be at least partly responsible for the transformation of skeletal morphology observed. Furthermore, the transformation of muscle and distal tendon attachments could cause the apparent footlike flexure we describe above (Fig. 2). As with the skeletal transformations, the effect of *Pitx1* on muscle pattern is specific to the hindlimb gene *Pitx1* because parallel misexpression of a highly related control gene, *Pitx2*, did not have an effect on soft tissue morphology, in spite of the fact that this gene had a high biological activity in other contexts (*17*). Because comparatively little is known about the molecular basis for muscle patterning, the ability of *Pitx1* to respecify muscle identity will provide a useful tool in this regard, in addition to being evidence for a role of *Pitx1* in the specification of hindlimb patterning.

In summary, we found that *Pitx1* acts upstream of *Tbx4* and regionally expressed *Hox* genes in a pathway that regulates limb-type identity. The correct induction of the forelimb- or hindlimb-specific genes in the respective limb fields must depend on upstream genes that regionalize the rostral-caudal body axis, which is likely ultimately dependent on axial expression of *Hox* genes. Further gain- and loss-of-expression studies with other genes in this pathway will provide additional insight into the mechanism by which the common aspects of limb patterning are modified to produce limb-type–specific morphologies.

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Negative Regulation of Wingless Signaling by D-Axin, a *Drosophila* Homolog of Axin

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Wnt/Wingless directs many cell fates during development. Wnt/Wingless signaling increases the amount of β-catenin/Armadillo, which in turn activates gene transcription. Here the *Drosophila* protein D-Axin was shown to interact with Armadillo and D-APC. Mutation of *d-axin* resulted in the accumulation of cytoplasmic Armadillo and one of the Wingless target gene products, Distalless. Ectopic expression of *d-axin* inhibited Wingless signaling. Hence, D-Axin negatively regulates Wingless signaling by down-regulating the level of Armadillo. These results establish the importance of the Axin family of proteins in Wnt/Wingless signaling in *Drosophila*.

The Wnt/Wingless (Wg) signal-transduction pathway is involved in cell-cell signaling in many developmental processes (*1*). Wnt/Wg signaling promotes the stabilization of β-catenin/Armadillo (Arm, a *Drosophila* homolog of β-catenin) by negatively regulating the activity of glycogen synthase kinase–3β (GSK-3β). β-Catenin/Arm binds to transcription factors of the LEF/TCF family and thereby modulates expression of Wnt/Wg-responsive genes (*2*). The colorectal tumor-suppressor gene product APC induces down-regulation of β-catenin, and mutation of APC results in the accumulation of the latter (*3*). Mutations that activate β-catenin have also been detected in some tumors with intact APC (*4*). Therefore, regulation of the level of β-catenin is critical for Wnt/Wg signaling during development and tumorigenesis. Here, we show that the *Drosophila* protein D-Axin interacts with Arm and D-APC and is a negative regulator of Wg signaling.

To identify Arm-interacting proteins, we performed a yeast two-hybrid screen of a *Drosophila* embryo cDNA library using the Armadillo repeat domain of Arm as target and identified a protein that we designate D-Axin (*5*). Sequence analysis of the *d-axin* cDNA showed that it encodes a protein of 743 amino acids. A region near its NH_2-

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terminus (amino acids 51 to 171) shows similarity to the regulator of G protein signaling (RGS) domain, whereas its COOH-terminus contains a region (amino acids 687 to 734) homologous to a conserved sequence near the NH_2-terminus of Dishevelled (Dsh) (Fig. 1B). Thus, D-Axin has a domain structure very similar to that of proteins of the mammalian Axin family, Axin and conductin/Axil (*6–9*), suggesting that D-Axin is a *Drosophila* homolog of the Axin family of proteins.

We examined whether D-Axin produced by in vitro translation could interact with fragments of Arm fused to glutathione S-transferase (GST) (*10*). D-Axin specifically interacted with the Armadillo repeat domain of Arm (amino acids 140 to 713), but not with its NH_2-terminal (amino acids 1 to 139) or its COOH-terminal (amino acids 714 to 843) domain (Fig. 1C). Pull-down assays with a series of deletion fragments of D-Axin showed that a fragment of D-Axin containing amino acids 459 to 538 bound to Arm (Fig. 1D). This region corresponds to the β-catenin–binding domain of Axin and conductin/Axil and contains a small segment (amino acids 494 to 525) that is highly conserved, suggesting that it may function in binding to Arm.

Recently, we and others have shown that proteins of the mammalian Axin family interact not only with β-catenin but also with GSK-3β and APC (*6–9*). In line with these findings, we found that the RGS domain of D-Axin interacts with a fragment of D-APC (amino acids 757 to 1270) (Fig. 1E). This region of D-APC corresponds to the region of APC that interacts with β-catenin, conductin, and Axin (*7*, *11*, *12*). However, D-Axin did not bind to Zeste white-3/Shaggy (ZW3/Sgg, *Drosophila* GSK-3β) (Fig. 1E).

The *d-axin* gene was mapped to the 99D1-7 region of the third chromosome, and its genomic organization was determined (Fig. 1A) (*13*). Whole-mount in situ hybridization to embryos and imaginal discs and Northern (RNA) blotting with the coding region of the *d-axin* cDNA as a probe revealed that *d-axin* transcripts are provided by maternal contribution and expressed ubiquitously throughout all developmental stages (*11*). To analyze the biological function of D-Axin in vivo, we examined a series of lethal lines that have mutations in the 99D1-7 region and found one line, *l(3)S044230*, that contains a P-element inserted into the 5′-untranslated region of the first exon of *d-axin* (Fig. 1A) (*14*). Although *l(3)S044230* homozygous animals died during the first larval instar stage, normal adults were produced upon precise excision of the P-element. In addition, ubiquitous expression of the *d-axin* cDNA with an inducible heat shock GAL4 system rescued 5 to 10% of *l(3)S044230* homozygous animals, allowing them to survive until the late third larval instar or pupal stage (*15*). Furthermore, no signal was observed when *d-axin* cDNA probe was hybridized in situ to *l(3)S044230* homozygous embryos (*11*). Thus, *l(3)S044230* is considered to be a loss-of-function allele of the *d-axin* locus.

Wg is critical for patterning and cell fate determination in embryonic segmentation (*1*). Although embryos that are zygotically mutant for *d-axin* appeared to have almost normal segment patterning (Fig. 2B), embryos devoid of both maternal and zygotic *d-axin* gene products were completely naked, lacking all denticles on the ventral cuticle (Fig. 2C) (*16*). Embryos that lack the maternal *d-axin* product but have received one paternal wild-type copy of the gene had some denticles on the ventral cuticle, suggesting that the zygotic *d-axin* product can partially rescue the *d-axin* maternal deficiency (Fig. 2D). These phenotypes are similar to those of embryos derived from homozygous *zw3/sgg* female germ lines and to those of embryos ubiquitously expressing the wild-type Wg or constitutively active Arm (*17*). Thus, Wg signaling is constitutively activated in embryos lacking maternal *d-axin*.

Wg is required for the organization of wing blade development, especially for specification of the wing margin structure (*18*). We found that clones of *d-axin* mutant cells

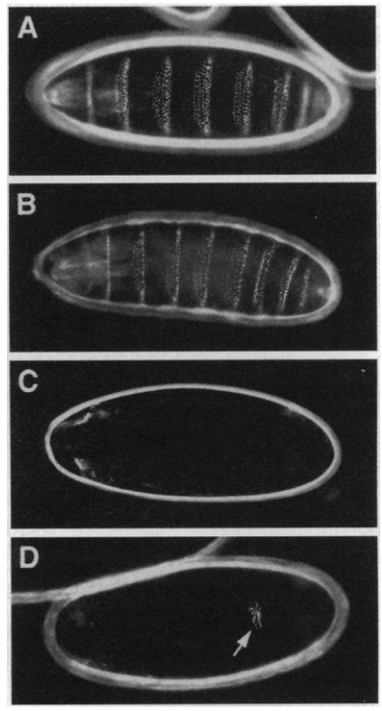

Fig. 2. Ventral cuticle phenotype of *d-axin* embryos. (**A**) Cuticle of a wild-type embryo. Each segment produces denticles in the anterior region and naked cuticle in the posterior region. (**B**) Cuticle of an embryo zygotically mutant for *d-axin* does not exhibit any major defects. (**C**) Cuticle of an embryo lacking both maternal and zygotic *d-axin* is entirely naked. Filzkorper is present. (**D**) Cuticle of an embryo that lacks maternal *d-axin* but has a wild-type paternal copy of the gene has occasional patches of denticles (indicated by the arrow). All embryos are shown with anterior oriented to the left.

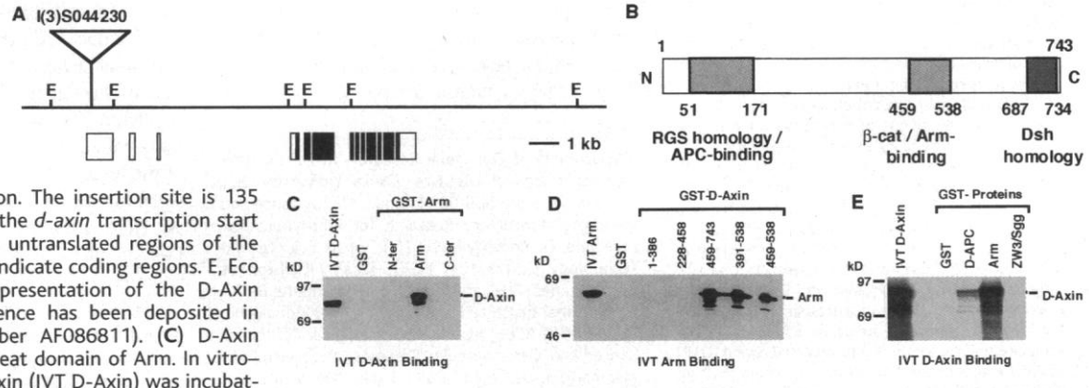

Fig. 1. Structures of the *d-axin* gene and its protein product and physical interaction of D-Axin with Arm and D-APC. (**A**) Genomic structure of the *d-axin* locus. The triangle indicates the location of the *l(3)S044230* P insertion. The insertion site is 135 base pairs downstream of the *d-axin* transcription start site. White boxes indicate untranslated regions of the *d-axin* cDNA. Black boxes indicate coding regions. E, Eco RI site. (**B**) Schematic representation of the D-Axin protein. The *d-axin* sequence has been deposited in GenBank (accession number AF086811). (**C**) D-Axin binds to the Armadillo repeat domain of Arm. In vitro–translated ^{35}S-labeled D-Axin (IVT D-Axin) was incubated with purified GST or GST-Arm fusion proteins (N-ter, amino acids 1 to 139; Arm, the Armadillo repeat domain, amino acids 140 to 713; C-ter, amino acids 714 to 843) immobilized to glutathione-Sepharose. The bound proteins were analyzed by SDS-PAGE and autoradiography. (**D**) Arm binds to amino acids 459 to 538 of D-Axin. The in vitro–translated ^{35}S-labeled Armadillo repeat domain of Arm (amino acids 140 to 713) was incubated with GST– or GST–D-Axin–Sepharose. (**E**) D-Axin interacts with D-APC, but not with ZW3/Sgg. In vitro–translated ^{35}S-labeled D-Axin was incubated with GST-, GST–D-APC (amino acids 757 to 1270)–, GST-Arm (the Armadillo repeat domain)–, or GST-ZW3/Sgg (full-length)–Sepharose.

marked with a *yellow* mutation produced ectopic marginal bristles cell autonomously (Fig. 3, A and B) (*19*). Wg also plays an essential role in organizing leg structures; ectopic activation of Wg signaling induces supernumerary outgrowth on the dorsal side of normal legs (*18*, *20*). The *d-axin* clone also produced a supernumerary leg from the dorsal side of the normal leg (Fig. 3C). Furthermore, Wg signaling is required for the formation of sternites in the ventral side of the adult abdomen, and its ectopic activation results in the appearance of ectopic sternite structures (*21*). The same phenotype was observed in an abdomen containing *d-axin* clones (Fig. 3D). During wing disc development, Wg signaling is induced along the dorsoventral compartment boundary in the wing imaginal disc. Arm accumulates in the cytoplasm, associates with its partner, Pangolin/DTcf, and activates expression of target genes such as *Distal-less* (*Dll*) (*1*, *2*, *22*). In these *d-axin* clones, the levels of Arm were markedly enhanced in a strictly cell-autonomous manner (Fig. 3, F and G) (*19*, *23*). In addition, Arm was localized predominantly in the cytoplasm and nuclei in the *d-axin* mutant clones, in contrast to the membrane localization observed in wild-type cells (Fig. 3G). The levels of *Dll* expression were also elevated in the *d-axin* clones in a cell-autonomous manner (Fig. 3H). These results suggest that D-Axin negatively regulates Wg signaling by down-regulating intracellular levels of Arm and that this regulatory mechanism is essential for Wg signaling.

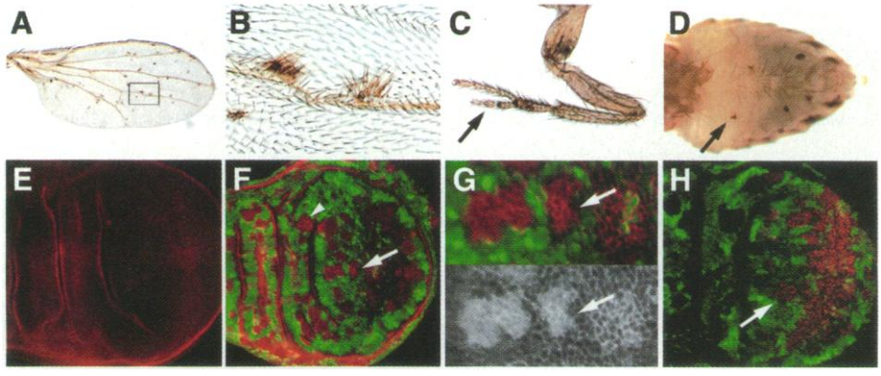

Fig. 3. Constitutive activation of Wg signaling in *d-axin* mutant clones. (**A**) A wing carrying *d-axin* mutant clones. Wing-margin structures are ectopically produced by the *d-axin* clones. Cells belonging to the clones are marked with the *yellow* mutation. (**B**) A close-up view of the ectopic wing-margin bristles in (A) (marked by the rectangle). (**C**) A leg carrying the *d-axin* clones. The supernumerary leg (indicated by the arrow) branches out from the dorsal surface of the normal leg. (**D**) Ventral surface of an abdomen carrying the *d-axin* clones. Ectopic bristles are formed in the pleura by the *d-axin* clones (indicated by the arrow). (**E** to **G**) A wild-type wing disc (E) and a wing disc carrying the *d-axin* clones (F and G) stained with anti-Arm (red). The *d-axin* clones are marked by the absence of green Myc-GFP expression (indicated by arrows). In the wild-type wing disc, Arm is ubiquitously expressed at low levels in the wing disc and accumulates preferentially at the cell membrane (E). In the *d-axin* clones, high levels of Arm accumulate in a cell-autonomous manner (F and G). Single-channel view indicates that Arm in the *d-axin* clones accumulates preferentially in the cytoplasm and nuclei, whereas Arm in normal cells is localized to the cell membrane (G). (**H**) A wing disc carrying the *d-axin* clones stained with anti-Dll (red). The *d-axin* clones are marked as in (F) and (G) by the absence of green Myc-GFP expression (indicated by the arrow). In the wild-type wing disc, Dll is expressed along the dorsoventral compartment border (Fig. 4D). In the *d-axin* clones, Dll is ectopically expressed at high levels in a cell-autonomous manner.

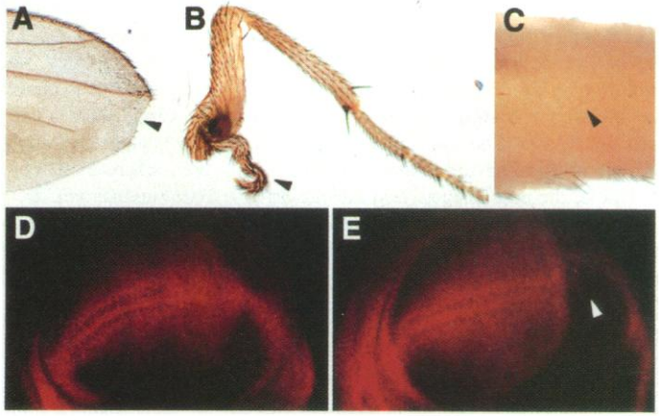

Fig. 4. Ectopic overexpression of *d-axin* causes inhibition of Wg signaling. (**A**) A wing of a 71B-GAL4:UAS–*d-axin* fly. Notches are indicated by the arrowhead. (**B**) A leg of a *tsh*-GAL4:UAS–*d-axin* fly. The supernumerary leg (indicated by the arrowhead) branches out from the ventral surface of the normal leg. Compare with the leg carrying the *d-axin* clones in Fig. 3C. (**C**) Sternites of a *tsh*-GAL4:UAS–*d-axin* fly. Sternite bristles are missing (indicated by the arrowhead), and the entire ventral surface is composed of pleura. Compare with the abdomen carrying the *d-axin* clones in Fig. 3D. (**D** and **E**) A wild-type wing disc (D) and a wing disc of an *engrailed*-GAL4:UAS–*d-axin* fly (E) stained with anti-Dll (red). Dll expression is repressed in the posterior region of the dorsoventral compartment border where *d-axin* is ectopically expressed by the *engrailed*-GAL4 expression system (indicated by the arrowhead). Wing discs are shown with anterior oriented to the left.

To further examine the function of D-Axin, we ectopically expressed the *d-axin* gene using the GAL4/UAS system (*15*). In contrast to the phenotypes observed with the *d-axin* mutant clones (Fig. 3, A to D), ectopic expression of *d-axin* induced notches in the wing (Fig. 4A), generation of a supernumerary leg from the ventral side of the normal leg (Fig. 4B), and loss of the sternite structure in the abdomen (Fig. 4C). In addition, when *d-axin* was expressed in the posterior compartment under the control of *engrailed*-GAL4, *Dll* expression was severely repressed in the posterior region of the dorsoventral compartment border (Fig. 4, D and E). Thus, ectopic expression of *d-axin* exerts an inhibitory effect on Wg signaling.

Using genetic analysis in *Drosophila*, we have demonstrated that D-Axin is required in vivo for the negative regulation of Wg signaling. Of particular interest is the finding that the levels of cytoplasmic Arm are highly and uniformly elevated wherever *d-axin* clones are located in the wing discs (Fig. 3). For example, the accumulation of Arm in *d-axin* clones was observed not only around the region where Wg is secreted (indicated by the arrow in Fig. 3F) but also in the region where Wg is not supposed to reach (indicated by the arrowhead in Fig. 3F) (*22*). Together with the fact that *d-axin* is ubiquitously expressed, these findings suggest that Wg activity is not required for the effect of D-Axin. We speculate that the Axin family of proteins functions to establish a threshold to prevent premature signaling events caused by Wg/Wnt and to restrict areas that are capable of responding to Wg/Wnt.

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embryo cDNA library fused to the transcription activation domain of yeast GAL4 in the vector pGAD10 and isolated three independent partial clones of the *d-axin* cDNA. A larger partial *d-axin* cDNA clone (2.7 kb) was isolated by rescreening a λgt11 *Drosophila* embryo cDNA library with the smaller cDNAs, and the remaining 5′-end region was obtained by the 5′ RACE (rapid amplification of cDNA ends) technique (Clontech). Multiple independent polymerase chain reaction (PCR) products were analyzed to exclude the possibility of PCR-induced errors. Sequence comparison analysis was performed with the *blastp* program [S. F. Altschul *et al.*, *Nucleic Acids Res.* **25**, 3389 (1997)].
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13. Using in situ hybridization to polytene chromosomes, we mapped the *d-axin* gene to 99D in the third chromosome. The genomic P1 clone DS00026 from the Berkeley *Drosophila* Genome Project contained the genomic region corresponding to the *d-axin* cDNA. The genomic organization of the *d-axin* locus and the intron-exon structure were determined by Southern (DNA) blot analysis and subsequent sequencing across the exon-intron boundaries.
14. A *Drosophila* strain containing the P-element–induced lethal line *l(3)S044230* was obtained from the European *Drosophila* P-element–induced lethal stock center (Szeged Center, Hungary). The precise point of P-element insertion was determined by genomic sequencing. Association of the observed lethal phenotype with the P-element insertion was confirmed by mobilization of the P-element with a Ki, p^{p}, Δ2-3 strain [H. M. Robertson *et al.*, *Genetics* **118**, 461 (1988)].
15. The GAL4/UAS system was used for overexpression of *d-axin* [A. H. Brand and N. Perrimon, *Development* **118**, 401 (1993)]. The entire *d-axin* open reading frames of various constructs were subcloned into the pUAST vector and introduced into the *w* germ line by the standard P-element transformation method. These UAS-*d-axin* lines were crossed with several GAL4 drivers [Y. Shiga, M. Tanaka-Matakatsu, S. Hayashi, *Dev. Growth Differ.* **38**, 99 (1996); E. J. Rulifson, C. A. Micchelli, J. D. Axelrod, N. Perrimon, S. S. Blair, *Nature* **384**, 72 (1996)].
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Truncated RanGAP Encoded by the *Segregation Distorter* Locus of *Drosophila*

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***Segregation Distorter* (*SD*) in *Drosophila melanogaster* is a naturally occurring meiotic drive system in which the *SD* chromosome is transmitted from *SD/SD^{+}* males in vast excess over its homolog owing to the induced dysfunction of *SD^{+}*-bearing spermatids. The *Sd* locus is the key distorting gene responsible for this phenotype. A genomic fragment from the *Sd* region conferred full distorting activity when introduced into the appropriate genetic background by germline transformation. The only functional product encoded by this fragment is a truncated version of the RanGAP nuclear transport protein. These results demonstrate that this mutant RanGAP is the functional *Sd* product.**

Examples of meiotic drive, in which a particular allele or chromosome of a heterozygous pair is preferentially transmitted to the offspring, have been described in natural populations of fungi, plants, insects, and mammals (*1*). This violation of the fundamental principle of Mendelian genetics can subvert the evolutionary process, which is contingent on the unbiased exposure of competing genes to selective forces. The molecular mechanisms of meiotic drive have remained elusive.

One of the best characterized meiotic drive systems is *Segregation Distorter* (*SD*) in *Drosophila melanogaster* (*2*). *SD* chromosomes are transmitted from *SD/SD^{+}* males to more than 95% of the progeny; transmission from females is normal. *Sd*, a dominant gain-of-function mutation, is the primary gene on *SD* chromosomes required for distortion. Strong distortion also requires several linked modifier loci, including *Enhancer* [*E(SD)*], *Modifier* [*M(SD)*], and *Stabilizer* [*St(SD)*] (*2–4*). The target of distortion is the *Responder* (*Rsp*) locus, which consists of an array of repeated satellite sequences whose copy number is correlated with sensitivity (*4, 5*). Chromosomes carrying *Rsp^{s}* (sensitive) or *Rsp^{ss}* (supersensitive) loci are subject to distortion, whereas *SD* chromosomes, which carry *Rsp^{i}* (insensitive), are resistant. Distortion ultimately involves sperm dysfunction, first visibly apparent as failed chromatin condensation in half of the developing spermatids (*6*).

The *Sd* locus was isolated by positional cloning and found to be associated with a tandem duplication that replaces the wild-type (*Sd^{+}*) 6.5-kb Eco RI fragment with an 11.5-kb Eco RI fragment (*7*). When introduced into appropriate genetic backgrounds by germline transformation, the 11.5-kb fragment confers full distortion, which indicates that *Sd* activity is contained entirely within this fragment (*8*). Transformants that have

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