-Myc and anti-plakoglobin antibodies (lanes 3 and 4). C, region of rAxin that interacts with plakoglobin. The lysates (250 µg of protein) expressing HAPlakoglobin with Myc-rAxin (full length) (lane 1). Myc-rAxin-(1–353) (lane 2), Myc-rAxin-(298–506) (lane 3), Myc-rAxin-(508–832) (lane 4), or Myc-rAxin-(713–832) (lane 5) were immunoprecipitated with the anti-Myc antibody, and the immunoprecipitates were probed with the anti-HA antibody. IP, immunoprecipitation; PG, plakoglobin; Ig, immunoglobulin; Ab, antibody. The arrows, large arrowhead, and small arrowheads indicate the positions of the Myc-rAxin, endogenous plakoglobin, and HA-plakoglobin, respectively. The results shown are representative of three independent experiments.
FIG. 2. Direct interaction of Axin with plakoglobin. A, kinetics of the binding of plakoglobin to rAxin. MBP-rAxin (5 pmol) immobilized on amyllose resin was incubated with the indicated concentrations of GST-plakoglobin. MBP-rAxin was precipitated by centrifugation, and the amounts of interacted GST-plakoglobin were quantified by densitometric tracing. B, purification of GST-plakoglobin and MBP-rAxin. GST-plakoglobin (full length) (lane 1), GST-plakoglobin-(1–121) (lane 2), GST-plakoglobin-(185–399) (lane 3), GST-plakoglobin-(399–744) (lane 4), MBP-rAxin (full length) (lane 5), MBP-rAxin-(1–229) (lane 6), MBP-rAxin-(298–832) (lane 7), MBP-rAxin-(298–506) (lane 8), or MBP-rAxin-(713–832) (lane 9) (500 ng of each protein) was visualized with Coomassie Brilliant Blue staining. C, regions of plakoglobin and rAxin that are necessary for their interaction. After 250 nM GST-plakoglobin (full length) (lane 1), GST-plakoglobin-(1–121) (lane 2), GST-plakoglobin-(185–399) (lane 3), or GST-plakoglobin-(399–744) (lane 4) was incubated with 500 nM MBP-rAxin (full length) for 1 h, GST-plakoglobin and its deletion mutants were precipitated with glutathione-Sepharose 4B. The precipitates were probed with the anti-plakoglobin antibody. After MBP-rAxin (full length) (lane 5), MBP-rAxin-(298–506) (lane 6), MBP-rAxin-(298–832) (lane 7), MBP-rAxin-(298–506) (lane 8), or MBP-rAxin-(713–832) (lane 9) (7.5 pmol of each) immobilized on amyllose resin was incubated with 500 nM GST-plakoglobin (full length) for 1 h, MBP-rAxin and its deletion mutants were precipitated with glutathione-Sepharose 4B. The precipitates were probed with the anti-MBP antibody. After MBP-rAxin (full length) (lane 1), MBP-rAxin-(298–506) (lane 2), MBP-rAxin-(298–832) (lane 3), MBP-rAxin-(713–832) (lane 4) (500 ng of each protein) was visualized with Coomassie Brilliant Blue staining.

FIG. 3. Effect of β-catenin on the interaction of plakoglobin with Axin. MBP-rAxin (full length) (5 pmol) immobilized on amyllose resin was incubated with 500 nM GST-plakoglobin (full length) (○) and the indicated concentrations of GST-β-catenin (full length) (□). After MBP-rAxin was precipitated by centrifugation, the precipitates were probed with the anti-β-catenin and anti-plakoglobin antibodies (upper and middle panels). The amounts of interacted GST-plakoglobin or GST-β-catenin with MBP-rAxin were quantified by densitometric tracing (lower panel). PG, plakoglobin. The arrow and arrowheads indicate the positions of GST-β-catenin and GST-plakoglobin, respectively. The results shown are representative of three independent experiments.

(298–506) contains a minimal region that interacts with both GSK-3β and plakoglobin and the phosphorylation sites for GSK-3β (Ref. 45 and Fig. 2). MBP-rAxin-(298–506) also enhanced GSK-3β-dependent phosphorylation of plakoglobin (Fig. 4A). These results indicate that rAxin-(298–506) is sufficient for GSK-3β-dependent phosphorylation of plakoglobin. GST-GSK-3β-phosphorylated GST-plakoglobin in time- and dose-dependent manners in the presence of MBP-rAxin-(298–506) (Fig. 4, B and C). MBP-rAxin-(298–506) enhanced the phosphorylation of GST-plakoglobin by GST-GSK-3β in a dose-dependent manner (Fig. 4D). Thus, rAxin promotes GSK-3β-dependent phosphorylation of plakoglobin.

Down-regulation of Plakoglobin by Axin—Finally we examined whether Axin regulates the stability of plakoglobin. Although expression of Axin (full length) in SW480 cells induces the degradation of β-catenin (49, 52), the level of plakoglobin was not changed by expression of rAxin (full length) (Fig. 1B). It has been shown that deletion of the regulators of G protein signaling domain from Axin enhances its ability to down-regulate β-catenin (49). Therefore, we expressed EGFP-rAxin-(298–832), in which the regulators of G protein signaling domain is deleted, in SW480 cells and detected cytoplasmic plakoglobin by immunofluorescence staining. EGFP-rAxin-(298–832) formed irregular particles as shown in the previous reports (Fig. 5, A and C) (52, 54). EGFP-hEpsin that was used as a control showed diffuse expression (Fig. 5, E and G). β-Catenin was stained homogeneously in cytoplasm (Fig. 5, B and F). Plakoglobin had a diffuse cytosolic pattern of expression with some areas of particular or vesicular staining (Fig. 5, D and H). Consistent with the previous observations (49, 52), more than 90% of rAxin-(298–832)-expressing cells exhibited a marked diminution of β-catenin immunofluorescence staining (n = 300) (Fig. 5B). rAxin-(298–832) also down-regulated plakoglobin (Fig. 5D), but its efficiency was less potent (23% of transfected cells) compared with that for β-catenin (n = 300). EGFP-hEpsin did not affect the level of either β-catenin or plakoglobin significantly (Fig. 5, F and H). These results suggest that the stability of plakoglobin is regulated by Axin as well as that of...
Regulation of Plakoglobin Stability by Axin

DISCUSSION

Regulation of the expression of β-catenin and plakoglobin is important in morphogenetic events during embryonic development (34, 35, 58, 60) and in the process of tumorigenesis (61–64). Although the molecular mechanism by which the stability of β-catenin is regulated has become clear, that for regulation of plakoglobin is not fully understood. In this study, we have addressed the regulation of the cellular plakoglobin level. We have demonstrated for the first time that Axin directly binds to plakoglobin, that it enhances GSK-3β-dependent phosphorylation of plakoglobin, and that it down-regulates plakoglobin. Taken together with the observations that Axin enhances GSK-3β-dependent phosphorylation of β-catenin, resulting in the degradation of β-catenin (45, 49, 51), these findings suggest that Axin regulates the stability of both β-catenin and plakoglobin.

The phosphorylation of serine/threonine residues 29–45 of β-catenin is necessary for its degradation, and deletion of this region results in the stabilization of β-catenin (23, 36, 37). In Xenopus embryos, plakoglobin behaves like β-catenin in that deletion of the 17 amino acids including the possible phosphorylation site for GSK-3β from the N terminus of plakoglobin leads to an increase in the stability of the protein (59). GSK-3β and β-catenin directly bind to separate, adjacent sites on Axin. Because β-catenin inhibits the binding of plakoglobin to Axin, β-catenin and plakoglobin may share the same binding site on Axin. Therefore, it is possible that Axin enhances GSK-3β-dependent phosphorylation of plakoglobin by simultaneously binding to GSK-3β and plakoglobin. It has been shown that the phosphorylation of β-catenin by GSK-3β in the presence of Axin is required for the complex formation with βTrCP/FWD, resulting in β-catenin being ubiquitinated and degraded by the proteasome pathway (50). Ubiquitination of plakoglobin is also observed in A431 cells treated with a proteasome inhibitor (38). Taken together, these results imply that the phosphorylation of plakoglobin by GSK-3β in the Axin complex could result in its degradation by the ubiquitin-proteasome pathway.

We have found that the armadillo repeats 2–7 of plakoglobin bind to Axin. This region of plakoglobin is also necessary to interact with the cytoplasmic tails of desmocollin, desmoglein, and cadherin (11, 17, 18). Expression of either desmoglein or desmocollin in mouse L cells decreases the rate of plakoglobin degradation (7). Expression of E-cadherin induces the accumulation of β-catenin (7, 65), and the binding region of β-catenin to Axin overlaps with that to E-cadherin (17, 45). These results indicate that the bindings of plakoglobin and β-catenin to desmoglein and desmocollin, and E-cadherin, respectively, rescue plakoglobin and β-catenin from the degradation pathway. It has been shown that plakoglobin interacts with desmoglein in the insoluble fraction of Madin-Darby canine kidney cells and that plakoglobin is more heavily phosphorylated in the soluble fraction than in the insoluble fraction (66). Therefore, plakoglobin complexed with desmoglein does not bind to Axin and may not be efficiently phosphorylated by GSK-3β and thereby be stabilized.

Although plakoglobin and β-catenin are structurally homologous, whether their functions are similar is not clear. Plakoglobin, like β-catenin, directly binds to cadherin (18), α-catenin (16), APC (17), and Tcf/Lef (67, 68). Expression of Lef-1 in Madin-Darby canine kidney cells induces nuclear translocation of β-catenin but not of plakoglobin, suggesting that these proteins may differ in their specificities for transcriptional factors (68). The ability of plakoglobin to activate Lef-1 is much lower than that of β-catenin (68). In the Drosophila female germ line, mammalian β-catenin and plakoglobin can complement an armadillo mutation (58). However, in embryonic signaling assays, plakoglobin has no detectable activity, whereas β-catenin has weak activity (58). Therefore, to definitively determine whether plakoglobin can induce transactivation, it is necessary to use β-catenin-null cells. Expression of plakoglobin in Xenopus embryos induces axis duplication (35) that was similar to that observed with β-catenin expression (34). These results suggest that plakoglobin and β-catenin have similar activities in at least axis formation in Xenopus embryos. However, it has been demonstrated that plakoglobin induces Wnt signaling in Xenopus embryos, even when it is tethered to the plasma membranes (60), suggesting that plakoglobin could stimulate the Wnt signaling by relieving the negative action on transcriptional activation of Wnt-responsive genes or by competition and displacement of endogenous β-catenin from its association with the degradation system, followed by stabilization and nuclear translocation of β-catenin. Our present results suggest that Axin degrades both β-catenin and plakoglobin in similar manners but that plakoglobin is less sensitive to this proteolysis. Overexpression of plakoglobin may lead to the accumulation of β-catenin by blocking the proteasome degradation system.

Because mutations in the phosphorylation site of β-catenin
for GSK-3β have been discovered in several human tumors, including colorectal cancers and melanoma (62–64), it is thought that β-catenin acts as an oncogene. However, plakoglobin suppresses the tumorigenesis of cells, and its expression level is often lost in tumor cells (19, 61). Although the exact roles of plakoglobin in tumorigenesis are not known, possible mechanisms whereby plakoglobin affects the functions of β-catenin have been suggested. For example, the plakoglobin/Tcf activates genes that are tumor suppressive or the plakoglobin/Tcf forms an inactive complex and thus inhibits transactivation by β-catenin. In addition to these possibilities, one more possibility is conceivable based on our results. Plakoglobin competes with β-catenin for the binding to cadherin and α-catenin. β-Catenin released from the adherens complex may bind to Axin, leading to β-catenin degradation. Indeed, it has been shown that overexpression of plakoglobin results in a decrease in the level of β-catenin (69). Further studies are necessary to understand the cellular functions regulated by β-catenin and plakoglobin, of which the stability is controlled by Axin.

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