Axin Contains Three Separable Domains That Confer Intramolecular, Homodimeric, and Heterodimeric Interactions Involved in Distinct Functions*

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Axin is a major scaffold protein, interacting with diverse molecules involved in a number of signaling pathways. Axin can undergo dimerization via its DIX domain. Here we show that whereas deletion of the DIX domain at the C terminus renders Axin incapable of forming dimers, a larger deletion of the C-terminal region restored the ability of Axin to form dimers. Detailed analyses revealed that Axin actually contains two separable domains (D and I) in addition to the DIX domain for homodimerization. The D, I, and DIX domains alone can form homodimers. Interestingly, D and I domains strongly interact with each other, suggesting that Axin can form an intramolecular structure through D and I interaction in the absence of DIX. We also found that DIX-DIX homodimeric interaction is weak but that point mutations in the DIX domain abolished Axin homodimerization. We propose a model to suggest that Axin forms homodimeric interactions through three domains, D, I, and DIX. More importantly, lack of DIX-DIX interaction caused by point mutations in the DIX domain or deletion causes Axin to form an intramolecular loop through the D and I domains, disallowing homodimer formation. Ccd1 interacts with Axin D domain yet fails to interact with AxinDIX, confirming that D is masked after D-I looping. The Axin mutants that are defective in homodimer formation fail to activate JNK but have no effect on β-catenin signaling. Our findings have thus provided a structural basis of conformational changes in Axin, which may underlie the diversity of Axin functions.

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§ The abbreviations used are: Dvl, dishevelled; aa, amino acid(s); MEKK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase; JNK, c-Jun N-terminal kinase; GFP, green fluorescent protein; HA, hemagglutinin; HEK, human embryonic kidney.
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information is lacking and even controversial (6, 12, 17, 22, 23). As mentioned previously, Axin formed homodimer via its DIX domain (23). We found recently that Axin actually did not utilize its DIX domain to interact with Cdc1; rather, a region N-terminal to the DIX domain is critical for Axin-Cdc1 interaction (15). Whereas it was shown that the DIX domain of Axin is sufficient for complex formation with Dvl1, the region of amino acid (aa) residues 530–712 of Axin, N-terminal to the DIX domain, was also important for its direct interaction with Dvl1 (6). Advance in understanding the structure and function of Axin has been hindered by lack of the crystal structure of the entire Axin protein, although the domains for interacting with APC and glycogen synthase kinase-3β have been resolved (24, 25).

In the course of characterization of the Axin DIX domain mutants identified from cancer tissues (26) and during domain mapping for HIPK2 binding that lies in region N-terminally adjacent to the DIX domain (27), we found that single mutations in the DIX domain could abolish dimer formation and observed perplexing or even contradictory patterns of dimerization among different C-terminal deletion mutants. As a result, we carried out thorough studies to identify the determinants involved in Axin dimer formation. In the present study, we unexpectedly found that Axin contains at least three separable domains for homodimer formation that are termed the D, I, and DIX domains, in order from N-proximal to C-proximal. The Axin DIX-DIX interaction is weak; nevertheless, it seems to augment or prime a conformation of D and I domains to form intermolecular homodimer. In the absence of the DIX domain, the I domain loops back to interact with D domain, explaining why AxinΔDIX cannot form homodimer. Furthermore, these three domains in fact also participate in interaction with the other two DIX domain proteins, Dvl1 and Cdc1. We have thus provided the structural basis that endows Axin with the ability to form diverse complexes in mediating multiple biological functions.

EXPERIMENTAL PROCEDURES

Cell Culture and Transient Transfection—Human embryonic kidney (HEK) 293T cells were maintained in Dulbecco’s modified Eagle’s medium as described previously (18). Transfection into 293T cells was carried out by the calcium method as described previously. The total amount of DNA was adjusted to 4 µg per 60-mm dish using empty vector when necessary.

Plasmid Construction—Plasmids pCMV5-HA-Axin, pCMV5-Myc-Axin, pCMV5-HA/Myc-Axin, and pcDNA3-Myc-Axin were constructed as described elsewhere (15, 18, 26). pCMV5-HA/Myc-AxinF759R, pCMV5-HA-Axin regions of aa 752-CT, were cloned into CMV5-HA-Axin digested with XbaI and BamHI to create pCMV5-HA-Axin MD1 and MD2, respectively. The oligonucleotides for PCR were 5’-ctattcaccgtctagaggctgctc-3’ and 5’-cctaggccctgagctctgctgctc-3’ for aa 600–667 and 5’-ctattcaccgtctagaggctgctc-3’ and 5’-cctaggccctgagctctgctgctc-3’ for aa 600–700. These fragments, together with the PCR-generated Axin region of aa 752-CT, were cloned into CMV5-HA-Axin digested with XbaI and BamHI to create pCMV5-HA-Axin MD1 and MD2, respectively. The oligonucleotides for aa 752-CT (C-terminal) were 5’-agcttggcttggtctacctt-3’ and 5’-agggcttcagggctgacctc-3’.

Point Mutagenesis—To construct full-length Axin constructs containing point mutations in the DIX domain (pCMV5-HA/Myc-AxinF759R, AxinF780R, AxinF796A, AxinF807R, and AxinLA177E), PCR-based site-directed mutagenesis was performed to generate C-terminal fragments, which were then fused to the wild-type 5’-flanking sequence.

Immunoprecipitation and Western Blotting—Cells were harvested at 40 h after transfection and lysed in lysis buffer (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM sodium orthovanadate, 1 µM leupeptin, and 1 mM phenylmethylsulfonyl fluoride), sonicated 10 times for 1 s each, and centrifuged at 13,000 rpm for 30 min at 4 °C. HA- or Myc-tagged proteins were immunoprecipitated from the cell lysate with anti-HA, anti-Myc (9E10), antibody-linked protein A/G Plus-agarose beads (Santa Cruz Biotechnology, Inc.). After separation on 10% SDS-polyacrylamide gels and electrotransfer onto polyvinylidene difluoride membrane (Roche Diagnostics), the proteins in immunoprecipitates or total cell lysates were analyzed by Western blotting using the respective antibodies as described previously.

Immunokinase Assays—Approximately 24 h after transfection, 293T cells were lysed, and assays for the kinase activity of immunoprecipitated FLAG-JNK1 protein were performed in a kinase buffer (25 mM Tris, pH 7.5, 5 mM β-glycerophosphate, 2 mM dithiothreitol, 0.1 mM Na3VO4, and 10 mM MgCl2) using 0.5 µg of glutathione S-transferase-c-Jun (–79) as substrate, as described previously (18). Phospho-c-Jun antibody was used in subsequent Western blotting to examine the phosphorylation of c-Jun.

TOPFLASH Luciferase Reporter Gene Assay—HEK 293T cells were transfected in 6-well dishes at 90% confluence with 0.5 µg of TOPFLASH luciferase reporter (Upstate Biotechnology) and 0.2 µg of LEF-1 expression plasmid (10 µg/ml of DNA). The cells were treated with Wnt3a-conditioned medium. At 12 h after the additional incubation, cells were washed with phosphate-buffered saline and lysed. Lysates were then collected and assayed for luciferase activity by using the dual luciferase reporter assay system (Promega) according to the manufacturer’s instructions. All transfections were carried out in triplicate at least five independent experiments, and error bars represent S.E.

RESULTS

DIX Domain Is Not Sufficient for Maximal Axin Homodimerization—Axin is a polypeptide with 832 amino acid residues for the short and most predominant form, with a DIX domain located at the extreme C terminus. It was found previously that Axin lacking the DIX domain failed to form homodimer/oligomer (17, 23, 28), suggesting that the DIX domain is critical for homodimerization. In order to clarify the importance of the DIX domain in homodimerization, we first tested whether DIX domain alone was sufficient for Axin dimer formation. Axin DIX domain was fused to HA tag and Myc tag generating HA-AxinDIX and Myc-AxinDIX, respectively. We transiently cotransfected both the HA-tagged and Myc-tagged AxinDIX into 293T cells and carried out immunoprecipitation. For comparison, we also cotransfected HA-tagged and Myc-tagged full-length Axin, or we cotransfected the full-length Axin with the DIX domain alone. Myc-tagged full-length Axin (Myc-Axin) was effectively communoprecipitated with HA-tagged full-length Axin (HA-Axin), whereas Myc-AxinDIX was poorly communoprecipitated with either HA-Axin or HA-AxinDIX (Fig. 1A). These results indicate that the DIX domain alone is a weak homodimerization domain.

Identification of Two Novel Domains for Axin Homodimerization—which Can Also Form Intramolecular Loop and Prevent AxinDIX from Forming Homodimer—We then created fine deletion mutants, as depicted on the top of Fig. 1B, and tested whether Axin contains other sequences that are
important for its homodimer formation. As expected, AxinC2 did not form homodimer. Removal of aa 701–832 made Axin regain its ability to form dimer; gradual deletions from the C-terminal (AxinCD3 to AxinCD1) increased homodimerization further until deletions reached amino acid residue 600 (AxinC1), at which point dimerization ability was lost. These results

FIG. 1. The DIX domain is not sufficient for maximal Axin homodimerization. A, Axin DIX domain alone confers weak Axin homodimerization. HA-Axin, Myc-Axin, HA-AxinDIX, and Myc-AxinDIX (2 μg of each) were cotransfected into HEK 293T cells in different combinations. Cells were lysed 40 h after transfection. Cell lysates were immunoprecipitated with anti-HA for 3 h. Proteins in immunoprecipitates and total cell lysates were separated using 4–10% SDS-polyacrylamide gels and electrotransferred to polyvinylidene difluoride membrane. After blocking for 1 h, the membrane was incubated with anti-HA for HA-tagged proteins or anti-Myc for Myc-tagged proteins. B, deletions with DIX and upstream DIX domains removed retained their ability to homodimerize. Top panel, the schematic representation indicates Axin mutants with different deletions used in transient transfection of 293T cells for Axin homodimerization. Cells were cotransfected with 2 μg of HA-tagged Axin deletions and 2 μg of corresponding Myc-tagged Axin deletions. Bottom panel, after transfection, cell lysates were immunoprecipitated with anti-Myc, followed by immunoblotting for HA-tagged proteins using anti-HA. C, homodimerization is enhanced with regard to Axin deletion mutants with the D domain removed. Cells were cotransfected with 2 μg each of HA-tagged Axin deletion mutants and 2 μg of Myc-Axin. Immunoprecipitation was carried out with anti-Myc.

FIG. 2. Axin contains two novel homodimerization domains that interact with each other. A, the regions of aa 601–633 (D domain) and aa 667–751 (I domain) are sufficient for mutual interaction and self-binding. HA- or Myc-tagged Axin aa 601–633 (AxinD) or aa 667–751 (AxinI) fragment, respectively, was fused with GFP by PCR. These GFP-fused Axin fragments were tested for their homodimerization with wild-type Axin as control. Cells were cotransfected with 2 μg of HA-tagged Axin together with 2 μg of corresponding Myc-tagged Axin deletions. B, deletions with DIX and upstream DIX domains removed retained their ability to homodimerize. Top panel, the schematic representation indicates Axin mutants with different deletions used in transient transfection of 293T cells for Axin homodimerization. Cells were cotransfected with 2 μg of HA-tagged Axin deletions and 2 μg of corresponding Myc-tagged Axin deletions. Bottom panel, after transfection, cell lysates were immunoprecipitated with anti-Myc, followed by immunoblotting for HA-tagged proteins using anti-HA. C, homodimerization is enhanced with regard to Axin deletion mutants with the I domain removed. Cells were cotransfected with 2 μg each of HA-tagged Axin deletion mutants and 2 μg of Myc-Axin. Immunoprecipitation was carried out with anti-Myc.
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FIG. 3. Axin DIX domain point mutations abolished Axin homodimer formation. A, DIX domain point mutations AxinF780R, AxinF807R, and AxinL817E lost their ability to form homodimer. HA- or Myc-tagged DIX domain point mutations were generated by site-directed mutagenesis using HA-Axin or Myc-Axin as template. Their abilities to form homodimer were tested by separately cotransfecting 2 μg of each of the HA- and Myc-tagged mutations into 293T cells. Cells lysates were immunoprecipitated with anti-Myc, immunoprecipitates and cell lysates were analyzed with anti-HA for HA-tagged Axin and anti-Myc for Myc-tagged Axin. B, AxinF780R, AxinF807R, and AxinL817E could not interact with GFP-AxinD fragment. Cells were cotransfected with 2 μg of each of HA-tagged DIX domain point mutations together with 2 μg of Myc-tagged GFP-AxinD. Cell lysates were subjected to immunoprecipitation with anti-Myc for Myc-tagged GFP-AxinD fragment, followed by immunoblotting analysis using anti-HA and anti-Myc. C, AxinDIX-(F780R), AxinDIX-(F807R), and AxinDIX-(L817E) could not form homodimer. Constructs encoding Axin DIX domain fragments harboring the above-mentioned amino acid alterations were generated by site-directed mutagenesis using HA- or Myc-AxinDIX as template. Cells were separately transfected with 2 μg of each of the HA- and Myc-tagged AxinDIX mutations; cell lysates were immunoprecipitated with anti-HA. After immunoprecipitation, immunoprecipitates and total cell lysates were subjected to immunoblotting with anti-HA and anti-Myc.

Axin and Ccd1 present an interaction pattern similar to that of Axin homodimerization. A, Axin utilizes D domain to interact with Ccd1. Cells were transiently transfected with 2 μg of HA-GFP-AxinD, HA-GFP-AxinI, or HA-AxinDIX together with 2 μg of Myc-Ccd1. Cell lysates were immunoprecipitated with anti-Myc for Ccd1 protein. Immunoblotting with anti-HA and anti-Myc was carried out as described previously. B, AxinC1 and C2 did not form complex with Ccd1. Cells were cotransfected with 2 μg of each of the HA-tagged Axin deletion mutants as indicated and 2 μg of Myc-Ccd1. At 36 h after transfection, cell lysates were subjected to immunoprecipitation with anti-Myc for Ccd1. After immunoprecipitation, immunoblotting with anti-HA and anti-Myc was carried out as described previously. C, AxinF780R, AxinF807R, and AxinL817E lost their ability to interact with Ccd1. Cells were cotransfected with 2 μg of each of the HA-tagged Axin DIX point mutations as indicated and 2 μg of Myc-Ccd1. Immunoprecipitation and immunoblotting were performed as described above.

indicate that the area of aa 601–633 contains the sequence for dimerization (defined as the D domain) and that a sequence in the region of aa 634–751 contains an inhibitory domain for homodimerization (hence the I domain), at least in the context of AxinC2. Consistent with a possible inhibitory role of the I domain, AxinMD1 with the I domain deleted increased homodimer formation (Fig. 1C). However, when both the D and DIX domains were deleted (AxinCD4), the mutant Axin could, albeit with slightly weaker affinity, readily form dimer, indicating that the I domain in fact is also a dimerization domain (Fig. 1B).

To further verify that the two newly identified areas are bona fide dimerization domains for Axin, we generated constructs that contain isolated D domain (GFP-Axin601–633 and GFP-AxinD) and I domain (GFP-Axin667–751 and GFP-AxinI), respectively. As shown in Fig. 2A, HA-GFP-AxinD was coprecipitated with Myc-GFP-AxinD, and the I domain was also capable of forming homodimer. Next, we asked whether the I domain exerted its inhibitory function by forming an intramolecular loop with the D domain. We cotransfected HA- and Myc-tagged GFP-AxinD, HA- and Myc-tagged GFP-AxinI, and HA-tagged DIX (HA-AxinDIX) in different combinations, as indicated in Fig. 2B. Results showed that the I domain (aa 667–751) could indeed interact with the D domain; however, neither D domain nor I domain could interact with the DIX domain. Because the D and I domains form dimer, we next tested whether the isolated D and I domains could mutually compete for homodimer formation. Whereas an increasing amount of D domain could not significantly compete against I-I interaction seems to have a higher affinity than the D-D interaction.

Point Mutations in the Axin DIX Domain Abolished Axin Homodimerization—We previously identified several point mutations that occurred to the DIX domain of the Axin1 gene from human cancer tissues (26). Axin mutants harboring those mutations or with other alterations in the conserved residues were generated and tested for their ability to form homodimer, among other assays. Among the point mutations tested, F759R formed homodimer as effectively as wild-type Axin, and K796A...
exhibited diminished ability to form homodimer (Fig. 3A). In contrast, F780R, F807R, and L817E virtually lost their ability to form homodimer (Fig. 3A). Loss of the ability to form homodimer in the three mutants with a single amino acid alteration in the DIX domain is reminiscent of the situation seen with AxinC2. In other words, these three point mutations have each further drastically weakened the ability of the respective DIX domain to form homodimer. Further inference is that these...
mutant DIX domains could not form homodimer because they instead allow the I domain to loop back to form an intramolecular D-I interaction. In support of these notions is the observation that the isolated DIX domains of these mutants (F780R, F807R, and L817E) alone failed to interact with the D domain alone (Fig. 3B) or form DIX-DIX homodimeric interaction (Fig. 3C).

The Axin D Domain Interacts with Ccd1—We showed previously that Axin does not utilize its DIX domain for heterodimeric interaction with Ccd1; rather, a region N-terminal to the DIX domain is critical for Axin-Ccd1 interaction. The Ccd1-interacting domain appeared to coincide with the D domain. We verified this by testing whether the D domain alone could interact with Ccd1. Indeed, the Axin D domain, but not the I or DIX domain, interacted strongly with Ccd1 (Fig. 4A). Next, we determined how Axin deletion constructs behave in the interaction with Ccd1. As shown in Fig. 4B, Ccd1 communoprecipitated well with full-length Axin but failed to interact with AxinC1 or AxinC2, displaying an interaction pattern that mirrored that of homodimeric interaction of these mutants exactly (compared with the data shown in Fig. 1). These results suggested that the Ccd1-interacting site in AxinC2 also might be masked by the I domain, as seen with AxinC2 homodimer formation, and that AxinC1 does not contain the Ccd1-interacting site.

We further utilized Axin point mutations in the DIX domain to test the intramolecular masking hypothesis in the Axin-Ccd1 interaction. The Axin point mutants that failed to form homodimer were also unable to interact with Ccd1 (Fig. 4C). Axin D, I, and DIX Domains Interact Separately with Distinct Domains of Dvl—Because some previous studies have indicated that domains other than the DIX domain of Axin may contribute to interaction with Dvl (12, 22), we also carried out interaction assays of the isolated Axin D and I domains with Dvl. Indeed, GFP-AxinD and GFP-AxinI each communoprecipitated with wild-type Dvl (Fig. 5A). Similarly to the Axin-DIX interaction with Axin, Axin-DIX also weakly interacts with Dvl, again indicating that the DIX domain in fact plays a minor role in protein interaction. In parallel, the three isolated Axin domains were tested for their ability to interact with different Dvl deletion mutants, as indicated in Fig. 5B. Axin-DIX communoprecipitated with wild-type Dvl, DvlPDZ, and DvlDEP, but not with DvlDIX, in line with previous studies showing that AxinDIX interacts with DvlDIX (Fig. 5B). Whereas the I domain of Axin seems to interact with a region not included in DIX, PDZ, or DEP, Axin D domain did not form complex with DvlDEP. Next, we assayed whether Axin constructs with point mutations in their DIX domain interact with Dvl. In drastic contrast to their interaction with Ccd1, all the Axin mutants effectively interacted with Dvl (Fig. 5C).

JNK Activation Requires Homodimerized Axin—Because homodimerization is required for Axin to activate JNK based on the observation that AxinC2 failed to activate JNK, we tested the effect of point mutations in the DIX domain of Axin, which could not form dimer, on JNK activation (Fig. 6A). Results showed that F780R, F807R, and L817E that fail to form homodimer completely lost their ability to activate JNK, displaying a remarkable positive correlation between the ability to form homodimer (Fig. 3A) and the ability to activate JNK (Fig. 6A). In contrast, these mutants had little effect, if any, on their ability to down-regulate β-catenin (Fig. 6B), in agreement with their intact ability to form heterodimer with Dvl. Finally, to determine whether an intact C-terminal region is required for JNK activation, we assayed for JNK activation by Axin1Δ and ΔD and found that they activated JNK as effectively as wild-type Axin (Fig. 6C), showing for the first time that amino acid sequences inside the C-terminal region can be dispensable for JNK activation.

**DISCUSSION**

Axin is a master scaffolding protein, regulating multiple signaling pathways, including the Wnt, JNK, tumor growth factor β, and p53 signaling pathways (reviewed in Refs. 4, 5, and 27). A critical question is how Axin forms distinct complexes with such a diverse array of proteins. In Wnt signaling, one possible mechanism to stabilize β-catenin is through recruitment of the Dvl-Axin complex by activated Frizzled-low density lipoprotein receptor-related protein receptor complex to the plasma membrane, where Axin is degraded (29). The rela-
cation of Axin to the plasma membrane depends on Dvl, emphasizing the importance of their mutual interaction (21). It has been shown that Dvl requires its DIX domain to inhibit Axin-promoted β-catenin degradation (6). We have also shown that Axin is in a homodimeric configuration for activation of JNK because AxinC2 that cannot form dimer fails to activate JNK. However, little information by systematic analysis of how DIX domain proteins form complex is available. In particular, our previous finding that Cdc1 does not interact with the DIX domain of Axin raises concerns that distinct modes of protein-protein interactions may exist among the DIX domain proteins. Here we show that Axin at its C-terminal region actually possesses three separable domains for homodimeric interaction, which are the D, I, and DIX domains, respectively. In fact, the DIX domain of Axin can only form weak homodimer, in agreement with the apparent lack of stable structure in solution structure of the Dvl DIX domain (30). The D and I domains can form D-D and I-I interactions, with the latter having higher affinity than the former based on a competition assay. Moreover, the D and I domains can form D-I intramolecular interaction, although the DIX domain cannot interact with D or I domain.

How do our current findings reconcile with the previous observation that deletion of the DIX domain alone renders Axin monomeric? The answer lies in the observation that the D and I domains can interact with each other. It is proposed here that in intact Axin, the DIX-DIX domain interaction serves to weaken the intramolecular interaction between the D and I domains, allowing for D-D and I-I homodimeric interactions (Fig. 7). In other words, when the DIX domain is absent, the D and I domains have a greater propensity to form an intramolecular loop. When I and DIX are deleted, Axin can resort to its ability to form dimer via the D domain alone. In support of this notion are the following two additional observations. First, D-I interaction is in fact stronger than D-D interaction because D fails to compete against D-I formation. Second, AxinC2 fails to interact with Cdc1, despite the fact that the actual interaction domain of Axin for Cdc1 is mapped to the D domain, confirming that the D domain in AxinC2 is masked by the I domain. In sum, to form an Axin homodimer, DIX interacts with DIX and generates a conformation that favors I to interact with I and D to interact with D, resulting in intramolecular Axin-Axin interaction (Fig. 7).

Another intriguing aspect of our current finding is that the D and I domains of Axin can also interact with Dvl, although Axin and Dvl do not have any apparent sequence similarity besides their DIX domains. This raises a provocative possibility that Axin may form many more as yet unidentified protein interactions. In fact, MEKK4 binds to the I domain, although the exact interaction interface has yet to be revealed by structural analysis. Moreover, our current finding that Axin possesses self-dimerization domains other than the DIX domain is consistent with the dominant negative phenotypes such as kinky tails seen in mature heterozygous Fused mice (31). The Fused mutation results from an insertion of intracisternal A particle retrotransposon into intron 6 of the Axin gene, which yields an aberrant protein product that lacks the DIX domain but not the D and I domain.

Our results also reinforce our previous notion that Axin requires homodimerization for JNK activation (17). Axin with point mutations in the DIX domain (F750R, F807R, and L817E) that cannot form homodimer fail to activate JNK, whereas Axin3D and AxinΔI can effectively activate JNK. The common denominator among these constructs is that those that can form homodimer can activate JNK. In the case of DvlΔDEP inhibition of Axin-mediated JNK activation (17), it is now understood that DvlΔDEP heterodimerizes with the I and DIX domains of Axin and effectively prevents Axin from forming homodimer. Moreover, DvlΔDEP does not compete for MEKK binding to inhibit Axin-mediated JNK activation, but it requires its DIX domain to form heterodimer with Axin (17). All lines of evidence point to the same scenario: Axin has to form a homodimer for JNK activation. Interestingly, the Axin point mutants that drastically affect DIX-DIX interaction and JNK activation had virtually no effect on down-regulation of β-catenin, indicating that Axin homodimeric formation might be not needed for β-catenin down-regulation. Questions still remain as to how Wnt regulators such as glycosyn synthase kinase-3β and casein kinase I, which bind to sites distinct from that for MEKK1, can prevent MEKK1 binding to Axin (20, 32).

In summary, we have identified two additional domains for protein interactions that confer upon Axin the ability to form intramolecular, homodimeric, and heterodimeric interactions. Given the fact that Axin can exert diverse functions through different signaling cascades, our data have provided novel structural insights for the ultimate understanding of how Axin can adopt versatile conformations for different functions, which inevitably involve distinct complex formations with diverse proteins.

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