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Osa associates with the Brahma chromatin remodeling complex and promotes the activation of some target genes

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The yeast SWI/SNF complex and its *Drosophila* and mammalian homologs are thought to control gene expression by altering chromatin structure, but the mechanism and specificity of this process are not fully understood. The *Drosophila osa* gene, like yeast SWI1, encodes an AT-rich interaction (ARID) domain protein. We present genetic and biochemical evidence that Osa is a component of the Brahma complex, the *Drosophila* homolog of SWI/SNF. The ARID domain of Osa binds DNA without sequence specificity *in vitro*, but it is sufficient to direct transcriptional regulatory domains to specific target genes *in vivo*. Endogenous Osa appears to promote the activation of some of these genes. We show evidence that some Brahma-containing complexes do not contain Osa and that Osa is not required to localize Brahma to chromatin. These data suggest that Osa modulates the function of the Brahma complex.
Keywords: brahma/chromatin/eyelid/SWI/SNF/transcription

Introduction

The role of specific transcription factors in activating or repressing gene transcription has been well established. However, more recently it has become clear that in order for these factors to gain access to their target sites *in vivo*, the organized chromatin structure surrounding the DNA must be altered. A number of large protein complexes have been implicated in remodeling chromatin to promote transcription factor access (reviewed by Kadonaga, 1998; Kingston and Narlikar, 1999).

In yeast, components of the SWI/SNF complex were originally identified genetically as factors required for the expression of the *HO* mating type gene or the *SUC2* sucrose fermentation gene, but have since been shown to affect the expression of a large number of other genes (reviewed by Peterson and Tamkun, 1995). A purified yeast or human SWI/SNF-related complex can facilitate transcription factor binding to nucleosomal DNA in an ATP-dependent reaction (Cote *et al*., 1994; Kwon *et al*., 1994). The SWI2/SNF2 component of the complex contains the DNA-stimulated ATPase activity (Laurent *et al*., 1992, 1993). The complex can bind to the minor groove of DNA, with an affinity not affected by sequence but influenced by DNA conformation (Quinn *et al*., 1996). Although the SWI/SNF complex is not essential for viability (Stern *et al*., 1984), another yeast chromatin remodeling complex, RSC, with an STH1 subunit related to SWI2/SNF2, is essential (Cairns *et al*., 1996).

In *Drosophila*, both genetic and biochemical approaches have led to the discovery of chromatin remodeling complexes. The *Polycomb* group of genes is required to maintain repression of homeotic genes such as *Ultrabithorax*; they are thought to do so by forming a repressive chromatin structure (reviewed by Pirrotta, 1997). Some members of the *trithorax* group of genes were subsequently identified by their ability to suppress dominant *Polycomb* phenotypes, suggesting that they might be involved in homeotic gene activation at the level of chromatin (Kennison and Tamkun, 1988). Indeed, two members of this group, *brahma* (*brm*) and *moira* (*mor*), have now been shown to encode proteins related to subunits of the SWI/SNF and RSC complexes, SWI2/SNF2/STH1 and SWI3/RSC8, respectively (Tamkun *et al*., 1992; Crosby *et al*., 1999). The products of two other members of the group, *absent, small and homeotic discs* 1 and 2 (*ash1* and *ash2*; Shearn, 1989), do not form part of this complex but are present in two distinct large nuclear complexes, the function of which has not been established (Papoulas *et al*., 1998). The products of the genes *trithorax* (*trx*) and *kismet* (*kis*) contain regions of homology to other proteins implicated in chromatin remodeling; Trx has a SET [Su(var)3–9, E(Z), Trx] domain and Kis has the domains characteristic of chromodomain-helicase (CHD) proteins (Mazo *et al*., 1990; Daubresse *et al*., 1999). Three other complexes, nucleosome remodeling factor (NURF), chromatin accessibility complex (CHRAC) and ATP-dependent chromatin assembly and remodeling factor (ACF), have been isolated from *Drosophila* embryo extracts using a biochemical assay for nucleosome array disruption (Tsukiyama and Wu, 1995; Ito *et al*., 1997; Varga-Weisz *et al*., 1997). All three contain imitation switch (ISWI), a protein with homology to the ATPase domain of SWI2/SNF2, but differ in their other subunits (Tsukiyama *et al*., 1995; Ito *et al*., 1997; Varga-Weisz *et al*., 1997).

Two human homologs of the SWI/SNF and RSC complexes can be distinguished by their SWI2/SNF2-related subunit; one complex contains human Brahma (hBRM) and the other contains Brahma-related gene 1 (*BRG1*) (Wang *et al*., 1996a). Five other subunits of these complexes are homologous to components of both the yeast and *Drosophila* complexes (Wang *et al*., 1996b). It has recently been shown that four components of the complex, BRG1, INI1 (the SNF5 homolog), BAF155 and BAF170

(SWI3 homologs), can reconstitute *in vitro* nucleosome remodeling activity almost equal to that of the full complex, suggesting that other subunits may play a modulatory role (Phelan *et al*., 1999).

An unanswered question is whether and how these complexes are targeted to specific genes. Although SWI/SNF has no apparent DNA sequence preference (Quinn *et al*., 1996), mutations in SWI2 affect only a small subset of the genome, causing the transcription of some genes to be up-regulated and others down-regulated (Holstege *et al*., 1998). It is possible that transcription factors showing specific promoter interactions, such as GAL4 or Trx, can recruit the complex to their target genes (Cote *et al*., 1994; Rozenblatt-Rosen *et al*., 1998). However, there is little understanding of what interactions might promote such recruitment.

The *osa* trithorax group gene of *Drosophila*, previously molecularly characterized under the name *eyelid* (Treisman *et al*., 1997; Vazquez *et al*., 1999), encodes a large protein with an AT-rich interaction (ARID) domain like that of SWI1, and a second domain that is conserved in multicellular organisms but is not present in yeast (Treisman *et al*., 1997). Although Osa was not found in the previously reported Brm complex (Papoulas *et al*., 1998), we show here that both biochemical and genetic interactions indicate that it is a component of a subset of Brm complexes. The ARID domain of Osa binds DNA apparently without sequence specificity *in vitro*, but it is sufficient to target specific genes for regulation *in vivo*. Analysis of transgenic flies containing constitutively activating or repressing forms of the Osa protein implies that *osa* is required for the activation of some target genes. We suggest that Osa is required for some functions of the Brm chromatin remodeling complex, and that both its DNA-binding capacity and other interactions contribute to promoter targeting.

Results

osa genetically interacts with trithorax group genes

It was recently reported (Vazquez *et al*., 1999) that the gene we had named *eyelid* (*eld*; Treisman *et al*., 1997) is allelic to the previously described *trithorax* group gene *osa* (Kennison and Tamkun, 1988); we will henceforth refer to our alleles as osa^{eld308} and osa^{eld616}. Vazquez *et al*. (1999) also showed that flies heterozygous for both *osa* and *brm*, which encodes a homolog of the yeast SWI2/SNF2 DNA-dependent ATPase (Tamkun *et al*., 1992), have a held-out wings phenotype. We have further characterized the genetic interactions of *osa* with *brm* and other members of the *trithorax* group.

Ectopic expression of a dominant-negative form of Brm with a mutation in the ATP binding site (UAS-brm^{K804R}) disrupts many developmental processes (Elfring *et al*., 1998). We used an *optomotor-blind* (*omb*)-GAL4 driver to direct expression of UAS-brm^{K804R} in the central region of the wing disc; this resulted in loss of the distal wing margin, formation of ectopic campaniform sensillae and wing margin bristles, and disruptions in wing vein morphology (Elfring *et al*., 1998; Figure 1B). These phenotypes were strongly enhanced in animals heterozygous for *osa* (Figure 1C). Expression of UAS-brm^{K804R} at the wing margin using *vestigial* (*vg*)-GAL4 resulted in the loss of the proximal, posterior wing margin, a phenotype that was again enhanced in *osa* heterozygotes (Figure 1D and E). We tested the effect of increasing *osa* dosage by co-expressing a full-length *osa* transcript under the control of the same *vg*-GAL4 driver, and found that this completely rescued the dominant-negative Brm phenotype (Figure 1F).

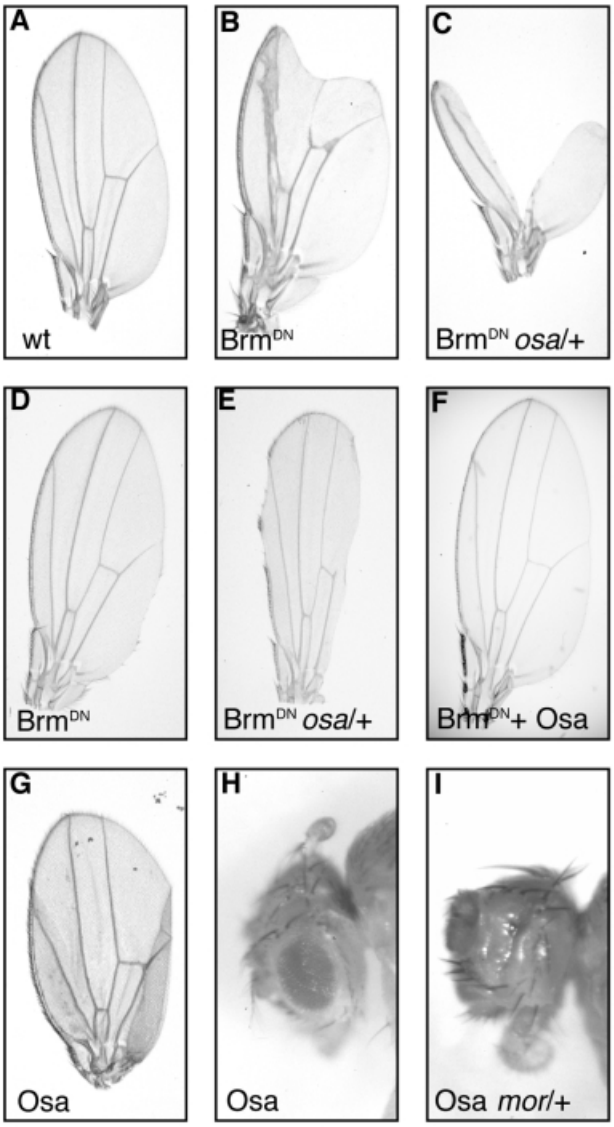

Fig. 1. Genetic interactions between *osa* and *brm*. Photomicrographs of adult wings (A–G) and adult heads (H and I) from wild-type (**A**), *omb*-GAL4/Y;UAS-brm^{K804R}/+ (**B**), *omb*-GAL4/Y;UAS-brm^{K804R}/osa^{eld308} (**C**), *vg*-GAL4/+;*UAS-*brm^{K804R}/+ (**D**), *vg*-GAL4/+;UAS-brm^{K804R}/osa^{eld308} (**E**), *vg*-GAL4/+;UAS-brm^{K804R},UAS-osa^{d3}/+ (**F**), *vg*-GAL4/+;UAS-osa^{d3}/+ (**G**), *ey*-GAL4/UAS-osa^{s2} (**H**) and *ey*-GAL4/UAS-osa^{s2};mor^{1}/+ (**I**) flies. Expression of dominant-negative *brm* with the *omb*-GAL4 (B) and *vg*-GAL4 (D) drivers induces the loss of the distal wing margin and a disruption of wing vein morphology, and the loss of the posterior wing margin, respectively. These phenotypes are strongly enhanced in flies heterozygous for *osa* (C and E). The loss of wing margin induced by expression of dominant-negative *brm* with *vg*-GAL4 (D) is rescued by co-expression of UAS-*osa* (F). Flies that express UAS-*osa* with *vg*-GAL4 have small wings that lack proximal hinge structures (G); these phenotypes are rescued by co-expression of dominant-negative *brm*. Flies that express UAS-*osa* from the *ey*-GAL4 driver have a small eye phenotype (H). This phenotype is enhanced in flies heterozygous for *mor*, occasionally resulting in flies with missing eyes (I).

Table I. Genetic interactions

Genotype	Number	Percentage <50%	Percentage missing
UAS-*osa*^{d3}/+;*ey*-GAL4/+	672	20.2 ± 3.0	1.0 ± 0.7
UAS-*osa*^{s2}/*ey*-GAL4	567	19.4 ± 3.3	–
UAS-*osa*^{d3}/*osa*^{eld308};*ey*-GAL4/+	581	11.0 ± 2.6	2.5 ± 1.3
UAS-*osa*^{d3}/+;UAS-*osa*^{s2}/*ey*-GAL4	123	39.0 ± 8.6	17.9 ± 6.8
UAS-*osa*^{s2}/*ey*-GAL4;*mor*^{1}/+	264	68.6 ± 5.6	12.1 ± 3.9
UAS-*osa*^{s2}/*ey*-GAL4;*snr1*^{F1}/+	191	45.0 ± 7.1	–
UAS-*osa*^{s2}/*ey*-GAL4;*brm*^{2}/+	331	29.6 ± 4.9	–
UAS-*osa*^{d3},UAS-*brm*^{K804R};*ey*-GAL4/+	384	25.0 ± 4.3	14.8 ± 3.7
UAS-*brm*^{K804R}/+;*ey*-GAL4/+	228	14.5 ± 4.6	7.9 ± 3.5
UAS-*osa*^{s2}/*ey*-GAL4;*ash1*^{22}/+	212	13.2 ± 4.6	–

UAS-*osa* expressed with *ey*-GAL4 causes a variable reduction in the size of adult eyes (see Figure 1H). The number of flies with eyes less than half the size of wild-type eyes (percentage <50%) and the number of flies with missing eyes (percentage missing) is significantly increased in flies heterozygous for genes that encode components of Brm complexes (*brm*^{2}, *mor*^{1} and *snr1*^{F1}) and that co-express dominant-negative Brm, but is unchanged in flies heterozygous for the *trithorax* group gene *ash1*^{22} that does not associate with Brm.

Interestingly, ectopic expression of *osa* alone with *vg*-Gal4 induced a dominant loss of proximal wing hinge structures (Figure 1G), and this phenotype was also rescued in animals co-expressing *osa* and dominant-negative *brm* (Figure 1F). This suggests that the functions of Osa and Brm are closely related, as a reduction in the activity of one can compensate for an excess of the other.

Ectopic expression of Osa in eye imaginal discs using *eyeless* (*ey*)-GAL4 resulted in a variable reduction in eye size (Figure 1H). Rather than the expected suppression, we observed an enhancement of this phenotype in flies that either co-expressed dominant-negative Brm or were heterozygous for *brm* (Table I). The eye phenotype was also enhanced by *mor* and *SNF5-related 1* (*Snr1*; Figure 1 and Table I), both of which encode components of the Brm complex (Dingwall *et al*., 1995; Crosby *et al*., 1999). However, reducing the dosage of the *trithorax* group genes *trx*, *ash1* or *ash2* did not enhance the Osa overexpression phenotype (Table I; data not shown). As expected, a reduction in *osa* dosage suppressed the small eye phenotype (Table I). Clones of *mor* mutant cells in the eye disc exhibited a severe reduction in growth (Brizuela and Kennison, 1997), which was partially rescued if the cells were also mutant for *osa* (data not shown). Taken together, these data demonstrate that *osa* shows strong and specific genetic interactions with components of the Brm complex. However, in the wing *osa* appears to act in concert with *brm*, whereas in the eye *osa* opposes the functions of *brm*, *snr1* and *mor*.

Osa physically interacts with the Brm complex

The Brm protein is thought to function in a large (~2 MDa) multiprotein complex that regulates gene expression through the alteration of chromatin architecture (Papoulas *et al*., 1998). The genetic interactions between *osa* and *brm* suggested that the encoded proteins might function together to alter chromatin structure. To investigate this we tested whether Osa and Brm could physically interact *in vivo*. Nuclear extracts from *Drosophila* Schneider cells were immunoprecipitated with anti-Osa antibody and blotted with antibodies against Brm or Snr1, or antibodies against ISWI or Ash2, components of different complexes that do not contain Brm (Papoulas *et al*., 1998). Brm and Snr1, but not ISWI or Ash2, were present in Osa immunoprecipitates (Figure 2). Similarly, Osa could be detected in nuclear extracts immunoprecipitated with either anti-Brm or anti-Snr1 antibody (Figure 2).

To determine whether Osa was associated with the high molecular weight Brm complex, we fractionated Schneider cell nuclear extracts through a glycerol gradient and immunoblotted with antibodies against the various proteins. Figure 3A shows that Osa, Brm and Snr1 co-sediment in the bottom third of the gradient, suggesting that they are part of a large protein complex. Although Osa and Brm are present in similar fractions, Snr1 sediments in the bottom half of the gradient and could also be part of another complex that does not contain Osa or Brm. Alternatively, the anti-Snr1 antibody might be much more sensitive, detecting very low levels of the Snr1 protein. When glycerol gradient fractions were immunoprecipitated with anti-Osa antibody, Osa, Brm and Snr1 were co-precipitated in the same region of the gradient in which they co-sediment (Figure 3B). ISWI and Ash2 both showed broad sedimentation patterns, appearing in the bottom half of the gradient, but neither protein was immunoprecipitated from the gradient fractions with anti-Osa antibody (data not shown). Thus, *in vivo*, Osa is found in a large complex with Brm and Snr1, but does not bind to proteins in other chromatin remodeling complexes.

To identify other proteins that co-sediment and co-immunoprecipitate with Osa, pooled gradient fractions 18–20 were immunoprecipitated with either anti-Osa or anti-Brm antibodies. Silver staining of the resulting purified protein complexes showed that all the bands in the Osa complex (Figure 3C, lane 3) were also present in the Brm complex (lane 4). We detected all the Brm complex components previously described (Papoulas *et al*., 1998) except BAP74. Two bands with molecular weights of 360 and 300 kDa were shown by mass spectrometry analysis to correspond to Osa. Although only a single *osa* RNA species has been detected on Northern blots (Vazquez *et al*., 1999), it is possible that two alternatively spliced products were not well resolved; alternatively, one of the bands could represent a proteolytically cleaved or post-translationally modified form of the protein. Two bands present at a molecular weight of 155–160 kDa were likewise shown by mass spectrometry analysis to correspond to Mor (BAP155; Papoulas *et al*., 1998; Crosby *et al*., 1999).

Interestingly, several protein bands present in the Brm

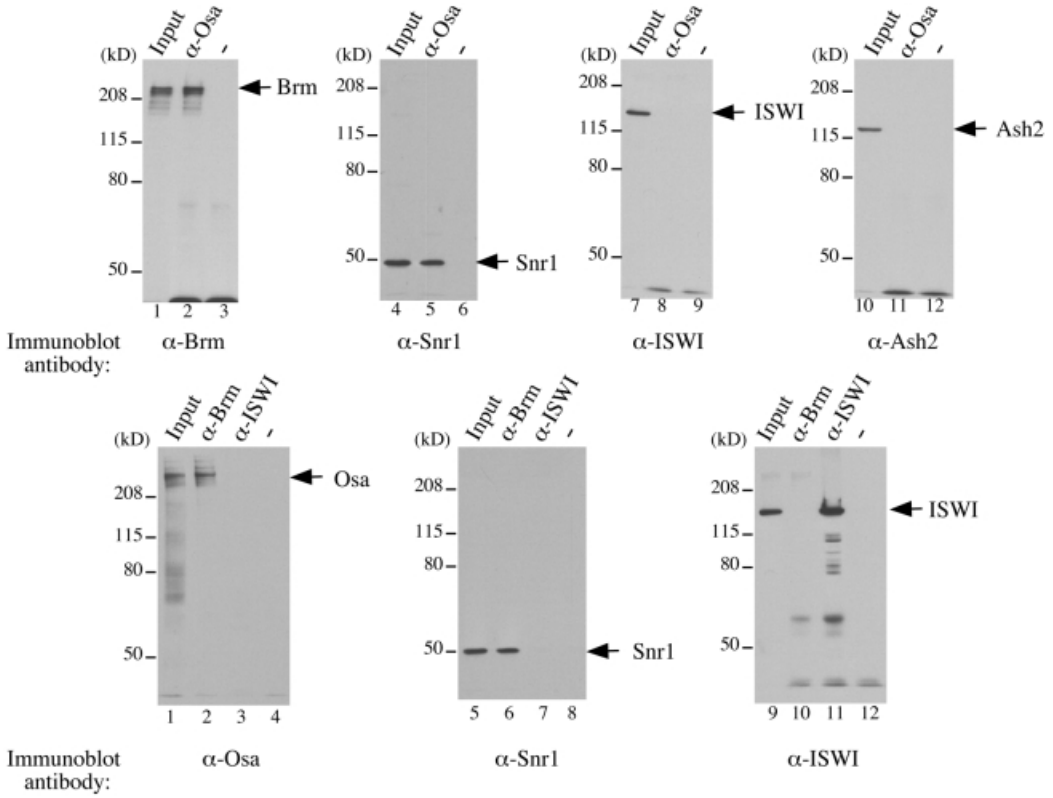

Fig. 2. Osa co-precipitates with Brm and Snr1. Immunoprecipitations of proteins from Schneider cell nuclear extracts (250 μg). The antibodies used for immunoprecipitation are indicated above each lane and the antibodies used for Western blotting are indicated below each panel. The input lane shows 5% of the nuclear extract used for the IP, and the (–) lane is a mock IP using protein A–Sepharose beads alone. Osa co-immunoprecipitates with Brm and Snr1, but not with ISWI or Ash2, and Brm co-immunoprecipitates with Osa and Snr1, but not with ISWI or Ash2 (data not shown).

complex were absent from the Osa complex. Proteins of ~190 and 380 kDa (P190* and P380*), as well as at least five additional very large proteins, depicted in Figure 3C, were detected in the complex immunoprecipitated with anti-Brm antibody but not in the complex immunoprecipitated with anti-Osa antibody. This suggests that Brm is a component of at least two distinct complexes, and that Osa is only present in a subset of these.

Osa is a non-specific DNA-binding protein

Osa contains a region that shares homology with the ARID family of DNA-binding proteins (Treisman *et al*., 1997). The ARID domain mediates the binding of DNA at AT-rich sequences, with contacts in the minor groove (Herrscher *et al*., 1995; Gregory *et al*., 1996; Yuan *et al*., 1998). Some ARID domain proteins, such as mouse Bright and *Drosophila* Dead ringer (Dri), recognize specific DNA sequences (Herrscher *et al*., 1995; Gregory *et al*., 1996), whereas others, such as human MRF, appear to interact with DNA without sequence specificity (Huang *et al*., 1996). The yeast SWI1 protein also contains an ARID domain and may mediate non-sequence-specific binding of the SWI/SNF complex to DNA (Quinn *et al*., 1996). We were interested in determining whether Osa could bind DNA and with what specificity.

Dri was isolated by its ability to bind to a multimer of the consensus binding sequence (NP) for the homeodomain of Engrailed (En) (Gregory *et al*., 1996). Because of the homology between Osa and Dri, we tested whether the predicted DNA-binding domain of Osa could also bind to NP. A fusion protein containing a region of Osa including its ARID domain linked to glutathione *S*-transferase (GST–osaDB; see Figure 6A), but not GST alone (data not shown), was able to retard the migration of a labeled oligonucleotide containing three copies of the NP sequence (NP_3) in a gel mobility shift assay (Figure 4A). This DNA–protein complex could be supershifted by the addition of an antibody specific to Osa, but not by an unrelated antibody. This demonstrated that the ARID domain of Osa encodes a functional DNA-binding domain, and that the anti-Osa antibody, which was generated against a peptide that partially overlaps the DNA-binding domain (Treisman *et al*., 1997; Figure 6A) is still able to recognize its epitope when Osa is bound to DNA. Osa also bound *in vitro* to the enhancer regions of two genes with altered expression in *osa* mutants, *even-skipped* (*eve*) and *Ultrabithorax* (*Ubx*; data not shown).

We next attempted to determine the sequence specificity of DNA binding by Osa using a PCR-based random oligonucleotide selection assay (Thiesen and Bach, 1990; Wilson *et al*., 1993). However, this produced no clear consensus sequence other than an apparent preference for AT-rich sequences (data not shown). As a more direct test of specificity, we allowed the Osa DNA-binding domain to bind to the 50 kb genome of phage lambda cut into 123 fragments. Osa bound to all these fragments with similar affinity at all salt and competitor DNA concentrations tested (Figure 4B; data not shown). Under the same

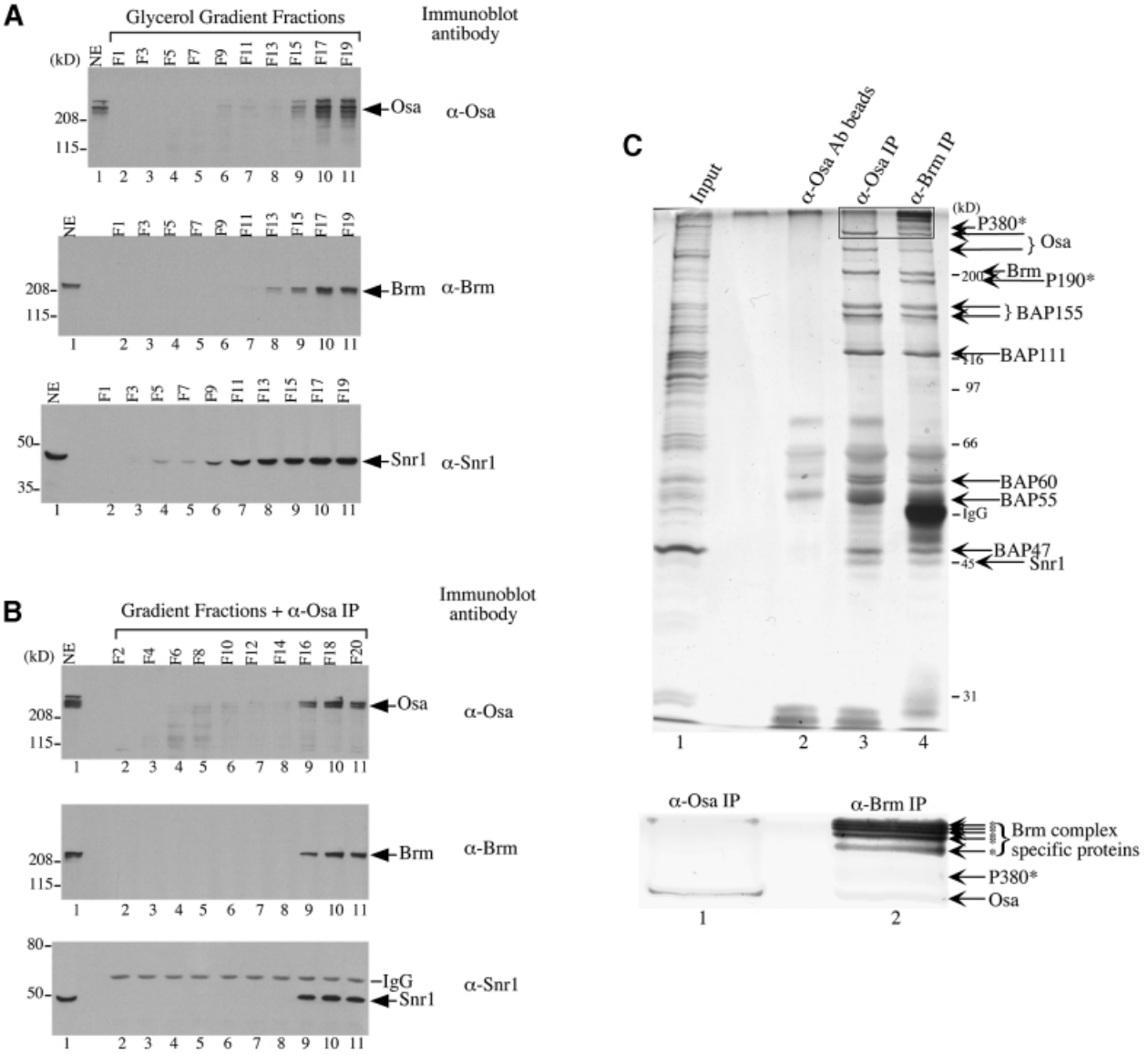

Fig. 3. Osa is present in a large complex with Brm and Snr1. (**A** and **B**) Nuclear extracts from *Drosophila* Schneider cells were fractionated by glycerol gradient sedimentation and immunoblotted with the antibodies indicated. Lane 1 of each immunoblot represents 8 μl (~40 μg) of nuclear extract, or 3% of the amount loaded on the gradient. Osa and Brm are present in the same fractions (F15–19), and Snr1 is present in these fractions but also extends into a lighter region of the gradient. (B) shows immunoprecipitations of the same glycerol gradient fractions with anti-Osa, blotted with anti-Osa, anti-Brm and anti-Snr1. The three proteins are present in the same high molecular weight complex. Immunoprecipitation of the gradient fractions with anti-Snr1 did not bring down Ash1, Ash2 or ISWI (data not shown). Molecular mass standards were run in a parallel gradient and their peak positions were: BSA (68 kDa, F6), aldolase (158 kDa, F9), catalase (240 kDa, F13). (**C**) Silver staining of proteins immunoprecipitated from glycerol gradient fractions 18–20 with either anti-Osa-conjugated protein A–Sepharose (lane 3) or anti-Brm and protein A–Sepharose (lane 4). Anti-Osa antibody-conjugated beads were loaded directly in lane 2 as a control for the background bands. The top of the gel in lanes 3 and 4 (boxed) is enlarged below for better viewing of the proteins of high molecular mass. All protein bands in the Osa complex (lane 3) were found to be present in the Brm complex (lane 4). The protein bands uniquely found in the Brm complex and absent in the Osa complex are indicated with asterisks. Interestingly, the intensities of the Osa bands are somewhat weaker in the Brm complex (lane 4) than in the Osa complex (lane 3) relative to other bands present in both complexes. The assignments of the BAP111, BAP60, BAP55, BAP47 and Snr1 protein bands were based on comparison with the Brm complex shown in Papoulas *et al.* (1998) and on immunoblotting for Snr1. The assignments of the Brm, Osa and BAP155 bands were based on mass spectrometry analysis. BAP74 (Papoulas *et al*., 1998) was not detected in our purified Osa and Brm complexes; it may not be co-purified by this method, or it may not stain as well with silver as with Sypro-Orange.

conditions, the sequence-specific DNA-binding protein E47 (Sun and Baltimore, 1991) selected only a few fragments (Figure 4B, lane 6). Furthermore, in gel mobility shift assays a mutated form of NP_3, in which the invariant central Ts were replaced by Cs, was able to compete with labeled NP_3 for binding to GST–osaDB as efficiently as the wild-type oligonucleotide (data not shown). Thus, the specific sequence of NP does not affect its ability to bind Osa.

We were not able to produce full-length Osa protein *in vitro*, but to examine the DNA-binding activity of the full-length protein *in vivo* we stained polytene chromosomes with anti-Osa antibody. Unlike many sequence-specific DNA-binding proteins that have been shown to recognize specific polytene bands (Kuzin *et al*., 1994; Serrano *et al*., 1995), Osa was localized along the entire length of all the chromosomes (Figure 5B). Osa antibody staining was present not only in the bands that stained

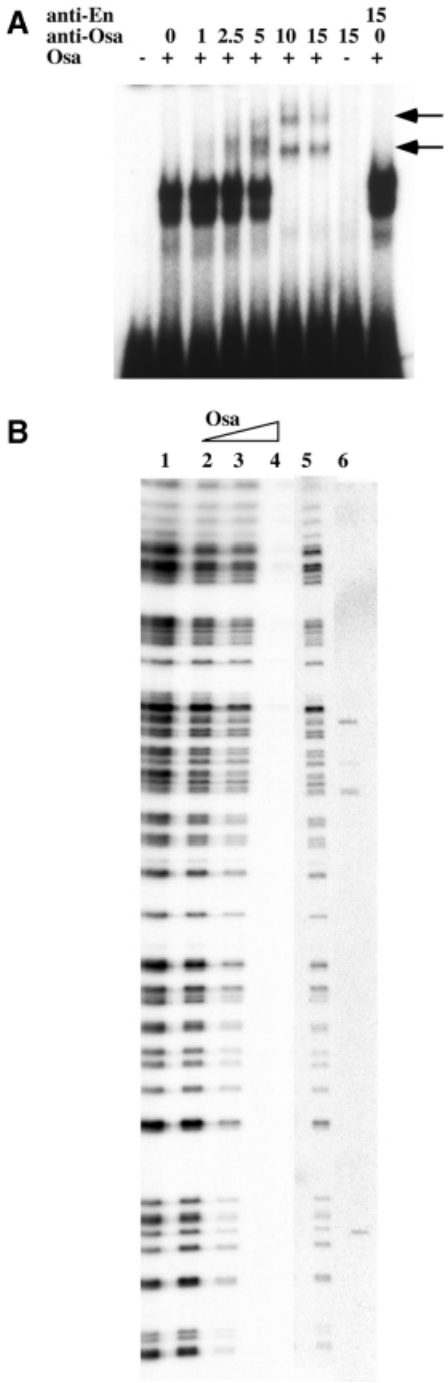

Fig. 4. Osa binds DNA without sequence specificity. (**A**) Gel shift of labeled NP_3 DNA with GST–osaDB (250 ng) and increasing amounts of anti-Osa or anti-En antibody as indicated (in µl). The complex formed with the Osa DNA-binding domain can be supershifted by addition of anti-Osa antibody (arrows). (**B**) Pulldown of labeled fragments of lambda DNA. Lane 1 shows the input DNA and lanes 2–4 show the DNA retained by GST–osaDB at increasing salt concentrations (200, 300 and 400 mM KCl). Lane 5 shows the DNA retained by GST–osaDB at 300 mM KCl and lane 6 shows the DNA retained by GST–E47 under the same conditions. Osa binds all fragments with equal affinity, while E47 selects specific fragments.

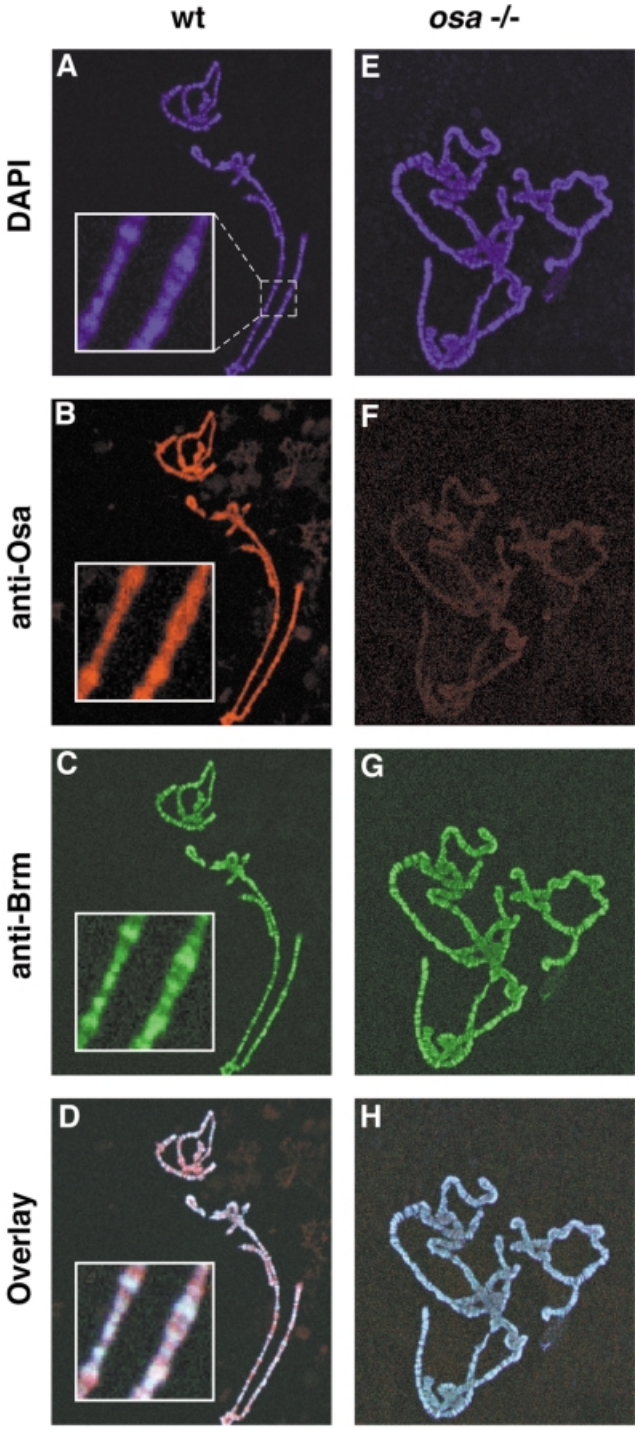

Fig. 5. Co-localization of Brm and Osa. Confocal images of wild-type polytene chromosomes (A–D) and chromosomes from *osa* mutant salivary gland clones (E–H). DAPI staining in blue (**A** and **E**), anti-Osa staining in red (**B** and **F**), anti-Brm staining in green (**C** and **G**) and the overlay (**D** and **H**). Anti-Osa and anti-Brm antibodies stain polytene chromosomes along their entire length (B and C). The staining pattern with anti-Brm is unchanged in *osa* mutant cells (G and H). Insets in (A–D) show higher magnification views of the boxed region.

strongly with DAPI, but also in the interband regions where DNA staining is less prominent (Figure 5A and D). As a control, we showed that antibodies directed against Male-specific-lethal-1 (Msl-1; Hilfiker *et al*., 1994) specifically stain the male X chromosome under the same conditions (data not shown). These data suggest that *osa* encodes a functional DNA-binding protein that binds DNA without sequence specificity *in vitro* and is associated extensively with chromosomal DNA *in vivo*.

Osa is not required for Brm localization to chromatin

Brm and its homologs in other species are components of large multiprotein complexes that associate with and remodel chromatin; however, it is not clear which components of these complexes are responsible for chromatin binding. It has been suggested that SWI1 functions to target the yeast SWI/SNF complex to DNA (Quinn *et al*., 1996). Osa has a DNA-binding domain related to that of SWI1 and, like the SWI/SNF complex, it binds DNA without sequence specificity. We therefore tested whether Osa might function to target Brm complexes to chromosomal DNA.

An antibody to Brm stains polytene chromosomes along their entire length in a pattern similar to that of DAPI staining for DNA (Figure 5C and D). We made clones of *osa* mutant cells in the salivary gland to determine whether Osa was required for this localization. Chromosomes from cells within these clones showed no anti-Osa staining, demonstrating the specificity of the antibody, but retained a wild-type pattern of staining with anti-Brm (Figure 5F, G and H). This demonstrates that Osa is not required for the association of Brm complexes with chromosomal DNA.

An activation domain is sufficient for some Osa functions

The above results show that *osa* interacts genetically with *brm*, and that Osa is a component of a large multiprotein complex containing Brm. Brm-related complexes are thought to promote transcription by altering the architecture of nucleosomal DNA, thus generating a conformation that is more favorable to binding by transcription factors and the basal transcriptional machinery. Some genes, such as *even-skipped*, show reduced levels of expression in *osa* mutant embryos, supporting the role of Osa as an activator of gene expression. However, other genes, such as *engrailed*, show expanded domains of expression in *osa* mutants (Treisman *et al*., 1997). These genes could be directly activated or repressed by Osa, or their changes in expression level could be secondarily due to the regulation of other transcription factors by Osa. The lack of specificity of DNA binding by Osa *in vitro* prevented us from demonstrating direct action by altering Osa binding sites in the promoters of potential target genes. As an alternative approach, we sought to preserve Osa's target specificity *in vivo* and to determine the effect of making it an obligate activator or repressor of transcription. We therefore fused either the exogenous activator domain of VP16 or the repressor domain of Engrailed to the DNA-binding domain of Osa (Figure 6A). The effects of misexpressing these activator (UAS-*osaAD*) and repressor (UAS-*osaRD*) forms of Osa under the control of the GAL4-responsive UAS sequences were compared with those caused by misexpressing the full-length wild-type Osa protein. The Osa DNA-binding domain appeared to be sufficient for chromosomal localization of these fusion proteins, as an antibody to VP16 detected the OsaAD protein along the length of polytene chromosomes (data not shown).

The notum of the adult fly contains a regular pattern of small (microchaetae) and large (macrochaetae) bristles (Figure 6B). Expression of the *osa* transgenes in the developing notum using a GAL4 insertion in the *pannier* (*pnr*) gene resulted in a dominant alteration of bristle formation. Ectopic expression of *osa* caused the loss of both micro- and macrochaetae, and defects in the midline of the notum, scutellum and abdomen (Figure 6C). Expression of UAS-*osaAD* with the same GAL4 driver led to a very similar phenotype (Figure 6D), and co-expression of UAS-*osa* and UAS-*osaAD* induced a stronger, apparently additive phenotype (Figure 6E). Expression of UAS-*osaRD* with *pnr*-GAL4 had the opposite effect, inducing the formation of ectopic macrochaetae on the notum (Figure 6F). Co-expression of UAS-*osa* with UAS-*osaRD* rescued the bristle loss phenotype caused by the expression of UAS-*osa* alone (Figure 6G). Thus, targeting an activation domain to Osa-regulated genes has an effect similar to overexpression of the full-length protein, while a repressor domain has the opposite effect.

In the wing, expression of UAS-*osaRD* with *omb*-GAL4 produced ectopic campaniform sensillae and wing margin bristles (Figure 6H). This phenotype was enhanced in flies heterozygous for *osa* (Figure 6I), suggesting that it results from interference with wild-type *osa* function. It is also very similar to the effect of expression of dominant-negative *brm* (Elfring *et al*., 1998). Expression of UAS-*osaAD* caused the opposite phenotype, loss of campaniform sensillae (Figure 6J). Expression of full-length *osa* with this driver resulted in dominant pupal lethality; although a small number of flies expressing *osa* did eclose, their wings were deformed, making a phenotypic comparison difficult.

The observation that UAS-*osaAD* and UAS-*osaRD* cause specific phenotypes in the developing wing disc, related to those caused by full-length Osa, implies that the DNA-binding domain of Osa has functional specificity in spite of its lack of DNA sequence specificity *in vitro*. Binding to other proteins could contribute to its ability to act on specific promoters. Expressing the DNA-binding domain alone had no effect (data not shown), suggesting that its promoter interactions are not strong enough to compete significantly with endogenous Osa. The similar effects of UAS-*osa* and UAS-*osaAD* and opposite effects of UAS-*osaRD* also indicate that, in the wing imaginal disc, Osa functions as an activator of gene expression.

Discussion

Osa associates with the Brm complex

We have found that the Osa protein co-immunoprecipitates and co-sediments with the Brm and Snr1 proteins, indicating that Osa associates with the Brm complex. Although Osa was not previously found as a component of the purified complex, some large unidentified proteins were co-purified and one of these could correspond to Osa (Papoulas *et al*., 1998). It has been reported that a human protein, p270, which has not been completely sequenced but also has an ARID domain, is associated with the BRG1 complex (Dallas *et al*., 1998). However, it is unlikely that Osa is an essential subunit of every Brm complex. A number of proteins are precipitated with anti-Brm but not anti-Osa. While it is possible that the binding of anti-Osa antibody to its epitope prevents the association of these proteins with Osa, or that these proteins bind the anti-Brm antibody rather than Brm itself, the simplest explanation is that Brm is a component of at least two distinct complexes and that these proteins are present in

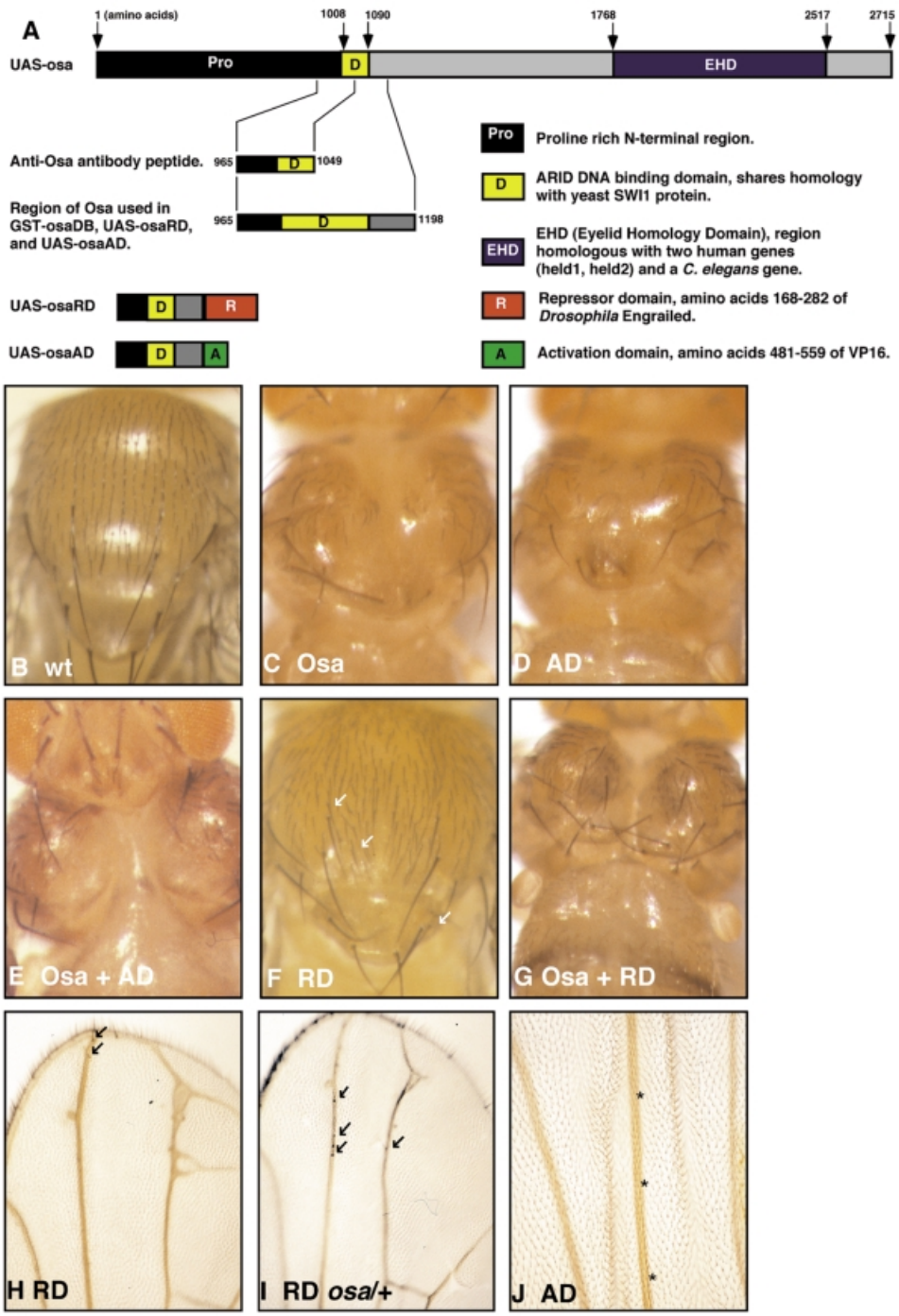

Fig. 6. Phenotypes induced by expression of *osa* transgenes. (**A**) Diagram of the primary structure of Osa protein and proteins generated by transgenic constructs. Also shown are the regions of Osa fused to GST for the generation of anti-Osa antibody and the GST–osaDB fusion protein. Photomicrographs of adult dorsal thorax (B–G) and wings (H–J) from wild-type (**B**), *pnr*-GAL4/UAS-osa^{d3} (**C**), UAS-$osaAD^{8b}$/+;*pnr*-GAL4/UAS-$osaAD^{20e}$ (**D**), UAS-$osaAD^{8b}$/+;*pnr*-GAL4/UAS-osa^{d3} (**E**), UAS-$osaRD^{13a}$/+;*pnr*-GAL4/+ (**F**), UAS-$osaRD^{13a}$/+;*pnr*-GAL4/UAS-osa^{d3} (**G**), *omb*-GAL4/+;UAS-$osaRD^{11c}$/+ (**H**), *omb*-GAL4/+;UAS-$osaRD^{11c}$/osa^{eld308} (**I**) and *omb*-GAL4/+;UAS-$osaAD^{5a}$/+ (**J**) flies. Ectopic expression of UAS-*osa* with the *pnr*-GAL4 driver induces the loss of microchaetae (small bristles) near the dorsal midline, and a loss of macrochaetae (large bristles), particularly the dorsocentrals and scutellars (C). Expression of two copies of UAS-*osaAD* with *pnr*-GAL4 induces a similar phenotype (D), and co-expression of UAS-*osa* and UAS-*osaAD* induces a stronger phenotype than either alone (E). The loss of macrochaetae induced by expression of UAS-*osa* with *pnr*-GAL4 is suppressed by co-expression of UAS-*osaRD* (G); all four dorsocentrals and all four scutellars are present. Expression of UAS-*osaRD* induces the formation of ectopic macro- and microchaetae (F). Expression of UAS-*osaRD* with *omb*-GAL4 induces the formation of ectopic wing margin bristles and a disruption of wing vein morphology (H). The margin bristle phenotype is enhanced in flies heterozygous for *osa* (I). Ectopic expression of UAS-*osaAD* with *omb*-GAL4 results in the loss of campaniform sensillae normally found on vein L3 (J). White arrows in (F) indicate ectopic macrochaetae, black arrows in (H) and (I) indicate ectopic margin bristles, and asterisks in (J) indicate the approximate positions where campaniform sensillae would normally be found.

Brm complexes from which Osa is absent. This would be consistent with the different phenotypic effects caused by *osa* and *brm* mutations. Mutations in *brm* or *mor* cause greatly reduced cell growth and viability, as well as oogenesis defects that prevent the production of maternally mutant embryos (Brizuela *et al*., 1994; Brizuela and Kennison, 1997; Elfring *et al*., 1998). In contrast, cells mutant for a null allele of *osa* are able to proliferate, and maternal *osa* is required for normal embryogenesis but not for oogenesis (Treisman *et al*., 1997; Vazquez *et al*., 1999). Genetic interactions between *osa* and components of the Brm complex show some tissue specificity; in the wing, loss of *osa* and loss of *brm* enhance each other, but in the eye, loss of *brm* enhances the effect of *osa* overexpression.

Osa is thus unlikely to be a strict functional homolog of SWI1, which is essential for the stability and function of the yeast SWI/SNF complex (Peterson and Herskowitz, 1992). Alternatively, other subunits of the *Drosophila* complex not present in yeast may be partially redundant with Osa; one possibility is the HMG box protein BAP111 (Papoulas *et al*., 1998), which has a vertebrate counterpart, BAF57 (Wang *et al*., 1998). Brm is still stable and localized to chromatin in the absence of Osa; it has recently been reported that an AT-hook DNA-binding domain is present in human and yeast homologs of Brm and contributes to their association with chromatin (Bourachot *et al*., 1999). Other possible explanations are that this localization could be mediated by the above DNA-binding proteins, or could be due to interactions of other subunits of the complex with protein components of chromatin; a third alternative is that the remaining Brm is present in complexes that do not contain Osa. As only SWI2/SNF2, SWI3 and SNF5-related subunits are essential for chromatin remodeling *in vitro* (Phelan *et al*., 1999), other DNA-binding proteins may play a specificity-determining or regulatory role.

The observation that overexpression of *osa* has effects opposite to those caused by its loss of function suggests that despite its widespread expression (Treisman *et al*., 1997) Osa is limiting *in vivo*; this is probably also the case for SWI1 (C.Peterson, personal communication). Interestingly, an excess of Osa can compensate for the presence of dominant-negative Brm in the wing, suggesting that other components are not limiting, so that increasing Osa is sufficient to restore the normal number of functional complexes. Overexpressing *osa* in a wild-type background may increase the number of Brm complexes carrying out Osa-regulated functions. Reducing *brm* function in the wing ameliorates the effects of excess *osa*; since ectopic *osa* requires *brm* for its activity, it is likely to be acting through the Brm complex rather than by an alternative mechanism. However, in the eye disc Osa antagonizes Brm function, perhaps redirecting Brm into the Osa-containing type of complex and thus reducing the number of complexes that do not contain Osa. The number of these complexes would be further reduced by lowering the level of Brm.

The Osa DNA-binding domain is not sequence-specific but has some target specificity in vivo

We have shown that Osa contains a functional DNA-binding domain that has no apparent sequence specificity beyond a preference for AT-rich regions. This DNA-binding domain thus resembles that of the related MRF (Huang *et al*., 1996), as well as reproducing some properties of the intact SWI/SNF complex that could reflect binding by SWI1; the major band that can be cross-linked to DNA has a molecular weight similar to that of SWI1 (Quinn *et al*., 1996). The similar domains present in Bright and Dri do have a consensus recognition sequence (Herrscher *et al*., 1995; Gregory *et al*., 1996); this specificity may be provided by an extended region of homology between the two proteins that is not conserved in Osa. Although we have not been able to produce full-length Osa protein *in vitro*, it is unlikely that another region of the protein confers sequence-specific recognition, as we observe binding of endogenous Osa protein along the entire length of the polytene chromosomes rather than to specific bands. However, it is possible that association of Osa with protein components of chromatin contributes to this staining pattern.

Several observations suggest that Osa has more specific effects on gene expression than its DNA-binding capability would predict. First, many genes are expressed normally in *osa* maternally and zygotically mutant embryos (Treisman *et al*., 1997), although we would expect Osa to be able to bind to every promoter region. Secondly, *osa* dosage affects the phenotype caused by *Antennapedia* (*Antp*) misexpression in the antennal disc only when this misexpression is driven by the *Antp* P2 promoter and not when it is driven by the *hsp70* or *sas* promoters, showing that Osa has promoter-specific effects (Vazquez *et al*., 1999). Finally, the Osa ARID domain, which is sufficient to bind any DNA *in vitro*, appears to target an activation or repression domain to only a specific subset of genes. This suggests that Osa's access to DNA *in vivo* may depend on interaction with other factors. The *in vivo* specificity observed for the isolated DNA-binding domain implies that this domain is sufficient for some such interactions. Interestingly, the ARID domain is the only region of homology between Osa and SWI1 and may therefore contain the determinants necessary for incorporation into the SWI/SNF or BRM complex. Alternatively, its interactions with other DNA-bound proteins could allow it to associate with specific promoters. Further experiments will be necessary to address this issue.

Osa can promote transcriptional activation

Lack of *osa* in the embryo leads to the loss of expression of certain genes but to the misexpression of others (Treisman *et al*., 1997). To investigate whether Osa can directly activate or repress transcription, we compared the effects of gain or loss of *osa* function with those caused by expressing the Osa DNA-binding domain fused to exogenous transcriptional activator or repressor domains. Although these Osa fusion proteins may not use the same mechanism as wild-type Osa to affect gene expression, the similarity of observed phenotypes allows us to infer the direction of the changes in gene expression normally caused by Osa. The results show that expression of the activator form in the wing or notum resembles overexpression of the full-length protein, while expression of the repressor form resembles and is enhanced by loss of *osa* function. This suggests that during wing disc development Osa functions to activate gene expression. However, the

effects of expressing these transgenes in other tissues suggest that Osa may also act to repress gene expression in some contexts (R.T.Collins, unpublished data).

The yeast SWI/SNF complex may likewise function both to activate and to repress transcription; genome-wide analysis shows that more genes show elevated rather than reduced expression in a *swi2* mutant strain (Holstege *et al*., 1998), although it is not known which are direct targets. The related yeast complex RSC appears to repress at least one identified target (Moreira and Holmberg, 1999). Human hBRM has also been shown to assist Rb to repress E2F1-regulated transcription (Trouche *et al*., 1997). It is also possible that Osa represses transcription by antagonizing the activity of the Brm complex rather than by facilitating its normal function. It is not clear how the multiple chromatin remodeling complexes present in the cell may interact. Although we did not detect any physical association of Osa with Ash2, which is in a complex smaller than the Brm complex (Papoulas *et al*., 1998), the absence of *osa* can rescue the oogenesis defect of *ash2* mutants (our unpublished data). Thus, the two complexes may have common target genes in addition to the homeotic genes.

We have found that many of the phenotypes of *osa* mutations resemble those caused by ectopic *wingless* (*wg*) expression, although *wg* itself is not ectopically expressed in *osa* mutant cells (Treisman *et al*., 1997). We now have additional evidence that *wg* target genes are activated in the absence of *osa* and repressed in the presence of excess *osa* (R.T.Collins and J.E.Treisman, manuscript in preparation). Because Wg signaling is transmitted by an HMG box transcription factor, dTCF (Brunner *et al*., 1997; Riese *et al*., 1997; van de Wetering *et al*., 1997), vertebrate homologs of which have been shown to act by bending DNA to bring other transcription factors into proximity (Giese *et al*., 1992), it is likely to be sensitive to chromatin structure (Sheridan *et al*., 1995). We suggest that Osa maintains a chromatin structure incompatible with transcription on *wg*-regulated genes, and that in the absence of Osa these promoters can be stimulated even without the activation domain contributed by Armadillo (van de Wetering *et al*., 1997). Interactions such as these between developmental signals and chromatin architecture are of great interest in understanding the different responses of different cells to such signals.

Materials and methods

DNA binding

A fragment of Osa encoding amino acids 965–1198 was amplified using PCR primers that introduced *Bam*HI and *Eco*RI sites, and was subcloned into pGEX-4T-1 (Pharmacia) to generate GST–osaDB. Recombinant protein was expressed in *Escherichia coli* and purified on glutathione–agarose beads. Purified protein was either retained on the beads or eluted with 5 mM glutathione in 50 mM Tris pH 8.0. For the gel mobility shift assay, 200 ng of ^{32}P end-labeled oligonucleotide consisting of three copies of the engrailed homeodomain consensus binding sequence (Desplan *et al*., 1988), NP_3 [$(GATCTCAATTAAT)_3$], incubated with 250 ng of purified GST–osaDB and increasing amounts of either anti-Osa (Treisman *et al*., 1997) or anti-En (DiNardo *et al*., 1985) antibody in gel shift buffer [20 mM HEPES pH 7.6, 1 mM EDTA, 10 mM $(NH_2)SO_4$, 1 mM dithiothreitol (DTT), 0.2% Tween-20 and 200 mM KCl] supplemented with 50 μg/ml sonicated salmon sperm DNA and 25 μg/ml bovine serum albumin (BSA) for 30 min at room temperature. Protein–DNA complexes were separated on an 8% non-denaturing polyacrylamide gel and exposed for autoradiography. For the GST pulldown assay, lambda DNA was digested with *Hin*dIII and *Sau*3AI and the fragments were end labeled with [^{32}P]dATP. Labeled DNA (0.8 μg) was incubated with 50 ng of GST–DB or GST–E47 (provided by Xiao-Hong Sun) bound to glutathione–agarose beads for 1 h at 4°C in binding buffer [gel shift buffer supplemented with 25 μg/ml each BSA, poly(dI–dC) and poly(dA–dT)]. The beads were washed three times with binding buffer at 4°C, and bound DNA was eluted by boiling in formamide loading buffer (90% formamide, 1× TBE, 0.04% bromophenol blue, 0.04% xylene cyanol) and separated on a 6% sequencing gel followed by autoradiography. The random oligonucleotide selection assay (SELEX) was performed as described (Jun and Desplan, 1996).

Immunoprecipitation and glycerol gradient sedimentation

Drosophila Schneider cell nuclear extracts were prepared as described (Pugh, 1995). Immunoprecipitations and washes were carried out in HEM buffer (25 mM HEPES–KOH pH 7.9, 0.1 mM EDTA, 12.5 mM $MgCl_2$) containing 0.3 M KCl and 20% glycerol. Glycerol gradient sedimentation was carried out according to Tanese (1997). Nuclear extract in buffer C (20 mM HEPES–KOH pH 7.9, 0.42 M NaCl, 0.2 mM EDTA, 1.5 mM $MgCl_2$, 0.5 mM DTT, 25% glycerol) was diluted to <10% glycerol. Approximately 1.25 mg (in 250 μl) of nuclear extract was applied to a 5 ml, 10–30% gradient of glycerol in HEM buffer plus 0.1 M KCl. Samples were centrifuged in a Beckman SW50.1 rotor at 43 000 r.p.m. for 13 h at 4°C. Twenty 250 μl fractions were collected from top (F1) to bottom (F20) of each gradient. Proteins in odd-numbered fractions (75 μl) were precipitated with trichloroacetic acid and separated by SDS–PAGE. Immunoblotting with α-Osa, α-Brm, α-Snr1, α-ISWI and α-Ash2 was performed on the protein samples prepared from the same gradient run. Molecular mass standards were run in a parallel gradient. For immunoprecipitations, 250 μl of even-numbered gradient fractions were used to immunoprecipitate proteins with α-Osa antibody followed by sequential immunoblotting with α-Osa, α-Brm and α-Snr1 antibodies. For silver staining of Osa and Brm complexes, glycerol gradient fractions F18–F20 from two parallel gradients were pooled (1200 μl), divided into three and each pooled fraction was incubated with either α-Osa antibody-conjugated protein A–Sepharose, α-Brm antibody and protein A–Sepharose, or protein A–Sepharose alone.

Immunohistochemistry

For immunostaining of polytene chromosomes, salivary glands from climbing third instar larvae were dissected into PBT [phosphate-buffered saline (PBS), 0.2% Triton X-100] and fixed for 30 s in a drop of 3.7% formaldehyde in PBS, 1% Triton X-100. Glands were then transferred to a drop of 3.7% formaldehyde, 50% acetic acid for 5 min and squashed under glass cover slips. Slides were frozen in liquid N_2 and cover slips flipped off. Chromosomes were blocked for 1 h at 4°C in PBT, 10% normal donkey serum (PBSTS) and incubated overnight at 4°C with anti-Osa antibody (diluted 1:1 in PBT), anti-Brm antibody (Elfring *et al*., 1998; diluted 1:5 in PBSTS), anti-VP16 antibody (Santa Cruz Biotechnology; diluted 1:50 in PBSTS) or anti-Msl-1 antibody (provided by Bruce Baker; diluted 1:100 in PBSTS). Slides were washed three times for 15 min at room temperature with PBT and incubated with FITC-conjugated donkey anti-rabbit (for Brm), or Texas Red-conjugated donkey anti-mouse (for Osa and VP16), or anti-rat (for Msl-1) secondary antibody (diluted 1:200 in PBSTS) for 1 h at room temperature. After three 15 min washes at room temperature in PBT slides were rinsed in 0.1 M phosphate buffer, 0.2% Triton X-100. Chromosomes were counterstained with DAPI and observed with a Leica TCS NT confocal microscope. Adult wings were mounted in Canada balsam:methyl salicylate (2:1).

Generation of transgenic strains

To generate UAS-*osa*, the sequence encoding the full-length Osa open reading frame with 453 bp of 5′UTR and 754 bp of 3′UTR was subcloned in three steps using *Eco*RI and *Xba*I sites into the P-element transformation vector pUAST. The other constructs, also in pUAST, contained 453 bp of 5′UTR and the first seven amino acids of Osa (MNEKIKS) followed by amino acids 965–1198. For UAS-*osaAD*, these Osa sequences were followed by amino acids 481–559 of VP16 (Sadowski *et al*., 1988). For UAS-*osaRD*, the same sequences were followed by amino acids 168–282 of *Drosophila* Engrailed (Tolkunova *et al*., 1998). All fragments were generated by PCR (details available on request). Transgenic strains carrying the transgenes UAS-*osa*, UAS-*osaDBD*, UAS-*osaRD* and UAS-*osaAD* were generated by P-element-mediated transformation as described (Rubin and Spradling, 1982). Several independent insertions of each construct were examined.

Genetics

Alleles and other transgenic lines used were *osa*^{eld308} (Treisman *et al*., 1997), *brm*^{2} (Tamkun *et al*., 1992), *mor*^{1}, *trx*^{E2} (Kennison and Tamkun, 1988), *snr1*^{P1} (Dingwall *et al*., 1995), *ash1*^{22} (Tripoulas *et al*., 1994), *ash2*^{1} (Adamson and Shearn, 1996), UAS-*brm*^{K804R} (Elfring *et al*., 1998), *omb*-GAL4 (Lecuit *et al*., 1996), *vg*-GAL4 (Simmonds *et al*., 1995), *ey*-GAL4 (Hazelett *et al*., 1998), *pnr*-GAL4 (Calleja *et al*., 1996) and UAS-*FLP* (Duffy *et al*., 1998). To make *osa* mutant clones in salivary glands, males of genotype *FRT82*,*osa*^{eld308}/*TM6B* were crossed to females of genotype *w,hsFLP122*; *FRT82*, *Sb*, *hs-[pi]myc* and the larvae were heat shocked at 38.5°C for 1 h during first instar to induce expression of *hsFLP*. Recombinant chromosomes carrying *FRT82*, *osa*^{eld308} and either *mor*^{1}or *ash2*^{1} were used for analysis of double-mutant phenotypes.

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