Suppressor of Fused Negatively Regulates β-Catenin Signaling*

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Suppressor of fused (Su(fu)) is a negative regulator of the Hedgehog signaling pathway that controls the nuclear-cytoplasmic distribution of Gli/Ci transcription factors through direct protein-protein interactions. We show here that Su(fu) is present in a complex with the oncogenic transcriptional activator β-catenin and functions as a negative regulator of T-cell factor (Tcf)-dependent transcription. Overexpression of Su(fu) in SW480 (APCmut) colon cancer cells in which β-catenin protein is stabilized leads to a reduction in nuclear β-catenin levels and in Tcf-dependent transcription. This effect of Su(fu) overexpression can be blocked by treatment of these cells with leptomycin B, a specific inhibitor of CRM1-mediated nuclear export. Overexpression of Su(fu) suppresses growth of SW480 (APCmut) tumor cells in nude mice. These observations indicate that Su(fu) negatively regulates β-catenin signaling and that CRM1-mediated nuclear export plays a role in this regulation. Our results also suggest that Su(fu) acts as a tumor suppressor.

The oncogenic transcriptional activator β-catenin is a major mediator in Wnt signaling (1–4). A large multiprotein complex that includes APC3 and axin normally facilitates the phosphorylation of β-catenin by GSK3β. Phosphorylated β-catenin binds to the P-box protein βTrCP and is then modified by ubiquitination and subjected to proteasome-mediated protein degradation. When cells are exposed to the Wnt signal, β-catenin phosphorylation and its subsequent ubiquitination are blocked. β-Catenin is thus diverted from the proteasome; instead, β-catenin accumulates and translocates to the nucleus, where it interacts with members of the Tcf/Lef family of transcription factors and activates transcription of Wnt-responsive genes. In tumors, β-catenin degradation is blocked by mutations of APC, axin, or β-catenin itself. As a result, stabilized β-catenin enters the nucleus and β-catenin-Tcf complexes activate oncogenic target genes.

Nuclear translocation of β-catenin is of key importance in its ability to regulate transcription, yet little is known about the factors important in controlling the nuclear versus cytoplasmic distribution of β-catenin. β-Catenin lacks a nuclear import signal, and it docks to the nuclear membrane by a mechanism that is Ran-independent and does not require importins (5). Nuclear import of β-catenin is also independent of its association with the Tcf transcription factors because mutant forms of β-catenin that do not bind Tcf proteins can enter the nucleus (6). Microinjection studies show that β-catenin rapidly exits the nucleus, suggesting a role for nuclear export in the regulation of the intracellular distribution of β-catenin (7).

Several studies demonstrate that APC is a nuclear-cytoplasmic protein with export from the nucleus inhibited by LMB, a specific inhibitor of CRM1-mediated nuclear export (8–10). CRM1, also called exportin-1, is an export karyopherin that binds to a leucine-rich nuclear export signal on its target protein and mediates nuclear-cytoplasmic trafficking of proteins as well as RNA through the nuclear pore. LMB binds directly to CRM1 and inactivates its nuclear export activity (11). In addition to the regulatory role of APC in β-catenin degradation, these studies suggest that APC promotes nuclear export of β-catenin (8–10). However, several recent studies demonstrate that β-catenin is exported from the nucleus in a CRM1- and Ran-independent manner (12, 13). Because the subcellular distribution of β-catenin is affected by many of its interacting proteins, it is possible that some of these β-catenin binding partners are regulated by CRM1-mediated nuclear export and that LMB treatment affects the nuclear localization of β-catenin indirectly.

Genetic screens in Drosophila first identified Su(fu) as a suppressor of the Fused kinase, a positive regulator of Hh signaling (14). Recent studies demonstrate that Su(fu) acts as a negative regulator of Hh signaling by directly interacting with the Ci/Gli zinc finger transcription factors, which are transducers of Hh signaling (15–17). Although the mechanisms by which Su(fu) functions remain unclear, Su(fu) is thought to control the nuclear-cytoplasmic distribution of Gli/Ci transcription factors through direct protein-protein interactions (18, 19). In mammalian cells, overexpression of Su(fu) causes Gli1 to be concentrated in the cytoplasm. Inhibition of CRM1-dependent nuclear export by LMB treatment counteracts these effects of Su(fu) overexpression restoring the nuclear distribution of Gli1, thus suggesting that Su(fu) promotes CRM1-dependent nuclear export of Gli1 (20).

Here we report that Su(fu) and β-catenin are found in the same complex. In the human colon cancer SW480 cell line,
overexpression of Su(fu) results in a reduction of nuclear β-catenin and Tcf-dependent transcription, suggesting that Su(fu) can act as a negative regulator of Wnt signaling. Studies with LMB treatment indicate that the ability of Su(fu) to regulate β-catenin is mediated by CRM1-dependent nuclear export mechanism. We propose that Su(fu) can negatively regulate Wnt signaling by promoting nuclear export of β-catenin. 293 cells were transfected with a β-catenin construct giving the same result. The β-catenin construct was generated using polymerase chain reaction mutagenesis, converting the four serine or threonine phosphorylation sites (codons 33, 37, 41, and 45) to alanine using the full-length β-catenin gene (obtained from S. Hirohashi (21)), and was subcloned into the pCMV5β vector. All polymerase chain reaction-generated constructs were verified by sequencing. The GluGlu-tagged ΔN89-β-catenin (obtained from P. Polakis) was subcloned into the pCMV5β vector, and the wild type APC gene in a CMV vector (obtained from B. Vogelstein and K. Kinzler (22)) was subcloned into a FLAG-tagged vector.

**Immunoprecipitations**—Human 293T cells (kidney epithelial cell line) were passaged to 50% confluence, and transfection was carried out with DNA using the Superfect transfection reagent (Qiagen) according to the manufacturer’s instructions. Cells were harvested 36 h after transfection. Lysates were prepared in immunoprecipitation buffer (50 mM Tris, pH 7.0, 150 mM NaCl, 0.5% Triton X-100, 1 mM EDTA, and protease inhibitors). Immunoprecipitations were performed using anti-β-catenin antibody, anti-GluGlu antibody, anti-Myc antibody, or anti-HA antibody, and protein G-agarose beads. The immunoprecipitates were separated on 8% SDS-polyacrylamide gel electrophoresis, and Western analysis was performed using the indicated primary and corresponding secondary horseradish peroxidase-conjugated antibodies (Jackson ImmunoResearch). Enhanced chemiluminescence detection (Pierce) was performed according to the manufacturer’s instructions. The β-catenin antibody was obtained from BD Transduction Laboratory; the Su(fu) antibody was a rabbit polyclonal antibody raised against a synthesized peptide fragment from amino acid residues 440–457 of Su(fu); the anti-GluGlu and anti-HA antibodies were obtained from Babco; and the anti-Myc antibody was obtained from Santa Cruz Biotechnology. Immunoprecipitation of native proteins was also performed using lysates from the SW480 colon cancer cell line in an identical manner.

**Tcf Transcriptional Activation**—Cells plated on 35-mm dishes were transiently transfected in triplicate with 1 μg of the luciferase reporter construct pTOPFLASH or pFOPFLASH (23). In addition, some cell dishes were also co-transfected with 1 μg of ΔN89-β-catenin, 1 μg of mutant β-catenin, 1 μg of the full-length APC gene, or empty vector controls. Where indicated, some of the cells were treated with 20 ng/ml LMB (obtained from M. Yoshida) for 90 min or 16 h before measuring Tcf transcriptional activation. Superfect transfection reagent (Qiagen) was utilized according to the manufacturer’s instructions, and in all cases, a Rous sarcoma virus β-galactosidase expression vector was used as a control.

**FIG. 2. β-Catenin-dependent Tcf transcriptional activation is inhibited by Su(fu)**. NIH3T3 cells have a low basal level of Tcf transcriptional activation, which is not significantly altered by overexpression of Su(fu). Expression of a stabilized form of β-catenin (either ΔN89-β-catenin or mut-β-catenin, in which the amino-terminal serine and threonine phosphorylation sites are mutated to alanine) results in a significant increase in Tcf transcriptional activation. Coexpression of Su(fu) attenuates these β-catenin-dependent Tcf transcriptional activation. Expression of either β-catenin construct gives the same result. The results are presented as luminescence of the pTOPFLASH and pFOPFLASH reporters after normalization for transfection efficiency. Below each set of bars is the ratio of pTOPFLASH to pFOPFLASH activities. The inhibitory effects of Su(fu) on β-catenin-dependent Tcf transcriptional activation are statistically significant (p < 0.05 t test) when a stabilized form of β-catenin is expressed in the cell cultures.

**MATERIALS AND METHODS**

**Plasmids**—A plasmid encoding mouse Su(fu) was constructed by subcloning the open reading frame of mouse Su(fu) cDNA (19) into the pCMV5β vector with an amino-terminal Myc tag or with an amino-terminal HA tag. A Myc-tagged Su(fu) construct used for stable transfection studies was also generated in the pcDNA3 vector. Su(fu) deletion mutants were generated by polymerase chain reaction mutagenesis and subcloned into the pCMV5β vector with an amino-terminal Myc epitope tag. The stabilized β-catenin mutant (Mut-β-catenin) was generated using polymerase chain reaction mutagenesis, converting the four serine or threonine phosphorylation sites (codons 33, 37, 41, and 45) to alanine using the full-length β-catenin gene (obtained from S. Hirohashi (21)), and was subcloned into the pCMV5β vector. All polymerase chain reaction-generated constructs were verified by sequencing. The GluGlu-tagged ΔN89-β-catenin (obtained from P. Polakis) was subcloned into the pCMV5β vector, and the wild type APC gene in a CMV vector (obtained from B. Vogelstein and K. Kinzler (22)) was subcloned into a FLAG-tagged vector.

**Western blot analysis confirmed their expression** complex. HA-tagged Su(fu) was expressed in 293 cells (lanes 2 and 4). Immunoprecipitation followed by Western blot analysis revealed the presence of Su(fu) in the immunocomplex of β-catenin, in which the amino-terminal serine and threonine phosphorylation sites are mutated to alanine (lanes 8–11). After immunoprecipitation (IP) with an anti-β-catenin antibody, Western blot analysis using an anti-Myc antibody revealed the presence of β-catenin bound to the Su(fu) antibody. Lanes 5 and 6 show the results of co-immunoprecipitation experiments in 293 cells in which either a GluGlu-tagged Su(fu) (lane 5) or without Myc-tagged Su(fu) (lane 6). Immunoprecipitation of native proteins was also performed using anti-HA antibody, and protein G-agarose beads. The immunoprecipitates were separated on 8% SDS-polyacrylamide gel electrophoresis, and Western blot analysis was performed using the indicated primary and corresponding secondary horseradish peroxidase-conjugated antibodies (Jackson ImmunoResearch). Enhanced chemiluminescence detection (Pierce) was performed according to the manufacturer’s instructions. The β-catenin antibody was obtained from BD Transduction Laboratory; the Su(fu) antibody was a rabbit polyclonal antibody raised against a synthesized peptide fragment from amino acid residues 440–457 of Su(fu); the anti-GluGlu and anti-HA antibodies were obtained from Babco; and the anti-Myc antibody was obtained from Santa Cruz Biotechnology. Immunoprecipitation of native proteins was also performed using lysates from the SW480 colon cancer cell line in an identical manner.

**FIG. 1. Su(fu) and β-catenin are present in a complex.** Co-immunoprecipitation experiments revealed that Su(fu) and β-catenin form a complex. HA-tagged Su(fu) was expressed in 293 cells (lanes 2 and 4). Western blot analysis using an anti-HA antibody detected the overexpressed Su(fu) protein (lane 2), and the anti-Su(fu) antibody detected both the endogenous (lanes 3) and overexpressed Su(fu) proteins (lane 4). In lane 5, Western blot analysis using the anti-Su(fu) antibody revealed the presence of Su(fu) in the immunocomplex of β-catenin. 293 cells were transfected with (lane 7) or without Myc-tagged Su(fu) (lane 6). After immunoprecipitation (IP) with an anti-β-catenin antibody, Western blot analysis using an anti-Myc antibody revealed that Myc-Su(fu) and endogenous β-catenin are present in a complex. Lanes 8–11 show the results of co-immunoprecipitation experiments in 293 cells in which either a GluGlu-tagged ΔN89-β-catenin (lane 9), Myc-tagged Su(fu) (lane 10), or both (lane 11) were overexpressed. Immunoprecipitation followed by Western blot analysis revealed that GluGlu-tagged ΔN89-β-catenin and Myc-Su(fu) form a complex (top two panels). Western blot analysis confirmed their expression (bottom two panels).
Su(fu) Regulates β-Catenin Signaling

β-Catenin in a subcellular location similar to the wild type Su(fu) (K). Although wild type Su(fu) decreases nuclear β-catenin, the carboxy-terminal Su(fu) deletion mutants lose the ability to decrease endogenous nuclear β-catenin. Merged views show co-localization of Su(fu) and β-catenin. Panel M shows Tcf transcriptional activation in SW480 cells with overexpression of Su(fu) or its deletion mutants. An asterisk next to the pTOPFLASH/pPOPLASH ratio indicates a statistically significant difference compared with transfection with an empty vector (p < 0.05 t test). Su(fu) expression decreases Tcf transcriptional activation, but the carboxy-terminal deletion mutants of Su(fu) do not alter Tcf transcriptional activation.

**RESULTS**

β-Catenin and Su(fu) Are Present in the Same Complex—To determine whether Su(fu) interacts with β-catenin, epitope-tagged Su(fu) was transiently expressed, either alone or in combination with a stabilized form of β-catenin (ΔN89-β-catenin, which lacks the first 89 amino acid residues important for protein degradation (26)), in 293 cells. As shown in Fig. 1, Myc-tagged Su(fu) co-precipitates with endogenous β-catenin (lane 7) as well as GluGlutagged ΔN89-β-catenin (lane 11), when Myc-tagged Su(fu) is immunoprecipitated with an anti-Myc antibody. Conversely, Myc-Su(fu) is also present in the immunocomplex of β-catenin precipitated with either an anti-β-catenin or anti-GluGlu antibody (data not shown). Interactions between endogenous Su(fu) and β-catenin were also verified using a polyclonal antibody raised against Su(fu) in co-immunoprecipitation experiments in 293 cells (Fig. 1, lane 5) and the human colon cancer SW480 cell (data not shown). These results show that Su(fu) interacts with β-catenin and that this interaction occurs in the absence of the first 89 amino acids of β-catenin, which have previously been shown to be required for binding of the F-box protein, βTrCP.

Su(fu) Overexpression Inhibits Tcf-dependent Transcriptional Activation—In response to the activation of the Wnt signaling pathway, stabilized β-catenin translocates to the nucleus where it regulates transcription of Wnt-responsive genes via interactions with the Tcf/Lef family of transcription factors. To determine whether Su(fu)/β-catenin interactions have any effects on the transcriptional function of β-catenin, a Tcf-luciferase reporter assay was employed. Tcf-dependent transcriptional activation was measured using the pTOPFLASH (luciferase reporter with optimized Tcf-binding sites) and pPOPLASH (luciferase reporter with mutant Tcf-binding sites) constructs in transient expression assays (23). In NIH2T3 cells, where endogenous β-catenin levels are low, only weak Tcf-dependent transcriptional activation was detected (Fig. 2). Expression of either of the stabilized forms of β-catenin (either ΔN89-β-catenin or Mut-β-catenin) in these cells resulted in a 2-3× increase in Tcf-dependent transcription, whereas Su(fu) itself had no obvious effects. Co-expression of Su(fu) with either of the stabilized forms of β-catenin resulted in a slight but significant reduction of Tcf-dependent transcription. The inhibition of Tcf-dependent transcription by Su(fu) is weaker than that by APC but is comparable with the level of inhibition as reported in several β-catenin-interacting proteins, such as duplin, pontin52, and reptin52 (27, 28). These observations suggest that Su(fu)/β-catenin interactions result in a down-regulation of Tcf-dependent transcription.

Su(fu) Overexpression Reduces Nuclear β-Catenin Levels—To further investigate the action of Su(fu) on Tcf-dependent transcription and β-catenin levels, we studied the human colon cancer cell line SW480, which exhibits elevated levels of β-catenin.
\(\beta\)-catenin because of a truncating mutation in \(APC\) (29). The subcellular distribution of \(\beta\)-catenin (\(A, B, E, F, I, J, M, and N\)) and transfected \(Su(fu)\) (\(C, G, K, and O\)) in transiently transfected SW480 cells (\(B, D\) and \(F-H\)) and stable SW480 transformants (\(J, L\) and \(N-P\)) were detected by immunofluorescence staining using anti-\(\beta\)-catenin and anti-Myc antibodies, respectively. Panels \(A\) and \(E\) are SW480 cells transfected with a control plasmid, and \(I\) and \(M\) represent untreated parental SW480 cells. Panels \(E-H\) and \(M-P\) represent LMB-treated cells, and \(A-D\) and \(I-L\) represent untreated cells. \(D, H, L, \) and \(P\) are merged images. \(Su(fu)\) expression in stable SW480 transformants results in decreased endogenous nuclear \(\beta\)-catenin (\(J\)). LMB treatment increases endogenous nuclear \(\beta\)-catenin in cells expressing \(Su(fu)\) (\(N\) and \(F\)) to levels approximating those in parental SW480 cells (\(A\) and \(I\)). \(Su(fu)\) is present primarily in the cytoplasm in the stable SW480 transformants (\(K\)), but its nuclear levels increase with LMB treatment (\(O\)). Merged views show co-localization of \(Su(fu)\) and \(\beta\)-catenin. Panel \(Q\) shows the effects of LMB treatment on \(Tcf\) transcriptional activation. LMB treatment results in a significant increase (\(p < 0.05\) t test) in the \(p\)-TOPFLASH/\(p\)-FOPFLASH ratio in \(Su(fu)\) overexpressing SW480 cells (indicated by an asterisk). Panel \(R\) shows \(\beta\)-catenin protein level in nuclear and cytoplasmic cell fractions as determined by Western analysis. Western analysis for tubulin or nuclear histone from the same blot is shown as a loading control. This shows a decrease in nuclear \(\beta\)-catenin with expression of \(Su(fu)\), and an increase with LMB treatment.

The effect of \(Su(fu)\) on the regulation of nuclear \(\beta\)-catenin was examined by using several \(Su(fu)\) deletion mutants. In SW480 cells, wild type \(Su(fu)\) was predominantly cytoplasmic (Fig. 3B), whereas, in contrast, \(Su(fu)\) mutants with carboxyl-terminal deletions showed significant nuclear accumulation (Fig. 3, \(E\) and \(H\)). Interestingly, these \(Su(fu)\) mutants have lost the ability to reduce nuclear \(\beta\)-catenin level (Fig. 3, \(F\) and \(J\)) and to down-regulate \(Tcf\)-dependent transcription (Fig. 3 \(M\)). In contrast, a \(Su(fu)\) mutant lacking only the amino-terminal region has activities similar to the wild type protein (Fig. 3, \(L\) and \(M\)). Although these mutant proteins exhibit distinct activities, they all retain their ability to form a complex with \(\beta\)-catenin as assayed by immunoprecipitation (data not shown). Taken together, these results indicate that the carboxyl-terminal region of \(Su(fu)\) is required for reduction of nuclear \(\beta\)-catenin levels and thus down-regulation of \(Tcf\)-dependent transcription.

**LMB Treatment Counteracts the Effect of \(Su(fu)\) on Reducing the Nuclear \(\beta\)-Catenin Level and Inhibiting \(Tcf\)-dependent Transcription**—Because \(APC\) is mutated in SW480 cells, we were intrigued by the possibility that \(Su(fu)\) might regulate the nuclear-cytoplasmic distribution of \(\beta\)-catenin level in an \(APC\)-independent manner. To address this, we treated transiently transfected SW480 cells with LMB, a specific inhibitor of CRM1-mediated nuclear export (11, 30) (Fig. 4). Strikingly, LMB treatment restored a high level of \(\beta\)-catenin staining in
the nucleus of Su(fu)-overexpressing cells, suggesting that the reduction of nuclear β-catenin level is mediated through a CRM1-mediated nuclear export mechanism (Fig. 4, B–D and F–H).

To further examine the CRM1-dependence of Su(fu) function, stable SW480 transformants overexpressing Su(fu) were established. As in our transient expression studies, Su(fu) overexpression resulted in a reduction of nuclear β-catenin levels (Fig. 4, J–L, and data not shown) and down-regulation of Tcf-dependent transcription in the stable transformants (Fig. 4Q). LMB treatment counteracted the effects of Su(fu) overexpression in these SW480 transformants (Fig. 4, N–Q, and data not shown). Similar results were observed in cells with LMB treatment for 90 min or 16 h. The subcellular distribution of Su(fu) itself is regulated by nuclear export, as Su(fu) could readily be detected in the nucleus of LMB-treated cells, suggesting that it is transiently present in the nucleus and actively exported (Fig. 4, G and O). These results suggest that the subcellular location of Su(fu) regulates β-catenin-mediated Tcf-dependent transcription.

To determine the subcellular distribution of β-catenin in a more quantitative manner, we examined protein levels in cytoplasmic or nuclear fractions using Western analysis. Extracts from SW480 cells and SW480 cells expressing Su(fu) (clone 7) with or without LMB treatment were separated into cytoplasmic and nuclear fractions. The purity of the fractions was determined by Western analysis using actin and histone as markers for cytoplasmic and nuclear fractions, respectively. Consistent with the results of confocal microscopy studies, SW480 cells with Su(fu) overexpression had a lower level of nuclear β-catenin, and LMB treatment increased the level of nuclear β-catenin in these cells to those of the parental SW480 cells (Fig. 4R). Together, these results indicate that Su(fu) reduces nuclear β-catenin levels through a CRM1-dependent nuclear export mechanism.

**Fig. 5.** Relative effects of APC and Su(fu) overexpression on the regulation of β-catenin in SW480 cells. A, Tcf transcriptional activation in SW480 cells with transient transfection of APC and Su(fu). APC overexpression results in a significantly greater inhibition of Tcf transcriptional activation than that of Su(fu). Subcellular distribution of endogenous β-catenin (B, E, H, K, N, O, R, and S), transfected Su(fu) (C and F), and transfected APC (I, L, P, and T) in transiently transfected SW480 cells (B–M) and stable SW480 transformants (O–Q and S–U) were detected by immunofluorescence staining using anti-β-catenin, anti-Myc, and anti-FLAG antibodies, respectively. Panels E–G, K–M, and R–U represent LMB-treated cells, and B–D, H–J, and N–Q are cells without LMB treatment. Panels N and R are parental SW480 cells, and D, G, Q, and U are merged images. Expression of wild type APC results in a dramatic decline in nuclear β-catenin in SW480 cells (H and K). Wild type APC expression in SW480 cells expressing Su(fu) results in a further decline in β-catenin, as illustrated by the cell in panel O containing an undetectable nuclear and cytoplasmic level of β-catenin. Treatment with LMB increases the cytoplasmic level of β-catenin in cells expressing both wild type APC and Su(fu) (S) but not to as great a degree as in SW480 cells expressing wild type APC alone (L). Merged views show that both Su(fu) and APC co-localize with β-catenin.

**Su(fu) Overexpression Attenuates Tumor Formation by SW480 Cells in Nude Mice**—To determine whether Su(fu) al-
these tumors. There was also a correlation between the level of Su(fu) expression and the effect on tumor growth; Clone 7, which expresses higher levels of Su(fu), showed less tumor growth than clone 2, which has lower Su(fu) expression. These results indicate that Su(fu) overexpression can suppress the oncogenic functions of β-catenin.

**DISCUSSION**

We have shown that Su(fu) and β-catenin reside in the same complex and that Su(fu) negatively regulates Tcf-dependent transcription by reducing nuclear β-catenin levels. These inhibitory activities of Su(fu) are sensitive to LMB treatment indicating that the reduction of nuclear β-catenin is accomplished through a CRM1-mediated nuclear export mechanism. Interestingly, although APC is implicated in both nuclear export and cytoplasmic degradation of β-catenin, Su(fu) promotes β-catenin nuclear export in SW480 cells, which lack normal APC function. Thus, our results indicate that Su(fu) regulates Wnt signaling through APC-independent mechanism in which CRM1-mediated nuclear export plays a role.

We also showed that the action of APC is dominant over that of Su(fu); LMB treatment failed to block the reduction of nuclear β-catenin level in cells overexpressing both APC and Su(fu) (see Fig. 5). In normal cells, once β-catenin is synthesized in the cytoplasm, it will be targeted for degradation by the destruction complex; this targeting involves APC, axin, and GSK3β (1–4). Although a role for Su(fu) in the cytoplasmic compartment cannot be excluded, our results suggest that the major role of Su(fu) is after nuclear translocation of β-catenin. In APC-overexpressing SW480 cells, because most β-catenin will be degraded in the cytoplasm, and very little β-catenin is imported into the nucleus; the role of Su(fu) in β-catenin signaling thus becomes minimal.

The accumulation of Su(fu) in the nucleus of LMB-treated cells suggests that the intracellular distribution of Su(fu) itself is regulated by CRM1-mediated nuclear export. We propose that there is a nuclear-cytoplasmic exchange of Su(fu) itself and that Su(fu) interacts with β-catenin in the nucleus, facilitates the nuclear export of β-catenin, and eventually leads to a down-regulation of β-catenin-mediated Tcf-dependent transcription. Because mutant forms of Su(fu) lacking the carboxyl-terminal region are enriched in the nucleus, this carboxyl-terminal region may be essential for the efficient nuclear export of Su(fu). Elevated β-catenin-mediated transcription in this case indicates that either these same regions of Su(fu) are important for the ability of Su(fu) to regulate β-catenin-mediated transcription or that nuclear export of Su(fu) is necessary for it to reduce nuclear β-catenin levels. In this latter possibility, Su(fu) might act in a complex to export β-catenin from the nucleus.

**What is the role of Su(fu) in β-catenin signaling in vivo?** Numerous studies have suggested that nuclear localization of β-catenin is a key step in Wnt signaling and that the intracellular localization of β-catenin is controlled by the availability and affinity of its binding partners (1–4). We propose that Su(fu) is one of these binding partners and that Su(fu) functions to control the level of nuclear β-catenin in cells receiving Wnt signals. As β-catenin signaling is over-activated in many benign and malignant tumors, Su(fu) would thus be expected to act as a tumor suppressor. Consistent with this notion, we find that overexpression of Su(fu) in SW480 cells suppresses tumor growth in nude mice. It will now be important to examine whether inactivating mutations of Su(fu) are found in tumors with activated β-catenin signaling.

The primary focus here has been on the role of Su(fu) in β-catenin signaling. However, it is also clear that Su(fu) acts as a negative regulator of Hh signaling in both flies and mammals. Several studies have suggested that Su(fu) might possess multiple functions controlling the activities of Ci/Gli transcription factors, including cytoplasmic sequestration (17–20, 31, 32) and increase of DNA binding (16). The present study has revealed a novel role for Su(fu) in the regulation of β-catenin signaling through CRM1-mediated nuclear export. Because Ci/Gli transcription factors possess nuclear export signals and LMB treatment has been shown to increase nuclear localization of Ci in Drosophila (33) and to inhibit cytoplasmic sequestration of Gli1 (20), we propose that Su(fu) also regulates the activity of β-catenin and Ci/Gli transcription factors through a similar CRM1-mediated mechanism. In both cases, it is possible that Su(fu) acts to regulate the nuclear location of these transcriptional regulators through an export-related mechanism.

Finally, the observation that Su(fu) is involved in the regulation of β-catenin signaling provides further evidence that multiple levels of cross-talk exist between Wnt and Hh signaling during development and cancer. In Drosophila, the F-box protein and β-TrCP homolog, Slim, is known to regulate the degradation of Armadillo (β-catenin homolog) and Ci (34). The transcriptional cofactor p300 can interact with both β-catenin and Ci/Gli, promoting their transcriptional activities (35–39). Our results suggest that Su(fu) may regulate the activities of both β-catenin and Gli1 through a CRM1-mediated nuclear export mechanism.

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