Osa-containing Brahma chromatin remodeling complexes are required for the repression of Wingless target genes

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The Wingless signaling pathway directs many developmental processes in *Drosophila* by regulating the expression of specific downstream target genes. We report here that the product of the *trithorax* group gene *osa* is required to repress such genes in the absence of the Wingless signal. The Wingless-regulated genes *nubbin*, *Distal-less*, and *decapentaplegic* and a minimal enhancer from the *Ultrabithorax* gene are misexpressed in *osa* mutants and repressed by ectopic Osa. Osa-mediated repression occurs downstream of the up-regulation of Armadillo but is sensitive both to the relative levels of activating Armadillo/Pangolin and repressing Groucho/Pangolin complexes present and to the responsiveness of the promoter to Wingless. Osa functions as a component of the Brahma chromatin-remodeling complex; other components of this complex are likewise required to repress Wingless target genes. These results suggest that altering the conformation of chromatin is an important mechanism by which Wingless signaling activates gene expression.

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The *Drosophila* segment polarity gene *wingless* (*wg*) encodes a secreted signaling molecule that provides positional information for the growth and patterning of numerous structures during both embryonic and imaginal development. Wg and its vertebrate homologs (Wnt proteins) activate a conserved signal transduction pathway and influence cell fate decisions by regulating the expression of specific downstream target genes (Cadigan and Nusse 1997).

Binding of Wg/Wnt proteins to cell surface receptors of the Frizzled (Fz) family (Bhanot et al. 1996; Yang-Snyder et al. 1996; Bhat 1998) triggers the stabilization and accumulation of the effector proteins Armadillo (Arm) in *Drosophila* (Riggleman et al. 1990; Peifer et al. 1994) and β-catenin (β-cat; Larabell et al. 1997) in vertebrates. In the absence of a Wg/Wnt signal, Arm and β-cat are maintained at low levels through the activity of a complex consisting of the serine/threonine kinase Shaggy/Zeste-white 3 (Sgg) or glycogen synthase kinase 3β (GSK-3β) in vertebrates (Siegfried et al. 1992; Peifer et al. 1994; Yost et al. 1996), the scaffold protein Axin (Zeng et al. 1997; Behrens et al. 1998; Hamada et al. 1999; Willert et al. 1999), and the product of the *adenomatous polyposis coli* tumor suppressor gene (APC; Rubinfeld et al. 1996; McCartney et al. 1999). Reception of the Wg/Wnt signal inhibits the kinase activity of Sgg/GSK-3β, preventing the complex from phosphorylating Arm/β-cat and targeting it for degradation (Ruel et al. 1999).

The posttranslational up-regulation of Arm and β-cat permits these proteins to translocate to the nucleus (Orsulic and Peifer 1996; Yost et al. 1996) and bind the HMG box proteins Pangolin/dTCF (Pan; Brunner et al. 1997; van de Wetering et al. 1997) in *Drosophila* and Lef-1/TCF (Behrens et al. 1996; Molenaar et al. 1996) in vertebrates. These Pan/Arm and TCF/β-cat complexes are thought to comprise bipartite transcription factors that regulate the expression of Wg- and Wnt-responsive genes, respectively. However, the mechanism by which these complexes activate gene expression is poorly understood.

It has recently been reported that β-cat can interact in vitro with the TATA binding protein (TBP; Hecht et al. 1999), as well as with Pontin52 (Bauer et al. 1998), a protein that interacts with TBP. This suggests that Pan/Arm and TCF/β-cat complexes may regulate gene expression by targeting components of the basal transcriptional machinery to promoters of Wg/Wnt-responsive genes. However, these complexes do not appear to be sufficient to activate the expression of target genes in vivo (Riese et al. 1997; Prieve and Waterman 1999). Thus, regulation of gene expression by Pan/Arm and TCF/β-cat complexes is likely to be far more complex than the simple recruitment of TBP.

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An interesting common theme emerging from recent research is the connection between the regulation of chromatin architecture and the expression of Wg/Wnt target genes. Pan, TCF, and Lef-1 are members of the HMG family of transcription factors. The HMG box is a DNA-binding domain that induces a sharp bend in DNA; this DNA-bending activity has been shown to be important for the regulation of gene expression by many HMG box proteins (for review, see Bustin 1999), including Lef-1 (Giese et al. 1997). Interestingly, Lef-1 bound to β-cat induces a sharper bend in DNA than does Lef-1 alone (Behrens et al. 1996). β-cat can also bind to CBP/p300 histone acetyltransferases, and this interaction stimulates β-cat dependent gene expression in vitro (Hecht et al. 2000). Furthermore, it has been shown that Pan and TCF are required for the repression of target gene expression in the absence of the Wg/Wnt signals (Brannon et al. 1997; Riese et al. 1997; Yang et al. 2000). Pan and TCF mediate this repression though an interaction with the *Drosophila* Groucho (Gro) and vertebrate TLE transcriptional corepressors (Cavallo et al. 1998; Roose et al. 1998). Gro has been shown to repress gene expression by directly interacting with the amino tail of histone H3 (Palaparti et al. 1997) and by recruiting the histone deacetylase Rpd3 (Chen et al. 1999). These data indicate that altering chromatin architecture may be an important mechanism for the regulation of target gene expression by the Wg and Wnt signaling pathways.

Eukaryotic organisms have evolved a number of multiprotein complexes that remodel chromatin structure to regulate gene expression, including the SWI/SNF and RSC complexes in yeast (for review, see Kadonaga 1998; Kingston and Narlikar 1999). Homologs of the SWI/SNF and RSC complexes have been identified in other organisms, including the hBRM and BRG1 complexes in humans (Wang et al. 1996) and the Brahma (Brm) complex in *Drosophila* (Papoulas et al. 1998). A purified yeast SWI/SNF or related human complex can catalyze an ATP-dependent reversible reaction that alters the structure of nucleosomal DNA, rendering it more accessible to transcription-factor binding (Cote et al. 1994; Kwon et al. 1994). Whereas chromatin remodeling by these complexes is generally thought to promote transcription of target genes, an increasing body of evidence suggests that they are also required for the direct repression of a subset of genes (Trouche et al. 1997; Holstege et al. 1998; Moreira and Holmberg 1999; Sudarsanam et al. 2000).

We have shown that the *trithorax* group gene *osa* encodes an ARID domain protein that is a component of Brm chromatin-remodeling complexes (Treisman et al. 1997; Collins et al. 1999). It has also been demonstrated that a human homolog of Osa, p270, is a component of the BRG1 complex (Dallas et al. 1998, 2000). Osa and Brm complexes have been shown to regulate the expression of a wide variety of genes including the segmentation gene *even-skipped* (Treisman et al. 1997) and the homeotic gene *Antennapedia* (Vasquez et al. 1999). Brm complexes have also been shown to be required for activation of expression by the *trithorax* group protein Zeste (Kal et al. 2000). Interestingly, loss of *osa* function in embryos and imaginal discs often induces phenotypes similar to those caused by ectopic activation of the *wg* pathway, although *wg* itself is not ectopically expressed (Treisman et al. 1997). This prompted us to suggest that *osa* functions to antagonize *wg* signaling (Treisman et al. 1997); however, it was not known whether this antagonism is direct or the result of the regulation of the expression of other components of the *wg* pathway by *osa*.

We show here that loss of *osa* function induces ectopic expression of Wg target genes and that overexpression of *osa* can repress the endogenous expression of the same genes. Furthermore, the lack of an effect of Osa on Arm up-regulation, the activity of an Osa-repressor domain fusion, and the specificity with which *osa* affects the Wg response of a minimal enhancer suggest that *osa* is required to directly repress the expression of these genes. Interestingly, loss of function of other components of the Brm complex also induces ectopic expression of Wg target genes. Thus, the repression of Wg target genes in vivo requires chromatin remodeling mediated by Osa-containing Brm complexes. These data provide further evidence that regulation of chromatin structure is an important mechanism for the control of target gene expression by the Wg and Wnt pathways.

Results

Osa is required for the repression of a Wg target gene

We have previously reported that loss of *osa* can induce phenotypes similar to those caused by ectopic *wg* expression (Treisman et al. 1997). Conversely, overexpression of full-length, wild-type Osa (UAS-Osa) results in dominant, gain-of-function phenotypes that often resemble those caused by loss of *wg* function (Collins et al. 1999; data not shown). However, *osa* appears to be epistatic to *wg*, and loss of *osa* function does not induce ectopic expression of *wg* (Treisman et al. 1997). Therefore, the *wg* gain-of-function phenotypes caused by *osa* loss of function are likely to result from de-repression of downstream target genes of Wg. To investigate this, we examined the regulation of *nubbin* (*nub*). *nub* encodes a POU domain protein that is required for the growth and patterning of the wing and is expressed throughout the wing primordium (or wing pouch) in third-instar wing discs (Fig. 1B; Ng et al. 1995, 1996; Cifuentes and Garcia-Bellido 1997). *wg* signaling is both necessary and sufficient for the expression of *nub*, as ectopic expression of *wg* (Ng et al. 1996) or ectopic activation of the *wg* pathway (Fig. 1D,K) can induce ectopic expression of *nub*, whereas blocking transmission of the *wg* signal in the wing pouch represses the endogenous expression of *nub* (Fig. 1F,H).

We found that *nub* was ectopically expressed in wing discs that were transheterozygous for null and hypomorphic alleles of *osa* (osa^{e1d308} and osa^{4H}, respectively; Fig. 1C). Similar ectopic *nub* expression was caused by ectopic activation of the *wg* pathway by an activated form of Armadillo (ΔArm; Zecca et al. 1996; Fig. 1D). Conversely, the endogenous expression of *nub* was reduced along the anterior/posterior (A/P) boundary when UAS-

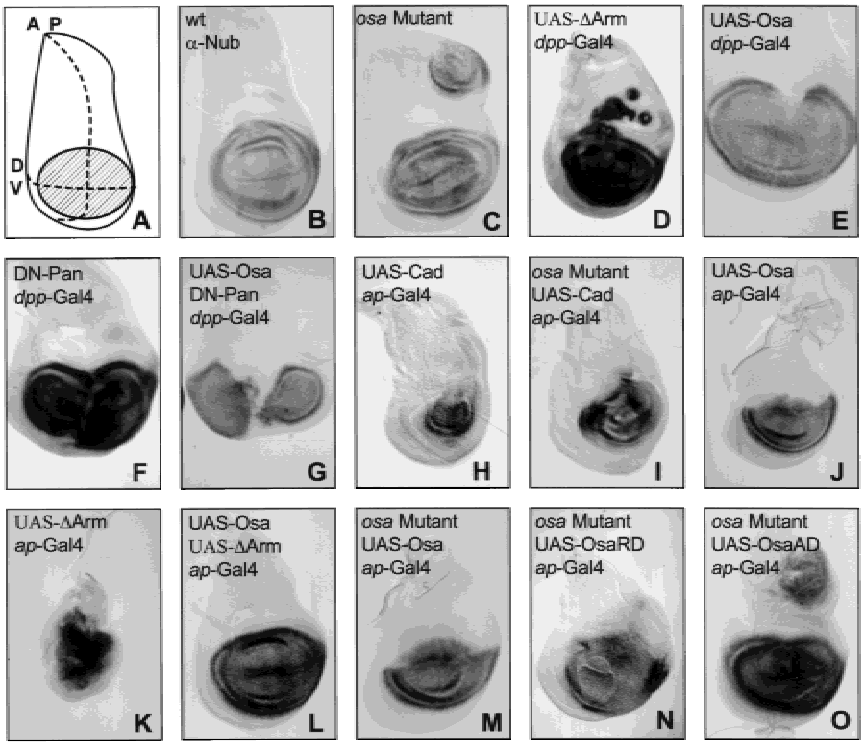

Figure 1. Osa regulates the expression of a *wg*-dependent gene. (*A*) shows a diagram of the third-instar wing disc indicating the anterior/posterior (AP) and dorsoventral (DV) compartment boundaries. The shaded region represents the wing pouch, while the rest of the disc gives rise to the notum. The remaining panels show third-instar wing discs stained with anti-Nubbin antibody from wildtype (*B*), osa^{eld308}/osa^{4h} (*C*), UAS-Δ*arm*/*dpp*-Gal4 (*D*), UAS-osa^{d3}/+; *dpp*-Gal4/+ (*E*), UAS-$dTCF\Delta N^{1}$/+; *dpp*-Gal4/+ (*F*), UAS-$dTCF\Delta N^{1}$, UAS-osa^{d3}/+; *dpp*-Gal4/+ (*G*), UAS-cad^{h7}/*ap*-GAL4 (*H*), osa^{eld308}/osa^{4h}; UAS-cad^{h7}/*ap*-GAL4 (*I*), UAS-osa^{d3}/+; *ap*-GAL4/+ (*J*), UAS-Δ*Arm*/*ap*-GAL4 (*K*), UAS-osa^{d3}/+; UAS-Δ*Arm*/*ap*-GAL4 (*L*), osa^{eld308}/osa^{4h}; UAS-osa^{s2}/*ap*-GAL4 (*M*), osa^{eld308}, UAS-$osaRD^{11c}$/osa^{4h}; *ap*-GAL4/+ (*N*), and osa^{eld308}/osa^{4h}; UAS-$osaAD^{8c}$/*ap*-GAL4 (*O*) larvae. *osa* mutant wing discs have ectopic *nub* expression (*C*) similar to that caused by ectopic activation of the *wg* pathway (*D*). Overexpression of *osa* along the anterior/posterior boundary causes a reduction in *nub* expression (*E*) similar to that caused by blocking the transmission of the *wg* signal (*F*). UAS-Osa and dominant negative Pan act synergistically to strongly reduce *nub* expression (*G*). The expression of UAS-Cad in the dorsal compartment results in the loss of *nub* expression in the dorsal wing pouch (*H*), and this expression is partially rescued in wing discs mutant for *osa* (*I*). Furthermore, the ectopic *nub* expression induced in *osa* mutant discs (*B*) is also rescued by the expression of UAS-Cad (*I*). Overexpression of *osa* in the dorsal compartment almost eliminates *nub* expression (*J*), whereas expression of activated Arm causes *nub* to be expressed in nearly all of the wing disc (*K*). More normal *nub* expression is restored when UAS-Osa and activated Arm are coexpressed (*L*). The ectopic *nub* expression induced in *osa* mutant wing discs (*B*) is rescued by expression in the dorsal compartment of UAS-Osa (*M*) or UAS-OsaRD (*N*) but not by UAS-OsaAD (*O*).

Osa was expressed there with a *decapentaplegic* (*dpp*)–Gal4 driver (Fig. 1E). A similar loss of *nub* expression was caused by the expression of a dominant negative form of Pangolin (Pan) that can no longer bind Arm to activate gene expression (DN-Pan; van de Wetering et al. 1997; Fig. 1F). When Osa and DN-Pan were coexpressed with *dpp*–Gal4, they acted synergistically to cause a severe reduction in *nub* expression (Fig. 1G).

In addition to its role in transmitting the *wg* signal, Arm binds directly to cadherins and is required for the formation of adherens junctions (Cox et al. 1996). Overexpression of *Drosophila* E-Cadherin (DE-Cadherin) can sequester Arm at the plasma membrane and prevent it from participating in Wg signaling; this results in the induction of *wg*-like phenotypes (Sanson et al. 1996). When DE-Cadherin (UAS-Cad) was overexpressed in the dorsal compartment of the wing disc with an apterous (*ap*)-Gal4 driver, dorsal expression of *nub* was lost and the growth of the wing pouch was reduced (Fig. 1H). Reduction of *osa* function in discs expressing UAS-Cad restored more normal *nub* expression and growth (Fig. 1I). Furthermore, the ectopic *nub* expression normally seen in osa^{eld308}/osa^{4H} discs (Fig. 1C) was suppressed by the expression of UAS-Cad in the dorsal compartment (Fig. 1I). Thus, the level of *nub* expression is determined by the relative levels of Arm and Osa when either of these levels is reduced. To increase the levels, we expressed UAS-Osa with *ap*-Gal4, causing a strong reduction of *nub* expression in the dorsal wing pouch (Fig. 1J). Expression of ΔArm with the same Gal4 driver caused *nub* to be expressed in almost the entire wing disc (Fig. 1K). The normal domain of *nub* expression was restored when UAS-Osa and ΔArm were coexpressed (Fig. 1L).

Taken together, these data demonstrate that Osa is required for the repression of a *wg*-dependent gene in vivo. Alterations in the dosage of *osa* can modulate the expression of *wg*-dependent genes even in the presence of an activated form of Arm or a dominant negative form of Pan, suggesting that Osa does not act upstream of Arm. Alterations in the level of active Pan/Arm complexes can also modulate *nub* expression in *osa* mutants; thus, lack of *osa* does not make Wg target genes entirely independent of Arm.

We have shown previously that the ARID DNA-binding domain of Osa fused to the repressor domain of Engrailed (UAS-OsaRD) or the activation domain of VP-16 (UAS-OsaAD) can target these domains to genes normally regulated by *osa* in vivo (Collins et al. 1999). The ectopic expression of *nub* in osa^{eld308}/osa^{4H} wing discs could be prevented by expression of either UAS-Osa or

UAS-OsaRD with *ap*-GAL4 (Fig. 1M,N) but not by expression of UAS-OsaAD (Fig. 1O). This suggests that Osa functions as a repressor of transcription in the regulation of Wg target genes.

Osa function is independent of the up-regulation of cytosolic Arm

To test whether Osa was acting directly on Wg target genes or regulating the expression of some other gene that interacts with the *wg* pathway, we sought to determine at what level in the *wg* pathway Osa acts. In third-instar wing discs, *wg* is expressed in a narrow stripe of cells that straddle the dorsal/ventral (DV) boundary of the wing pouch and directs growth and patterning of the wing blade with respect to the DV axis (Neumann and Cohen 1997). Cells adjacent to the DV boundary respond to the *wg* signal by posttranscriptionally up-regulating cytosolic Arm (Fig 2A,C; Riggleman et al. 1990; Peifer et al. 1994). Arm then translocates to the nucleus and binds to Pan (Orsulic and Peifer 1996; Brunner et al. 1997; van de Wetering et al. 1997) to activate the expression of downstream target genes such as *Distal-less* (*Dll*; Fig. 2B,C; Zecca et al. 1996; Neumann and Cohen 1997).

When an activated form of the protein kinase Sgg that constitutively targets Arm for degradation (UAS-Sgg*; Hazelett et al. 1998) was expressed in the dorsal compartment using the *ap*-Gal4 driver, Arm was not up-regulated (Fig. 2D,F) and *Dll* was not expressed (Fig. 2E,F). Expression of UAS-Osa in the dorsal compartment similarly prevented the expression of *Dll* on the dorsal side of the DV boundary (Fig. 2H,I). However, these cells still responded to the Wg signal by up-regulating cytosolic Arm (Fig. 2G,I). Therefore, Osa represses Wg target genes without affecting the up-regulation of Arm. This places the activity of Osa in the nucleus and argues that Osa may directly repress the expression of Wg target genes.

Osa specifically represses the Wg response of a defined enhancer

To test the requirements for Osa to repress the expression of Wg target genes, we examined the expression of a *lacZ* reporter gene driven by a well-characterized *wg*-responsive enhancer. The midgut enhancer (UbxB) of the *Ultrabithorax* (*Ubx*) promoter drives *lacZ* expression in the embryonic midgut in a pattern that is dependent on

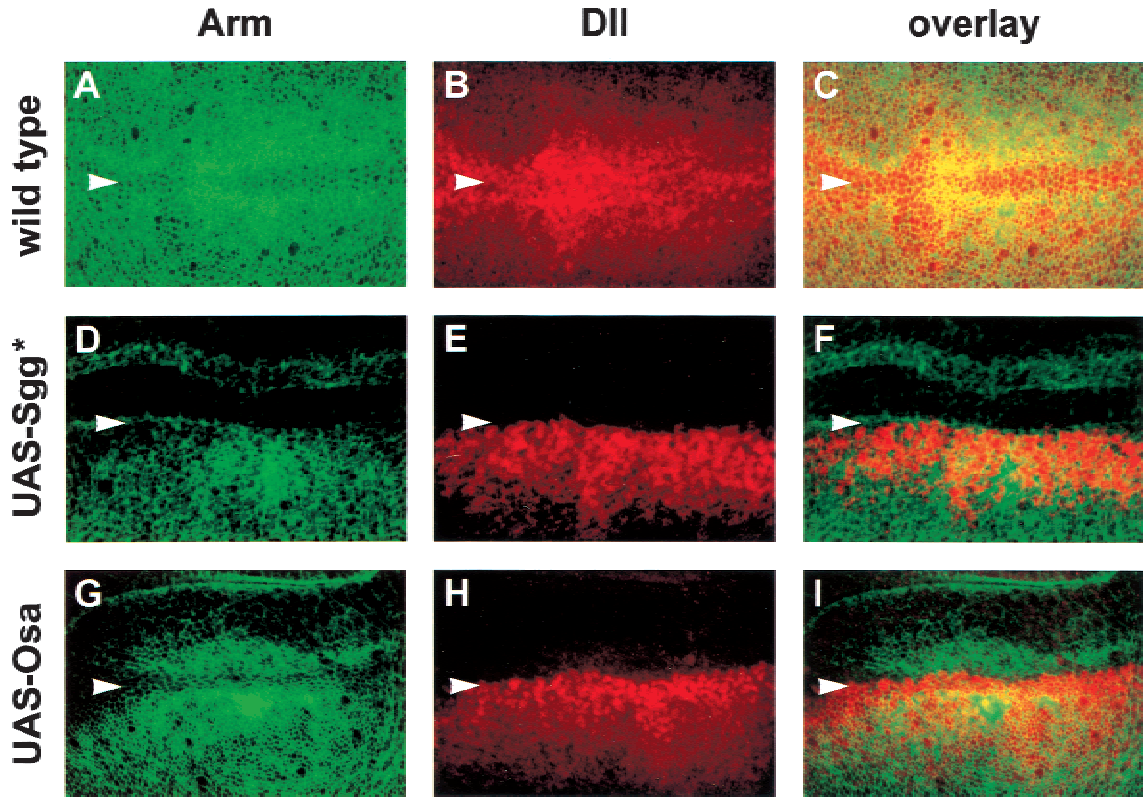

Figure 2. Osa repression of Wg target genes is independent of Armadillo up-regulation. Confocal images of wing imaginal discs stained with anti-Arm (green) and anti-β-galactosidase to reflect *Dll-lacZ* expression (red) from *Dll-lacZ*/+ (*A–C*), UAS-*sgg*^{a7}, *Dll*-lacZ/*ap*-GAL4 (*D–F*), and UAS-*osa*^{d3}/+; *Dll*-lacZ/*ap*-GAL4 (*G–I*) third-instar larvae. *wg* expressed at the dorsal/ventral boundary of the wing pouch (indicated by arrowheads) signals the up-regulation of Arm in adjacent cells (*A*,*C*), which activates the expression of *Dll* (*B*,*C*). Expression of activated Sgg (UAS-Sgg*) in the dorsal compartment blocks the up-regulation of Arm (*D*,*F*) and as a result, these cells fail to express *Dll* (*E*,*F*). Expression of UAS-Osa in the dorsal compartment also represses the expression of *Dll* (*H*,*I*). However, these cells still respond to the Wg signal by up-regulating Arm (*G*,*I*).

both *wg* and *decapentaplegic* (*dpp*; Thuringer et al. 1993). In wild-type embryos UbxB–*lacZ* is expressed primarily in parasegment (ps) 6,7, and 8 with weaker expression in ps 3 (Fig. 3A; Thuringer et al. 1993). This expression was de-repressed in embryos lacking the maternal contribution of *osa*, such that the expression of *lacZ* expanded anteriorly as far as ps 3 (Fig. 3B). Similarly expanded expression was induced by ectopic expression of *wg* in the mesoderm using 24B-Gal4 (Fig. 3C; Thuringer et al. 1993). Conversely, expression of UAS-Osa (Fig. 3D) or UAS-DN-Pan (Fig. 3I) in the mesoderm repressed the expression of UbxB–*lacZ*. However, neither *wg* nor *dpp* was ectopically expressed in the midgut in embryos lacking maternal *osa* (data not shown).

When the *dpp* response element in UbxB is mutated (UbxBC; Eresh et al. 1997), the expression of the *lacZ* reporter is severely reduced; only weak levels of *lacZ* expression are detectable in ps 8 (Fig. 3E; Eresh et al. 1997). Expression of UbxBC–*lacZ* was unchanged in the absence of maternal *osa*, suggesting that the *dpp* response element is still required for the expression of the reporter construct in the absence of Osa. When one of the two *wg* response elements in UbxB is mutated (UbxB4; Riese et al. 1997; Yu et al. 1998), the expression of *lacZ* is reduced in wild-type embryos (Fig. 3G; Riese et al. 1997). However, removal of maternal *osa* allowed an expansion of UbxB4–*lacZ* expression (Fig. 3H). This suggests that lack of *osa* can compensate for a reduction in the responsiveness of the promoter to Wg but not to Dpp. Furthermore, the expression of wild-type UbxB–*lacZ* was also de-repressed in embryos lacking maternal *osa* even in the presence of DN-Pan (Fig. 3J). These data argue that Osa functions specifically to repress the activation of the UbxB enhancer by the Wg pathway.

brahma *and* moira *are also required to repress Wg target genes*

Osa functions as a component of Brm chromatin-remodeling complexes (Collins et al. 1999) and might be acting through the Brm complex to repress Wg target genes. We therefore tested other components of the Brm complex for genetic interactions with the *wg* pathway.

Blocking Wg signaling at the wing margin by expressing UAS-Sgg* with *vg*-Gal4 caused a reduction in wing growth and a loss of the wing margin (Fig. 4A,B). These phenotypes were strongly enhanced in flies heterozygous for *wg* (Fig. 4C) or that coexpressed UAS-Osa (Fig. 4D) and were suppressed in flies heterozygous for *axin* (a negative regulator of Wg signaling; Hamada et al. 1999) or *osa* (Fig. 4E,F). The effects of UAS-Sgg* expression were also suppressed by the loss of one copy of *brm* or

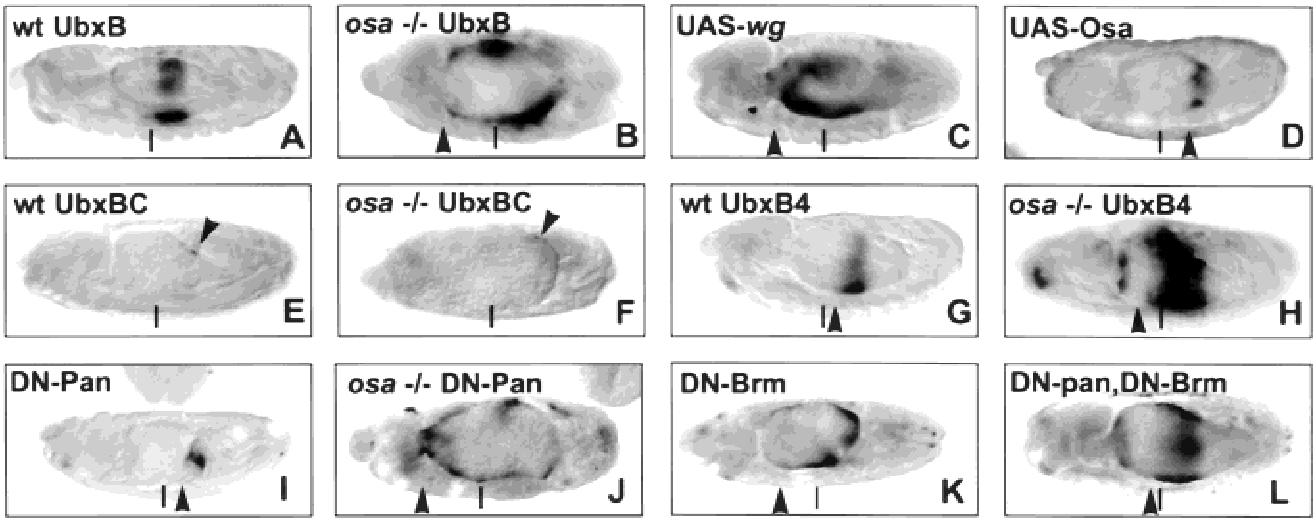

Figure 3. Osa specifically represses a *wg*-responsive enhancer. Photomicrographs of stage-14 embryos stained with anti-β-Gal antibody. All embryos carry *24B*-GAL4; (*A–D*,*I–L*) have *UbxB*-lacZ; (*E*,*F*) have *UbxBC*-lacZ; (*G*,*H*) have *UbxB4*-lacZ; (*C*) has UAS-*wg*; (*D*) has UAS-*osa*; (*I*,*J*,*L*) have UAS-*dTCF*Δ*N*; and (*K*,*L*) have UAS-brm^{K804R}. Embryos derived from osa^{e1d308} germ-line clones are shown in *B*,*F*,*H*, and *K*. In wild-type embryos, *UbxB-lacZ* is expressed in the embryonic midgut in parasegments (ps) 6, 7, and 8 (*A*). In embryos lacking the maternal contribution of *osa*, *UbxB-lacZ* expression is de-repressed and expands anteriorly to ps 3 (*B*); similarly expanded expression is induced by ectopic expression of *wg* in the mesoderm (*C*). Conversely, overexpression of *osa* causes a dramatic reduction in *UbxB-lacZ* expression (*D*). A mutation in the Dpp response element in *UbxB* (*UbxBC*) causes a strong reduction of *lacZ* expression (*E*), and this expression is unchanged in embryos derived from *osa* mutant germ-line clones (*F*). A mutation in one of the Wg response elements in *UbxB* (*UbxB4*) also causes a reduction in the expression of *lacZ* (*G*). However, the expression of *UbxB4-lacZ* is de-repressed in embryos derived from *osa* mutant germ-line clones. Expression of a dominant negative form of Pan (DN-Pan) causes a reduction in *UbxB-lacZ* expression (*I*), whereas expression is expanded in embryos lacking maternal *osa* that also express DN-Pan (*K*). Expression of a dominant negative form of Brm also causes an expansion in *UbxB-lacZ* expression (*K*) and rescues the loss of expression caused by DN-Pan (*L*). Arrowheads indicate the position of the anterior extent of *UbxB-lacZ* expression. Vertical bars indicate 50% embryo length and mark the approximate position of the anterior extent of *UbxB-lacZ* expression in a wild-type embryo. Embryos derived from *osa* mutant germ-line clones have additional abnormalities, and constrictions fail to form in the midgut.

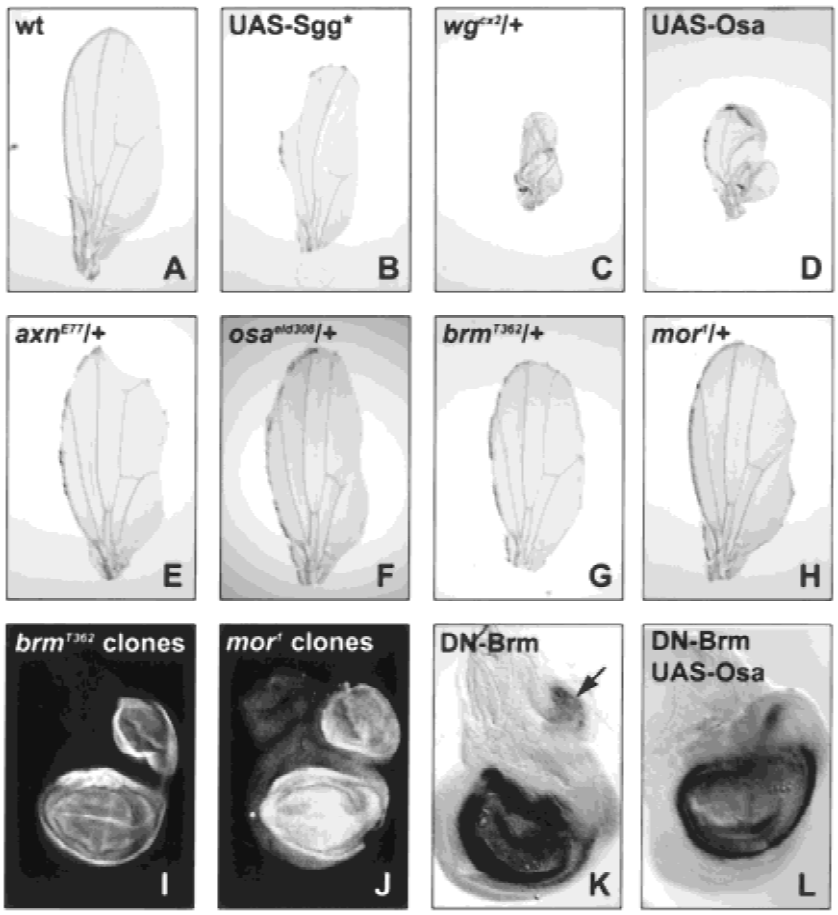

Figure 4. Brm and Mor are required for the repression of Wg target genes. Photomicrographs of adult wings (*A–H*) and third-instar wing discs stained with anti-Nub antibody (*I–L*) from wild-type flies (*A*), UAS-sgg^{a11}/*vg*-Gal4 (*B*), UAS-Sgg^{a11}, wg^{cx2}/*vg*-Gal4 (*C*), UAS-Osa^{d3}/+; UAS-Sgg^{a11}/*vg*-Gal4 (*D*), axn^{E77}/+; UAS-Sgg^{a11}/*vg*-Gal4 (*E*), osa^{e1d308}/+; UAS-Sgg^{a11}/*vg*-Gal4 (*F*), brm^{T362}/+; UAS-Sgg^{a11}/*vg*-Gal4 (*G*), mor^{1}/+; UAS-sgg^{a11}/*vg*-Gal4 (*H*), *FRT80*, brm^{T362}/*FRT80*, *M(3)67C*; *ap*-Gal4, UAS-*FLP*/+ (*I*), *FRT82*, mor^{1}/*FRT82*, *M(3)96C*; *ap*-Gal4, UAS-*FLP*/+ (*J*), UAS-brm^{K804R}/+; ap-Gal4/+ (*K*), and UAS-osa^{d3}/UAS-brm^{K804R}; *ap*-Gal4/+ (*L*). Expression of an activated form of Sgg along the developing wing margin with the *vg*-Gal4 driver causes a reduction in the growth of the wing and a loss of the wing margin (*B*). These phenotypes are enhanced in flies that are heterozygous for *wg* (*C*) or that coexpress UAS-Osa (*D*) and are suppressed in flies heterozygous for *axn* (*E*) or *osa* (*F*). The growth and loss of margin phenotypes are also suppressed in flies heterozygous for *brm* and *mor* (*G*,*H*, respectively). *nub* is ectopically expressed in wing discs with clones of *brm* and *mor* that occupy a large part of the dorsal compartment (*I*,*J*, respectively). Expression of dominant negative Brm in the dorsal compartment induces ectopic expression of *nub* (arrow in *K*). Coexpression of dominant negative Brm also rescues the loss of endogenous expression of *nub* caused by expression of UAS-Osa alone (cf. *L* with Figs. 1J or 5A).

moira (*mor*), which encodes an essential component of the Brm complex (Crosby et al. 1999), or by coexpression of a dominant negative form of Brm (DN-Brm; Elfring et al. 1998; Fig. 4H,I; data not shown). In contrast, two other *trithorax* group genes, *trithorax* (*trx*) and *absent, small, or homeotic discs 2* (*ash2*), which encode components of other nuclear complexes thought to regulate chromatin structure (Mazo et al. 1990; Adamson and Shearn 1996; Papoulas et al. 1998), failed to modify the UAS-Sgg* phenotype (data not shown).

This demonstrates that there is a specific genetic interaction between the *wg* pathway and components of Brm complexes and suggests that these complexes are required for the repression of Wg target genes. Indeed, we found that the *wg*-dependent gene *nub* was ectopically expressed in wing discs that contained large clones of cells mutant for *brm* or *mor* (Fig. 4I,J) or that expressed DN-Brm in the dorsal compartment (Fig. 4K). Furthermore, the loss of *nub* expression caused by expression of UAS-Osa with *ap*-Gal4 was rescued by coexpression of DN-Brm (cf. Fig. 4L with Figs. 1J and 5A), indicating that Brm activity is required for the repression of Wg target genes by Osa. The Wg-dependent UbxB–*lacZ* reporter was also de-repressed in embryos that expressed DN-Brm (Fig. 3K), and coexpression of DN-Brm could rescue the loss of UbxB–*lacZ* expression caused by DN-Pan (Fig. 3L). These results suggest that Osa acts through the Brm chromatin-remodeling complex to prevent the expression of Wg target genes.

osa *interacts genetically with* gro *and* rpd3

In addition to transducing the *wg* signal in a complex with Arm (Brunner et al. 1997; van de Wetering et al. 1997), Pan is also required for the active repression of Wg target genes in the absence of the Wg signal (Cavallo et al. 1998). This repression requires the association of Pan with the corepressor Groucho (Gro; Cavallo et al. 1998). Gro functionally interacts with the histone deacetylase Rpd3, and this interaction is important for at least some of the repressive activity of Gro (Chen et al. 1999). Thus, both Osa-containing Brm complexes and Pan/Gro/Rpd3 complexes repress the expression of Wg target genes and probably mediate this repression by altering the local chromatin architecture at the promoters of these genes. Consistent with this, we found that reduction of *gro* or *rpd3* dosage reduced the ability of Osa to repress *nub*. The loss of *nub* expression caused by expression of UAS-Osa with *ap*-Gal4 (Fig. 5A) was significantly rescued in wing discs homozygous for a hypomorphic allele of *rpd3* ($rpd3^{04556}$; Fig. 6B). Also, larvae transheterozygous for osa^{e1d308} and gro^{E48} often ectopically expressed *nub* in the wing disc (Fig. 5C), and 40% (n = 253) of transheterozygous adults had notum-to-wing transformations (Fig.

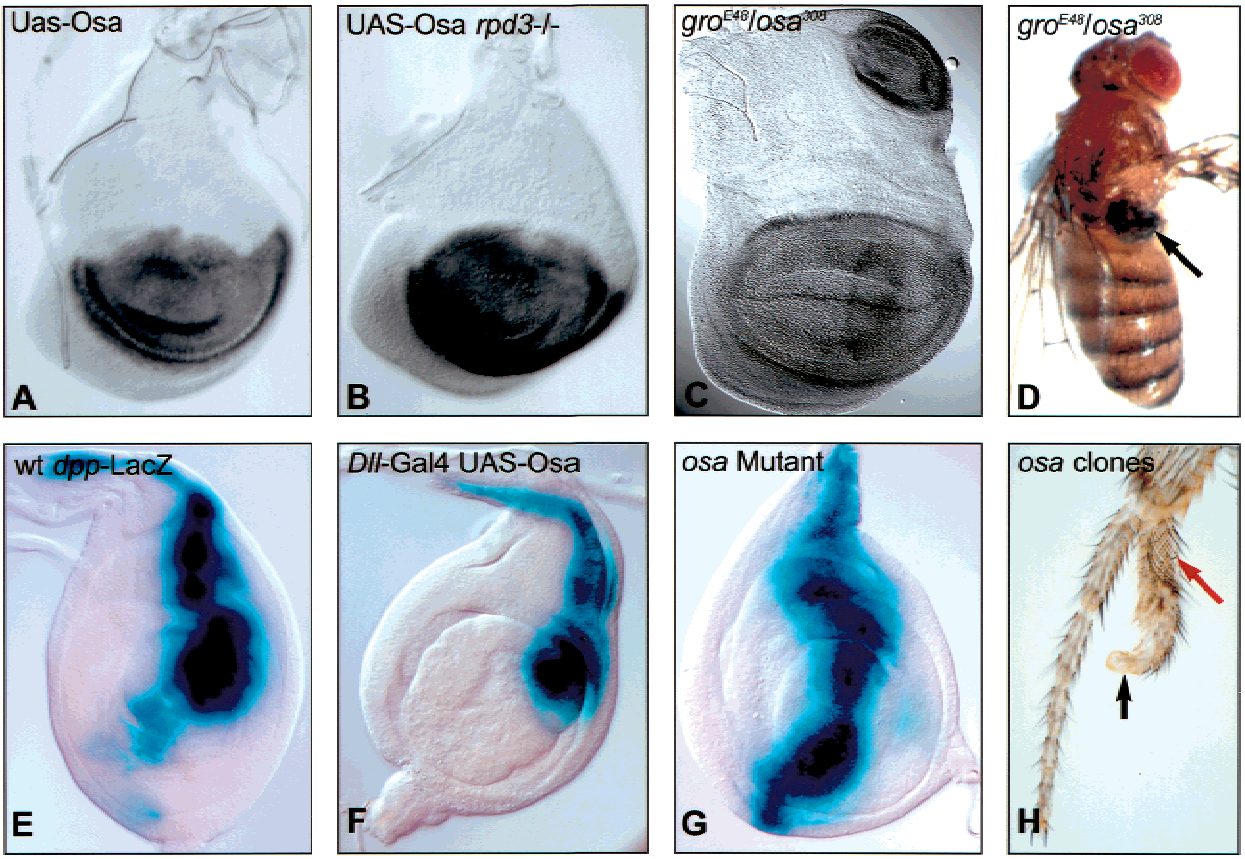

Figure 5. Osa cooperates with Gro and Rpd3 to repress Wg target genes and is required for the Wg-dependent repression of *dpp*. Photomicrographs of third-instar wing discs stained with anti-Nub antibody (*A–C*) from larvae of genotype UAS-osa^{d3}/+; *ap*-Gal4/+ (*A*), UAS-osa^{s2}/*ap*-GAL4; $rpd3^{04556}$/$rpd3^{04556}$ (*B*), and osa^{e1d308}/gro^{E48} (*C*). (*D*) osa^{e1d308}/gro^{E48} adult. The loss of *nub* expression in the dorsal wing pouch caused by the expression of UAS-Osa with *ap*-Gal4 (*A*) is partially rescued in larvae homozygous for a hypomorphic allele of *rpd3* (*B*). Flies that are transheterozygous for *osa* and *gro* often have ectopic expression of *nub* in wing discs (*C*) and notum-to-wing transformation in adults (arrow in *D*). (*E–G*) show leg discs stained for *dpp*-lacZ expression from larvae of genotype *dpp*-lacZ/+ (*E*), UAS-osa^{d3}/+; *dpp*-lacZ/*Dll*-Gal4 (*F*), osa^{e1d308}/osa^{4h}; *dpp*-lacZ/+ (*G*). (*H*) adult leg from FRT82, osa^{e1d616}/FRT82, P(y^{+}); hs*FLP122*/+ fly. *dpp* is expressed along the anterior/posterior boundary in the dorsal compartment of the leg disc (*E*). The expression of UAS-Osa represses the endogenous expression of *dpp* (*F*), whereas *dpp* is ectopically expressed in *osa* mutant leg discs (*G*). Clones of cells mutant for *osa* (marked with *yellow*) generated in leg discs can induce leg duplications in the ventral compartment (*H*). The black arrow indicates the *yellow*, *osa* mutant cells, and the red arrow points to transverse bristle rows, normally found ventrally, within the duplication.

5D). These phenotypes were not seen when *osa* or *gro* single mutants were crossed to wild-type flies.

Osa represses the expression of a gene that is repressed by Wg

Whereas many of the genes regulated by the *wg* pathway require *wg* for their expression, several genes appear to be repressed by high levels of *wg* signaling (Johnston and Schubiger 1996; Theisen et al. 1996; Cadigan et al. 1998; Yu et al. 1998; Payre et al. 1999). To determine the effect of Osa on the expression of genes that are normally repressed by *wg*, we examined the expression of *dpp* in leg discs with altered dosage of *osa*.

In third-instar leg discs, *wg* and *dpp* are expressed along the A/P boundary in the ventral and dorsal compartment, respectively, and mutually antagonize each other's expression (Brook and Cohen 1996; Jiang and Struhl 1996; Johnston and Schubiger 1996; Theisen et al. 1996). We found that *dpp* expression was repressed when UAS-Osa was expressed in a broad central domain of the leg disc with a *Dll*-Gal4 driver (Fig. 5F) and that *dpp* was ectopically expressed in the ventral compartment in osa^{e1d308}/osa^{4H} leg discs (Fig. 5G). Clones of cells mutant for *osa* could also induce leg duplications in the ventral compartment of the leg (Fig. 5H), consistent with the ectopic expression of *dpp* (Brook and Cohen 1996; Jiang and Struhl 1996). Thus, in addition to repressing the expression of genes that are normally activated by the *wg* signal, Osa is also required for the repression of least one of the genes that are repressed by *wg*.

Discussion

Osa directly represses Wg target genes

We show here that the loss of *osa* function in the wing disc results in the ectopic expression of *wg*-dependent genes and that overexpression of *osa* can block the endogenous expression of these genes. Several lines of evidence suggest that Osa directly represses the expression of these genes. Firstly, up-regulation and nuclear accumulation of Arm is both necessary and sufficient for the response of a target gene to the Wg signal (for review, see Cadigan and Nusse 1997). Altering the dosage of *osa* modulates the expression of a Wg target gene without affecting the up-regulation of Arm. This indicates that Osa does not act upstream of Arm and that Osa activity is required in the nucleus for the repression of Wg target genes. In addition, Osa appears to act as a repressor rather than activating the expression of another repressor, as replacement of most of the protein with an exogenous repressor domain preserves its function in Wg target-gene regulation.

Second, increasing *osa* dosage suppresses the ectopic expression of Wg target genes induced when cytosolic-nuclear Arm is maintained at artificially high levels, and reducing *osa* dosage restores Wg target gene expression when Arm is sequestered at the plasma membrane or when a form of Pan that is unable to bind to Arm is overexpressed. Therefore, the expression of a Wg target gene is determined by the relative levels of Osa and activating Pan/Arm complexes.

Finally, the *UbxB* enhancer contains two Wg response elements that are required for full expression of a reporter gene (Yu et al. 1998). A mutation in one of the Wg response elements prevents the recruitment of Arm to that site by Pan and results in a reduction in reporter gene expression (Riese et al. 1997). Loss of *osa* function can compensate for the reduced responsiveness of the enhancer to the Wg signal, allowing it to be more broadly expressed. This suggests that endogenous Osa is able to repress an enhancer lacking this Pan-binding site. However, the remaining Pan-binding site may be necessary for Osa to act on the enhancer; ectopic Osa can only repress the expression of a Wg response element from the *optomotor-blind* gene (K. Hofmeyer and G. Pflugfelder, pers. comm.) when its Pan-binding sites are intact (data not shown).

The most likely explanation of these data is that Osa functions to directly repress Wg target gene expression, with such target genes being defined by their inclusion of a Pan-binding site. Osa function is not exclusive to the Wg signaling pathway; Osa also functions as a promoter specific activator of *Antennapedia* expression and as a coactivator for Zeste and likely represses E2F-mediated gene expression (Staehling-Hampton et al. 1999; Vasquez et al. 1999; Kal et al. 2000). Furthermore, the expression of *even-skipped* is perturbed in embryos lacking maternal *osa*, a phenotype that precedes the expression of *wg* in the embryo (Treisman et al. 1997). However, the strong correlation of the expression of Wg target genes with the level of Osa suggests that counteracting Osa activity is an important function of the Wg pathway.

Brm complex chromatin remodeling activity is required to repress Wg target genes

Osa functions as a component of Brm chromatin-remodeling complexes (Collins et al. 1999). These complexes and closely related complexes in other species such as the yeast SWI/SNF complex catalyze an ATP-dependent alteration in the structure of nucleosomal DNA that can run in either direction to render the DNA either more or less accessible to binding by transcription factors (Cote et al. 1994; Kwon et al. 1994). Whereas chromatin-remodeling complexes are generally thought to promote gene expression, recent reports have demonstrated that they are also required for the repression of some genes. Genome-wide analysis shows that more genes have elevated than reduced expression in a *swi2* mutant yeast strain, and some of these genes are directly repressed by SWI/SNF (Holstege et al. 1998; Sudarsanam et al. 2000). The hBRM complex in humans has been shown to cooperate with the retinoblastoma protein (Rb) to repress E2F-1-mediated activation (Trouche et al. 1997). Furthermore, *brm*, *mor*, and *osa* were identified as enhancers of an E2F gain-of-function phenotype, suggesting that Brm complexes also repress E2F activation in *Drosophila* (Staehling-Hampton et al. 1999).

Because we had found that Osa can antagonize Brm complex function in some tissues (Collins et al. 1999), it was possible that Brm complex activity could be required for the expression of Wg target genes and that Osa might be a negative regulator of Brm complex function. However, our findings that the effects of blocking the *wg* pathway at the wing margin can be suppressed by reducing the dosage of *brm* or *mor* and that *nub* and UbxB–*lacZ* are ectopically expressed when *brm* or *mor* function is lost suggest that Brm complexes are required for the repression, rather than the activation, of Wg target genes. Furthermore, expression of a form of Brm that has a mutation in its ATP-binding site also induces ectopic expression of *nub* and UbxB–*lacZ* and can rescue the loss of *nub* expression caused by overexpression of Osa. Because the ATPase activity of Brm is required for the chromatin-remodeling activity of the Brm complex (Elfring et al. 1998), this suggests that chromatin remodeling by the Brm complex is necessary for Osa to repress the expression of Wg target genes.

Osa cooperates with Pan and Gro to repress Wg target gene expression

In addition to activating gene expression by recruiting Arm to the promoters of *wg*-responsive genes, Pan also represses these same genes (Riese et al. 1997; Yang et al. 2000) by recruiting the transcriptional corepressor Gro (Cavallo et al. 1998). Interestingly, Gro has been shown to interact with the N-terminal tail of histone H3 and with the histone deacetylase Rpd3, and it has therefore been proposed that Gro mediates repression by altering chromatin structure (Palaparti et al. 1997; Chen et al.

1999). Consistent with this, we found a strong genetic interaction between *osa* and *gro* that suggests that their activities in repressing Wg target genes are closely related. Although it has not previously been reported that Rpd3 functions in the repression of *wg* target genes, we have shown that reducing the function of *rpd3* can partly rescue the loss of *nub* expression caused by the overexpression of Osa. Rpd3 is therefore important for the repression of Wg target genes; testing whether it is essential awaits the isolation of null alleles.

The loss of either *osa* or *gro* leads to ectopic expression of Wg target genes; thus, the activity of one is not sufficient to repress the expression of these genes without the activity of the other. Osa and Gro may, therefore, be mediating the same repressive event rather than acting in parallel. Interestingly, Zhang et al. (2000) have reported that human SWI/SNF forms a repressor complex with Rb and the histone deacetylase HDAC. This complex interacts with the *cyclin E* promoter through the binding of Rb to E2F-1 and represses E2F-1 activation of *cyclin E* expression. This suggests the intriguing possibility that Osa and the Brm complex function in a larger repressor complex containing Gro and Rpd3 and that this complex is recruited to Wg target genes though the binding of Gro to Pan. However, Gro acts as a corepressor for a large number of transcription factors (for review, see Fisher and Caudy 1998), and Osa cannot be required for all repression mediated by Gro because loss of *osa* does not result in neurogenic phenotypes like those caused by the loss of *gro* (Treisman et al. 1997). Further research is needed to determine if Gro and/or Rpd3 can directly interact with components of the Brm complex and, if so, what determines the specificity of this interaction.

The mechanism by which Wg signaling leads to the active repression of genes such as *dpp* is not fully understood, although it is counteracted by Sgg (Jiang and Struhl 1996). However, our observation that *dpp* expression is repressed by Osa suggests that other factors may allow Wg signaling to reinforce repressive chromatin modeling by the Brahma complex on such promoters.

Model for the regulation of gene expression by the Wg pathway

The requirement of chromatin remodeling complexes for the repression of Wg target-gene expression clearly demonstrates that regulating chromatin architecture is important for the repression of these genes. However, it is becoming increasingly evident that altering the chromatin conformation at the promoters of Wg target genes is also important for the activation of these genes.

Pan and its vertebrate homologs Lef-1 and TCF belong to the high-mobility group, or HMG, family of proteins. The HMG domain is known to induce a sharp bend in DNA, and this DNA-bending activity has been shown to be important for the activation of gene expression by HMG box transcription factors (for review, see Bustin 1999). It is thought that DNA bending promotes activation by bringing distantly spaced transcription factors into proximity, thus promoting the formation of higher-order activation complexes. Interestingly, it has been reported that Lef-1 bound to the vertebrate homolog of Arm, β-catenin, can induce a sharper bend in DNA than Lef-1 alone (Behrens et al. 1996).

Hecht et al. (2000) have reported that β-cat binds to the histone acetyltransferase (HAT) proteins p300 and CBP and that this interaction can promote β-cat-dependent gene expression. They also report that the HAT activity of CBP is dispensable for this activation. However, these experiments were performed using the transient transfection of an artificial β-cat-responsive reporter gene that may not reflect the true requirement for CBP HAT activity for the expression of an endogenous gene in the context of chromosomal DNA. Alternatively, p300 may promote expression by recruiting other HATs or components of the basal transcriptional machinery (for review, see Grant and Berger 1999).

Drosophila CBP (dCBP) has also been shown to interact with Wg signaling; dCBP appears to negatively regulate Wg signaling by acetylating Pan and disrupting its association with Arm (Waltzer and Bienz 1998). Thus, CBP and dCBP may play opposing roles in Wnt and Wg signaling, respectively. However, in the case of the Interferon beta (IFNβ) enhanceosome complex, CBP HAT activity is not only required for activation by the complex (Merika et al. 1998) but also promotes the dissociation of the complex through the acetylation of the HMG I(Y) component of IFNβ (Munshi et al. 1998). Similarly, dCBP could promote expression of Wg target genes through acetylation of the core histones and later repress expression by acetylating Pan. While the full extent of the role of dCBP remains to be determined, the requirement of chromatin remodeling (and the apparent requirement of Rpd3) for the repression of Wg target gene expression makes it likely that Arm activates target-gene expression by recruiting the HAT activity of dCBP.

Our current model for the regulation of gene expression by components of the Wg pathway is depicted in Figure 6. The chromatin remodeling activity of the Osa-Brm complex is required to maintain the chromatin at the promoters of *wg*-responsive genes in a repressive conformation. This would prohibit the association of other transcription factors with their binding sites and prevent the recruitment of components of the basal transcription machinery. Osa/Brm complexes may be recruited to Wg-responsive genes through an association with Pan/Gro/Rpd3 complexes. In response to the Wg signal, Arm is stabilized and accumulates in the cytosol. This accumulation of cytosolic Arm permits Arm to translocate to the nucleus and displace Gro from Pan and, in so doing, relieve the repression mediated by Gro, Rpd3, and Osa/Brm complexes. Arm may also promote a more open chromatin conformation by recruiting the HAT activity of dCBP, thus permitting the association of other transcription factors with their binding sites. Also, the stimulation of the DNA-bending activity of Pan by Arm may bring distantly spaced transcription factors into juxtaposition to promote the activation of gene expression.

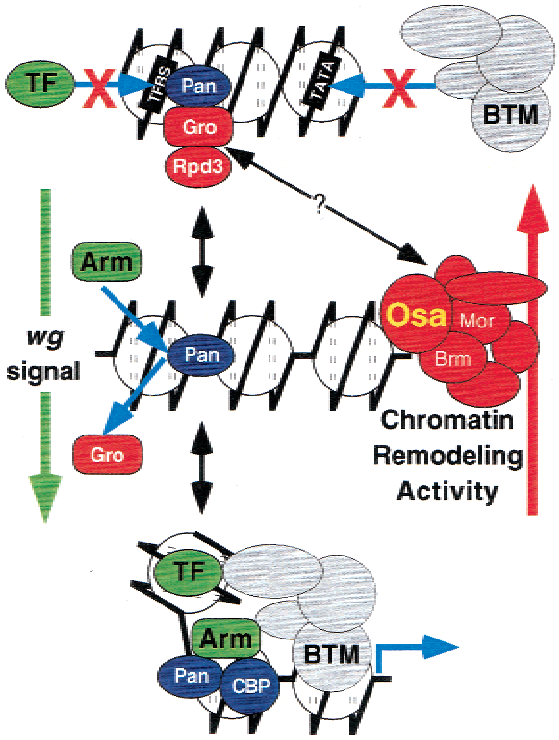

Figure 6. Model for the regulation of gene expression by the Wg pathway. See text for details. TF indicates a transcription factor that requires Wingless signaling for access to its binding site (TFBS). BTM indicates components of the basal transcriptional machinery.

In the absence of *osa*, the chromatin is maintained in a more open and less repressive conformation. This would permit other transcription factors to interact with their binding sites at lower concentrations than would otherwise be possible. Under these conditions, the low levels of Arm that are always present in the cell may be sufficient to promote the activation of gene expression without the Wg signal.

Materials and methods

Genetics

Alleles used were *ash2*^{1}, *rpd3*^{04556}, *wg*^{CX2}, *osa*^{eld308}, *osa*^{eld616}, *gro*^{E48}, *mor*^{1}, *brm*^{2} (Flybase), *brm*^{T362}, *brm*^{T808} (J.E. Treisman, unpubl.), *axn*^{E77} (gift of J. Lee, New York University), and *osa*^{4H} (gift of T. Lebestky, UCLA). The reporters were *Dll*^{01092} (Flybase), *dpp-lacZ* (Blackman et al. 1991), UbxB–*lacZ* (Thuringer et al. 1993), UbxB4–*lacZ* (Riese et al. 1997), and UbxBC–*lacZ* (Eresh et al. 1997) Gal4 driver lines used were *how*$^{24B\text{-}Gal4}$, *ap*^{md544}, *Dll*^{md23} (Flybase), *dpp*-Gal4, *ey*-Gal4 (Hazelett et al. 1998), and *vg*-Gal4 (Simmonds et al. 1995). Transgenic lines used were UAS-*brm*^{K804R} (Elfring et al. 1998), UAS-*Cad* (Sanson et al. 1996), UAS-*dTCFΔN* (van de Wetering et al. 1997), UAS-*FLP* (Duffy et al. 1998), UAS-*fluΔArm* (Zecca et al. 1996), UAS-*osa*, UAS-*osaRD*, UAS-*osaAD* (Collins et al. 1999), UAS-*Sgg*^{S9A} (Hazelett et al. 1998), and UAS-*wg* (Azpiazu et al. 1996). To make mutant clones of *brm* and *mor*, flies of genotype *FRT-80, brm*^{T362}*/TM6B* were crossed to flies of genotype *hs-FLP122; FRT-80, M(3)67C/TM6B* and flies of genotype *FRT-82, mor*^{1}*/TM6B* were crossed to flies of genotype *hs-FLP122; FRT-82, M(3)96C/TM6B*, respectively. Larvae were heat shocked for 1 h at 38.5°C during first and second instars to induce expression of *hs-FLP*. To make germ-line clones mutant for *osa*, *FRT-82, osa*^{eld308}*/TM6B* females were crossed to *hsFLP122*/Y; *FRT82, ovoD/TM3* males, and larvae were heat shocked for 1 h at 38.5°C during first and second instars. The resulting females with germ-line clones were crossed to *24B-GAL4, UbxB(BC, B4)* males. The controls for genetic interactions with UAS-sgg* were the FRT80 and FRT82 chromosomes, on which the other mutations were generated or onto which they were recombined.

Immunohistochemistry

Wing imaginal discs and embryos were stained as described by Treisman et al. (1997). The antibody dilutions were mouse anti-Nub (Ng et al. 1996) 1:5, mouse anti-Arm (Peifer et al. 1994) 1:1, and rabbit anti-β-Gal (Cappel) 1:500. Leg discs from late third-instar larvae were dissected into PBS and fixed for 10 min in 1% glutaraldehyde in PBS and washed twice with PBS. β-Gal activity was detected by incubating the discs in X-gal staining buffer. Embryos and adult wings were mounted in Canada balsam: methyl salicylate (2:1).

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