Wnt Signaling to β-Catenin Involves Two Interactive Components

GLYCOCEN SYNTHASE KINASE-3β INHIBITION AND ACTIVATION OF PROTEIN KINASE C

Received for publication, July 8, 1999, and in revised form, February 28, 2000
Published, JBC Papers in Press, April 3, 2000, DOI 10.1074/jbc.M905336199

Rui-Hong Chen‡, W. Vivianne Ding, and Frank McCormick§

From the University of California, San Francisco, School of Medicine, Cancer Research Institute, San Francisco, California 94115

Wnt signaling involves inhibition of glycogen synthase kinase-3β (GSK-3β) and elevation of cytoplasmic β-catenin. This pathway is essential during embryonic development and oncogenesis. Previous studies on both Xenopus and mammalian cell lines indicate that lithium mimics Wnt signaling by inactivating GSK-3β. Here we show that serum enhances accumulation of cytoplasmic β-catenin induced by lithium in both 293 and C57MG cell lines and that growth factors are responsible for this enhancing activity. Growth factors mediate this effect through activation of protein kinase C (PKC), not through Ras or phosphatidylinositol 3-kinase. In addition, Wnt-induced accumulation of cytoplasmic β-catenin is partially inhibited by PKC inhibitors and by chronic treatment of cells with phorbol ester. Both calphostin C, a PKC inhibitor, and a dominant negative PKC exhibit partial inhibition on Wnt-mediated transcriptional activation. We therefore propose that Wnt signaling to β-catenin consists of two interactive components: one involves inhibition of GSK-3β and is mimicked by lithium, and the other involves PKC and serves to augment the effects of GSK-3β inhibition.

β-Catenin was originally identified as a cytoplasmic protein that binds to the cell adhesion molecule cadherin in cell-cell junctions where it connects cadherin to the actin cytoskeleton (1–3). It is also involved in the Wnt signaling pathway to regulate developmental processes in a variety of organisms, and in tumorigenesis (4–6). In normal or non-stimulated cells, the majority of β-catenin protein is present in cell-cell junctions with very little in cytoplasmic or nuclear fractions. This is due to rapid turnover of β-catenin promoted by complexes containing the adenomatous polyposis coli protein, GSK-3β, and Axin/Conductin (7–14). The N-terminal clusters of serine and threonine residues of β-catenin are putative GSK-3β phosphorylation sites (8), which serve as signals for ubiquitin-mediated degradation (15–17). Recently, an F-box protein β-TrCP in a ubiquitin-ligase complex has been shown to be involved in the proteasome-mediated degradation of phosphorylated β-catenin (18–20). However, in the presence of a Wnt signal, a Frizzled family receptor and the downstream component Dishevelled are activated. Dishevelled in turn leads to the inactivation of GSK-3β, causing the accumulation of cytoplasmic β-catenin. High levels of β-catenin in the cytosol result in its translocation into the nucleus and subsequent interaction with the Tcf/Lef family of transcription factors to activate expression of Wnt-responsive genes (6). Recently, c-myc and cyclin D1 have been identified as target genes of β-catenin (21, 22). In colon cancer cells, adenomatous polyposis coli protein is frequently mutated resulting in elevated levels of cytoplasmic β-catenin (23). In addition, β-catenin mutations have been detected in colon cancer cell lines with wild-type adenomatous polyposis coli protein and other types of cancer (24–28). The majority of these mutations are located in the putative GSK-3β phosphorylation sites (29, 30). Similarly, these mutations result in stabilization of the β-catenin protein. Intracellular levels of β-catenin are therefore regulated by different signaling pathways and play a central role in both development and tumorigenesis.

Lithium has marked effects on the embryonic development and patterning in different organisms including Xenopus and Dictyostelium (31). In Xenopus embryos, lithium causes axis duplication, resembling a phenotype caused by overexpression of Wnt, dominant negative GSK-3β, or β-catenin (32–35). In Dictyostelium, lithium alters cell fate determination, resulting in transformation of pre-spore cells into pre-stalk cells (36). In isolated rat adipocytes, treatment with lithium leads to increased glycogen synthesis (37). The action of lithium has been previously attributed to depletion of inositol, based on the observation that lithium inhibits inositol monophosphatase (38). However, recent observations suggest that lithium may mimic Wnt signaling by direct inhibition of GSK-3β, leading to the accumulation of cytoplasmic β-catenin (39–41). This is consistent with the fact that GSK-3β is a highly conserved serine/threonine protein kinase in evolution and plays a central role in diverse biological processes including those affected by lithium (34, 42–48).

Normally, GSK-3β is highly active in resting cells. However, many extracellular stimuli result in the inhibition of its activity. For example, stimulation by insulin and growth factors activates the phosphatidylinositol 3-kinase (PI3K) pathway and the subsequent protein kinase B (PKB) activation leads to inhibition of GSK-3β, probably via the N-terminal Ser-9 phosphorylation (49). Interestingly, Wnt stimulation also leads to similar degrees of inhibition of GSK-3β yet does not involve PI3K pathway, p70 S6 kinase, or MAP kinase pathway (50). Instead, TPA-sensitive isoforms of protein kinase C (PKC) have been shown to be involved in Wnt-induced inactivation of GSK-3β (50). PKC has been shown to phosphorylate and inac-
tivate GSK-3β in vitro (51). However, the role of PKC activation in Wnt-induced β-catenin accumulation remains unknown. Inactivation of GSK-3β by Wnt causes slower cytoplasmic turnover and increased nuclear translocation of β-catenin, and leads to activation of Wnt target gene expression. However, there is no evidence that GSK-3β inhibition by insulin or growth factor stimulation leads to β-catenin accumulation and Tcf/Lef-dependent transcriptional activation.

Here we provide evidence that growth factors can enhance lithium-induced β-catenin accumulation and transactivation. This enhancement is through a distinct signaling pathway that involves TPA-sensitive isoforms of PKC. More importantly, our study suggests that two different components, one mimicked by TPA-sensitive isoforms of PKC. More importantly, our study suggests that two different components, one mimicked by TPA-sensitive isoforms of PKC, and the other mediated by PKC, contribute to Wnt-induced β-catenin stabilization and activation of gene expression.

EXPERIMENTAL PROCEDURES

Materials—EGF, calphostin C, GF-109203X (bisindolylmaleimide I), and TPA (12-0-tetradecanoylphorbol-13-acetate) were obtained from Calbiochem. Insulin, platelet-derived growth factor, insulin-like growth factor-1, and l-cysteine were purchased from Sigma. The monoclonal antibodies against β-catenin and actin were purchased from Transduction Laboratories and Amersham Pharmacia Biotech, respectively. C57MG, C57MG-MV, C57MG-Wnt-1, Rat2-MV, and Rat2-Wnt-1 cell lines were generous gifts from Dr. Anthony Brown (Cornell University Medical College, New York) (52, 53). Conditioned Wnt media were made as described previously (67). 293-Wnt-1 cells were made from the pLNCX-Wnt-1 plasmid generously provided by Dr. Roel Nusse (Stanford University, CA). p110-CAAX, gag-PKB mammalian expression plasmids, and packaging cell lines for vector and RasVal-12 retroviruses (Stanford University, CA) were obtained from Transduction Laboratories and Amersham Pharmacia Biotech, respectively. The monoclonal antibodies against β-catenin, Aβ, a dominant negative form of PKCα, and CD43 were gifts from Dr. Peter Parker (Imperial Cancer Research Fund, United Kingdom) (55). AP-1-luciferase (AP-1-Luc) and p53-luciferase (p53-Luc) reporter plasmids were purchased from Stratagene. Cells were maintained in Dulbecco’s modified Eagle’s medium with 10% fetal calf serum and 1% penicillin/streptomycin. 1 mg/ml G418 (Life Technologies, Inc.) was added where selection was necessary.

Cellular Fractionation, Electrophoresis, and Immunoblotting—Cytoplasmic and membrane fractions were prepared according to a protocol described previously (56). Briefly, cells were washed twice and scraped in phosphate-buffered saline and the cell pellets were resuspended in ice-cold hypotonic buffer (20 mM Tris, pH 7.5, 25 mM sodium fluoride, and 1 mM EDTA) containing a protease inhibitor mixture tablet (Roche Molecular Biochemicals). Cells were lysed by incubating on ice for 20 min, followed by 30 strokes in a Dounce homogenizer. The lysates were subject to ultracentrifugation at 100,000 g for 10 min at 4 °C. The supernatant and the pellet were collected as the cytoplasmic fraction (S100 fraction) and the membrane fraction (P100 fraction), respectively. Protein concentration of the cytoplasmic extracts was estimated by using Bradford reagent (Bio-Rad). Equal amounts of protein from each lysate was analyzed by 10 or 7.5% SDS-polyacrylamide gel electrophoresis. The protein bands were transferred to polyvinylidene difluoride membranes (Millipore), blocked in 2% bovine serum albumin, 1× TBS, 0.05% Tween 20. Membranes were incubated with appropriate primary antibodies for 1 h at room temperature or overnight at 4 °C and then probed with horseradish peroxidase-conjugated secondary antibodies. ECL reagents (Amersham Pharmacia Biotech) were used to visualize the protein bands on the membranes, Semi-Quantitation by densitometry.

RESULTS

Serum Stimulates Accumulation of Cytoplasmic β-Catenin in the Presence of Lithium—Regulation of cytoplasmic β-catenin is critical for its signaling function. In colon carcinomas, cytoplasmic β-catenin levels and Tcf/Lef-dependent transcription can be up-regulated by either the loss of adenomatous polyposis coli protein function or mutations of the putative GSK-3β-phosphorylation sites in the N-terminal region of β-catenin (27, 29, 30). Wnt stimulation also specifically causes repression of GSK-3β activity and subsequent β-catenin accumulation in the cytosol and nucleus (6). We asked whether serum stimulation, which has been shown to decrease GSK-3β activity to a similar extent as Wnt stimulation (50, 57), would induce cytoplasmic β-catenin accumulation. To test this possibility, cells were stimulated with serum, lysed in hypotonic buffer, and fractionated by ultracentrifugation. Normalized amounts of S100 lysates were separated by 10% SDS-polyacrylamide gel electrophoresis and cytoplasmic β-catenin was visualized by immunoblotting. As shown in Fig. 1A, serum stimulation alone did not lead to cytoplasmic accumulation of β-catenin. However, lithium did increase cytoplasmic β-catenin levels, which is consistent with previous observations that lithium acts as a non-competitive inhibitor of GSK-3β and leads to the cytoplasmic accumulation of β-catenin (39–41). Surprisingly, when cells were stimulated with both serum and lithium, a much more pronounced accumulation of cytoplasmic β-catenin in C57MG and 293 cells was observed (Fig. 1A and data not shown). Furthermore, serum and lithium also synergistically enhanced transcriptional activation of a reporter gene with multiple copies of Tcf-binding sites (TOPFlash) (Fig. 1B). Whereas lithium activated the reporter gene only up to 3-fold, lithium plus serum gave rise to 10-fold activation in 293 cells. These results indicate that serum by itself does not have a significant effect on β-catenin levels, yet can potentiating the effect of lithium on cytoplasmic β-catenin accumulation and β-catenin-dependent transcriptional activation.

Growth Factors Activate the Wnt/β-Catenin Signal Transduction Pathway in the Presence of Lithium—In order to find out what is responsible for the serum enhancement of lithium-induced β-catenin stabilization, we used l-α-l-lysophosphatidic acid and different growth factors, instead of serum, to stimulate cells. l-α-Lysophosphatidic acid neither caused the accumulation of β-catenin by itself nor had any synergistic effect with lithium (data not shown). Combinations of growth factors (insulin, platelet-derived growth factor, insulin-like growth factor-1, and EGF) could substitute for serum stimulation (Fig. 2A). Among them, EGF partially substituted serum in β-catenin accumulation (data not shown) and promoted β-catenin-dependent transcriptional activation (Fig. 2B). Therefore, growth factors play a major role in the cooperation of serum with lithium on the cytoplasmic β-catenin stabilization and β-catenin-dependent transcriptional activation.

Serum Effect on Cytoplasmic Levels of β-Catenin Does Not Involve PI3K and Ras—Since growth factors contributed to the serum effect on β-catenin accumulation, we reasoned that...
growth factors might elicit their functions by feeding into 
GSK-3β via PI3K/PKB pathway (49, 58). Therefore, we 
examined if a constitutively active form of PI3K (p110-CAAX) (59) 
would substitute for serum in the reporter gene assay described 
earlier. p110-CAAX, co-transfected with the reporter TOP- 
Flash, neither activated the reporter gene by itself nor syner-
gized with lithium. Moreover, lithium-induced reporter gene 
activity could be further increased by serum stimulation in the 
p110-CAAX-transfected 293 cells (Fig. 3A). Likewise, co-
transfection of gag-PKB, an activated form of PKB, with TOPFlash 
failed to enhance lithium-induced reporter gene activation 
(data not shown).

Ras is a major mediator in the growth factor signaling path-
ways (60, 61). We wondered if Ras might be involved in syner-
gizing with lithium in β-catenin accumulation. We made a 
stable C57MG-V12 cell line expressing activated RasVal-12. The 
C57MG-V12 cells showed typical morphology of Ras-trans-
formed cells (data not shown). We then examined if RasVal-12 
could substitute for serum in potentiation of β-catenin accumu-
lation by lithium. The C57MG-V12 cells did not accumulate 
more cytoplasmic β-catenin than the vector cells when treated 
with lithium. In addition, the cytoplasmic β-catenin levels in 
both the vector and the C57MG-V12 cells could be further 
elevated to a similar extent by treatment with serum and 
lithium (Fig. 3B). Therefore, expression of RasVal-12 cannot 
substitute for serum in potentiation of β-catenin accumulation.

PKC Is Necessary for Serum Potentiation of Lithium-induced 
β-Catenin Accumulation—One of the other candidate path-
ways activated by serum and growth factors is the PKC path-
way. To investigate if PKC is involved in the accumulation of 
β-catenin, we tested TPA, a potent activator of certain isoforms 
of PKC, in conjunction with lithium to induce β-catenin accu-
mentation. TPA alone had little effect on the cytoplasmic β-cate-
nin levels. Interestingly, TPA plus lithium stimulated further 
the accumulation of β-catenin (Fig. 4A). The level of stimula-
tion by TPA is comparable to that of serum. These results 
suggest that although activation of PKC alone is not sufficient 
for activation of Wnt signaling pathway, it can enhance Wnt 
signaling by cooperating with lithium. We postulated that the 
signal cooperation might be due to the activation of PKC. To 
test this, we pretreated cells with calphostin C, a PKC inhibi-
tor, before being stimulated with serum and lithium. As shown 
in Fig. 4B, treatment of calphostin C inhibited serum potenti-
ation of β-catenin accumulation. Furthermore, long-term treat-
ment of cells with TPA ablated the serum potentiation of lith-
ium-stimulated β-catenin accumulation (data not shown).

Chronic treatment of cells with TPA has been shown to down-
regulate the TPA-sensitive isoforms of PKC. Therefore, the 
TPA-sensitive isoforms of PKC might be responsible for the
enhancement of lithium-induced β-catenin accumulation by serum.

TPA-sensitive PKCs Are Necessary But Not Sufficient for the Full Accumulation of β-Catenin Induced by Wnt-1—Wnt signaling also leads to cytoplasmic β-catenin accumulation by inhibiting GSK-3β activity (62, 63). Since lithium mimics the Wnt signaling in terms of inhibiting the activity of GSK-3β, we examined if serum could also enhance the Wnt signaling by increasing Wnt-stimulated β-catenin accumulation. C57MG-Wnt-1 cells showed elevated levels of cytoplasmic β-catenin (52, 53). However, serum starvation or serum stimulation of C57MG-Wnt-1 cells did not affect the levels of cytoplasmic β-catenin accumulation (Fig. 5A). Therefore, serum does not seem to further enhance β-catenin accumulation induced by Wnt-1 expression. This observation suggests that lithium is not the surrogate for the Wnt signaling, and serum or growth factors can provide additional signal(s) that leads to further accumulation of β-catenin.

PKC has been shown to participate in the inactivation of GSK-3β by Wnt (50). To investigate whether PKC is involved in the β-catenin accumulation induced by Wnt-1, we treated the Wnt-1 cells with different PKC inhibitors and examined the cytoplasmic β-catenin accumulation by immunoblotting. Treatment of the C57MG-Wnt-1 or 293-Wnt-1 cells with increasing concentrations of calphostin C for 3 h resulted in gradual reduction of the cytoplasmic β-catenin (Fig. 5B and data not shown). Similarly, chronic treatment of C57MG-Wnt-1 or 293-Wnt-1 cells with TPA or treatment of the C57MG-Wnt-1 cells with GF-109203X (1 μM) also led to partial inhibition of β-catenin accumulation (data not shown). Furthermore, β-catenin accumulation stimulated by conditioned Wnt media was also partially inhibited by treatment of calphostin C, as well as by chronic treatment of TPA in C57MG and 293 cells (Fig. 5C and data not shown). To test whether the inhibition of β-catenin accumulation by these inhibitors has any functional significance, we assayed the Tcf reporter activity in 293-Wnt-1 cells. The reporter activity was 10-fold higher in 293-Wnt-1 cells compared with 293-Vector cells and was partially inhibited by calphostin C in a dose-dependent manner in 293-Wnt-1 cells (Fig. 6A). To further strengthen the conclusion from the PKC inhibitor experiments, we employed PKCα(T/A), a dominant negative PKC (DN-PKC), to test whether it has any effect on Wnt-mediated transcriptional activation. In control experiments, DN-PKC blocked 70% of TPA-stimulated AP-1 luciferase reporter activity (Fig. 6B, left panel) and it had no signifi-
Our data further demonstrated that Wnt/lithium and the other involves PKC.

The activity is expressed as a percentage of maximum luciferase activity. The activity is expressed as a percentage of maximum luciferase activity.

**Fig. 6. PKC participates in Wnt-mediated activation of transcription.** A, calphostin C partially inhibits Wnt-associated transcriptional activation. 293-Wnt-1 or 293-Vector cells were transfected with the reporter plasmid TOPFlash and the internal control plasmid pRL-TK for 24 h. Cells were treated with increasing concentrations of calphostin C (0.1 to 1 μM) for another 16 h and then harvested for luciferase assay. The activity is expressed as a percentage of a maximum luciferase activity. B, in 12 wells, 293-Wnt-1 cells were co-transfected with 0.2 μg of each reporter plasmid (AP-1-Luc, TOPFlash, or p53-Luc) and 1 μg of the dominant negative PKC (DN-PKC) and 1 μg of the dominant negative Tcf4 (DN-Tcf4) was co-transfected; for p53 luciferase assay, 0.3 μg of p53 was used as an activator. The luciferase activity is expressed as a percentage of the maximum activity in each assay and represents the average of triplicates of three independent experiments.

The role of PKC in the Wnt signaling pathway remains controversial. In vitro, PKC has been shown to phosphorylate and inactivate GSK-3β (51). An earlier report showed that a PKC inhibitor Ro31-8220 or chronic treatment with TPA resulted in complete block of Wnt-mediated inhibition of GSK-3β in mouse 10T1/2 fibroblasts (50). In that study, the authors did not determine the effect of these PKC inhibitors on Wnt-induced β-catenin accumulation. On the other hand, others reported that treatment with the PKC inhibitors GF-109203X or Ro31-8220 caused β-catenin accumulation in certain human breast epithelial cell lines, whereas treatment with the PKC inhibitor calphostin C or TPA-induced down-regulation of wortmannin treatment (50).

Lithium has profound effects on morphogenesis in diverse organisms (31). One action of lithium has been recently shown to mimic Wnt signaling by direct inhibition of GSK-3β causing the accumulation of cytoplasmic β-catenin (39–41). In this study, we confirmed the lithium effect on GSK-3β inhibition (data not shown) and β-catenin accumulation in both 293 and C57MG cells. We further showed that serum or growth factors could cooperate with lithium to induce β-catenin stabilization (Figs. 1 and 2). This raised a possibility that Wnt might also synergize with serum or growth factors to induce maximum accumulation of β-catenin. However, our results indicated that Wnt-induced β-catenin accumulation is independent of serum (Fig. 5). These observations imply that lithium might only partially mimic the Wnt signaling pathway.

Which serum-activated pathways are involved in the cooperation with lithium to induce cytoplasmic β-catenin accumulation? We ruled out the involvement of PI3K/PKB and Ras in the synergistic stabilization of β-catenin (Fig. 3). Since PKC is implicated as a candidate component in the Wnt signal transduction, we examined PKC inhibitors on serum enhancement of lithium-induced β-catenin accumulation. Indeed, PKC inhibitors completely block the serum enhancement function. The role of PKC is further supported by the observation that TPA, a potent activator of certain PKC isoforms, can also cooperate with lithium (Fig. 4). In conclusion, we identified a pathway involving PKC that enhances lithium-induced β-catenin accumulation. It is noteworthy that a synergy between lithium and insulin-like growth factor-1 is reported in the stimulation of granule neuron progenitor proliferation, a process involving inhibition of GSK-3β (69). Questions emerge as what is the target(s) of growth factors/PKC pathway. One possibility is that growth factors/PKC may cooperate with lithium in the inactivation of GSK-3β. To test this hypothesis, we immuno-precipitated GSK-3β kinase from resting cells or serum-stimulated cells and performed in vitro kinase assay in the presence of increasing amounts of lithium. We did not observe any cooperative inhibition of GSK-3β by serum and lithium (data not shown). However, considering the nature of the in vitro kinase assay, we still cannot rule out the possibility of cooperative inhibition of GSK-3β by serum and lithium in vivo. Further study is needed to dissect the converging point from activation of PKC to β-catenin accumulation.

The role of PKC in the Wnt signaling pathway remains controversial. In vivo, PKC has been shown to phosphorylate and inactivate GSK-3β (51). An earlier report showed that a PKC inhibitor Ro31-8220 or chronic treatment with TPA resulted in complete block of Wnt-mediated inhibition of GSK-3β in mouse 10T1/2 fibroblasts (50). In that study, the authors did not determine the effect of these PKC inhibitors on Wnt-induced β-catenin accumulation. On the other hand, others reported that treatment with the PKC inhibitors GF-109203X or Ro31-8220 caused β-catenin accumulation in certain human breast epithelial cell lines, whereas treatment with the PKC inhibitor calphostin C or TPA-induced down-regulation of wortmannin treatment (50). Instead, Wnt-1-induced GSK-3β inactivation is completely blocked by certain PKC inhibitors (50). Therefore, the pathway from GSK-3β inhibition to cytoplasmic β-catenin accumulation appears to be fundamentally different between Wnt signaling and growth factor signaling. In the case of insulin signaling, GSK-3β inhibition is mediated by phosphorylation of Ser-9 on GSK-3β (66–68); in Wnt signaling, GSK-3β activity is inhibited to 50% of its normal activity but little is known about the mechanism of the inhibition. It is of interest to study whether Wnt-mediated GSK-3β inhibition is accomplished via covalent modification and whether different pools of GSK-3β are involved in Wnt signaling.

From genetic and biochemical studies of Drosophila and Xenopus, a linear pathway has been elucidated from the extracellular ligand Wnt to the transcription factor Tcf/lef. Wnt binds to its receptor Frizzled, triggering the activation of Dishevelled, which in turn leads to the inactivation of GSK-3β (6). Inactivation of GSK-3β abolishes the phosphorylation and ubiquitin-mediated degradation of β-catenin (18–20). Nevertheless, the molecular mechanisms underlying the inactivation of GSK-3β and β-catenin accumulation have not been fully understood. It has been proposed that PKC might be involved in the inactivation of GSK-3β because certain inhibitors of PKC are capable of antagonizing Wnt-mediated GSK-3β inactivation (50). Our data further demonstrated that Wnt/β-catenin pathway could comprise two components, one is mimicked by lithium and the other involves PKC.

It is interesting that Wnt-1 and growth factors inactivate GSK-3β to a similar extent (50, 57), yet only Wnt-1 can induce cytoplasmic β-catenin accumulation (Figs. 1 and 5). Earlier studies have indicated that signaling from growth factors (e.g., insulin) to GSK-3β is wortmannin sensitive and involves PKB (49, 64, 65) whereas that from Wnt to GSK-3β is insensitive to
PKCs has no significant effect on β-catenin accumulation (15). Our data showed that Wnt-dependent β-catenin stabilization was partially inhibited by certain PKC inhibitors (Fig. 5 and data not shown). The discrepancy may be explained by the differences of these inhibitors on various isoforms of PKCs or their nonspecific effects on other kinases. It is possible that TPA-sensitive PKCs are involved in the Wnt-induced GSK-3β inactivation whereas atypical PKCs contribute to β-catenin degradation (15, 50). However, the most plausible explanation is from the recent publication by Hers and colleagues (70), who showed that the PKC inhibitors GF-109203X and Ro31-8220 also inhibit GSK-3β, potentially slowing down β-catenin degradation.

The importance of PKCs in colon cancer has been demonstrated in transgenic PKC β2 mice that exhibit hyperproliferation and increased Wnt signaling in the colonic epithelium (71). Recently, certain Wnt and Frizzled homologs have been reported to modulate PKC but have no effect on expression of β-catenin target genes, whereas other Frizzled homologs capable of activating β-catenin target genes do not activate PKC (72). Consistent with the report, we found that molecules such as growth factors or TPA that activate PKCs do not stimulate any detectable β-catenin accumulation and transcriptional activation. In contrast, using inhibitors and the dominant negative PKC mutant, we showed that PKCs do participate in Wnt/β-catenin signaling, although they are not the main components of this pathway. For example, calphostin C-mediated PKC inhibition or TPA-induced PKC down-regulation results in partial inhibition of Wnt-induced β-catenin accumulation. Furthermore, both calphostin C and the dominant negative PKC inhibit partially the Wnt-mediated Tcf/LEF reporter activation. These results strengthened the notion that PKC activation alone is not sufficient to cause stabilization of β-catenin, but it is a component of Wnt pathway that leads to maximum accumulation of β-catenin and transcriptional activation. Elevation of β-catenin levels in the cytosol and nucleus as well as its transcriptional activity is strongly correlated with tumorigenesis. Therefore, understanding the regulation of free β-catenin pools by different signaling pathways could facilitate the development of new strategies to regulate β-catenin signaling.

Acknowledgments—We thank Dr. P. Polakis, Dr. H. Clevers, Dr. A. Brown, Dr. P. Parker, Dr. P. Rodríguez-Viciana, and Dr. H. Jiang for plasmids, retroviruses, and cell lines. We thank Dr. D. Stokoe and Dr. P. Sambatini for critical reading of the manuscript. We also thank members of Dr. Frank McCormick’s laboratory for discussions and help.

REFERENCES

1. Kemler, R. (1993) Trends Genet. 9, 317–321
2. Aberle, H., Schwartz, H., and Kemler, R. (1996) J. Cell. Biochem. 61, 514–523
3. Gumbiner, B. M. (1996) Curr. Opin. Genet. Dev. 6, 345–357
4. Polakis, P. (1999) Curr. Opin. Genet. Dev. 9, 15–21
5. Eastman, Q., and Grosschedl, R. (1999) Curr. Opin. Cell Biol. 11, 233–240
6. Cadigan, K. M., and Nusse, R. (1997) Genes Dev. 11, 2286–2305
7. Ornitz, D. M., Albert, S., Iovinelli, L., Rubinfeld, B., and Polakis, P. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 3046–3050
8. Rubinfeld, B., Albert, I., Porfirii, E., Fiol, C., Munemitsu, S., and Polakis, P. (1996) Science 272, 1023–1026
9. Behrens, J., Jerchow, B. A., Wartele, M., Grimm, J., Asbrand, C., and Raffel, C. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 959–968
10. Hart, M. J., de los Santos, R., Albert, I. N., Rubinfeld, B., and Polakis, P. (1999) Cell 97, 370–383
11. Hart, M., Concortel, J. P., Lassot, I., Albert, I., del los Santos, R., Durand, H., Perrett, C., Rubinfeld, B., Margottin, F., Benarous, R., and Polakis, P. (1999) Curr. Biol. 9, 207–210
12. Leves, E., Chiaru, D. S., and Pagano, M. (1999) Oncogene 18, 849–854
13. 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
14. Ludes, O., and McCormick, F. (1999) Nature 398, 422–426
15. Kinzler, K. W., and Vogelstein, B. (1996) Cell 87, 159–170
16. Cross, D. A., Alessi, D. R., Cohen, P., Andjelkovich, M., and Hemmings, B. A. (1997) Science 275, 1787–1790
17. de la Coste, A., Romagnolo, B., Biliuart, P., Renard, C. A., Buendia, M. A., Soubré, O., Fabre, M., Chelly, J., Beldjord, C., Kahn, A., and Perrett, C. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 8847–8851
18. Zarrawal, R. H., Chiappa, S. A., Allen, C., and Raffel, C. (1998) Cancer Res. 58, 896–899
19. Ilyas, M., Tomlinson, I. P., Rowan, A., Pignatelli, M., and Bodmer, W. F. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 10330–10334
20. Chan, E. F., Gat, U., McNeill, J. M., and Fuchs, E. (1999) Nat. Genet. 21, 410–413
21. Morin, P. J., Sparkes, A. B., Korinek, V., Barker, N., Clevers, H., Vogelstein, B., and Kinzler, K. W. (1997) Science 275, 1787–1790
22. Sparks, A. B., Morin, P. J., Vogelstein, B., and Kinzler, K. W. (1998) Cancer Res. 58, 1130–1134
23. Kao, K. R., and Elson, B. P. (1998) Biol. Cell 90, 585–589
24. Sokol, S., Christian, J. L., Moon, R. T., and Melton, D. A. (1991) EMBO J. 10, 378, 1537–1542
25. Stubble, F. K., and Cazabau, S. M. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 269–275
26. Welsh, G. I., and Proud, C. G. (1993) Curr. Biol. 3, 203–206
27. Chen, A. K., and Proud, C. G. (1994) Curr. Opin. Cell Biol. 6, 1664–1668
28. Woodgett, J. R. (1994) Semin. Cancer Biol. 5, 269–275
29. Korinek, V., Barker, N., Morin, P. J., van Wichen, D., de Weger, R., Kinzler, K. W., Vogelstein, B., and Clevers, H. (1997) Science 275, 1784–1787
30. Harwood, A. J., Gough, J. L., Montgomery, L. C., Lee, V. M., and Kinzler, K. W. (1997) Nature 389, 213–215
31. Cui, H., Meng, Y., and Bulleit, R. F. (1998) Brain Res. Dev. Brain Res. 112, 177–188
32. Steinbeck, V., Ruel, L., and Woodgett, J. R. (1996) Curr. Biol. 6, 1664–1668
33. Hedgepeth, C. M., Conrad, L. J., Zhang, J., Huang, H. C., Lee, V. M., and Klein, T. C. (1996) Curr. Biol. 6, 8498–8502
34. Siegfried, E., Chou, T. B., and Perrimon, N. (1992) Cell 71, 1167–1179
35. Shigemoto, R., Watanabe, H., and Kano, M. (1998) J. Cell Biol. 140, 1664–1679
36. Shaw, M., Cohen, P., and Alessi, D. R. (1997) J. Biol. Chem. 272, 4431–4438
37. Armellin, S., Albert, S., Rubinfeld, B., and Polakis, P. (1996) Mol. Cell. Biol. 16, 4088–4094
38. Winston, J. T., Strack, P., Beer-Romero, P., Chu, C. Y., Eldredge, S. J., and Halder, G. (1999) Science 284, 230–233
39. Orford, K., Crockett, C., Jensen, J. P., Weissman, A. M., and Byers, S. W. (1997) J. Biol. Chem. 272, 47385–47388
40. Aberle, H., Bauer, A., Stappert, J., Kispert, A., and Kemler, R. (1997) EMBO J. 16, 3797–3804
41. Munemitsu, S., Albert, I., Rubinfeld, B., and Polakis, P. (1996) Mol. Cell. Biol. 16, 2321–2328