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Structural basis of the recognition of the Dishevelled DEP domain in the Wnt signaling pathway

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Abstract

The DEP domain of Dishevelled (Dvl) proteins transduces signals to effector proteins downstream of Dvl in the Wnt pathway. Here we report that DEP-containing mutants inhibit Wnt-induced, but not Dvl-induced, activation of the transcription factor Lef-1. This inhibitory effect is weakened by a K434M mutation. Nuclear magnetic resonance spectroscopy revealed that the DEP domain of mouse Dvl1 comprises a three-helix bundle, a β -hairpin 'arm' and two short β -strands at the C-terminal region. Lys 434 is located at the tip of the β -hairpin 'arm'. Based on our findings, we conclude that DEP interacts with regulators upstream of Dvl *via* a strong electric dipole on the molecule's surface created by Lys 434, Asp 445 and Asp 448; the electric dipole and the putative membrane binding site are at two different locations.

The Wnt signaling pathway is a key regulatory pathway for cellular development and growth. Wnt signaling has been extensively studied in *Drosophila*, *Caenorhabditis elegans*, *Xenopus*, and mammalian systems^{1–5}. Through genetic studies, a working model of the Wnt signaling pathway has been established. Secreted Wnt proteins bind to Frizzled transmembrane receptors. The receptor–ligand complex activates the Dishevelled (Dsh and Dvl) proteins, which suppress the activity of glycogen synthase kinase-3 β (GSK-3 β). If its activity is not suppressed, GSK-3 β binds to adenomatous polyposis coli (APC) protein and axin and then phosphorylates specific Ser and Thr residues at the N-terminus of β -catenin. In turn, the ubiquitin-proteasome pathway rapidly degrades hyperphosphorylated β -catenin. Wnt activation prevents this process by promoting the cytosolic accumulation and subsequent translocation of β -catenin into the nucleus. Once in the nucleus, β -catenin binds to the transcription factors T-cell factor (Tcf) or lymphoid enhancer factor (Lef) and acts as a transcriptional coactivator⁵. The result is increased expression of Tcf or Lef regulated target genes, such as *Myc*⁶ and the gene encoding cyclin D⁷.

The Dsh protein in *Drosophila* and Dvl in mice play a key role in the transduction of the Wnt signal from the cell surface to the nucleus. Mutations in the Dsh protein cause patterning defects in *Drosophila* similar to those seen with mutated wingless (*wg*) genes⁸. The results of Dsh or Dvl overexpression can also mimic the effects of Wg in cultured cells^{9,10}, in the eye¹¹, and in the heart¹². In addition, recent results have provided exciting insight into the mechanisms by which activated Dsh and Dvl proteins lead to the suppression of GSK-3 β activity^{13–18}.

Dishevelled proteins also participate in other Wnt signaling pathways that do not involve β -catenin or Lef regulation. Dishevelled proteins control the planar cell polarity in *Drosophila* epithelia¹⁹ and cell polarity during gastrulation in zebrafish²⁰ and in *Xenopus*²¹. Although the signaling pathway has not been clearly defined, Dsh- and Dvl-mediated regulation of c-Jun N-terminal kinase (JNK) may regulate tissue polarity²². Indeed, Dishevelled proteins regulate JNK activity in flies and in mammalian cells^{10,22}. Dishevelled proteins may play a crucial role at the nexus of the Wnt and JNK signaling pathways^{21,23}.

Alignment of members of the Dishevelled protein family reveals three well-conserved domains, DIX, PDZ, and DEP. The term ‘DEP’ is derived from the three proteins (Dishevelled, Egl-10, and pleckstrin) in which the DEP domain was first defined (Fig. 1)²⁴. Through its regulator of G-protein signaling (RGS) domain, Egl-10 mediates G protein signaling in the *C. elegans* nervous system. Pleckstrin, which contains a central DEP domain that is flanked by pleckstrin homology domains on both sides²⁵, is an important substrate of protein kinase C in platelets. The pleckstrin homology domains may be involved in translocating proteins to membranes in phosphatidylinositol-dependent pathways^{26,27}. Currently, DEP domains have been identified in more than 50 proteins; they can be found not only in eukaryotic cells but also in bacteria.

The DEP domain of Dishevelled is required for the upregulation of β -catenin activity and the stimulation of Lef-1 mediated transcription in mammalian cells and for the activation of the JNK pathway^{10,22}. To better understand the role of the DEP domain in Wnt- and Dvl-mediated signaling, we have determined the solution structure of the DEP domain of mouse Dvl1 (mDvl1). Based on this structure, we predict that the electrostatic dipole on the protein surface that includes Lys 434, Asp 445, and Asp 448 is crucial for the interaction of DEP with upstream regulators of Dvl, because the K434M mutation inhibits the Wnt-induced effect. This hypothesis is further supported by the finding that D445I and D448I mutations attenuate the ability of the Dvl1 DEP domain to inhibit Wnt induced effects.

DEP domain interacts with upstream regulators of Dvl

We and others have demonstrated that Wnt-1 or Dvl1, when expressed in NIH3T3 cells, can activate the transcription regulator Lef-1 (refs 10,16,28,29). This activation depends on β -catenin and is suppressed by GSK-3 β and axin. To characterize the role of the mDvl1 DEP domain in Wnt signaling, we examined the effect of the Dvl1 N-terminal truncation mutant, DvlC1, on Wnt-induced activation of the transcription factor Lef-1. In Lef dependent reporter gene assays, DvlC1 inhibited Wnt-1 induced Lef-1 activation but not Dvl1 induced Lef-1 activation (Fig. 2). The ability of DvlC1 but not Dvl1 to inhibit Wnt signaling

suggests that DvlC1 inhibits the Wnt signal by interacting with upstream regulators of Dvl. To further characterize the specific sequences that inhibit the Wnt-induced activation, we generated two additional mutants of mDvl1: DvlDEP, which contains only the DEP domain; and DvlC2, which encompasses the sequence downstream of the DEP domain. When expressed in NIH3T3 cells, DvlDEP, like DvlC1, blocked the Wnt-induced, but not the Dvl-induced, activation whereas DvlC2 had no effect on either (Fig. 2). Thus, the DEP domain is instrumental in the inhibition of Wnt-induced activation. These findings, together with other studies^{10,22}, clearly suggest that the DEP domain of Dishevelled proteins is involved in the recognition of upstream molecules in the Wnt signalling pathway and the transduction of Wnt signaling.

Boundaries of the DEP domain of mDvl1

To establish a better understanding of the structure and function of the DEP domain and its role in the Wnt and other signaling pathways, we determined the solution structure of the DEP domain of mDvl1 using NMR. Because the sequence homology among the DEP domains is relatively low and because no structural information about the domains was available, determining its correct boundaries has been difficult, and the results have been inconsistent²⁵. For these reasons, we initially analyzed a relatively large construct of the mDvl1 DEP domain, mDvl1(390–498), to ensure that all of the residues of the domain were present in a single construct. After completing the backbone assignments of this construct, heteronuclear ¹⁵N-¹H nuclear Overhauser effects (NOEs) were measured using standard methods³⁰. The results indicated that residues 402–495 are well folded and that the N-terminal 10 amino acids is unstructured.

To verify this, the original construct was modified by inserting a region that encodes a thrombin cleavage site between residues 399 and 400 in the protein product. The modified protein was treated with thrombin and the ¹⁵N-HQSC spectra of this and the original protein were superimposable; the only difference between the two spectra was the resonances from the N-terminal residues that is present in the large construct. This superimposition demonstrates that the unstructured N-terminal region does not affect the structure of the folded DEP domain. We conclude, therefore, that the DEP domain of mDvl1 consists of residues 402–495 (Fig. 1).

Solution structure of the DEP domain of mDvl1

A family of solution structures, determined from NMR constraints, reveals that the DEP domain consists of a helix bundle with three α -helices (H1–H3), a β -hairpin ‘arm’ composed of two β -strands (B1 and B2) between H1 and H2, and two short β -strands (B3 and B4) in the C-terminal region. Backbone traces of 20 structures were calculated using the program DYANA³¹ and superimposed (Fig. 3a); these 20 structures had the lowest target functions of the 1,000 structures calculated. A ribbon representation of the DEP domain is shown in Fig. 3b. The structure has no distance violations >0.3 Å and no angle violations $>5.5^\circ$.

The central architectural feature of the DEP domain is the core formed by the three α -helices. Highly conserved, hydrophobic amino acids are involved in key interactions that stabilize this core structure (Fig. 4). The first helix, H1 (residues 411–419), contains three

conserved hydrophobic amino acids, Met 411, Ile 414, and Met 418. Ile 414 forms a hydrophobic contact with Leu 450 from the second helix, H2 (residues 442–453), while Met 418 is very close to Met 470, which is in the third helix, H3 (residues 460–473). The side chain of Val 447 in H2 is also in close contact with Ala 467 in H3. The extensive hydrophobic interactions among the helices maintain the tertiary structure of the DEP domain; all three helices are required to form the stable structure because they are all heavily involved in the hydrophobic interaction network. However, only one helix of DEP may be required in intermolecular interactions. A recent study found that the DEP domain of *Xenopus* Dishevelled protein (Xdsh) is required for secondary axis formation. Furthermore, replacing the entire Xdsh DEP domain with the H1 helix did not affect the axis formation³². It is likely, therefore, that the H1 helix provides the key interactions between Xdsh DEP and domains of other proteins involved in *Xenopus* axis induction.

The β -hairpin ‘arm,’ which consists of B1 (residues 429–432) and B2 (residues 435–437), is located between H1 and H2. This arm is relatively isolated from the core, and few NOE crosspeaks between them were observed. Furthermore, although J-coupling experiments indicate that the structure of this region consists of stretches of β -sheets, the typical hydrogen bonds between two antiparallel β -strands were not observed in a proton/deuterium (H/D) exchange experiment. These observations explain why certain residues are highly conserved. Gly 423 and Gly 443 are present in all DEP domains and contribute to the flexible bends in the polypeptide chain at the two ends of the β -hairpin (between H1 and B1 and between B2 and H2). The flexibility conferred by the conserved Gly residues is large enough so that the hydrogen bonds between the two β -strands were not observed in the H/D exchange experiment.

The C-terminal portion of the DEP domain contains several highly hydrophobic residues that form the two antiparallel β -strands B3 (residues 476–477) and B4 (residues 492–493). These two β -strands serve to cap the hydrophobic core; we observed contacts between the core residues Val 415, Ile 442, and Leu 471 and the β -cap residues Leu 476 and Phe 493 (Fig. 4b).

Membrane localization

The DEP domains found in Dishevelled³³ and other proteins such as Egl-10 have a membrane targeting function³⁴. An examination of the surface of the mDvl1 DEP domain revealed a region where several basic residues (Lys 408, Lys 458, Arg 461, Arg 464, Lys 465, Lys 472 and Lys 482) cluster together (Fig. 5a). It has been well documented both experimentally and theoretically that, for membrane bound proteins, electrostatic interaction between a cluster of basic residues on the protein and acidic lipids in the membrane provides the driving force for protein association with membranes^{35,36}. We speculate, therefore, that the surface of the DEP domain shown in Fig. 5a is likely to be responsible for its membrane targeting activity. Further site-directed mutagenesis studies investigating the effects of these basic residues on the membrane targeting function of the DEP domain are currently under way. Studies of the interactions between lipid micelles and the DEP domain are also under way.

Protein recognition and interaction

An examination of the electrostatic potential of the DEP domain surface revealed that a strong electric dipole is formed by Lys 434, Asp 445, and Asp 448 (Fig. 5b). This dipolar nature suggests that the DEP domain surface may be involved in protein–protein interactions^{37,38} because the presence of a significant population of charged and polar residues at protein–protein interfaces has been well documented. The K434M mutation found in the naturally occurring *Drosophila* allele *dsh*¹ causes a well-defined phenotype. To test whether this mutation affects the inhibition of Wnt signaling by the DEP domain, we introduced a K434M substitution into DvlC1. The ability of the mutated DvlC1, designated C1KM, to inhibit Wnt-induced activation of Lef-1 was significantly reduced. Because Lys 434 is located at the tip of the β -turn region of the β -hairpin arm and because the entire β -hairpin arm is missing in Dvl1 of *C. elegans*³⁹, the replacement of Lys with Met is not expected to alter the overall fold of the DEP domain. Instead, the K434M mutation is expected to destroy the electric dipole. Thus, the electric dipole probably mediates molecular recognition and interaction.

If the electric dipole on the surface is indeed involved in molecular recognition and interaction, then mutation of the Asp residues that compose the negative pole of the surface should also produce an effect similar to that produced by the K434M mutation. To test this hypothesis, we generated a second DvlC1 mutant, C1DI, by introducing two amino acid substitutions, D445I and D448I. When C1DI was expressed in NIH3T3 cells, its Wnt-1-induced activation of Lef-1 was attenuated, a finding similar to that for C1KM (Fig. 6). These results suggest that the dipolar surface of the DEP domain plays an important role in molecular recognition and interaction. In addition, Lys 434 is not located within the putative membrane binding site. This finding is consistent with that of a previous study that showed that mutation of it did not affect the membrane targeting function of the DEP domain³³.

Overall fold of the DEP domain of mDvl1

The overall fold of the mDvl1 DEP domain is unique among the proteins in the structural data base. Analysis with the software DALI⁴⁰ showed that only the N-terminal portion of the structure — that is, the helix core and the β -hairpin arm — bears minor similarity to the globular domain of histone H5 (Z-score 5.3)⁴¹. The structure of the globular domain of histone H5 belongs to the superfamily of winged-helix DNA binding proteins. Most of the members of this superfamily are eukaryotic transcription factors⁴². However, because the location of the Dvl proteins is far from the nucleus and because no study so far has indicated that DEP domains bind to DNA, we do not think that the function of the DEP domain is similar to that of the proteins in the winged-helix superfamily. Comparing the β -hairpin arm in our structure with the β -sheet in the globular domain of histone H5 revealed that these structural components are located in very different positions. In our structure, the β -hairpin arm is located between H1 and H2 of the helix core, whereas the β -sheet in the globular domain of histone H5 is the C-terminal wing extension.

The greatest similarity between the two structures lies in the helix cores. The relative orientations of the three helices are similar (Table 1). In our structure, the angle between H1

and H2 is 88.19°, that between H1 and H3 is 99.83°, and that between H2 and H3 is 123.86°. The angles between the helices of the globular domain of histone H5 are 109.94°, 81.42° and 123.05°. In addition, the degrees to which the helices are packed are similar. The distances between the midpoints of the three helices of the DEP domain are 11.3 Å, 10.1 Å and 11.9 Å. In the globular domain of H5, the distances are 13.3 Å, 9.5 Å and 12.5 Å. Unlike members of the winged-helix superfamily, the DEP domain is found in both eukaryotes and bacteria. Therefore, the three-helix core represents a common protein fold that is probably found in other protein motifs as well, and it may be conserved because of the structural stability it provides.

Functional implications of the DEP domain structure

The DEP domain appears to be involved in many of the functions of Dishevelled proteins. First, it is required for activating the Wnt pathway; such activation leads to the stabilization of β -catenin and the activation of Lef-1 (ref. 10). Second, the DEP domain is required for the activation of JNK^{10,22}. Third, the DEP domain may also be responsible for membrane translocation of Dvl in the presence of overexpressed Frizzled^{33,43}. Finally, in this report, our finding that DvlC1 and DvlDEP inhibited Wnt-1 induced, but not Dvl induced, Lef-1 activation shows that the DEP domain may also interact with upstream regulators of Dvl.

Examination of the surface electrostatic potential of the DEP domain revealed that the dipolar surface of the domain consists of Lys 434, Asp 445, and Asp 448. Because charged surfaces often play a role in protein interaction, we predict that these residues interact with Dvl upstream regulators or downstream effectors. Although no specific interaction partners have been identified for the DEP domain of Dvl, the ability of the DEP domain to inhibit Wnt-1 induced, but not Dvl induced, Lef-1 activation suggests that DEP may interact with molecules that function as upstream regulators of Dvl. Substitution of either Lys 434 or the two Asp residues (Asp 445 and Asp 448) in DvlC1 reduced, although it did not completely eliminate, the ability of the protein to inhibit Wnt signaling. These results indicate that these residues may be involved in interaction with upstream regulators of Dvl.

The replacement of Lys with Met (Lys 419 in the *Drosophila* protein Dsh) occurs in the *dsh*¹ allele in *Drosophila*, and the homozygous *dsh*¹ mutant displays a phenotype of strong planar cell polarity (PCP) that is absent in *wg* mutants¹⁹. This Lys residue is highly conserved in all of the DEP domains of Dishevelled, but not in the DEP domains of other proteins (Fig. 1). It was suggested that the Lys to Met mutation renders Dvl unable to activate JNK²². However, evidence showed that the level of JNK activation caused by this mutant Dvl was similar to the level caused by wild type Dvl¹⁰. In light of our new finding that C1KM partially blocks Wnt signaling, we hypothesize that the *dsh*¹ allele is partially permissive to Wg signaling and that the partial permissiveness is sufficient to cause the planar cell polarity phenotypes associated with *dsh*¹, but not enough to cause *wg*-like phenotypes.

Our findings are the first to reveal the structure of the novel DEP domain at the atomic level. These findings not only provide detailed structural information but also shed light on the mechanisms underlying the involvement of Dishevelled in signal recognition in the Wnt

pathway. In light of our structure, the electronic dipolar surface, which consists of Lys 434, Asp 445 and Asp 448, is predicted to be involved in molecular interaction between Dishevelled and other proteins and to play an important role in signal transduction.

Methods

Cell culture, transfection, and luciferase assay

Most experimental methods were similar to those described^{10,16,18}. Briefly, NIH3T3 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. The cells were maintained at 37 °C under 5% CO₂. For transfection, NIH3T3 cells that had been seeded the day before were transfected with 0.5 µg of DNA per well by using LipofectAMINE Plus (Life Technologies, Inc.). After 3 h, the transfection was stopped by switching to normal growth medium. Cell extracts were collected 24 h later for luciferase assays, kinase assays, and western blot analysis. Luciferase assays were performed with the Boehringer Mannheim Constant Light Luciferase Assay Kit (Boehringer Mannheim), according to the manufacturer's instructions. The intensity of the green fluorescence protein (GFP) in cell lysates was first assayed in a Wallac multiscint counter. Luciferase substrate was then added to the cell lysates, and luciferase activities were determined by measuring luminescence intensity with the same counter. Luminescence intensity was normalized against fluorescence intensity. The wild type and deletion mutants of mDvl1 were generated by polymerase chain reaction. The mDvl1 cDNA was used as the template with high fidelity, thermostable *Pfu* DNA polymerase (Stratagene). HA epitope tags were introduced to the C-termini of the full length and mutant Dvl molecules (DvlC1, 380–695; DvlC2, 495–695; DEP, 377–503). The expression of Wnt-1, wild type Dvl, and Dvl1 mutants was driven by a cytomegalovirus promoter.

Expression and purification of the mDvl1 DEP domain

N-terminally His₆-tagged DEP domains were expressed in *E. coli* XL-1 Blue. Transformed cells were grown in Luria broth, and induced with 1 mM isopropyl-1-thio-β-D-galactoside (IPTG) when the OD₆₀₀ was ~0.7; 6 h after induction, the cells were resuspended in lysis buffer, sonicated, and centrifuged. The supernatant was then transferred to a column of Ni-NTA beads, and the column was washed with 50 mM imidazole. The protein was eluted by the addition of 200 mM imidazole in 20 mM phosphate buffer (pH 7.8) and 300 mM NaCl. The DEP domain was cleaved from the His₆-tag with thrombin (250 µg l⁻¹ of original culture). The protein was further purified by using a Superdex 75-pg column (Amersham Pharmacia) and was eluted with 100 mM phosphate buffer (pH 6.8). For radiolabeled samples, MOPS-buffered medium was used with 1 g l⁻¹ ¹⁵NH₄Cl as the source of ¹⁵N and 2.5 g l⁻¹ ¹³C₆-glucose as the source of ¹³C. The NMR samples consisted of 0.8–1.5 mM of the DEP domain in 100 mM phosphate buffer (pH 6.8), 10% (v/v) D₂O, 0.1 mM NaN₃, and 3 mM 1,4-dithiothreitol-d₁₀.

NMR spectroscopy

NMR experiments were performed with a Varian INOVA 600 NMR spectrometer at 27 °C. Both T₂ and diffusion experiments indicated that at such a temperature, the DEP domain is in a monomeric state in solution. For backbone assignment of the ¹H, ¹³C, and ¹⁵N

resonances, three pairs of triple resonance experiments were performed with uniformly ^{13}C , ^{15}N labeled protein: [HNCA, HN(CO)CA], [HNCACB, CBCA(CO)NH], and [HNCO, HN(CA)CO]⁴⁴. NOE distance constraints were obtained from NOE peaks in 2D NOESY, 3D ^{15}N HSQC-NOESY and 3D ^{13}C HSQC-NOESY experiments. Selective ^{15}N labeling of Val was used to confirm the sequential assignment. Side chain proton resonances were assigned with HCCH-TOCSY and HCCH-COSY^{44,45}. The $^3J_{\text{HN-H}\alpha}$ coupling constant was measured with J-HMQC⁴⁶. The exchange between labile amides and D_2O was evaluated by monitoring the ^1H - ^{15}N crosspeak intensities in a series of ^1H - ^{15}N HMQC experiments. Crosspeak intensities were collected at increasing time intervals by using an NMR sample in 100% D_2O . NMR spectra were processed with FELIX (Molecular Simulations, Inc.) and analyzed by XEASY⁴⁷.

Structure calculation

Structure calculations were performed with the software program DYANA³¹. The structure calculation was mainly based on a set of 2,009 NOE distance restraints. When 50 structures were calculated based on the NOE distance constraints, the root mean square (r.m.s.) deviation of the 25 best structures for the backbone atoms of all residues in the protein was 0.81 Å; the success rate, defined by the percentage of acceptable structures among the total calculated structures, was more than 50%. In addition to the NOE constraints, 54 hydrogen bond distance restraints were introduced on the basis of the results of the H/D exchange experiments, and 40 dihedral angle restraints were obtained from J-coupling experiments. In the final structural calculation, 1,000 structures were calculated, and 20 structures with the lowest target functions were selected. The structural statistics are listed in Table 2.

Coordinates

The coordinates of the mDv11 DEP domain have been submitted to the Protein Data Bank (accession code 1FSH).

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Fig. 1.

Structure-based amino acid sequence alignment of selected DEP domains. The alignment was produced by ClustalW and manually modified. The gene identification numbers for these proteins are: 930347 (mDvl1), 2291006 (hDvl1), 2291008 (hDvl2), 2291010 (hDvl3), 1706527 (Dsh), 1706530 (XDsh), 1796621 (Egl-10), 3309252 (RGS6), 3914633 (RGS7), 3746656 (RGS9), 3641493 (RGS11), 4505879 (pleckstrin), and 7522640 (pleckstrin2). The consensus secondary structure of the mDvl-1 DEP domain as determined by MOLMOL⁴⁸ is shown above the sequence.

Fig. 2.

Effect of Dvl C-terminal region on Lef-1 activation. **a**, Schematic representation of mDvl and its mutants. **b**, NIH3T3 cells in 24-well plates were transfected with 0.05 μ g of Lef-1 expression plasmid, 0.1 μ g of Lef-1 luciferase reporter plasmid, 0.15 μ g of GFP expression plasmid and 0.1 μ g of *LacZ*, DvlC1, DvlC2 or DEP in the presence (filled bars) or absence (open bars) of 0.1 μ g of Wnt-1 (left) or Dvl-1 (right). *LacZ* plasmid was added to make the total amount of DNA equal (0.5 μ g per transfection). After one day of growth, cells were lysed, and GFP levels and luciferase activities were determined. The luciferase activities presented are normalized against the levels of GFP expression. Each experiment was carried out in triplicate, and error bars represent the standard deviation. **c**, The expression of DvlC1, DvlC2 and DEP were determined by western blot analysis using an anti-HA antibody that recognizes the HA epitope tags carried by these recombinant proteins.

Fig. 3. Solution structure of the DEP domain of mDvl1. **a**, Stereo view of the peptide backbone (N, Cα, C') of 20 superimposed structures of the DEP domain (residues 402–495). β-strands are yellow, and α-helices are magenta. **b**, Ribbon diagram of the DEP structure with the lowest target function. The images in (a) were generated using MOLMOL⁴⁸, and that in (b) was generated using MOLSCRIPT⁴⁹ and render⁵⁰.

Fig. 4. The hydrophobic core of the DEP domain. *a*, The loop between H1 and B1 has been removed for clarity. Selected distances between carbon atoms are shown. *b*, The interactions between hydrophobic residues in H1, H2, H3, B3 and B4.

Fig. 5. Surface potential of the DEP domain. *a*, A cluster of basic residues that indicates a putative membrane binding site shown. *b*, Front view of the image that has been rotated 90° with respect to (*a*). The circled area shows the electrostatic dipole formed by Lys 434, Asp 445 and Asp 448. The images were produced using the program GRASP⁵¹. In the surface map, red regions represent negative potential, blue positive and white neutral.

Fig. 6.

Mutagenesis study of DvlC1. **a**, NIH3T3 cells in 24-well plates were transfected with 0.05 μ g of Lef-1 expression plasmid, 0.1 μ g of luciferase reporter plasmid that contained Lef-1 binding sites in the promoter, 0.15 μ g of GFP expression plasmid, and varying amounts of *LacZ*, DvlC1, DvlC1KM, or DvlC1DI expression plasmid in the presence or absence of 0.1 μ g of Wnt-1 expression plasmid. *LacZ* plasmid was added to make the total amount of DNA equal in each transfection (0.5 μ g per transfection). One day later, cells were lysed, and GFP levels and luciferase activities were determined as described in Fig. 2. **b**, The expression of DvlC1, DvlC1KM, and DvlC1DI was examined by western blot analysis with an antibody specific to the HA tag of these recombinant proteins.

Table 1

Interhelical angles and distances in the DEP domain and histone H5 (GLH)

	Interhelical angle ¹ (°)	Interhelical distance ² (Å)
DEP		
H1–H2	88.19	11.3
H1–H3	99.83	13.3
H2–H3	123.86	10.1
GLH		
H1–H2	109.94	9.5
H1–H3	81.42	11.9
H2–H3	123.05	12.5

¹ Interhelical angles and distances were calculated using the program interhlx (K. Yap, University of Toronto). Values for GLH are based on Protein Data Bank entry 1HST.

² Interhelical distance is defined as the distance between midpoints of the helices.

Table 2

Structural statistics for the mDvl1 DEP domain

Number of NOE distance restraints		
Intraresidue	1,055	
Interresidue		
Short range	456	
Medium range	230	
Long range	268	
Total	2,009	
Hydrogen bonds	27 × 2	
Number of dihedral angle restraints		
φ	20	
ψ	20	
R.m.s. deviations from the mean ¹	overall structure	secondary structure
Backbone (Å)	0.52	0.32
Heavy atoms (Å)	1.00	0.83
Average r.m.s. deviations from experimental restraints		
Distance restraints (Å)	0.0037	
Dihedral angle restraints (°)	0.22	
Residues in Ramachandran plot		
Most favorable regions (%) ²	54.9	77.8
Additionally allowed regions (%)	32.6	18.0
Generously allowed regions (%)	12.4	2.2
Nonallowed regions (%)	0.1	0.0

¹The average r.m.s. deviation between the 20 structures of the lowest target functions and the mean coordinates of the protein.

²Excluding Gly and Pro.