and unoccupied states with the wave-function overlap of the tip and sample. A narrow local density of states at the tip may also explain why these electron scattering patterns could be imaged in a topographic, rather than spectroscopic, mode.

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Antagonistic Interactions Between Wingless and Decapentaplegic Responsible for Dorsal-Ventral Pattern in the *Drosophila* Leg

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Subdivision of the limb primordia of *Drosophila* into anterior and posterior compartments triggers cell interactions that pattern the legs and wings. A comparable compartment-based mechanism is used to pattern the dorsal-ventral axis of the wing. Evidence is presented here for a mechanism based on cell interaction, rather than on compartment formation, that distinguishes dorsal from ventral in the leg. Mutual repression by Wingless and Decapentaplegic signaling systems generates a stable regulatory circuit by which each gene maintains its own expression in a spatially restricted domain. Compartment-independent patterning mechanisms may be used by other organisms during development.

Short-range interactions between cells in adjacent compartments control growth and patterning in developing limbs [reviewed in (*1*)]. The secreted protein Hedgehog (HH) is expressed by cells of the posterior compartment of the wing imaginal disc and induces expression of another secreted signaling molecule Decapentaplegic (DPP) in nearby anterior cells (*2–5*). DPP serves as a long-range signal that relays information from the anterior-posterior (A-P) interaction to pattern the wing in a symmetric manner (*4–8*). A similar signal-relay system is used in the leg disc, but the situation is complicated by the fact that HH induces DPP expression in dorsal anterior cells while Wingless (WG) and lower levels of DPP are induced in ventral anterior cells (*2, 9*) (Fig. 1, A and B). The different interpretation of the HH signal in dorsal and ventral leg regions reflects an underlying subdivision of the leg disc into dorsal and ventral quadrants, in which DPP and WG play central roles in patterning the leg (*1, 10*). The distinction between dorsal and ventral fates in the leg is made differently from that in the developing wing. Expression of the selector gene *apterous* specifies the dorsal compartment of the wing (*11, 12*). Evidence for a comparable subdivision of the leg into dorsal and ventral lineage compartments is not compelling (*13*), raising the question of whether this distinction depends on a selector gene.

WG activity specifies ventral cell fate in the leg. In larvae mutant for *wg* or for genes in the WG signal transduction pathway, ventral structures are lost and are replaced by a symmetric duplication of dorsal structures (*10, 14–18*). Furthermore, ectopic expression of WG or activation of the WG signal transduction pathway in cells on the dorsal side of the leg respecifies dorsal cells to ventral fate and causes dorsal-ventral (D-V) axis duplication (*9, 19–22*). WG is expressed in a wedge of anterior cells adjacent to the A-P compartment boundary on the ventral side of the leg imaginal disc (Fig. 1A) (*14, 16*). *dpp* is expressed in a stripe of anterior cells adjacent to the A-P boundary, but at higher levels in dorsal cells, which do not express WG (Fig. 1B) (*23, 24*). We found that the pattern of *dpp* expression is altered in wg^{cx3}/wg^{cx3} leg discs, which have reduced WG activity (*25*) (Fig. 1D). Expression of a *dpp-lacZ* reporter gene is increased on the ventral side, resulting in a symmetric pattern of expression at a level equivalent to that on the dorsal side. Consistent with this pattern of DPP expression, larvae homozygous for the wg^{cx3} mutation develop legs that have dorsal-dorsal symmetry (*10, 14*). These results suggest that one function of WG in specifying ventral cell fate is to reduce the levels of *dpp* expression in ventral cells.

DPP activity is required to specify dorsal cell fate in the leg. In weak *dpp* mutants, dorsal structures are replaced by symmetrically duplicated ventral structures (*10*). We found that reducing DPP activity in dpp^{d6}/dpp^{d12} leg discs (*26*) alters the pattern of WG expression from its normal ventral domain into an expanded domain along the dorsal A-P boundary (Fig. 1, A and C) (*27*). The domain of ectopic WG expression corresponds to the region of the disc that is transformed to ventral fate in this mutant (*10*). Thus, one function of DPP is to repress WG expression in dorsal cells. This suggestion is supported by the recent observation that clones of cells mutant for *Mad*, a component of the DPP signal transduction pathway, express WG when such clones are located on the dorsal side of the antennal disc near the A-P compartment boundary (*28*).

Taken together, these results suggest that an important aspect of D-V fate specification involves mutual repression by the WG and DPP signaling systems. To further test this model, we asked whether ectopic expression

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of WG or DPP using the yeast Gal4 system (*29*) would affect expression of the other gene. Transgenic flies carrying a *P* element containing a wild-type *wg* cDNA under the control of a Gal4 response element (*UAS-wg*) (*30*) were crossed to flies carrying the *dpp-GAL4 P* element, which express the Gal4 protein in the *dpp* pattern (*22*). Expressing WG in the *dpp* domain reduces dorsal expression of the *dpp-lacZ* reporter to a level equivalent to that on the ventral side of the leg (Fig. 1F). We noted that repression of *dpp* by ectopic WG allows the expression of the endogenous *wg* gene in dorsal cells, as monitored by expression of a *wg-lacZ* reporter gene (Fig. 1E). This is analogous to the effect on WG expression caused by mutants with reduced *dpp* activity (Fig. 1C) and confirms that one function of DPP is to repress *wg* transcription in dorsal cells. Further confirmation was obtained by overexpression of DPP in its normal domain in larvae of genotype *dpp-GAL4/UAS-dpp*. Overexpression of DPP represses WG expression in the ventral leg (Fig. 1G), indicating that the lower level of DPP normally found on the ventral side of the disc is not sufficient to repress WG but that increasing the concentration of DPP can do so. Reducing WG expression in ventral cells by overexpression of DPP leads to increased expression of the endogenous *dpp* gene, analogous to the effect of reducing WG activity in *wg* mutant discs (Fig. 1D).

To assess the extent of D-V fate transformation in discs where WG or DPP were ectopically expressed, we examined the expression of two downstream target genes: *optomotor-blind* (*omb*) and *H15*. The *omb* gene encodes a predicted T-box protein (*31*) that is expressed on the dorsal side of the leg disc (Fig. 2E). The domain of *omb* expression is essentially coincident with the region of high *dpp* expression in the dorsal side of the leg (Fig. 2F) and depends on *dpp* activity (*32*). *omb* is also regulated by DPP in the wing (*7, 8, 33*). *H15* is an enhancer-trap that expresses *lacZ* in the ventral side of the disc (Fig. 2A) (*34*). *H15* expression is centered on the WG domain but is broader (Fig. 2B), suggesting that *H15* is a target for regulation by WG. *H15* is activated by ectopic WG expression (Fig. 2C) and in clones lacking the activity of *shaggy/zeste-white3* kinase, a negative regulator of the WG pathway (*22*). *shaggy/zeste-white3* mutant cells assume ventral fate regardless of their location in the leg disc (*21*); thus, *H15* expression is correlated with ventral fate. *H15* encodes a predicted T-box protein closely related to OMB (*34*).

Both *omb* and *H15* serve as molecular markers to assess the positive readout of WG and DPP signaling. We found that when WG is ectopically expressed in dorsal cells, *H15* is ectopically expressed (Fig. 2C) and *omb* is repressed (Fig. 2G), indicating that the discs have ventral-ventral symmetry. Ectopic DPP expression has the reciprocal effect. Ventral expression of *H15* is repressed (Fig. 2D) and is replaced by *omb* expression (Fig. 2H), indicating that the discs have dorsal-dorsal symmetry. These results indicate that WG and DPP specify ventral and dorsal fate through the activation of region-specific target genes in addition to repressing each other's expression.

In this study, the use of a *UAS-wg* construct expressing the wild-type WG protein allowed us to respecify dorsal cells to ventral fate. This result contrasts with a previous report in which equivalent ectopic expression of a temperature-sensitive mutant form of WG failed to respecify dorsal cell fate or to ectopically activate *H15* in the dorsalmost cells of the imaginal disc [*UAS-wg*^{IL114} (*22*)]. This observation taken together with our data indicates that, even at the permissive temperature, *wg*^{IL114} mutant protein does not have normal WG activity, despite the high levels of WG antigen produced. The difference between WG and WG^{IL114} suggests that low levels of WG activity are not sufficient to specify ventral fate in the presence of DPP (and by inference, to repress DPP expression). We confirmed this suggestion using an *Act5C>wg* transgene to produce a uniform low level of WG expression (*19*). This low level of wild-type WG is sufficient to induce D-V axis bifurcation but is at the limit of detection by antibody staining (*19*). *Act5C>wg* expression directs *H15* expression throughout the leg disc except in cells close to the dorsal A-P boundary, where DPP is expressed (Fig. 3, A and B). To ask if the spatial limitation on *H15*

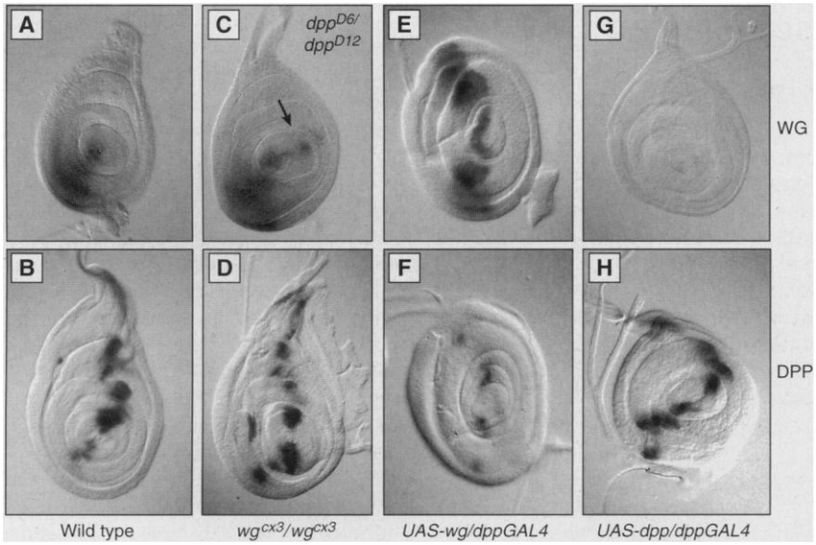

Fig. 1. WG and DPP repress each other's expression. All discs are oriented so that anterior is to the left and dorsal is to the top of the panel. (**A**) WG protein expression in a mature wild-type leg disc visualized by antibody staining. Mouse monoclonal antibody 4E6 was raised to a fragment of WG protein (*41*). WG expression is restricted to a ventral anterior wedge of cells and appears graded, with highest levels of expression in the center of the domain. (**B**) *dpp-lacZ* reporter gene expression visualized by X-Gal staining in a mature wild-type leg disc. *dpp-lacZ* expression is restricted to a relatively narrow stripe of anterior cells and is higher in the dorsal side of the disc, where WG is not expressed [compare with (A)]. The *dpp-lacZ* domain is a relatively narrow stripe, whereas the WG domain is wedge-shaped. This difference is also apparent when a *wg-lacZ* reporter gene is assayed [compare with (E); see also (*16*)]. (**C**) Ectopic expression of WG protein on the dorsal side of a *dpp*^{d6}/*dpp*^{d12} mutant leg disc. Expression is limited to the central region of the disc, which corresponds to the tarsal segments of the leg. In this mutant combination, the dorsal tarsal segments are transformed to ventral identity (*10*). (**D**) Ectopic expression of *dpp-lacZ* in *wg*^{cx3}/*wg*^{cx3} mutant leg discs. The level of *dpp-lacZ* expression in the ventral leg is equivalent to that on the dorsal side. In this mutant combination, ventral leg segments are transformed to dorsal identity (*10, 16*). WG protein is present at reduced levels, but in a normal spatial pattern in *wg*^{cx3} homozygous leg discs, suggesting that *wg*^{cx3} is a hypomorphic allele for the leg (*32*). (**E**) Ectopic expression of WG in the *dpp* domain in *UAS-wg/dppGAL4* leg discs activates the endogenous *wg* gene along the dorsal A-P boundary. Expression of the endogenous gene was distinguished from expression of the transgene with the use of a *wg-lacZ* enhancer-trap. (**F**) Ectopic expression of WG in the *dpp* domain in *UAS-wg/dppGAL4* leg discs represses *dpp-lacZ* reporter gene expression on the dorsal side, resulting in a level and pattern of expression resembling normal ventral expression. The resulting adult legs show ventral-ventral symmetry (see Fig. 4). Ectopic expression of DPP in the *dpp* domain [see (B)] of *UAS-dpp/dppGAL4* leg discs represses WG expression (**G**) and activates *dpp-lacZ* to dorsal-like levels in ventral cells along the A-P boundary (**H**). *UAS-dpp* is described in (*42*).

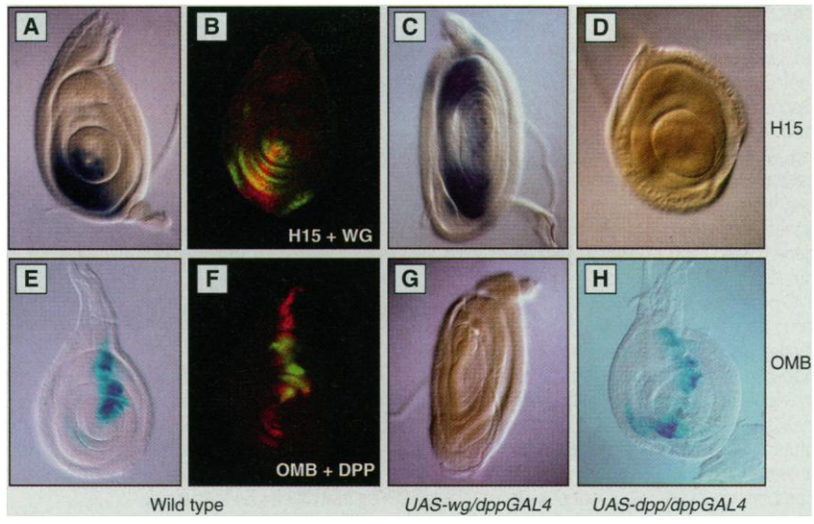

Fig. 2. Ectopic expression of DPP and WG results in reciprocal fate transformations in the D-V axis. All discs are oriented so that anterior is to the left and dorsal is to the top of the panel. (**A**) *H15-lacZ* is expressed in the ventral leg disc (*33*). (**B**) Double immunofluorescent labeling shows that the *H15-lacZ* domain is centered on the domain of highest WG expression. *H15-lacZ* [anti–β-galactosidase (anti–β-Gal): green]; anti-WG (red). (**C**) Expression of *H15-lacZ* on the dorsal side of a *UAS-wg/dppGAL4* disc. Expression is symmetric on dorsal and ventral sides. Note also that this disc shows partial axis bifurcation in the tarsus. (**D**) *H15 lacZ* expression is repressed in ventral cells when DPP is overexpressed in *UAS-dpp/dppGAL4* discs. (**E**) *omb-lacZ* is expressed in dorsal cells (*33*). (**F**) Double immunofluorescent labeling shows that the *omb* domain is essentially coincident with the domain of *dpp-lacZ* expression. *omb* expression (green) was detected with an *omb-GAL4* enhancer-trap insertion to direct expression of a UAS-GFP transgene, as described in (*8*). *dpp-lacZ* expression (red) was detected by anti–β-Gal. Although *omb* expression is *dpp*-dependent in the leg, *omb* is not activated nonautonomously in a broad domain as in the wing (*7, 8*). (**G**) *omb* expression is repressed on the dorsal disc of a *UAS-wg/dppGAL4* disc. The tarsus is slightly elongated along the D-V axis, reflecting a partial axis bifurcation in the tarsus. Partial axis bifurcation was observed in half of the discs treated in this way. (**H**) Ectopic *omb* expression directed on the ventral side of a *UAS-dpp/dppGAL4* disc.

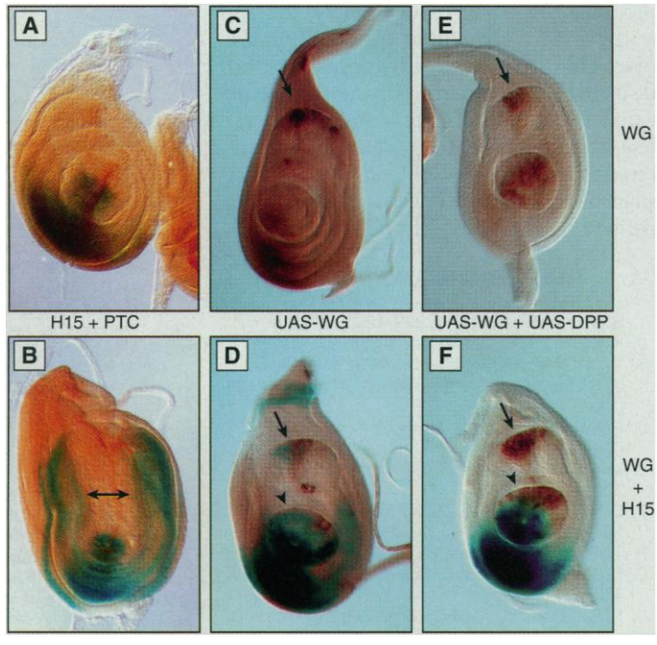

Fig. 3. DPP antagonizes the ability of WG to direct *H15* expression. All discs are oriented so that anterior is to the left and dorsal is to the top of the panel. (**A** and **B**) *H15-lacZ* expression (blue). The elevated level of Patched (PTC) expression (brown) marks the position of the A-P compartment boundary (*43*). A wild-type leg disc is shown in (A), and a leg disc in which WG is expressed at low levels in all cells (*43*) is shown in (B). Note that *H15* expression is expanded into the dorsal side of the disc but is excluded from a stripe centered on the A-P boundary (arrow), corresponding to the region where DPP is expressed. (**C** and **D**) Misexpression of WG under control of the *GAL30A* driver. WG protein is ectopically expressed in a patch of dorsal cells in the femur (arrow), and in the dorsal 5th tarsal segment (arrowhead). WG expression is shown in (C), and WG expression plus *H15-lacZ* is shown in (D). Note that *H15-lacZ* is expressed in dorsal cells in the tarsus and near the patch of WG expression in the dorsal femur. *Hl5-lacZ* expression is stronger in cells located on the side of the WG patch away from the A-P boundary. (**E** and **F**) Misexpression of WG and DPP together under *GAL30A* control. The pattern of ectopic WG expression in (E) is the same as that in (C), but expression of the endogenous gene has been partly repressed by the ectopic DPP. Note that very low levels of WG are sufficient to activate *H15-lacZ* expression [see (B); the level of WG produced by the *Act5C>wg* transgene is almost undetectable by antibody staining]. Though reduced, the level of ventral WG is still sufficient to direct *H15-lacZ* expression. In (F), co-misexpression of DPP and WG prevents WG from activating *H15-lacZ* expression (*43*).

expression in these two experiments is due to DPP activity, we tested whether DPP could block the ability of WG to activate *H15* when both proteins are expressed together. When WG is misexpressed alone, *H15* is expressed in nearby cells (Fig. 3, C and D). Simultaneous misexpression of DPP and WG blocks the ability of WG to activate *H15*, even though WG is abundantly expressed (Fig. 3, E and F). Note that WG and DPP cannot affect each other's expression in this experiment because both are under Gal4 control.

Our results suggest that the distinction between dorsal and ventral fates is maintained through mutual repression by DPP and WG. Expression of WG and DPP in their normal domains depends on the HH signal (*2*). However, ectopic HH expression can direct WG in all ventral anterior cells and DPP in all dorsal anterior cells (*2*). This finding indicates that cells that are not likely to be within range of the WG or DPP signals have a different capacity to respond to HH, depending on whether they are dorsal or ventral, and suggests that dorsal and ventral cells are preprogrammed to respond differently to HH. A possible explanation for this apparent preprogramming might be subdivision into compartments by a selector gene; however, clonal analysis does not provide strong support for a D-V compartmental subdivision of the leg (*13*).

To address the genetic basis for the preprogrammed response, we asked how the choice to express DPP or WG in a dorsal cell is determined. Clones of cells mutant for *pka* behave as though they have received the HH signal (*35–38*). *pka* mutant cells express *dpp* in the dorsal leg and *wg* in the ventral leg. Clones of cells in the dorsal leg or antenna that are simultaneously mutant for *pka* and *dpp* can express WG (Fig. 4). This result suggests that the ability of a dorsal cell to express *dpp* determines whether it will respond to the HH signal by expressing *dpp* or *wg*. A similar conclusion is suggested by the finding that clones mutant for *wg* and *pka* and located in the ventral anterior leg occasionally produce bifurcations (*36*). This differs from single-mutant

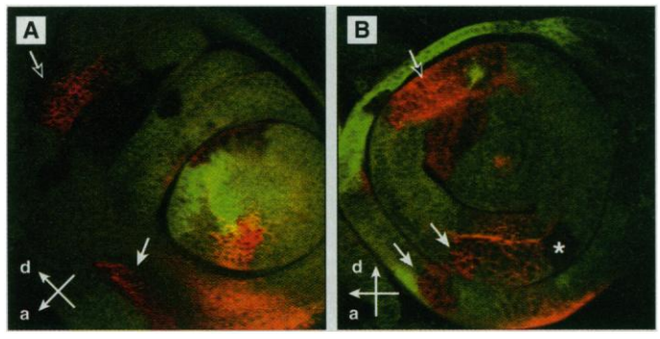

Fig. 4. WG can be expressed in dorsally located *dpp-pka* double-mutant clones in (**A**) the leg disc and (**B**) the antenna disc. WG antibody staining is in red. Clones of cells mutant for *dpp* and *pka* were marked by the absence of a *lacZ* reporter gene [green, see (*39*) for details]. The overlap of WG protein (in its endogenous domain) and the cell marker appears orange. *dpp-pka* mutant clones in the ventral anterior region of the leg and antenna discs express WG (white arrows; WG label appears red in the absence of the cell marker). Clones in the posterior compartment do not express WG [asterisk in (B)]. Dorsally located *dpp-pka* mutant clones can express WG (open arrows). About half of the mutant clones examined in the dorsal anterior leg do not express WG, whereas most dorsal clones in the antenna do so. The variability in whether WG is expressed may be a consequence of the fact that the genetic combination used here is not completely null for DPP activity [see (*39*) for details].

pka clones, which must include both dorsal and ventral cells to cause bifurcations (*35–37*). Given that both *dpp* and *wg* expression are needed for axis bifurcation (*9*), and that ventral *pka* clones only express *wg*, it is probable that ventral *wg-pka* double-mutant clones express *dpp* rather than *wg* and cause axis bifurcation if they are situated near WG-expressing cells. Taken together, these results suggest that the predisposition of dorsal cells to express *dpp* and of ventral cells to express *wg* depends primarily on the activity of the *dpp* and *wg* genes themselves. The observation that dorsal anterior cells can express *wg* if they have reduced *dpp* activity (*39*) suggests that there need not be a deeper level of preprogramming that distinguishes dorsal from ventral in the leg and antenna discs. Viewed in this context, the observation that ectopic expression of DPP or WG is sufficient to reprogram D-V identity argues against the idea that the dorsal and ventral quadrants are defined by a selector gene acting upstream of *dpp* or *wg*.

If we allow for an initial asymmetry to determine whether HH will direct WG or DPP expression, subsequent mutual repression by DPP and WG is sufficient to maintain the distinction between dorsal and ventral fates in the leg. Is there an initial asymmetry? We have shown previously that WG signaling recruits cells to form the imaginal disc primordia in the embryo and that these primordia arise straddling the A-P compartment boundary at the junction between rows of WG- and DPP-expressing cells (*40*). A consequence of this arrangement is that WG is already expressed in the presumptive ventral anterior cells at the time when these cells are recruited to form the disc primordium (*16*, *40*). We propose that the requisite asymmetry in ventral expression of WG is directly inherited from the embryonic ectoderm and that this asymmetry provides the bias toward the ability of cells to express WG under the influence of the HH signal. Implicit in this proposal is the suggestion that in the absence of WG, HH will activate DPP in the disc. Thus, the D-V subdivision of the leg disc may be inherited from the embryonic ectoderm in the form of a stable genetic-regulatory circuit based on antagonistic interaction between WG and DPP, rather than in the form of lineage compartments defined by expression of a selector gene.

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27. Double immunofluorescent labeling with antibodies to WG and Ci proteins shows that the ectopic expression of WG on the dorsal side of the dpp^{d6}/dpp^{d12} disc runs along the anterior side of the A-P compartment boundary, as it normally does on the ventral side (*32*).
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34. The *H15* locus was identified as a *P* element enhancer-trap insertion at 25D6 [W. J. Brook *et al.*, *Development* **117**, 1287 (1993)]. Genomic DNA flanking the insertion was cloned by plasmid rescue. Coding sequences expressed in the *H15* pattern were identified with genomic DNA fragments as in situ hybridization probes on leg discs. The sequence of a partial cDNA revealed an open reading frame encoding a predicted T-box protein (accession number: X98766). OMB (M81796), human TBX2 (U28049), and mouse TBX2 (U15566) were selected by a BLAST search as the nearest relatives of H15. *omb-lacZ* is a *P* element enhancer-trap insertion at the *omb* locus (*8*, *33*). Expression of *omb* is absent in leg discs where *dpp* activity is reduced, indicating that *omb* expression is DPP-dependent in the leg (*32*). Loss-of-function mutants in either gene develop with only slight defects in the leg (*32*).
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39. Clones of cells mutant for *dpp* and *pka* were induced in second instar (48 to 72 hours at 25°C) by heat shock at 38°C for 90 min in larvae of genotype *Tn(JA1, dpp⁺)*/w hsFLP; dpp^{H61} *pka-C1*^{n2} *FRT40A/ arm-lacZ FRT40A*. Comparable results were obtained with clones induced in early third instar [72 to 96 hours (*32*)]. Strains are described in (*28*) and (*35*). We ob-

served that some *dpp-pka* mutant clones in the dorsal-anterior quadrant of the leg do not express WG. Possible explanations include the following: (i) repression by endogenous DPP, affecting clones located near the A-P boundary; or (ii) the combination of dpp^{H61} $pka\text{-}C1^{h2}$ with *Tn(JA1, dpp⁺)* does not behave as a complete loss of function for *dpp*. Clones of cells mutant for dpp^{H61} $pka\text{-}C1^{h2}$ that carry *Tn(JA1, dpp⁺)* exhibit some DPP activity, in that they have a low level of expression of *omb* in the wing. Comparable dpp^{H61} $pka\text{-}C1^{h2}$ clones that lack the transgene do not express *omb* (*32*). For technical reasons, the clones examined here were generated in the presence of the *Tn(JA1, dpp⁺)* transgene. Although not sufficient to repress WG in all cases, it is possible that the low level of DPP activity provided by the transgene prevents WG expression in some clones.

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43. *H15 lacZ* expression was visualized by X-Gal staining. *Act5C>y⁺>wg* is described in (*19*). Larvae of genotype *y HSFlp;Act5C>y⁺>wg/H15-lacZ* were subjected to a 90-min heat shock at 38°C at 60 ± 12 hours of age (second instar). This treatment causes excision of the *flip*-out cassette (a segment of DNA bounded by direct FRT repeats) in virtually all cells, resulting in low-level ubiquitous WG expression. This level of WG expression is sufficient to direct *H15* expression but does not repress DPP expression (*20*). The *GAL30A* driver is described in (*29*). Larval genotypes: (Fig. 3C) *GAL30A/+; UAS-wg/+*; (Fig. 3D) *GAL30A/H15-lacZ; UAS-wg/+*; (Fig. 3E) *GAL30A/+; UAS-wg UAS-dpp/+*; and (Fig. 3F) *GAL30A/H15-lacZ; UAS-wg UAS-dpp/+*.
44. We thank C. Pfeifle for help in molecular characterization of *H15* and for isolation of *H15* mutants; A.-M. Voie for producing the WG monoclonal antibody; W. Norris and P. Ingham for mouse antibody to PTC protein; F. Diaz-Benjumea and V. Wiersdorff for sharing unpublished results; and S. Eaton, M. Averof, M. Ng, and an anonymous reviewer for suggesting improvements to the manuscript. W.J.B. is the recipient of an EMBO long-term fellowship in molecular biology.

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Purification and Molecular Cloning of Plx1, a Cdc25-Regulatory Kinase from *Xenopus* Egg Extracts

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Cdc2, the cyclin-dependent kinase that controls mitosis, is negatively regulated by phosphorylation on its threonine-14 and tyrosine-15 residues. Cdc25, the phosphatase that dephosphorylates both of these residues, undergoes activation and phosphorylation by multiple kinases at mitosis. Plx1, a kinase that associates with and phosphorylates the amino-terminal domain of Cdc25, was purified extensively from *Xenopus* egg extracts. Cloning of its complementary DNA revealed that Plx1 is related to the Polo family of protein kinases. Recombinant Plx1 phosphorylated Cdc25 and stimulated its activity in a purified system. Cdc25 phosphorylated by Plx1 reacted strongly with MPM-2, a monoclonal antibody to mitotic phosphoproteins. These studies indicate that Plx1 may participate in control of mitotic progression.

In dividing eukaryotic cells, the entry into mitosis is governed by M phase–promoting factor (MPF). MPF, which consists of the Cdc2 protein kinase and cyclin B, acts by phosphorylating substrates that are essential for the execution of mitotic processes (*1*). Before mitosis, the activity of Cdc2–cyclin B is suppressed through phosphorylation of its Thr^{14} and Tyr^{15} residues by the Wee1 and Myt1 kinases (*2*). Cdc25, a dual-specificity phosphatase that dephosphorylates both Thr^{14} and Tyr^{15}, has a pivotal role in the cell cycle by activating Cdc2 at the G_2-M boundary (*3*, *4*). In higher eukaryotes, there are at least three members of the Cdc25 family (A, B, and C) that appear to act at different points in the cell cycle (*5*), but presently the role of Cdc25C in the G_2-M transition is best understood.

In *Xenopus*, Cdc25C (Xcdc25) undergoes extensive phosphorylation of its NH_2-terminal regulatory domain at mitosis (*3*, *4*). This phosphorylation strongly stimulates the Cdc2-specific phosphatase activity of Cdc25 and conceivably could have additional roles in its localization or stability. The NH_2-terminal region of Xcdc25, like that of Cdc25 proteins in other species, is rich in Ser-Pro and Thr-Pro motifs, which constitute part of the consensus recognition site for Cdc2 and other cell cycle–regulated kinases (*1*). Although Cdc2 appears to participate in the regulation of Xcdc25, there is at least one other mitotic kinase that phosphorylates Xcdc25 (*6*, *7*). In principle, this kinase or kinases could act upstream of Cdc2 to trigger its activation. Alternatively, it could act downstream of Cdc2 and thereby facilitate the progression through and eventual exit from mitosis.

One clue about the identity of this Xcdc25-specific kinase is that the phosphorylated form of Xcdc25 found at mitosis interacts strongly with the MPM-2 antibody (*8*). The MPM-2 antibody binds to a phosphopeptide epitope present in various mitotic phosphoproteins, including other regulators of mitosis such as Wee1, Myt1, NimA, and the anaphase-promoting complex (*2*, *9*). Thus, one or more kinases that phosphorylate the MPM-2 epitope are most likely to participate in mitotic control (*10*).

A major Xcdc25-specific kinase distinct from Cdc2–cyclin B (*7*) associated with exogenously added recombinant Xcdc25 in M-phase egg extracts from *Xenopus*. This kinase bound well to a histidine-tagged fusion protein containing the NH_2-terminal domain of Xcdc25 (His6-Xcdc25-N, residues 1 to 264) (*11*). We used a His6-Xcdc25-N affinity column and several conventional chromatographic steps (Superdex 200, phosphocellulose, and Mono Q) to enrich this Cdc25-specific kinase activity approximately 2500-fold (Table 1) (*12*). Upon separation of this highly enriched fraction in a sucrose density gradient, a 67-kD polypeptide (p67) comigrated closely with the kinase activity (Fig. 1) (*13*). We isolated several micrograms of p67 and sequenced four of its tryptic peptides (*14*). Polymerase chain reaction (PCR) primers designed for two of these peptides were used to amplify an 850–base pair (bp) segment of the cDNA encoding p67 (*15*). This fragment was used to isolate a full-length 2.4 kb cDNA encoding an open reading frame that contains all four tryptic peptide sequences (Fig. 2) (*16*). The complete amino acid sequence indicates that p67 is a typical

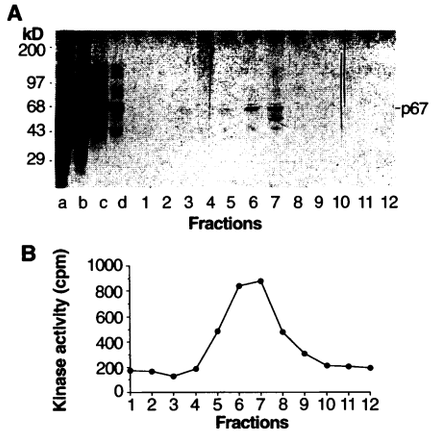

Fig. 1. Purification of a mitotic kinase that phosphorylates the NH_2-terminal domain of Xcdc25. (**A**) A cytosol fraction from an M-phase egg extract that had been eluted from a His6-Xcdc25-N agarose column (lane a) was subjected to chromatography on Superdex 200 (lane b), phosphocellulose (lane c), and Mono Q (lane d). The Mono Q fraction was separated in a sucrose gradient. Fractions from the columns and sucrose gradient (lanes 1 through 12, bottom to top) were subjected to SDS gel electrophoresis and silver staining. (**B**) Xcdc25-specific kinase activity of the sucrose gradient fractions.

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