Figures and figure supplements

Conformational change of Dishevelled plays a key regulatory role in the Wnt signaling pathways

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Figure 1. The C-terminal tail of Dishevelled (Dvl) is a PDZ domain binding motif. Sequence alignment of the C-terminus of Dvl/Dsh from selected species (Wallingford and Habas, 2005), showing residue numbers.
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Figure 2. Competitive binding experiments. The $K_D$ value of the fluorescence-labeled Dapper (Dpr)-derived peptide Rox-DprC was obtained by plotting $1/\Delta mP$ vs $1/[PDZ]$, where $\Delta mP$ is the fluorescence polarization change ($\times 1000$) of Rox-DprC and [PDZ] is the concentration of the PDZ domain of Dvl. The $K_i$ values of the Dvl-C and Dsh-C peptides were obtained by using the equation $K_i = (K_D/(1 + [I]/K_I))$.
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Figure 3. Isothermal titration calorimetry experiment. The MicroCal Auto-ITC-200 was used to obtain the binding affinity of Dvl-C peptide and Dvl PDZ protein in 50 mM phosphate buffer. The concentration of Dvl-C peptide in the syringe was 1.05 mM and the concentration of Dvl PDZ domain in the cell was 0.114 mM. The $K_D$ value was averaged from two independent experiments at 25°C.
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Figure 4. Direct interaction of the Dvl C-terminus and PDZ domain. (A) Overlap of $^1$H-$^{15}$N HSQC spectra of $^{15}$N-labeled PDZ domain without (blue) and with the unlabeled peptide (SEFFVDVM) derived from the extreme C-terminus of Dvl. Free: blue; final: red; ratio of peptide:protein = 20:1. (B) Overlap of $^1$H-$^{15}$N HSQC spectra of $^{15}$N-labeled PDZ domain without (blue) and with unlabeled peptide (QDVVSNYVL) derived from the C-terminus of Drosophila Dsh (Dsh-C). Blue: free; red: final; ratio of peptide:protein = 20:1. DOI: 10.7554/eLife.08142.006
Figure 5. Solution NMR structure of Dvl PDZ domain in complex with Dvl-C peptide. (A) 2D plane of 3D $^{13}$C-F1-half-filtered F2-edited NOESY-HSQC spectrum (mixing time, 300 ms) at 15°C. The ratio of peptide: protein was 10:1. [13C, 15N-PDZ] = ∼1 mM. (B) A stereo view of the backbone of 15 superimposed structures of the Dvl PDZ–Dvl-C peptide complex. (C) Ribbon diagram of the lowest-energy structure of the Dvl PDZ/Dvl-C peptide complex. (D) Surface of Dvl-1 PDZ bound to Dvl-C peptide (carbon, green; nitrogen, blue; sulfur, yellow; oxygen, red; hydrogen atoms are omitted for clarity). (E) Structural details of the Dvl-C peptide–PDZ Figure 5. continued on next page
Figure 5. Continued

The side chain of Asp(-2) in the Dvl-C peptide forms a hydrogen bond with the side chain of Arg322 on the αB-structure.

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Figure 5—figure supplement 1. The mutant Dvl-1 PDZ (R322A) domain binds more weakly than wild-type Dvl-1 PDZ domain to the Dvl-C peptide. Overlap of the ¹H-¹⁵N HSQC spectra of the labeled mutant Dvl PDZ (R322A) domain and the Dvl-C peptide. The mutation dramatically weakens interaction with the Dvl-C peptide (free: blue; final titration: red; final peptide:protein ratio = 4:1).

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Figure 6. The binding pocket of the Dvl PDZ domain can be occupied by its intrinsic C-terminus. (A) Schematic representation of protein constructs mC1 (residues 251–695) and mC1-CΔ7 (residues 251–688) numbered according to the mouse Dvl-1 protein sequence. (B) Polarization change of the fluorescence-labeled peptide Rox-DprC (Rox-SGSLKLMTTV, derived from the C-terminus of Dpr) after addition of mC1-CΔ7 and mC1 proteins in 50 mM phosphate with 0.3 M NaCl and 6 mM β-mercaptoethanol. For the binding of Rox-DprC to mC1, KD is 3.8 ± 0.5 μM the value was obtained by fitting the titration data with the equation: ΔmP = ΔmP_max × [P]/([P] + K_D), where ΔmP is the polarization change of Rox-DprC, [P] is the concentration of protein, and both K_D and ΔmP_max are the fitting variables. For the binding of Rox-DprC to mC1-CΔ7, K_D was estimated as 68 ± 5 μM. Because of the limitation in the titration study, to estimate the K_D value, although we used the same equation to fit the titration data, in the fitting, K_d was the only variable and the maximum polarization change, ΔmP_max, was fixed to the value that was obtained in the titration study of Rox-DprC binds to mC1-CΔ7.

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Figure 7. Effect of wild-type XDsh and XDsh-CΔ8 activity on canonical Wnt signaling. Luciferase assay using a Siamois promoter reporter (Sialuc). Sialuc DNA (200 pg) was injected alone or with myc-tagged Xdsh or XDsh-CΔ8 mRNA (500 pg) into the animal pole region of 2-cell Xenopus embryos. Ectodermal explants were dissected at the early gastrula stage for luciferase assay. Values are the means ±SD from four independent experiments (p < 0.05). Inset shows a representative western blot using anti-myc antibody (9E10) to control for XDsh and XDsh-CΔ8 protein expression in the four experiments.

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Figure 8. The open conformation of Dvl significantly enhances gain-of-function planar cell polarity (PCP) signaling. (A) Xenopus embryonic abnormal convergent extension (CE) phenotypes induced by injection of wild-type XDsh and XDsh-CΔ8 mRNA at three increasing concentrations (arrows at bottom represent 80 pg, 200 pg and 500 pg of injected mRNA; above are numbers of embryos injected from two independent experiments). Phenotypes are severe (green), mild (red), and normal (blue). (B) Comparison of phenotypes induced by dose-equivalent injections (500 pg mRNA) of XDsh, XDsh-CΔ8, and Xdd1 (a well-established dominant-negative XDsh mutant). XDsh-CΔ8 and Xdd1 induced similar phenotypes. The numbers of embryos injected from three independent experiments are listed on the top of each column.
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The open conformation of Dvl disrupts CE by activating Jun N-terminal kinase (JNK). (A) Western blot of phosphorylated JNK in ventral mesoderm cells overexpressing wild-type XDsh or its mutants. At equivalent protein level, Xdd1 and XDsh-CΔ8 more potently induce JNK phosphorylation than wild-type XDsh. (B) Xenopus 4-cell stage embryos were dorsally coinjected with equal quantities of wild-type XDsh mRNA or XDsh-CΔ8 mRNA (500 pg) and the AP1-luciferase reporter DNA (200 pg); luciferase activity was assayed at the late gastrula stage. Inset shows a representative Western blot using anti-myc antibody to control XDsh and XDsh-CΔ8 protein levels. Values are the mean and SD from three independent experiments (XDsh vs XDsh-CΔ8, p < 0.05). (C–H) The dominant negative JNK mutant (dnJNK) rescues activin-induced explant elongation blocked by overexpression of XDsh-CΔ8 or Xdd1. (C) Uninjected explants treated with activin show extensive elongation. (D) XDsh-injected explants treated with activin show moderate inhibition of explant elongation. (E) Injection of XDsh-CΔ8 strongly inhibits explant elongation. (F) Injection of Xdd1 similarly inhibits explant elongation as injection of XDsh-CΔ8. (G, H) dnJNK rescues Figure 9. continued on next page

Figure 9. continued on next page

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Figure 9. Continued

Explant elongation inhibited by XDsh-CΔ8 or Xdd1. (I) dnJNK also rescues CE defects produced by overexpression of XDsh-CΔ8 or Xdd1 in whole embryos. Phenotypes are severe (green), mild (red), and normal (blue). Numbers on the top indicate total embryos scored from three independent experiments.

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Figure 10. The open conformation of Dvl induced by targeting the Dvl PDZ domain potentiates Wnt/JNK signaling. (A) Regulation of XDsh-mediated PCP signaling by a PDZ-binding small molecule or peptide is shown by the gain-of-function CE phenotypes of whole embryos that were uninjected (controls) or injected with XDsh mRNA with or without treatment with the Dvl inhibitors 3209–8625 or coinjected with XDsh and TMEM88-C mRNAs. (B) Inhibiting the Dvl PDZ domain blocks canonical Wnt signalling induced by Dvl overexpression. Wild-type XDsh mRNA was injected alone or coinjected with an equal quantity of TMEM88-C mRNA in the animal pole region of two-cell stage Xenopus embryos, and ectodermal explants were dissected at the late blastula stage. TOPFLASH luciferase activity values are the mean and SD from three independent experiments (p < 0.05). (C) Inhibition of the Dvl PDZ domain by TMEM88 opens the conformation of Dvl and potentiates Wnt/JNK signaling induced by Dvl overexpression. Xenopus 4-cell stage embryos were injected dorsally with wild-type XDsh mRNA or coinjected with equal quantities of wild-type XDsh mRNA and TMEM88-C mRNA. AP1 luciferase activity was assayed at the late gastrula stage. Values are the mean and SD from three independent experiments (p < 0.05).

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