Mechanisms of Signal Transduction:

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Dapper 1 Antagonizes Wnt Signaling by Promoting Dishevelled Degradation*

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Wnt signaling plays pivotal roles in the regulation of embryogenesis and cancer development. *Xenopus* Dapper (Dpr) was identified as an interacting protein for Dishevelled (Dvl), a Wnt signaling mediator, and modulates Wnt signaling. However, it is largely unclear how Dpr regulates Wnt signaling. Here, we present evidence that human Dpr1, the ortholog of *Xenopus* Dpr, inhibits Wnt signaling. We have identified the regions responsible for the Dpr-Dvl interaction in both proteins and found that the interaction interface is formed between the DEP (Dishevelled, Egl-10, and pleckstrin) domain of Dvl and the central and the C-terminal regions of Dpr1. The inhibitory function of human Dpr1 requires both its N and C terminus. Overexpression of the C-terminal region corresponding to the last 225 amino acids of Dpr1, in contrast to wild-type Dpr1, enhances Wnt signaling, suggesting a dominant negative function of this region. Furthermore, we have shown that Dpr1 induces Dvl degradation via a lysosome inhibitor-sensitive and proteasome inhibitor-insensitive mechanism. Knockdown of Dpr1 by RNA interference up-regulates endogenous Dvl2 protein. Taken together, our data indicate that the inhibitory activity of Dpr on Wnt signaling is conserved from *Xenopus* to human and that Dpr1 antagonizes Wnt signaling by inducing Dvl degradation.

Wnt signaling plays a key role in embryogenesis, and its dysregulation leads to cancer development (1, 2). Dishevelled (Dvl)[2] is a central mediator of Wnt signaling in both the canonical and the noncanonical pathways. It inhibits glycogen synthase kinase 3β-induced degradation of β-catenin and therefore activates the expression of target genes via the β-catenin-T-cell factor-lymphoid enhancer factor complex (3, 4). Dvl is also involved in a β-catenin-independent noncanonical pathway by influencing calcium influx, the activity of c-Jun N-terminal kinase and GTP-binding proteins (5).

Dapper (Dpr), originally identified as a Dvl-interacting protein, has been shown to inhibit both Dvl-mediated canonical and noncanonical Wnt pathways and is required for formation of the notochord and head structures in *Xenopus* embryos (6). Several Dpr-related proteins have been identified, and they may have distinct functions. Frodo, which shares 90% identity with Dpr at the amino acid level, promotes Wnt signaling in axis formation and in eye and neural development in *Xenopus* embryos (7). Frodo has also been shown to associate with T-cell factor via its N terminus and may directly link Dvl signaling to T-cell factor in a β-catenin-independent way (8). The zebrafish Dpr1, similar to *Xenopus* Frodo, was suggested to be a positive regulator of Wnt/β-catenin signaling (9), whereas the fish Dpr2, which exhibits a high similarity at both the N and C terminus, albeit having an overall low similarity to Dpr1, specifically antagonizes transforming growth factor-β/Nodal signaling (10). Both Dpr1 and Dpr2 are highly conserved from fish to human (11–13). Dpr1 not only regulates embryo development but may have an important role in tumorigenesis, and a computer analysis predicted that both Dpr1 and Dpr2 may be involved in the formation of certain tumors (11). Indeed, a recent study demonstrated that down-regulation of human Dpr1, an ortholog of *Xenopus* Dpr, was observed in hepatocellular carcinoma, and this down-regulation significantly correlated with the cytoplasmic accumulation of β-catenin (14).

To further define the functions of Dpr1, we have cloned human Dpr1 and mouse Dpr1. In this report, we have shown that mammalian Dpr1 inhibits expression of the Wnt-responsive reporters lymphoid enhancer factor (LEF)-luciferase and TopFlash-luciferase. We have further demonstrated that Dpr1 binds Dvl and induces its degradation in a lysosome inhibitor-sensitive pathway.

EXPERIMENTAL PROCEDURES

Construction of Plasmids—Human Dpr1 cDNA was assembled from two human EST clones WF08 (GenBank™ accession number gi:24420531) and BX356369 (gi:46285645) and subcloned into pCMV-Myc with EcoRI and XhoI and into pCMV-HA with HindIII and XbaI. Mouse Dpr1 cDNA was constructed by assembling the mouse expressed sequence tag BE952137 (gi:10592199), a reverse transcription-PCR product from mouse thymus covering the very 5′-end and PCR product from mouse genomic DNA covering the 3′-end. Myc-tagged Dpr1 deletions were generated by restriction digestions and PCR and subcloned into pCMV-Myc. All of the sequences were verified by DNA sequencing. Dvl constructs were kindly provided by Dr. Xi He (Children's Hospital, Harvard Medical School) (15). LEF-luciferase and TopFlash-luciferase were obtained from Dr. Zhijie Chang (Tsinghua University).

Cell Culture—HeLa and HEK293T cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Hyclone), nonessential amino acids, L-glutamine, and penicillin/streptomycin in a 5% CO_2-containing atmosphere at 37 °C.

Generating the Dpr1 Antibody—Dpr1 polyclonal antibody was generated by immunizing rabbit with human Dpr1 (282–516)-glutathione *S*-transferase fusion protein.

Transfection, Immunoprecipitation, and Immunoblotting—HeLa cells or HEK293T cells were transiently transfected using the calcium phosphate method or Lipofectamine (Roche Applied Science). At 40 h post-transfection, the cells were lysed with 1 ml of lysis buffer (20 mM

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[2] The abbreviations used are: Dvl, Dishevelled; Dpr, Dapper; LEF, lymphoid enhancer factor; HA, hemagglutinin; CMV, cytomegalovirus; siRNA, small interfering RNA; RNAi, RNA interference; DEP, Dishevelled, Egl-10 and pleckstrin; DIX, Dishevelled and Axin; PDZ, PSD-95, Discs-large, and ZO-1.

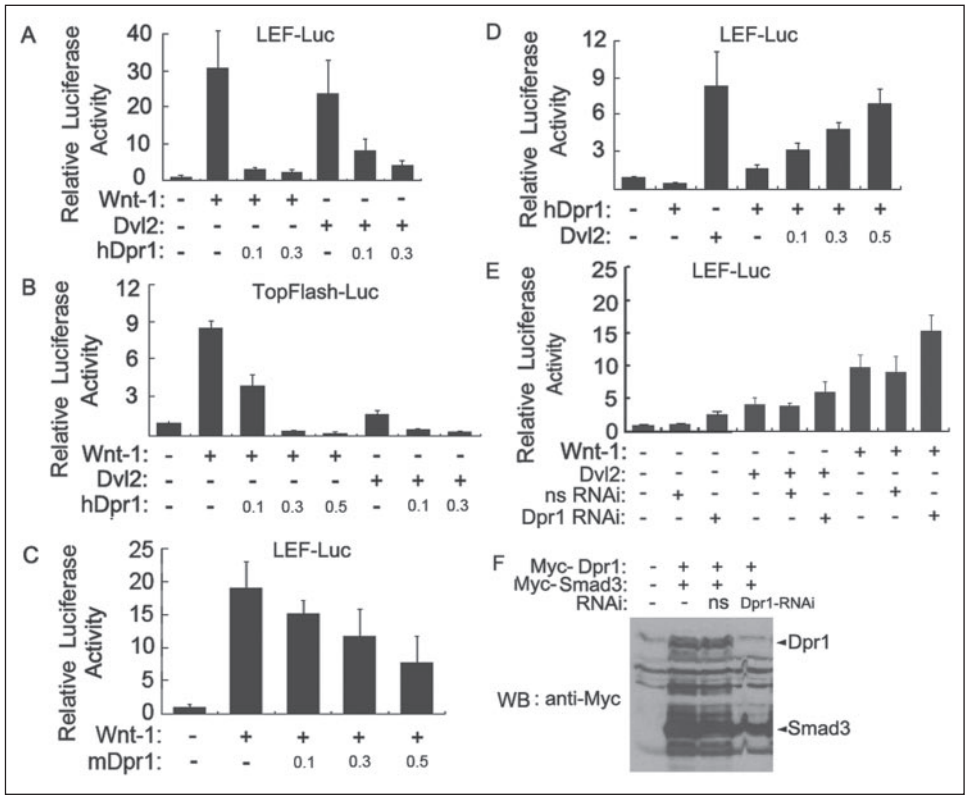

FIGURE 1. **Mammalian Dpr1 antagonizes Wnt signaling through Dvl.** Human Dpr1 inhibits Wnt1- or Dvl2-activated expression of LEF-luciferase reporter (*A*) or TopFlash-luciferase reporter (*B*) in a dose-dependent manner. HeLa cells were co-transfected with reporter plasmid (0.4 μg), and the constructs encoded Wnt1 (0.3 μg) or Dvl2 (0.3 μg) with or without human Dpr1 (0.1–0.3 μg). *C*, mouse Dpr1 blocks Wnt1-induced expression of the LEF-luciferase reporter. HeLa cells were co-transfected with LEF-luciferase reporter (0.4 μg) and Wnt1 (0.3 μg) with or without mouse Dpr1 (0.1–0.5 μg). *D*, inhibitory effect of Dpr1 can be reversed by increasing the expression of Dvl. HeLa cells were co-transfected with LEF-luciferase reporter (0.4 μg) and human Dpr1 (0.1 μg) with or without Dvl2 (0.1–0.5 μg). *E*, knockdown of endogenous Dpr1 results in enhanced expression of the LEF-luciferase reporter. HEK293T cells were co-transfected with the constructs as indicated with or without Dpr1-specific siRNA (0.1 μg) or a nonspecific siRNA (*ns RNAi*). All of the experiments were performed by co-transfection of *Renilla*-luciferase (20 ng) as an internal control. The transfected cells were lysed for luciferase assay at 30 h post-transfection. Each experiment was performed in triplicate, and the data represent the mean ± S.D. of three independent experiments after normalized to *Renilla* activity. *F*, Myc-Dpr1 (1 μg) and Myc-Smad3 (1 μg) were co-transfected into HEK293T with nonspecific siRNA (2 μg) or Dpr1-specific siRNA (2 μg). At 40 h post-transfection, the cells were harvested for anti-Myc immunoblotting.

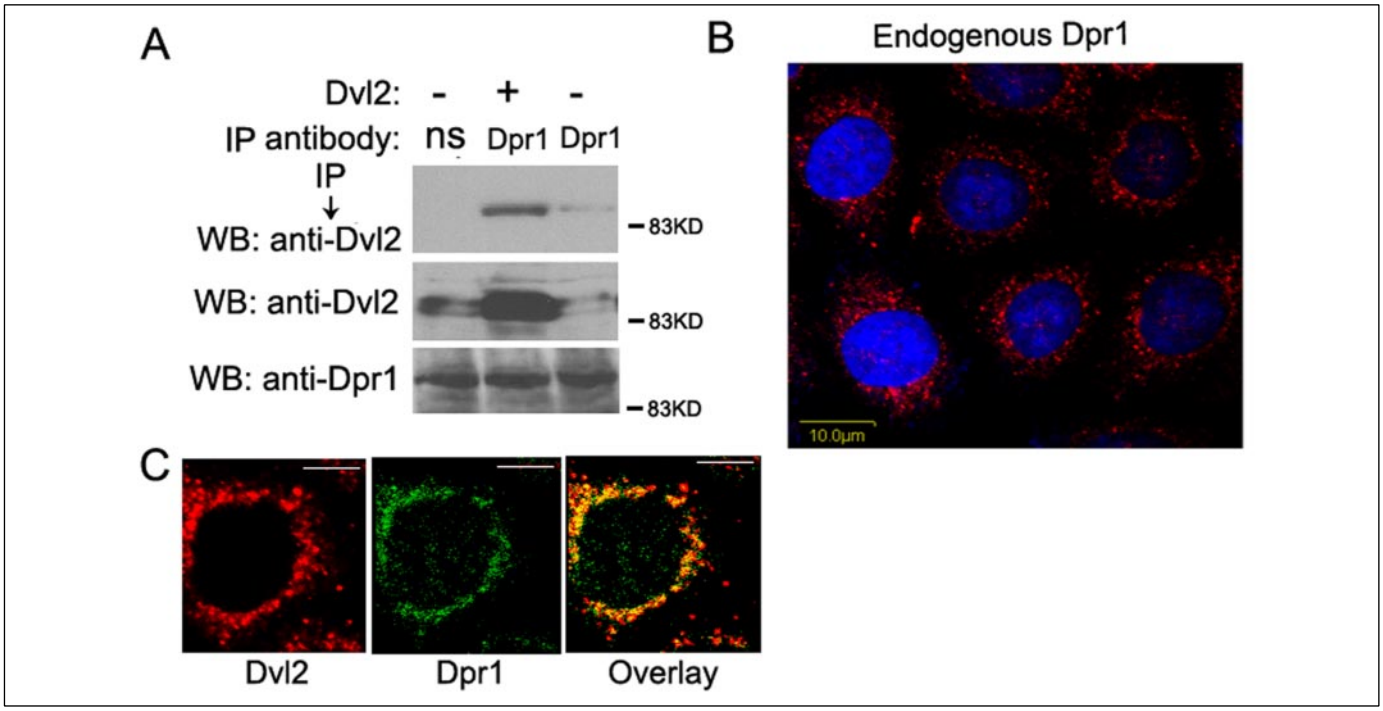

FIGURE 2. **Human Dpr1 interacts with Dvl2 endogenously.** *A*, interaction between Dpr1 and Dvl2 at the endogenous levels. HeLa cells were transfected with or without Dvl2 as indicated. At 40 h post-transfection, the cells were harvested for immunoprecipitation with anti-Dpr1 antibody or a nonspecific antibody (*ns*). Dpr1-associated Dvl2 was revealed by anti-Dpr1 immunoblotting (*upper panel*). The protein expression was confirmed by anti-Dvl2 or -Dpr1 immunoblotting with the total cell lysates (*middle* and *lower panels*). *B*, subcellular localization of endogenous Dpr1 in HeLa cells shown by immunofluorescence. Paraformaldehyde-fixed and Triton X-100-permeabilized HeLa cells were incubated with rabbit anti-Dpr1 primary antibody and then rhodamine-conjugated anti-rabbit secondary antibody. The nuclei were stained in *blue* with 4,6-diamidino-2-phenylindole. *Scale bar*, 10 μm. *C*, subcellular co-localization of endogenous Dpr1 and Dvl2 in HeLa cells. Paraformaldehyde-fixed and Triton X-100-permeabilized HeLa cells were incubated with rabbit anti-Dpr1 and goat anti-Dvl2 primary antibodies and then fluorescein isothiocyanate-conjugated anti-rabbit or rhodamine-conjugated anti-goat secondary antibodies. *Scale bar*, 10 μm.

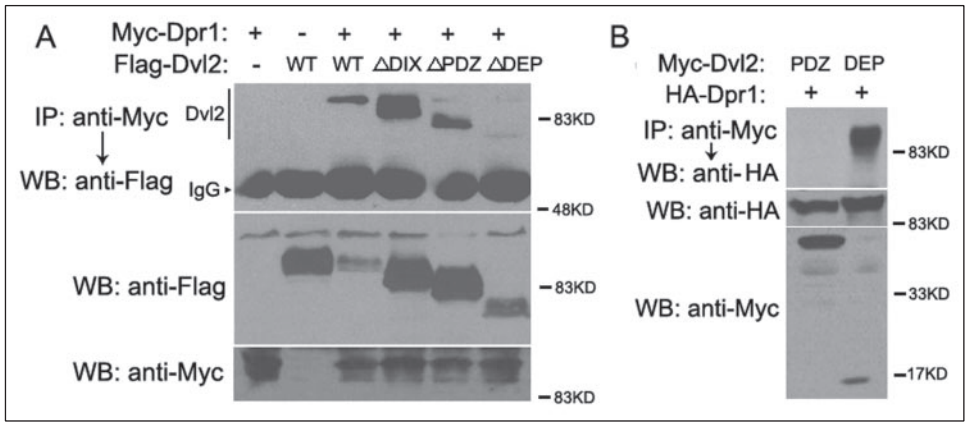

FIGURE 3. **The DEP domain of Dvl mediates the interaction with Dpr1.** *A*, HEK293T cells were transfected with Myc-tagged human Dpr1 and wild-type or truncation mutants of FLAG-tagged Dvl2 as indicated. At 40 h post-transfection, the cells were harvested for anti-Myc immunoprecipitation (*IP*). Dpr1-associated Dvl was revealed by anti-FLAG immunoblotting (*upper panel*). The protein expression was confirmed by anti-Myc or anti-FLAG immunoblotting (*WB*) with the total cell lysates (*middle* and *lower panels*). *B*, HEK293T cells were transfected with HA-tagged human Dpr1 and Myc-tagged Dvl2 fragments as indicated. At 40 h post-transfection, the cells were harvested for anti-Myc immunoprecipitation. Dpr1-associated Dvl was revealed by anti-HA immunoblotting (*upper panel*). The protein expression was confirmed by anti-Myc or anti-HA immunoblotting with the total cell lysates (*middle* and *lower panels*).

Tris-HCl, pH 7.4, 2 mM EDTA, 25 mM NaF, 1% Triton X-100) plus protease inhibitors (Sigma) for 30 min at 4 °C. After 12,000 × *g* centrifugation for 15 min, the lysates were immunoprecipitated with specific antibody and protein A-Sepharose (Zymed Laboratories Inc.) for 3 h at 4 °C. Thereafter, the precipitants were washed three times with washing buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, and 0.1% SDS) and the immune complexes eluted with sample buffer containing 1% SDS for 5 min at 95 °C and analyzed by SDS-PAGE. Immunoblotting was performed with primary antibodies against the HA tag (made in-house), Myc tag (made in-house), and FLAG tag (Sigma, M2) and secondary anti-mouse antibodies were conjugated to horseradish peroxidase (Amersham Biosciences). Proteins were visualized by chemiluminescence. Most experiments were carried with human Dpr1 unless mouse Dpr1 was indicated. Goat anti-Dvl2 (sc-7399) and mouse anti-β-catenin (sc-7963) antibodies was purchased from Santa Cruz Biotechnology.

Luciferase Reporter Assays—HEK293T cells were transfected with various plasmids as indicated in the figures. Two days after transfection, the cells were harvested and luciferase activities were measured by a luminometer (Berthold Technologies). Reporter activity was normalized to the control *Renilla*. Experiments were repeated in triplicate.

Immunofluorescence—HeLa cells grown on glass coverslips were washed twice with phosphate-buffered saline, fixed with 4% paraformaldehyde in phosphate-buffered saline for 15 min, permeabilized with 0.1% Triton X-100 for 10 min, and blocked with 3% nonfat dry milk in phosphate-buffered saline for 60 min. The cells were then incubated with primary antibodies diluted in TBST (20 mM Tris-HCl, pH 7.6, 137 mM NaCl, 0.1% Tween 20) for 3 h and washed twice with phosphate-buffered saline and with fluorescein- or rhodamine-conjugated anti-mouse or anti-rabbit antibodies (Jackson Immunoresearch Laboratories) for an additional 40 min. The nuclei were counterstained with 4,6-diamidino-2-phenylindole (Sigma).

RNA Interference—pSUPER.retro (pSR) (OligoEngine) was used for the expression of siRNA. The target sequence of hDpr1 is 5′-ATCTGCAGATCTCATAGGATT-3′, corresponding to 713–733nt of hDpr1 (pSR01). A nonspecific siRNA expression vector, pNS, containing the sequence 5′-AGCGGACTAAGTCCATTGC-3′, was constructed as a negative control. These sequences were all analyzed by a BLAST search of the GenBank™ Data Base to avoid similar sequences found in the human genome. Oligonucleotides were synthesized (Bioasin, Shanghai, China) and inserted into the pSR vector in the SalI and KpnI sites.

RESULTS AND DISCUSSION

Dpr1 Antagonizes Wnt Signaling via Dvl—*Xenopus* Dpr has been shown to act as a negative regulator of Wnt signaling (6). To test whether the inhibitory function of Dpr on Wnt signaling is conserved in mammalian cells, the cDNAs encoding human and mouse Dpr1 were cloned and examined for their activities with Wnt-responsive reporters. Overexpression of human or mouse Dpr1 interfered with Wnt- and Dvl-induced expression of the reporters LEF-luciferase (16, 17) and Topflash-luciferase (18) in a dose-dependent manner (Fig. 1, *A–C*), whereas they had no effect on the control reporter Fopflash-luciferase (data not shown). Human Dpr1 also inhibited c-Jun-mediated reporter expression (data not shown), which is consistent with the previous results obtained with *Xenopus* Dpr and supports the notion that Dpr1 antagonizes both canonical and noncanonical Wnt pathways (6). We presumed that increased expression of Dvl should be able to reverse the inhibitory effect of Dpr1 if Dpr1 functions on Dvl to modulate Wnt signaling. Overexpression of Dvl2 indeed overcame Dpr1 inhibition (Fig. 1*D*).

To examine the activity of endogenous Dpr1 on Wnt signaling, we constructed a siRNA against human Dpr1, which could specifically knock down human Dpr1 expression but had no effect on Smad3 expression (Fig. 1*F*). Down-regulation of endogenous Dpr1 by RNAi not only led to a higher basal expression of the LEF-luciferase reporter, but also increased Dvl2- and Wnt1-promoted expression of this reporter, whereas a nonspecific siRNA had no effect (Fig. 1*E*). Our data suggest a conserved inhibitory function of Dpr1 in the Wnt signaling pathway from *Xenopus* to mouse and human. These results are also in agreement with a recent observation that down-regulation of Dpr1 in hepatocellular carcinoma leads to a high Wnt/β-catenin signaling activity (14).

Interaction between Mammalian Dpr1 and Dvl at the Endogenous Levels—It was reported that *Xenopus* Dpr1 can directly interact with *Xenopus* Dvl (6). To confirm this interaction with mammalian Dpr1, we performed a co-immunoprecipitation assay with the endogenous proteins. The antibody we generated against a human Dpr1 fragment (amino acids 282–516) could detect endogenous Dpr1 in HeLa cells (Fig. 2*A*, *lower panel*). Indeed, Dvl2 was found in anti-Dpr1 immunoprecipitant but not in the control with a nonspecific antibody (Fig. 2*A*, *upper panel*). To further consolidate this interaction, we studied subcellular localization of Dpr1 and Dvl2 in HeLa cells. Dpr1 was mainly localized in the cytoplasm as spots (Fig. 2*B*), and most Dpr1 proteins were co-localized with Dvl2 (Fig. 2*C*). These data further support that Dpr1 binds Dvl at the physiological condition.

Identification of the Regions Responsible for the Dpr1-Dvl Interaction—There are three conserved domains in Dvl proteins, the N-terminal DIX (Dishevelled and Axin) domain, the middle PDZ (PSD-95, Discs-large, and ZO-1) domain, and the C-terminal DEP domain, each of which mediates association with a variety of Dvl-associating proteins (19). To map the regions of Dvl responsible for

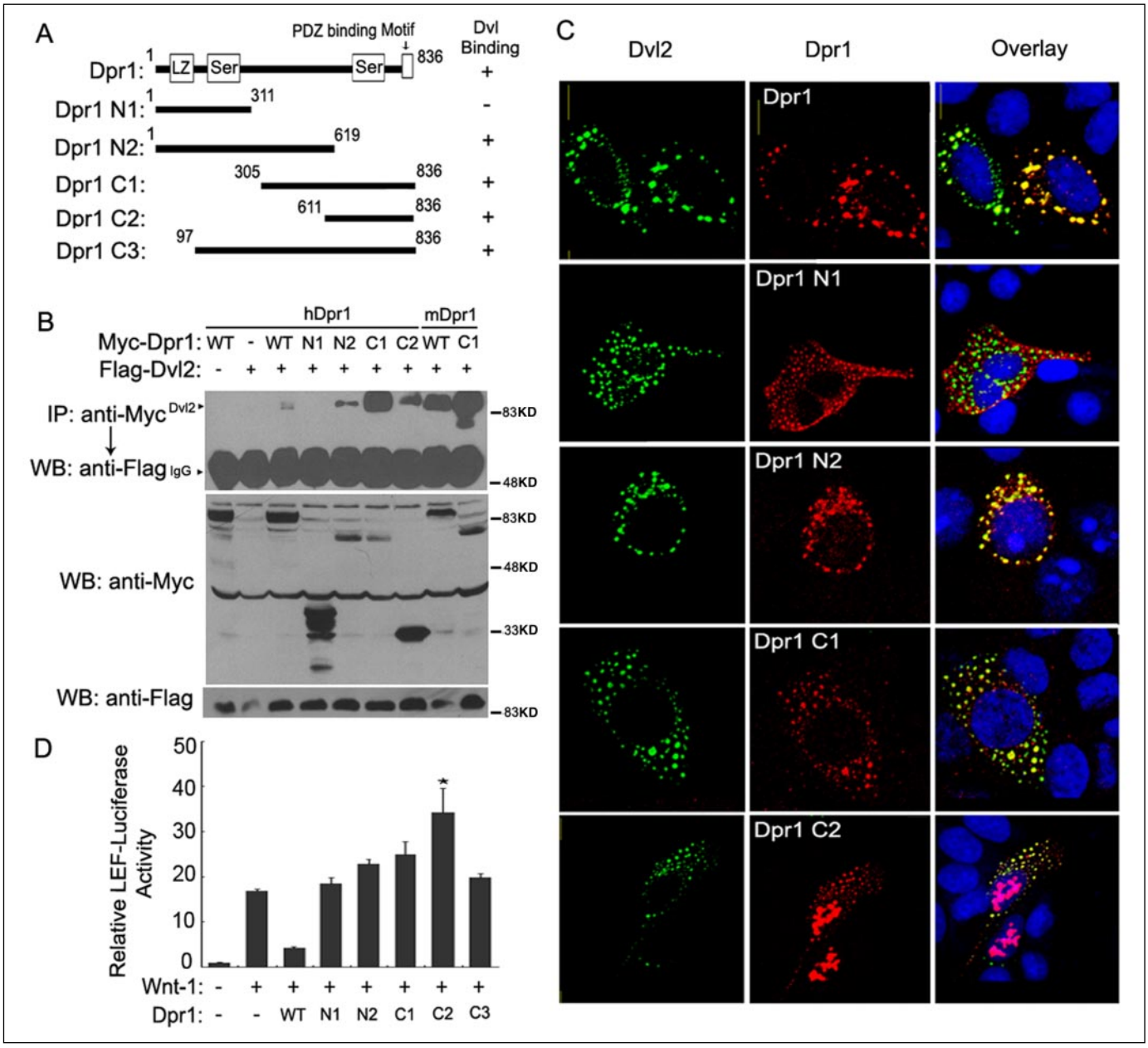

FIGURE 4. **The Dpr1-Dvl interaction is mediated by both the central and the C-terminal regions of Dpr1.** *A*, schematic representative of human Dpr1 deletion mutants. Dpr1 contains a leucine zipper domain (*LZ*), two serine-rich regions (*Ser*) and a C-terminal PDZ-binding motif. *B*, Dpr1 associates with Dvl with its central and C-terminal regions. HEK293T cells were transfected with wild-type or truncation mutants of Myc-tagged human (*hDpr1*) or mouse Dpr1 (*mDpr1*) and FLAG-tagged Dvl2 as indicated. Mouse Dpr1 C1 mutant lacks the N-terminal region of amino acids 1–304. Protein complexes were examined in a similar manner as described in the legend to Fig. 3*A*. *C*, subcellular co-localization of human Dpr1 and Dvl2. HeLa cells were transfected with wild-type FLAG-tagged Dvl2 together with wild-type or mutant Myc-tagged human Dpr1. Subcellular localization of Dvl2 (*left panels*) or Dpr1 (*middle panels*) were detected by indirect anti-FLAG or anti-Myc immunofluorescence. Their co-localization was shown in the merged images (*right panels*), and the nuclei were stained with 4,6-diamidino-2-phenylindole (*blue*). *D*, both the N and C termini of human Dpr1 are required for the inhibitory effect on Wnt signaling. Overexpression of the Dpr1 C2 mutant resulted in a statistically significant enhancement of Wnt signaling as analyzed by Student's *t* test ($p < 0.05$) (indicated by *asterisk*). HeLa cells were co-transfected with the constructs as indicated. The transfected cells were lysed for luciferase assay at 30 h post-transfection. The experiment was performed in triplicate, and the data represent the mean ± S.D. of three independent experiments after normalized to *Renilla* activity.

the interaction with Dpr1, plasmids encoding FLAG-tagged Dvl deletion mutants were transfected with or without Myc-tagged human Dpr1 into HEK293T cells. The protein complexes were analyzed by co-immunoprecipitation and immunoblotting. As shown in Fig. 3*A*, the DIX domain deletion (Δ1–109 amino acids) and PDZ domain deletion (Δ241–390 amino acids) mutants of Dvl2, similar to wild type, retained the ability to bind to Dpr1, whereas deletion of the DEP domain (Δ365–667 amino acids) virtually eliminated its association with Dpr1. Furthermore, Dpr1 interacted with a short region containing DEP domain (429–593 amino acids) but not with another long region containing PDZ domain (134–432 amino acids) (Fig. 3*B*), indicating that the DEP domain is sufficient for Dpr1 binding. This result suggests that Dvl binds to human Dpr1 and *Xenopus* Dpr via different regions, with the DEP domain binding to human Dpr1 and the PDZ domain to *Xenopus* Dpr (6, 20). Interestingly, the DIX domain of *Xenopus* Dvl has been reported to mediate its association with Frodo (7).

To identify the Dvl-associating regions of Dpr1, similar co-immunoprecipitation assays were performed in HEK293T cells with truncation mutants of Dpr1 (Fig. 4*A*). The mutants containing the C-terminal

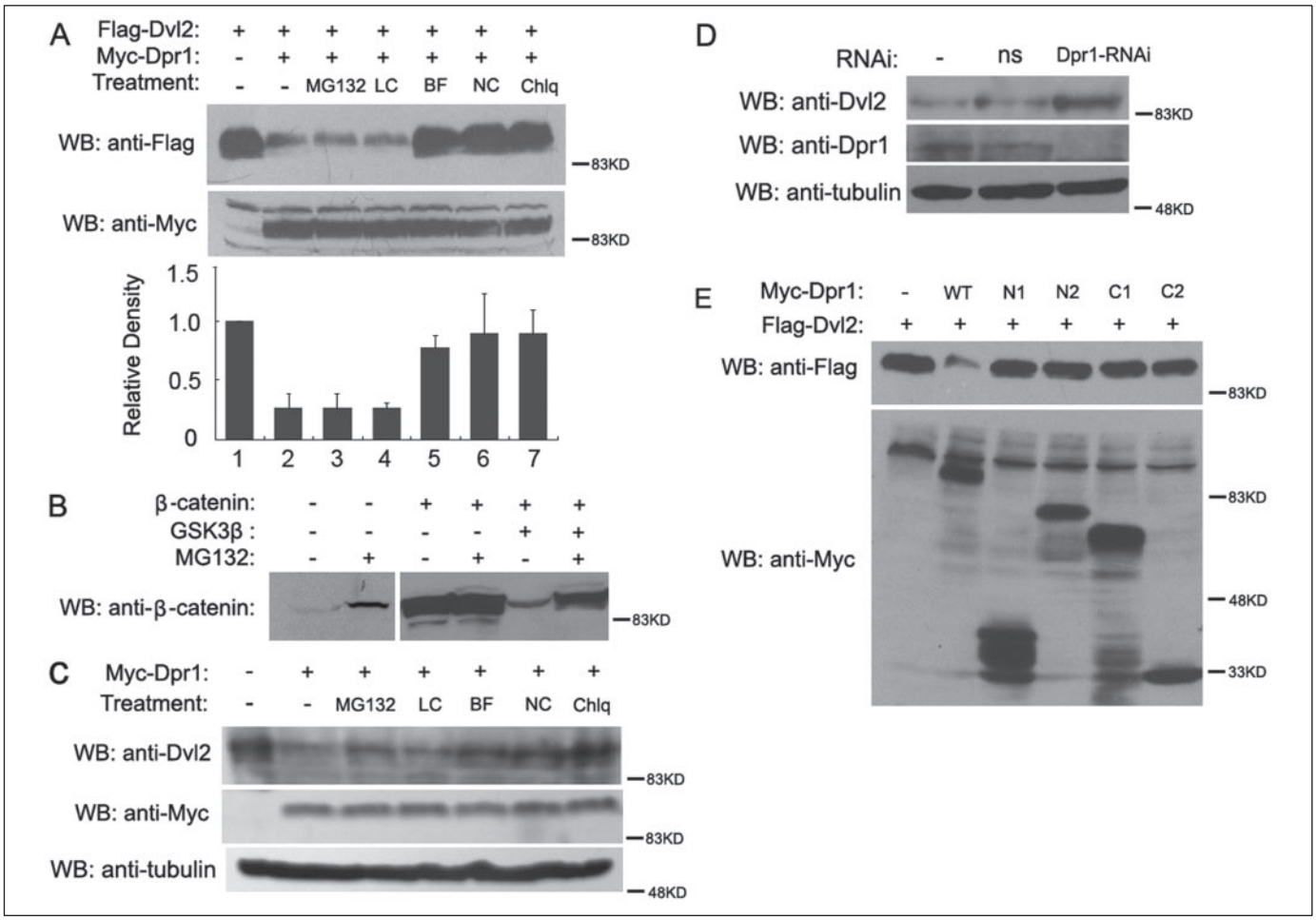

FIGURE 5. **Dpr1 induces Dvl degradation.** *A*, co-expression of Dpr1 with Dvl2 induce Dvl2 degradation in a lysosome inhibitor-sensitive manner. HEK293T cells were transfected with FLAG-Dvl2 together with or without Myc-tagged human Dpr1. At 35 h post-transfection, the cells were treated with or without proteasome inhibitors 25 μM MG132 or 30 μM lactacystin (*LC*) or lysosomal inhibitors 1 μM bafilomycin A1 (*BF*), 25 mM NH_4Cl (*NC*), or 100 μM chloroquine (*Chlq*) for 6 h and then harvested for examination of protein expression by immunoblotting. The histogram shows relative protein expression densities compared with the control Dvl2 expression, and the data were expressed as means ± S.D. of three independent experiments. *B*, MG132 is effective in preventing β-catenin degradation. HEK293T cells were transfected with or without β-catenin and glycogen synthase kinase 3β. At 35 h post-transfection, the cells were treated with or without MG132 and harvested for examination of β-catenin protein levels by immunoblotting (*WB*). *C*, overexpression of Dpr1 induces degradation of endogenous Dvl. HeLa cells were transfected with or without Myc-tagged human Dpr1. At 35 h post-transfection, the cells were treated with or without various inhibitors and harvested for examination of protein expression by immunoblotting. Loading control was verified by tubulin protein levels. *D*, knockdown of endogenous Dpr1 by RNAi results in a higher protein level of Dvl in HeLa cells. HeLa cells were transfected with pSUPER-encoding Dpr1-specific siRNA (*Dpr1-RNAi*) or a nonspecific RNA (*ns*). Thirty-six hours later, the cells were treated with 0.5 mg/ml puromycin for another 72 h to select the transfected cells before harvesting for examination of endogenous protein expression by immunoblotting. Loading control was verified by tubulin protein levels. *E*, Dpr1 deletion mutants have no effect on Dvl stability. HEK293T cells were transfected with FLAG-Dvl2 together with or without Myc-tagged human Dpr1 mutants. At 40 h post-transfection, the cells were harvested for examination of protein expression by immunoblotting.

regions of Dpr1 (C1 and C2 of human Dpr1 and C1 of mouse Dpr1) interacted with Dvl (Fig. 4*B*), consistent with the previous reports that the C terminus of *Xenopus* Dpr and Frodo is critical for Dvl binding (6, 7, 20). The C-terminal region of Dpr1 has been shown to mediate a direct binding to the PDZ domain of Dvl (6, 20). Furthermore, the peptides derived from the C-terminal tail of Dpr inhibits Wnt1-induced secondary axis formation in *Xenopus* embryos (20), highlighting the importance of the Dpr1 C-terminal tail-mediated interaction with Dvl. In addition, we found that the N2 mutant of Dpr1 (amino acids 1–619) that lacks the C-terminal region also associated with Dvl, whereas the N1 mutant (amino acids 1–331) did not. These data were confirmed by the subcellular co-localization of Dpr1 mutants with Dvl. The N1 mutant that does not bind to Dvl did not co-localize with Dvl (Fig. 4*C*). Taken together, these results indicate that Dpr1 can interact with Dvl via two regions, the central and the C-terminal regions.

Next, we attempted to investigate the functional significance of the Dpr1-Dvl interaction by examining the effect of Dpr1 truncated mutants on Wnt signaling with the LEF-luciferase reporter. Neither the N nor the C terminus of Dpr1 had inhibitory effects on Wnt-activated reporter expression (Fig. 4*D*). An N-terminal truncation mutant (Dpr1-C3) lacking the first 96 amino acids lost the ability to inhibit Wnt signaling, implying that both the N and the C terminus of Dpr1 is required for its modulating activity on Wnt signaling. Notably, overexpression of the Dpr1 C-terminal C2 mutant resulted in constantly increased Wnt signaling, suggesting that the C-terminal region may possess a dominant negative effect on endogenous Dpr1.

Dpr1 Induces Dvl Degradation—When Dvl was co-expressed with Dpr1, we noticed a low protein level of both endogenous and ectopically expressed Dvl (Figs. 2*A*, 3*A*, and 4*B*), suggesting that overexpression of Dpr1 may lead to Dvl degradation. To test this possibility, HEK293T cells were co-transfected with Dpr1 and Dvl and treated with or without different protein degradation inhibitors. Co-expression of Dpr1 with Dvl led to decreased protein levels of Dvl (Fig. 5*A*), and lysosome inhibitors bafilomycin A1, NH_4Cl, and chloroquine blocked Dpr1-induced degradation of Dvl. However, the proteasome inhibitors MG132 and lactacystin had no effect, although MG132 was effective in preventing

glycogen synthase kinase 3β-promoted degradation of β-catenin via the ubiquitination-proteasome pathway (Fig. 5*B*) (21). Overexpression of Dpr1 also induced degradation of endogenous Dvl2 in a similar manner (Fig. 5*C*).

To investigate the role of endogenous Dpr1 in regulating Dvl2 protein stability, HeLa cells were transfected with pSUPER-encoding Dpr1-specific siRNA or a nonspecific siRNA and treated with puromycin for 72 h to select the transfected cells. Thereafter, the cells were harvested for the examination of endogenous protein expression by immunoblotting. Expression of Dpr1-specific siRNA effectively knocked down the endogenous Dpr1 protein level (Fig. 5*D*, *middle panel*). Importantly, endogenous Dvl2 protein levels were evidently increased when Dpr1 protein was knocked down (Fig. 5*D*, *upper panel*). This result clearly shows that Dpr1 plays an important role in modulating Dvl protein levels in the physiological condition. We further examined whether any of the Dpr1 deletion mutants has an effect on Dvl stability when they were co-expressed with Dvl2 and found that none of them induced Dvl2 degradation (Fig. 5*E*). These results together suggest that Dpr1 interferes with Wnt signaling by directly binding to and targeting Dvl for lysosome inhibitor-sensitive degradation.

Dpr proteins belong to a newly defined protein family. Although much more work is needed to elucidate their physiological and pathological functions in embryos and adult tissues, it appears that Dpr1 and Dpr2 play distinct roles by modulating Wnt signaling and transforming growth factor-β signaling, respectively. Here, we show that Dpr1 interacts with Dvl and enhances Dvl degradation in a lysosome inhibitor-sensitive and proteasome inhibitor-insensitive manner in an analogous way to the activity of Dpr2 that associates with transforming growth factor-β type I receptors and targets them for lysosomal degradation (10). Dvl shuttles between the cytoplasm and the nucleus (22) and can also be recruited to the plasma membrane (23, 24). How Dpr1 promotes Dvl degradation awaits further investigation. A recent study demonstrated that PR72, a protein phosphatase type 2A regulatory subunit, can induce Dvl degradation probably in a proteasome inhibitor-sensitive manner (25). Therefore, the stability of Dvl can be regulated by distinct mechanisms possibly according to its different subcellular localizations.

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